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The genetic control of antibody affinity

EVIDENCE FROM BREEDING STUDIES WITH MICE SELECTIVELY BRED FOR EITHER HIGH OR LOW AFFINITY ANTIBODY PRODUCTION

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Summary. The genetic control of antibody affinity has been studied in mice selectively bred on the basis of the affinity of antibody they produce to protein antigens injected in saline. Two lines of mice have been obtained, one producing predominantly high and the other predominantly low affinity antibody. Breeding experiments have been performed with these two lines after ten generations of selection and the level and affinity of antibody to protein antigens measured in parents, F_1 hybrids and backcross offspring. The results indicate that antibody affinity is a genetically controlled parameter of the immune response and that this control is exerted independently of that controlling antibody levels. Furthermore, high and low affinity line mice have been typed for major histocompatibility complex antigens and the results show that the two lines are not significantly different. This therefore suggests that genes controlling antibody affinity are not linked to the major histocompatibility locus.

INTRODUCTION

The immune response is subject to a variety of genetic controls which, in many instances, are linked to the major histocompatibility locus (McDevitt & Benacerraf, 1969; Benacerraf & McDevitt, 1972; Benacerraf & Katz, 1975). The functional expression of such control is mediated through the co-operative interaction of macrophages and B and T lymphocytes. A further aspect of genetic control has been described (Biozzi, Stiffel, Mouton, Bouthillier & Decreusefond, 1968) in which the characteristic of general responsiveness is controlled by a group of approximately ten loci. This control seems to be generally antigen non-specific and is expressed primarily at the level of the macrophage. Previous work from this and other laboratories (Soothill & Steward, 1971; Petty, Steward & Soothill, 1972; Imanshi & Mäkelä, 1974, 1975; Ruscetti, Kunz & Gill, 1974; Segre & Segre, 1974) has indicated that antibody affinity is an additional genetically controlled parameter of the immune response. Furthermore, it appears that this control is exerted independently of that regulating antibody levels (Katz & Steward 1975, 1976; Steward & Petty, 1976; Kim & Siskind, 1978).

We have described the generation by selective breeding of two lines of mice, one producing high and the other low affinity antibody (Katz & Steward, 1975). Ten generations of selective breeding have now been completed and this report describes the levels and affinity of antibody produced against protein antigens injected in saline in generation ten high and low line mice, their F_1 hybrids and backcross offspring and

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provides further evidence for the genetic control of antibody affinity by mechanisms independent of those controlling antibody levels.

MATERIALS AND METHODS

Mice

Random bred T.O. mice were initially obtained from the Scientific Animal Service, Elstree, Hertfordshire and bred as described below.

Antigens

Human serum albumin (HSA) obtained from Miles Ltd and human serum transferrin (HST) obtained from Sigma Ltd were used as received.

Immunizations

Mice were immunized at approximately 8 weeks of age with either 1 mg HSA or HST in 0·1 ml saline once-weekly for 4 weeks. Serum was obtained 2 weeks after the last injection from blood drawn from the retro-orbital venous plexus.

Radiochemicals

$Na^{125}I$ and $^{22}NaCl$ were obtained from the Radiochemical Centre, Amersham. Radio-iodination of the HSA and HST was carried out by the solid-phase lactoperoxidase method (David, 1972)

Antibody assay

The level (Ab_t, picomoles of binding sites per 10 μl serum) and relative affinity (K_R, l/mol) of antibody to either HSA or HST was measured by an ammonium sulphate globulin precipitation method which incorporated a ^{22}Na volume marker (Gaze, West & Steward, 1973). Free and antibody-bound antigen was determined at equilibrium over a range of antigen concentrations and Ab_t obtained by extrapolation to infinite free antigen concentration, of a Langmuir plot of the reciprocal of the bound antigen the reciprocal of the free antigen:

$$\frac{1}{b} = \frac{1}{K} \cdot \frac{1}{c} \cdot \frac{1}{Ab_t} + \frac{1}{Abt}$$

where b=bound antigen; c=free antigen and K=affinity.

A curvilinear plot was obtained and antibody affinity calculated by linear regression analysis using data points where approximately 30–80% of the total antibody binding sites were antigen-bound. Affinity was expressed as the reciprocal of the free antigen concentration when 50% of the total binding sites were bound to antigen (Steward, 1977).

Selection and breeding

Mice were selected for breeding on the basis of the affinity of antibody they produced to HSA or HST. Mice producing antibody of K_R values greater that 10^6 l/mol were mated at each generation to give 'high affinity line' mice and those with K_R values between 10^4 and 7×10^5 l/mol were mated to produce 'low affinity line' mice (Katz & Steward, 1975). In order to avoid possible effects of passively transferred maternal antibody, HSA and HST were used alternately as immunogens at each generation since they do not cross-react in the mouse and the affinity of antibody produced to both in inbred mice is similar (Petty *et al.*, 1972). F_1 hybrids and backcrosses were bred from mice at the tenth generation of selective breeding.

Histocompatibility

Histocompatibility typing was performed on cells obtained from high affinity and low affinity mice at the seventh and ninth generation of selective breeding.

All antisera with the prefix 'S' were prepared by skin grafting followed by multiple i.p. injections of spleen or thymus cells (Staines, Ashton, Cuthbertson & Davies, 1976). Antisera prefixed 'D' and 'C' were provided by the Serum Bank of the National Institute of Health, Washington D.C.

Typing of animals was carried out as described by Staines & Archer (1975) using [^{51}Cr]-sodium chromate-labelled lymph node lymphocytes in the complement-dependent cytolysis technique. Tests were performed in V-bottomed microtitre trays, using pooled guinea-pig serum as a complement source. Each antiserum was tested for its reaction with lymphocytes at three dilutions: 1/5, 1/50 and 1/500. Typing reactions were analysed by comparison with known reactions of the antisera. Twenty-three of the antisera between them cover all H-2 specificities, except public specificities H-2·6, 10, 14, 27, 29, 40, 46, 47, 49, 50 and the private specificity H-2·21. Two antisera, S66 and S76 were used to type for Thy-1 alleles and three others to detect multiple Ia specificities of the k, s and q haplotypes (S182·1, S183W, S184W).

RESULTS

The levels (Ab_t, p moles per 10 μl serum) and relative affinity (K_R, l/mol) of anti-protein antibodies pro-

duced by generation 10 high and low affinity line mice, their F_1 hybrids and backcross offspring are presented in Fig. 1 and Table 1. The results of statistical analysis by the Student's t test of the differences between the various populations of animals are presented in Table 2.

Generation 10 high affinity line mice produced anti-HSA antibody of significantly higher average K_R than did the low line mice ($P=0{\cdot}0005$). In addition, high affinity line mice produced Ab_t levels which were significantly higher ($P=0{\cdot}0005$) than those produced by low line mice but there was no correlation between K_R and Ab_t (Table 3). The distribution of affinities of F_1 hybrid mice immunized with HST was not significantly different from that of the high line parents but was significantly different from the low line. K_R values obtained in the F_1 mice, however, extended over almost the entire range of values in the parental populations from which mice used for breeding the F_1 hybrids were drawn. The distribution of affinity values in the F_1 hybrids suggests that in these animals the characteristic of high affinity antibody production is dominant. In addition, there was a correlation

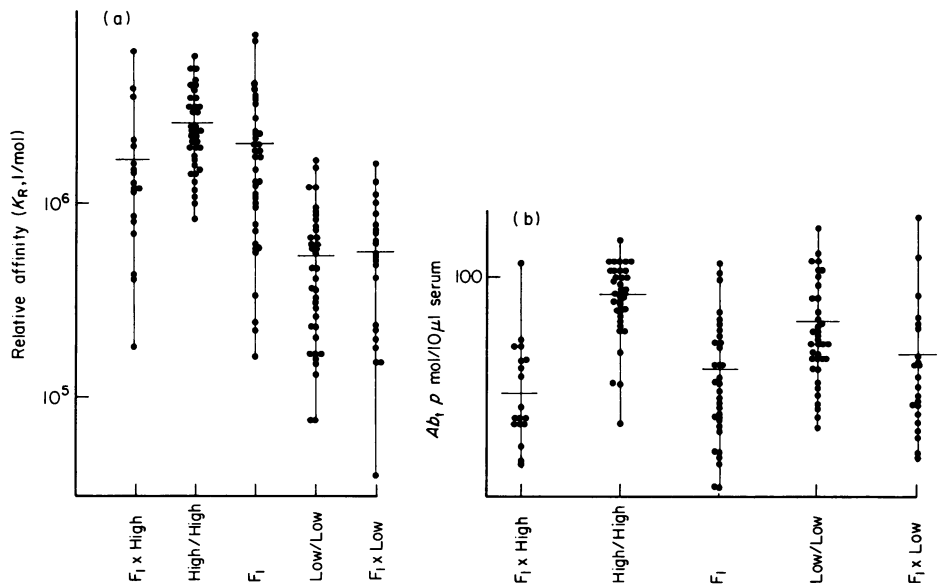

Figure 1. (a) Affinity (K_R, l/mol) and (b) levels (Ab_t, p moles/10 µl serum) of anti-protein antibody in T.O. high and low affinity line mice, their F_1 hybrids and backcrosses.

Table 1. Levels and affinity of antibody in parents, F_1 hybrids and backcrosses of tenth generation genetically-selected high and low affinity mice

Mice	Antigen	Number of animals	NAD*	Mean K_R (l/mol × 10^6 ± SD)	Mean Ab_t (p moles binding sites per 10 µl serum ± SD)
High affinity	HSA	46	9	2·59 ± 1·24	85·1 ± 25·0
Low affinity	HSA	43	5	0·53 ± 0·38	61·7 ± 33·0
F_1 hybrids	HST	34	8	2·10 ± 1·81	29·6 ± 14·7
(F_1 × High) backcrosses	HST	26	8	1·70 ± 1·50	34·8 ± 23·1
(F_1 × Low) backcrosses	HST	31	9	0·57 ± 0·40	47·2 ± 40·1

* NAD, no antibody detectable.

between Ab_t and K_R (Table 3) an observation which is not consistent with our previous findings in inbred mice and their progeny (Steward & Petty, 1972, 1976; Katz & Steward 1975, 1976). The F_1 × High backcross offspring produced antibody with an average K_R of $1{\cdot}7 \times 10^6$ l/mol which was very significantly different ($P=0{\cdot}0005$) from that produced by the (F_1 × Low) backcrosses of $5{\cdot}7 \times 10^5$ l/mol.

The average Ab_t values in the two backcross populations were not significantly different and the K_R and Ab_t values were not correlated (Table 3). Furthermore, the average K_R in the (F_1 × Low) backcrosses was not significantly different from the Low line parents ($P=0{\cdot}475$) but the differences in K_R between the (F_1 × high) backcrosses and the high line parents did achieve statistical significance ($P=0{\cdot}025$). Other statistical comparisons are recorded in Table 2 which further indicate that K_R and Ab_t are unrelated. For example, K_R values in (High × F_1) and (Low × F_1) backcrosses are significantly different ($P=0{\cdot}0005$) whereas Ab_t values are not ($P=0{\cdot}15$). Figure 2 represents graphically the distribution of K_R values in parents, F_1 hybrids and backcrosses. From this it can be seen (i) that the distribution of the K_R values in the F_1 hybrids covers the range of values in the parents; (ii) the (F_1 × High) backcrosses have a distribution of K_R values similar to that of the high line parents and (iii) the distribution of K_R values in the (F_1 × low) backcrosses is similar to that of the low line parents.

H-2 typing of both high and low line mice was carried out during the course of the selective breeding programme. The results of such measurements performed on generations 7 and 9 mice are shown in Table 4.

Table 2. Statistical analysis by Student's t test of differences between K_R and Ab_t values in parents, F_1 hybrids and backcrosses of high and low affinity line mice

Comparison	P values	
	K_R	Ab_t
High *v.* Low	0·0005	0·0005
High *v.* F_1	0·10	0·0005
Low *v.* F_1	0·0005	0·0005
(High × F_1 *v.* High	0·025	0·005
(High × F_1) *v.* Low	0·0005	0·0025
(High × F_1) *v.* F_1	0·20	0·15
(High × F_1) *v.* (Low × F_1)	0·0005	0·15
(Low × F_1) *v.* Low	0·475	0·10
(Low × F_1) *v.* High	0·0005	0·0005
(Low × F_1) *v.* F_1	0·0005	0·025

Table 3. Correlation coefficients for the association of K_R and Ab_t in genetically selected mice and their offspring

Mice	Correlation coefficient, r	P
High affinity line	0·015	0·45
Low affinity line	0·019	0·45
High and Low lines	0·243	0·05
F_1 hybrids	0·440	0·0125
(F_1 × High) backcrosses	−0·240	0·20
(F_1 × Low) backcrosses	−0·310	0·10

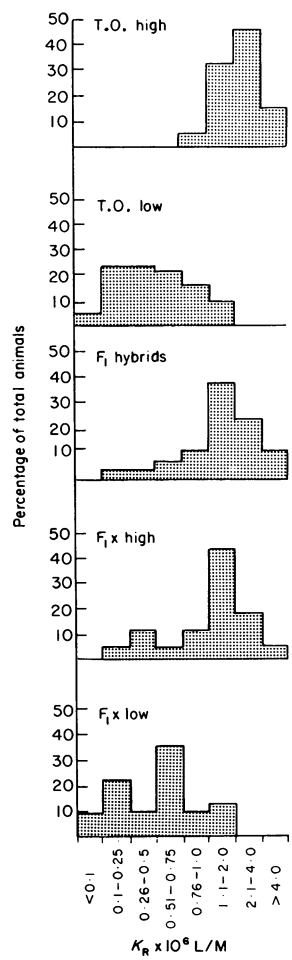

Figure 2. The distribution of antibody affinity (K_R, l/mol) in T.O. high and low affinity line mice, their F_1 hybrids and backcrosses.

Table 4. Histocompatibility typing of high and low affinity line mice

Serum number	Putative specificity	Typing reactions* High affinity line. Number positive/ number negative	Low affinity line. Number positive/ number negative	A.SW. strain
D2	D2,56	0/8	0/7	−
C8G	K8:K/D9	2/6	1/6	−
D11	K11,17:D30,55	0/15	4/9	−
D12	D12	14/1	10/3	+
D13	D4,13,41,42,43,44	10/5	7/6	+
D15	K15	0/8	0/7	−
D16†	K/D16:K38	0/15	0/13	−
D17	K17:D?	0/8	0/7	−
D18	K/D18:K54	8/0	6/1	+
D19	K19,51:D12	8/0	4/3	+
D20	K20,52,53	8/0	7/0	+
C28	K19,51:D12,28,36,42	8/0	7/0	+
D30	D30,55	0/8	1/6	−
D32	D32	0/8	0/7	−
D33	K33,53,54	3/5	1/6	trace
S145†	K31	0/8	0/7	−
S155W†	K33,35,36	0/8	1/6	−
S156†	K17,54:D30,55,56	3/5	2/5	−
S163W†	K/D7:K19,51:D12	14/1	13/0	+
S169†	K/D7,9	6/2	6/1	+
S170W	K17	0/8	0/7	−
S188W	D4,13,35,36,40,41,42,43,44	2/6	2/5	−
S191†	K1,3,5,11,23,24,25,45,52	6/2	6/1	+

* Reactions of seventh generation mice except for D11, D12, D13, D16 and S163W which are reactions with seventh and ninth generation mice.
† Contains anti-Ia activity.

All mice typed Thy-1.2$^+$, Thy-1.1$^-$ and with Ia antisera, most animals of both lines showed weak positive reactions for Ia^s and Ia^k but not for Ia^q specificities. Analysis of the results indicates an H-2 type in both high and low affinity mice which is very similar to that of H-2^s (A.SW) mice, i.e. positive reactions for H-2.1, 3, 5, 12, 19, 28, 36, 51, including the H-2^s private specificities K19 and D12. No other specificities were consistently represented. No significant and consistent differences between high and low affinity lines were found. Some antisera, however, reacted only with a proportion of animals of both lines suggesting that the two lines are segregating for some public H-2 specificities and that, as expected, they are not yet completely homogeneous for H-2.

DISCUSSION

Several years ago, the demonstration of inbred mouse strain-related differences in affinity of antibody to protein antigens injected in saline suggested that antibody affinity was under some form of genetic control (Soothill & Steward, 1971). Subsequently, detailed breeding studies with inbred mice provided considerable data to support the contention that antibody affinity is a genetically controlled parameter of the immune response and that such control is exerted by several genes (Steward & Petty, 1976). Conventional genetic analysis of the control of antibody affinity to provide information, for example, on the number of genes involved is difficult because of the marked heterogeneity of affinity even in inbred mice. This heterogeneity may arise, at least in part, from factors (e.g. dietary, environmental) which are not necessarily under genetic control.

The data reported in this paper were obtained from experiments performed on two lines of mice developed by a programme of selective breeding on the basis of

antibody affinity. This breeding procedure resulted in a progressive separation of the two lines of mice into one producing high affinity and the other low affinity antibody to protein antigens which again suggests multiple gene involvement in the control of affinity. Analysis of the antibody response in F_1 hybrids and backcrosses of these high and low affinity line mice confirms that affinity is genetically controlled and that this control is independent of that governing antibody levels. Whilst we and others (Petty *et al.*, 1972; Kim & Siskind, 1978) have found that some strains of mice producing high affinity antibody also produce high levels of antibody and some low affinity strains also produce low levels of antibody, there is not a direct relationship between affinity and level of antibody. Furthermore, we have not demonstrated a direct correlation between affinity and antibody levels in individual mice from inbred strains or from their F_1 and backcross offspring. In the (high × low) F_1 hybrids described here, however, a significant but not very marked correlation between K_R and Ab_t was obtained ($r=0{\cdot}44$; $P=0{\cdot}0125$) but for all other groups of mice these two parameters were not correlated (Table 3). The reason for this discrepancy with all other data is not clear but the overwhelming majority of data supports the independence of K_R and Ab_t. The apparent dominance of high K_R antibody production in these same F_1 animals may be one aspect of hybrid vigour in the progeny of two non-inbred parental lines resulting in more effective selection of high affinity B lymphocytes. These observations clearly highlight the complexity of the control of antibody affinity. Data presented in Table 4 indicate that there are no significant differences in H-2 type between high and low affinity line mice. It therefore appears that the genes controlling antibody affinity segregate independently of those controlling the major histocompatibility locus. It should be emphasized, however, that individual Ia specificities were not typed. In addition, as H-2 typing antisera able to discriminate between the two lines were not available, it was not possible to examine H-2 linkage in F_1, and backcross animals. Kim & Siskind (1978) have demonstrated the independence of the affinity of anti-DNP antibody and H-2 and inbred mice injected with DNP-BGG in Freund's complete adjuvant. In addition, they obtained some evidence that the control of antibody concentrations was linked to the major histocompatibility locus in certain breeding combinations.

The question still remains as to the level at which the genetic control of antibody affinity is exerted and it is possible that macrophages, T helper cells, T suppressor cells or other factors may be involved. Experiments by Passwell, Steward & Soothill (1974), Morgan & Soothill (1975) and Morgan & Steward (1976) implicate a role for macrophages in the control of affinity. Biozzi high and low responder mice (Biozzi *et al.*, 1968) exhibit macrophage function differences but interestingly, both lines produce antibody of the same affinity to both hapten (Del Guercio & Zola, 1972) and protein antigens (Katz & Steward, 1976). Further experiments are required to adequately define the level at which affinity is genetically controlled. The availability of the H-2 identical high and low affinity line mice described here provides an ideal model in which to investigate this question.

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