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Evidence for the genetic control of antibody affinity from breeding studies with inbred mouse strains producing high and low affinity antibody

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Summary. The amount (Ab_t) and relative affinity (K_R) of antibody produced in response to protein antigens injected in saline has been measured in the parents, F_1 hybrids and backcross offspring of inbred mice which produce high and low K_R antibody to these antigens. The results obtained support the view that antibody affinity is under polygenic control. Furthermore, strain related variation in Ab_t is independent of K_R and the breeding experiments indicate that these two parameters are under independent genetic control.

INTRODUCTION

The ability to respond to a range of natural and synthetic antigens by the production of specific immune responses is under autosomal dominant genetic control (McDevitt and Benacerraf, 1969) and in many instances there is an association between immune response genes and the histocompatibility antigens of the species concerned (Benacerraf and McDevitt, 1972). Biozzi and his colleagues (Biozzi, Stiffel, Mouton, Bouthillier and Decreusefond, 1968) have confirmed the existence of genetic control of the amount of antibody produced by selectively breeding two lines of mice from a random bred population which were either quantitatively good or poor at antibody production to erythrocyte antigens. We have recently reported evidence from selective breeding experiments which shows that antibody quality (or affinity) is a genetically controlled parameter of the immune response (Katz and Steward, 1975). In this paper we report the results of more extensive genetic studies which were carried out to investigate the genetic control of antibody affinity.

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MATERIALS AND METHODS

Mice. Inbreeding and production of F_1 and backcross offspring from AJAX; B10D2 new line; SWR/J; and Simpson mice was carried out in the Animal Unit of the Kennedy Institute of Rheumatology.

Antigens. Human serum albumin HSA (Pentex) and human serum transferrin (HST) (Sigma Chemical Company Ltd) were passed through a column of Sephadex G-200 and the peak fractions pooled and used for subsequent immunizations.

Immunizations. Mice aged 2–3 months of both sexes were injected intraperitoneally once a week for 4 weeks with 1 mg of either HSA or HST in 0·1 ml saline. In addition, 50 μg anthisan® was injected prior to the second, third and fourth immunizations to reduce deaths due to anaphylaxis particularly in

the SWR/J strain. Serum was obtained from blood drawn by cardiac puncture under ether anaesthesia 2 weeks after the last immunization and stored at −20° prior to assay.

Antibody determinations. The amount (Ab_t) and relative affinity (K_R) of antibodies produced to ^{125}I-labelled HSA or HST were determined by an ammonium sulphate globulin precipitation technique as previously described (Steward and Petty, 1972b). In the latter part of this study a modification of this technique utilizing a ^{22}Na volume marker was utilized (Gaze, West and Steward, 1973).

RESULTS

Antibody responses in F_1 hybrids of AJAX and B10D2 new strains

The K_R and Ab_t of antibody to HSA and HST produced by AJAX; B10D2 new and F_1 offspring of these two strains are shown in Figs 1 and 2 and Tables 1 and 2. The mean K_R and Ab_t of antibody in all F_1 mice for each antigen were intermediate between those of the two parents.

With both antigens the incidence of non-response was higher in those F_1 offspring where the B10D2 new strain was the male parent (Tables 1 and 2). The levels of antibody produced to both antigens in the F_1 hybrids are shown in Figs 1(b) and 2(b) and in Tables 1 and 2. It is apparent that the F_1 hybrids immunized with HSA or HST produced levels of antibody closer to those produced by the AJAX parent strain than to those of the B10D2 new parent strain.

For both antigens, the K_R of antibody was lower in the (AJAX♀ × B10D2♂)F_1 than in the (AJAX♂ × B10D2♀)F_1 hybrids although in the F_1 of all crosses there were no significant differences between male and female mice. Furthermore, these Ab_t values are independent of the K_R of the antibody (correlation coefficient = −0·15, for antibodies to HST and −0·30 for antibody to HSA; both values representing no significant correlation).

Antibody responses in F_1 hybrids of Simpson (high K_R) × SWR/J (low K_R) mice

Because of the relatively high incidence of animals not producing detectable antibody in the B10D2 new × AJAX crosses and the fact that the low K_R strain (B10D2, new) produced low levels of antibody, confirmation of the B10D2 × AJAX data was sought by studying the F_1 hybrids of a strain producing high levels of low K_R antibody (SWR/J) and another high K_R strain (Simpson). The K_R and Ab_t values in these F_1 hybrids were determined using the more

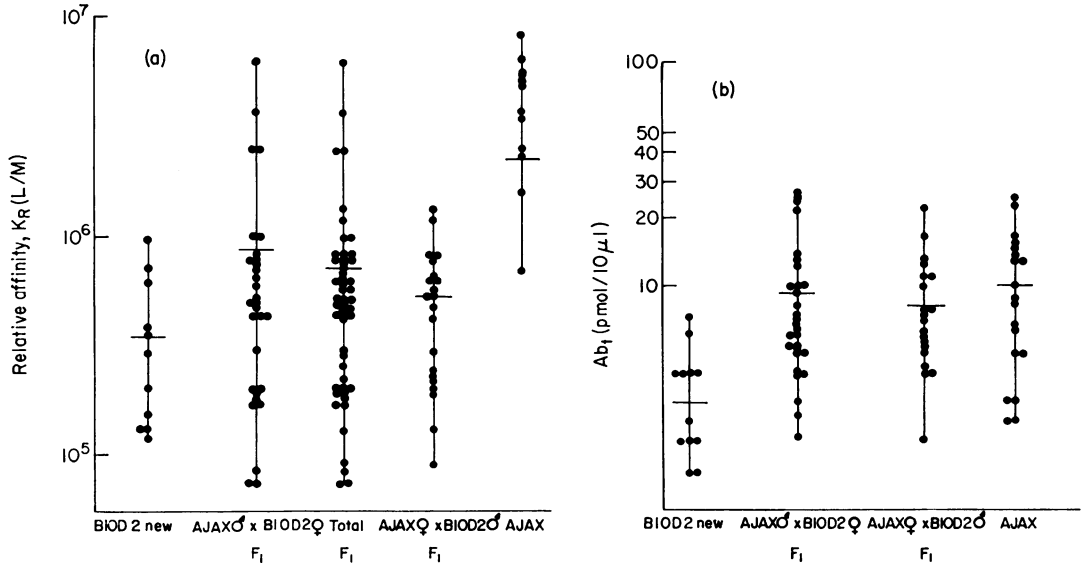

Figure 1. The antibody response to HST in AJAX and B10D2 new inbred mice and their F_1 hybrids: (a) relative affinity, K_R; (b) antibody levels, Ab_t.

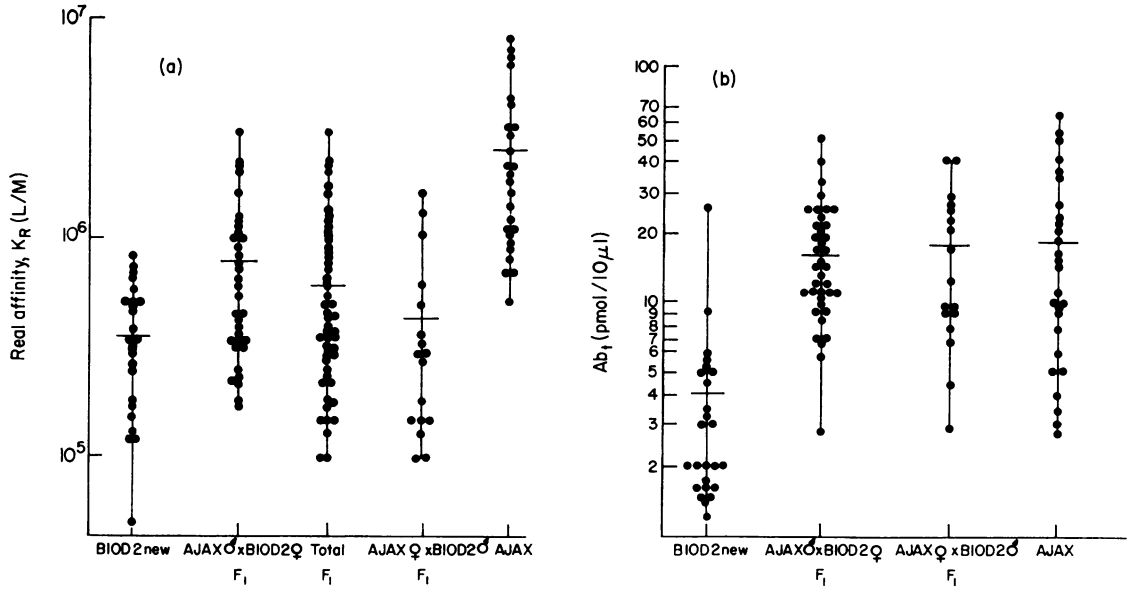

Figure 2. The antibody response to HSA in AJAX and B10D2 new inbred mice and their F_1 hybrids: (a) relative affinity, K_R; (b) antibody levels, Ab_t.

Table 1. The amount and relative affinity of antibody to HST in parents and F_1 hybrids of 'high K_R' and 'low K_R' mice

	No. of mice	No. of responders	No. NDA*	Mean Ab_t (pmol/10 μl)	Mean K_R (L/M)
AJAX	19	18	1 (5·3)	10·4	$3·4\times10^6$
B10D2 new	30	11	19 (63·3)	3·0	$3·6\times10^5$
(AJAX♂ × B10D2♀)F_1	43	30 (+8)†	5 (11·6)	9·4	$8·9\times10^5$
(AJAX♀ × B10D2♂)F_1	36	21 (+1)	14 (38·9)	8·3	$5·4\times10^5$
Total F_1 hybrids	79	51 (+9)	19 (24·0)	8·9	$7·2\times10^5$

* NDA: mice not producing detectable antibody. Values in parentheses refer to percentage of animals not producing detectable antibody.

† Numbers in parentheses refer to mice producing antibody but K_R values not calculable.

Table 2. The amount and relative affinity of antibody to HSA in parents and F_1 hybrids of 'high K_R' and 'low K_R' mice

	No. of mice	No. of responders	No. NDA*	Mean Ab_t (pmol/10 μl)	Mean K_R (L/M)
AJAX	24	23	1 (4·2)	19·1	$2·6\times10^6$
B10D2 new	58	26	32 (55·2)	4·0	$3·7\times10^5$
(AJAX♂ × B10D2♀)F_1	57	36 (+10)†	11 (19·3)	15·1	$7·9\times10^5$
(AJAX♀ × B10D2♂)F_1	32	17 (+5)	10 (31·3)	17·1	$4·5\times10^5$
Total F_1 hybrids	89	53 (+15)	21 (23·5)	16·1	$6·2\times10^5$

* NDA: mice not producing detectable antibody. Values in parentheses refer to percentage of animals not producing detectable antibody.

† Numbers in parentheses refer to mice producing antibody but K_R values not calculable.

Table 3. The amount and relative affinity of antibody to HST in parents and F_1 hybrids of Simpson and SWR/J mice

	No. of mice	No. of responders	No. NDA*	Mean Ab_t (pmol/10 μl)	Mean K_R (L/M)	Coefficient of correlation (r) of Ab_t and K_R
Simpson	22	22	0	28·0	$2{\cdot}7\times10^6$	0·26 (n.s.)‡
SWR/J	6	6	0	34·0	$6{\cdot}0\times10^5$	−0·35 (n.s.)
(Simpson♂ × SWR/J♀)F_1	49	45 (+1)	3	29·2	$1{\cdot}7\times10^6$	0·08 (n.s.)
(Simpson♀ × SWR/J♂)F_1	42	41	1	37·8	$2{\cdot}0\times10^6$	0·46 (P = 0·01)
Total (Simpson × SWR/J)F_1 hybrids	91	86 (+1)†	4 (4·4)	33·3	$1{\cdot}8\times10^6$	0·30 (P = 0·01)

* NDA: mice not producing detectable antibody. Values in parentheses refer to percentage of animals not producing detectable antibody.

† Number in parentheses refers to mouse producing antibody, but K_R incalculable.

‡No significant correlation.

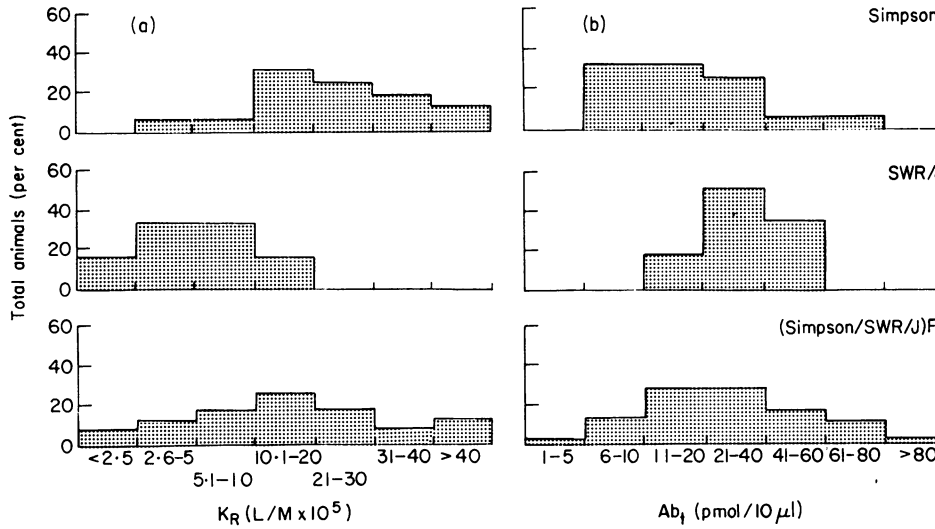

Figure 3. The antibody response to HST in Simpson and SWR/J inbred mice and their F_1 hybrids: (a) relative affinity, K_R; (b) antibody levels, Ab_t.

convenient double isotope technique (Gaze *et al.*, 1973). The data are shown in Table 3 and Fig. 3. As shown in Fig. 3(a), the range of values for K_R in the F_1 extended from the lower end of the low K_R parent strain values to the upper end of the high K_R parent strain values. The mean K_R of the F_1 hybrids was intermediate between that of the two parent strains. A similar range of values for Ab_t was also obtained (Fig. 3b) but the mean Ab_t of the F_1 hybrids was closer to that of the parent producing higher Ab_t levels (SWR/J). K_R values were independent of Ab_t values in the Simpson, SWR/J and (Simpson ♂ × SWR/J ♀)F_1 but were correlated in the (Simpson ♀ × SWR/J ♂)F_1 hybrids (Table 3).

Antibody responses in F_1 and backcrosses of AJAX × B10D2 new line mice

The results presented thus far indicate a genetic control of antibody affinity which is independent of the amount of antibody produced. A more extensive genetic study was carried out involving the determination of K_R and Ab_t of antibody to HST in F_1 and (F_1 × AJAX) and (F_1 × B10D2) backcross animals utilizing the double isotope assay. The results of these studies are shown in Fig. 4 and Tables 4 and 5.

When the F_1 hybrids were backcrossed to the high K_R parent (AJAX), anti-HST antibody produced by the resulting offspring had a distribution of affinities

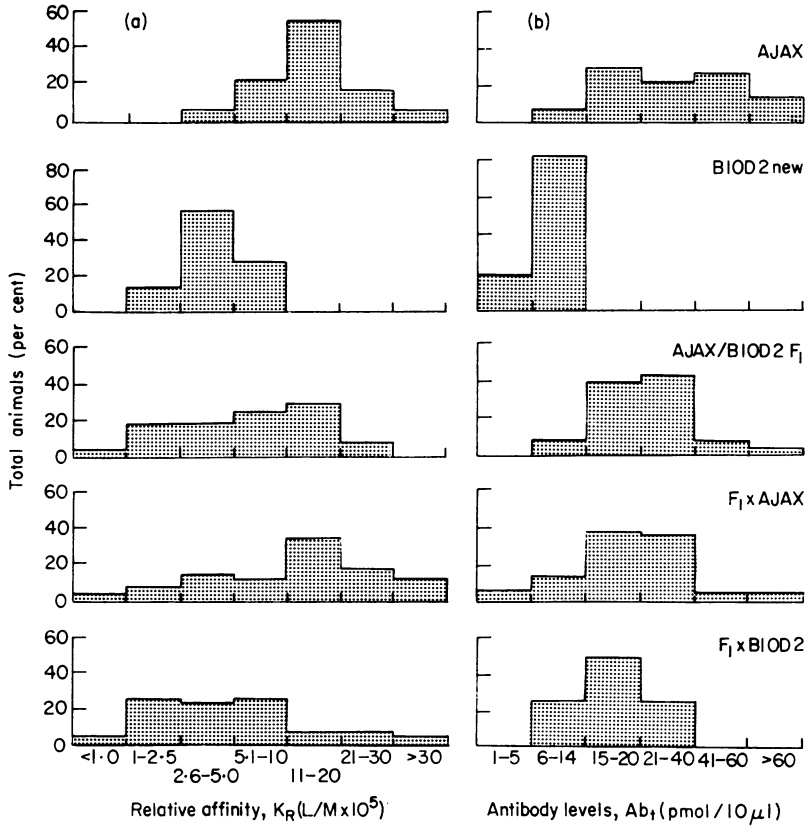

Figure 4. The antibody response to HST in AJAX and B10D2 new inbred mice and their F_1 hybrids and backcross offspring: (a) relative affinity, K_R; (b) antibody levels, Ab_t.

Table 4. The amount and relative affinity of antibody to HST in parents, F_1 hybrids and backcrosses of AJAX and B10D2 new mice

	No. of mice	No. of responders	No. NDA*	Mean Ab_t (pmol/10 μl)	Mean K_R (L/M)	Coefficient of correlation (r) of Ab_t and K_R
AJAX	28	27	1 (3·6)	37·0	$1·5 \times 10^6$	0·21 (n.s.)‡
B10D2 new	27	7	20 (74)	6·7	4.7×10^5	−0·5 (n.s.)
(AJAX × B10D2)F_1	52	19 (+14)†	19 (36·5)	27·8	$1·1 \times 10^6$	−0·36 (n.s.)
F_1 × B10D2 new	90	39 (+9)	42 (47)	15·8	$8·1 \times 10^5$	0·18 (n.s.)
F_1 × AJAX	61	58 (+1)	2 (3·3)	21·4	$1·7 \times 10^6$	0·03 (n.s.)

* NDA: mice not producing detectable antibody, values in parentheses refer to per cent NDA animals.
† Numbers in parentheses refer to mice producing antibody but K_R values not calculable.
‡ No significant correlation.

Table 5. *P* values from statistical analysis by Student's *t*-test of differences in K_R and Ab_t in parents, F_1 hybrids and backcrosses of AJAX and B10D2 mice

Comparison	K_R	Ab_t
AJAX *v.* B10D2	0·005	0·005
AJAX *v.* F_1	0·05	0·15
B10D2 *v.* F_1	0·025	0·01
(AJAX × F_1) *v.* F_1	0·05	0·10
(AJAX × F_1) *v.* AJAX	0·25	0·001
(B10D2 × F_1) *v.* F_1	0·05	0·005
(B10D2 × F_1) *v.* B10D2	0·20	0·005
(AJAX × F_1) *v.* (B10D2 × F_1)	0·001	0·025

(Fig. 4) which was not significantly different from that of the AJAX parents ($P = 0{\cdot}25$, Student's *t*-test) (Table 5). Anti-HST antibody produced by offspring of the F_1 × low K_R parent (B10D2) had a distribution of affinities (Fig. 4) which was very similar ($P = 0{\cdot}20$) to that of the B10D2 parents (Table 5). However, both AJAX × F_1 and B10D2 × F_1 backcrosses had anti-HST affinity distributions which were significantly different from that of the F_1 animals ($P = 0{\cdot}05$, Table 5).

Ab_t values, however, did not show the same trend as that observed for K_R and the results of statistical analysis of Ab_t values in F_1 and backcrosses bore no relationship to those obtained for K_R values (Table 5). The mean Ab_t values in the F_1 hybrids was similar to that of the high K_R parent, and confirms the results shown in Table 2 and Fig. 2. The (B10D2 × F_1) backcrosses had a mean Ab_t intermediate between the F_1 and B10D2 mice but the mean Ab_t of the (AJAX × F_1) backcrosses was lower than that of the F_1. The correlation coefficients of K_R and Ab_t in these experiments are shown in Table 4 and confirm the independence of these two parameters of the antibody response.

The incidence of animals not producing detectable antibody was greatest in male F_1 hybrids which confirms the previous observation with these mice (Table 2). In the backcross offspring, the incidence of such non-responders was greatest in the F_1 × B10D2 new backcross and particularly in the males.

DISCUSSION

On the basis of experiments with inbred mice selected according to their susceptibility or resistance to LCM virus induced immune complex disease (Oldstone and Dixon, 1969), we have suggested that susceptibility to immune complex disease may be an immunodeficiency phenomenon, in which susceptible individuals produce antibody of low affinity to the antigen(s) involved (Soothill and Steward, 1971). Such low affinity antibody would fail to eliminate antigen and thus the production and subsequent deposition of antigen excess complexes in the tissues would be favoured (Alpers, Steward and Soothill, 1972). The data on which this hypothesis was based showed that reproducible strain differences exist in the K_R of antibody produced to a range of antigens (Petty, Steward and Soothill, 1972) and indicated that this qualitative aspect of antibody production was genetically controlled.

We have therefore measured the K_R and Ab_t of antibody produced to protein antigens in F_1 hybrids and backcross offspring of inbred strains of mice which differ in these two parameters of the antibody response in order to investigate their mode of inheritance. The results of such measurements presented here confirm that the affinity of an antibody response is genetically controlled and indicate that such control is operating independently of that governing antibody levels.

If antibody affinity was genetically controlled by a single gene with two alleles, K for high affinity, and k for low affinity, then experiments of the type described in this paper would produce results shown in Table 6. However, application of this type of

Table 6. Theorectical genetic composition and generation mean affinity values in parents, F_1 hybrids and backcrosses if affinity was controlled by a single gene with two alleles K (high affinity) and k (low affinity)

Generation	Genetic composition	Expected mean K_R value
High K_R parent	KK	m+d
Low K_R parent	kk	m−d
F_1 hybrids	Kk	m+h
F_2	$\frac{1}{4}$KK+$\frac{1}{2}$Kk+$\frac{1}{4}$kk	m+$\frac{1}{2}$h
F_1 × high K_R	$\frac{1}{2}$Kk+$\frac{1}{2}$KK	m+$\frac{1}{2}$d+$\frac{1}{2}$h
F_1 × low K_R	$\frac{1}{2}$Kk+$\frac{1}{2}$kk	m−$\frac{1}{2}$d+$\frac{1}{2}$h

Where m = the average effect of other genes determining the phenotype, i.e. mean background effect; d = the total effect of allelic gene(s) K and k determining the characteristic, i.e. deviation from the mean background effect; h = dominance effect: if K is dominant h is positive if k is dominant, h is negative.

Table 7. Comparison of predicted and observed generation means of K_R and Ab_t on the basis of a single gene model

	(AJAX × F_1) backcross		(B10D2 × F_1) backcross	
	K_R (L/M)	Ab_t (pmol/10 μl)	K_R (L/M)	Ab_t (pmol/10 μl)
Predicted value	$1{\cdot}3 \times 10^6$	42·3	$0{\cdot}77 \times 10^6$	26·3
Observed value	$1{\cdot}7 \times 10^6$	21·4	$0{\cdot}81 \times 10^6$	15·8
Discrepancy	+0·4	−20·9	+0·04	−10·5

analysis to the data presented in this paper is difficult particularly in view of the considerable range of values of both K_R and Ab_t even in inbred mice. These variations are greater than methodological error (Steward and Petty, 1972a) and are perhaps not surprising since environmental factors such as diet (Passwell, Steward, and Soothill, 1974a), infection (Steward and Voller, 1973) and hormones Passwell, Steward and Soothill, 1974b) can influence both K_R and Ab_t. Nevertheless, values for K_R in the backcrosses predicted on the basis of the information in Table 6 were in very close agreement with observed values whereas the predicted and observed Ab_t values did not show agreement (Table 7). Although the results of such an analysis of generation means is consistent with K_R being controlled by a single gene, such an interpretation is unlikely for the following reasons: (a) the character d (Table 6) also measures the net effect of several allelic genes; (b) the two backcross generations do not show a biphasic distribution which is characteristic of a single gene effect (Table 6); (c) the progressive separation of two lines of mice (one 'high K_R' and the other 'low K_R') following selective breeding on the basis of antibody K_R (Katz and Steward, 1975) indicates that this function is under polygenic rather than single gene control—a single gene effect would have resulted in the separation of high and low K_R after one generation of selection; (d) since the measured value for antibody affinity is the mean of a heterogeneous distribution of affinities, it would seem unlikely that a single gene would be responsible for the production of a predominantly high or predominantly low K_R population of antibody molecules.

We consider that the following aspects of the data presented in this paper are consistent with the view that antibody affinity is under polygenic control: (1) the mean K_R of F_1 hybrids of high and low K_R strains was intermediate between that of the parents with both HSA and HST as antigens (Figs 1a, 2a and 3); (2) the range of values for K_R of anti-HST antibody in the F_1 hybrids was as great as the combined ranges of the parent strains and that for anti-HSA was only slightly restricted; and (3) the distribution of K_R values of antibody produced by offspring of (F_1 × low K_R parent) backcrosses was very similar to that of the low K_R parent and the distribution of K_R values produced by offspring of (F_1 × high K_R parent) backcrosses was similar to that of the K_R parent (Fig. 4a).

It appears that antibody levels (Ab_t) are controlled independently of K_R (Table 4) and that in crosses between a low Ab_t producer and a high Ab_t producer, the F_1 hybrids produce Ab_t levels close to those of the high Ab_t parent (Tables 1–4). Furthermore, in backcross offspring, the Ab_t values did not show the same trend as for K_R, although with (F_1 × low K_R) backcrosses, the mean Ab_t was intermediate between the F_1 and low K_R parents. Whilst there were no significant differences between K_R and Ab_t in male and female mice, in (AJAX × B10D2)F_1 and backcross offspring the incidence of animals not making detectable antibody was highest in males and particularly with crosses involving B10D2 males. The increased number of non-responders in males may be a result of hormonal differences but the effect of B10D2 male parents on the subsequent response in male offspring is hard to explain.

It is possible that animals which do not produce detectable antibody are in fact 'responders' but at an affinity level below the limit of detectability of the ammonium sulphate globulin precipitation assay. This view is supported in part by the results of experiments with F_1 hybrids of two low affinity mouse strains: B10D2 × CBA (which would be expected to yield F_1 offspring producing predominantly low K_R antibody). A very high incidence of non-responders was found using this assay and in the

few animals in which antibody was detected it was of low relative affinity (M. W. Steward, unpublished observation).

The mechanism by which the genetic control of antibody affinity in mice is achieved is not known. However, following immunization of low K_R mice with antigen in adjuvant, high K_R antibody is produced with values which are similar to those in high K_R mice immunized with antigen in saline. Antigen in adjuvant immunization of high K_R mice does not increase the K_R compared with animals immunized with antigen in saline (Soothill and Steward, 1971) and F_1 hybrid mice similarly immunized with antigen in adjuvant produce high K_R antibody (M. W. Steward, unpublished observation). This optimization of the K_R in low K_R mice by adjuvant immunization indicates that it is unlikely that the genetic control of antibody affinity is expressed at the level of the immunoglobulin variable region structural gene complex.

Recent evidence has suggested that the interstrain differences in antibody affinity following four once-weekly injections of antigen in saline may be due, at least in part, to the greater susceptibility of immunocompetent cells bearing high affinity receptors in the low K_R mice to tolerance induction than corresponding cells in high K_R mice (Steward, Gaze and Petty, 1974). The production of low K_R antibody is associated with either poor carbon clearance or poor recovery from carbon blockade (Passwell, 1974a, b) and poor clearance of ^{125}I-labelled polyvinylpyrrolidone (Morgan and Soothill, 1975) by macrophages. These observations suggest an alternative, but not necessarily mutually exclusive explanation for differences in K_R of antibody: that is, low K_R production may be a consequence of defective macrophage function at the level of antigen processing and presentation to immunocompetent cells (Unanue, and Cerrottini, 1970; Feldman, 1972). In addition to the possibility that the genetic control of antibody affinity may be expressed at the level of the macrophage it is possible that such control could arise as a consequence of genetically determined variations at the level of the T cell. The development of high affinity anti-hapten antibody appears to require the presence of T cells (Gershon and Paul, 1971) and New Zealand mice which are deficient in T cells (Denman and Denman, 1970) make low affinity antibody to protein antigens and double-stranded DNA (Petty and Steward, 1972; Steward, Katz and West, 1975). However, recent observations that New Zealand mice also have poor macrophage function as assessed by *in vivo* clearance of ^{125}I-labelled PVP (Morgan and Steward, in preparation) favours the conclusion that the genetic control of antibody affinity is exerted at more than one level of the immune system.

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