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Failure of affinity maturation leads to increased susceptibility to immune complex glomerulonephritis

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Summary. Mice, previously selected for the production of low affinity antibody after four injections of protein antigens in saline, fell into two groups on the basis of their antibody response after injection of adjuvantized antigen. One group produced antibody of sequentially rising affinity but the other produced only low affinity antibody. Mice from the latter group were interbred to produce low affinity non-maturing mice (low N/M mice). Daily injections in these mice produced a more rapid and severe glomerulonephritis than that observed in mice of the original low affinity line. Male low N/M mice were more severely affected than female low N/M mice. Susceptibility to the disease was associated not only with an inability to produce the maturational transition from low affinity to high affinity antibody with time but also with the production of low levels of antibody. It is suggested that these quantitative and qualitative defects in the antibody response may lead to increased susceptibility to immune complex disease.

INTRODUCTION

There is considerable evidence from experimental animal studies that susceptibility to chronic immune complex disease (ICD) is associated with the production of low affinity antibodies and a failure to eliminate antigen from the circulation (Alpers, Steward & Soothill, 1972; Koyama *et al.*, 1978; Steward, 1979; Germuth *et al.*, 1979; Devey & Steward, 1980; Germuth, Rodriguez & Wise, 1982). Selective breeding of mice on the basis of the affinity of antibody produced following four once-weekly injections of protein antigen in saline has resulted in the establishment of two lines of mice, one producing high affinity antibody and the other low affinity antibody (Katz & Steward, 1975).

We have shown that daily injection of protein antigens induces a chronic ICD, characterized by proteinuria, extensive immune complex deposition in the glomerular basement membrane and eventual death from renal failure, in a high proportion of low affinity mice but only rarely in high affinity mice (Steward, 1979; Devey & Steward, 1980; Devey *et al.*, 1982). In mice that did not develop ICD, the affinity of free antibody in the circulation rose with the increasing number of daily antigen injections. However, in low affinity mice that developed ICD, this increase in antibody affinity did not occur (Devey *et al.*, 1982). This suggested that, within the low affinity line, there was a subpopulation of mice that lacked the ability to produce the normal maturational transition from low to high affinity antibody and that it was this subpopulation that was particularly susceptible to chronic ICD.

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The purpose of the work described here was to identify this subpopulation and to determine whether, by selective breeding, a line of mice could be obtained with a greater susceptibility to chronic ICD than mice of the original low affinity line.

MATERIALS AND METHODS

Mice

Groups of male and female low affinity mice at the 20th and 21st generation of selective breeding were used.

Injection of antigen with adjuvant

Mice received a single intraperitoneal (i.p.) injection of 1 mg human serum albumin (HSA, Pentax Fraction V) or 1 mg human serum transferrin (HST, Sigma) in Freund's complete adjuvant (FCA, Difco). Blood was taken from the retro-orbital venous plexus after 2, 3·5 and 5 weeks. Some mice received a booster injection of 0·3 mg HSA in saline i.p. and were bled after 1 week.

Induction of chronic ICD

Mice were injected i.p. with 10 mg/kg HSA in saline five or six times a week for up to 17 weeks.

Renal function

Mice were housed in metabolic cages overnight and urine samples were collected. The mouse serum albumin levels in the urine were measured by rocket immunoelectrophoresis as described previously (Devey & Steward, 1980). An albumin content of greater than 3 mg/ml was considered to be an indication of proteinuria.

Immunofluorescence

Kidneys were snap-frozen in n-hexane cooled in alcohol/dry ice. Four micrometre cryostat sections were fixed in acetone and stained with FITC-conjugated antisera to mouse-immunoglobulin, HSA (kindly given by Dr A. Lew) and mouse C3 (Nordic). The localization and intensity of fluorescent deposits were scored on a 0 to 3+ scale using a Zeiss microscope.

Measurement of antibody levels and antibody affinity

Mice were bled from the retro-orbital plexus not less than 24 hr after the last injection of antigen. Blood samples were allowed to clot at room temperature and sera were separated and stored at $-70°$ prior to assay. The amount (Ab_t, pmoles/10 μl serum) and relative affinity (K_R, litres/mole) of antibody were measured by the double isotope ammonium sulphate precipitation method of Gaze, West & Steward (1973). The 'Antibody Index' was obtained as the product of the Ab_t and K_R values.

Statistics

The significance of differences between means were tested using Student's t-test and the χ^2 test for discrete variables.

RESULTS

The effect of adjuvant on the maturation of antibody affinity in low affinity mice

Forty-three mice were injected with HSA in FCA and bled after 2, 3·5 and 5 weeks. Mice were assigned to one of two groups on the basis of the affinity of their antibody responses. Those producing antibodies with affinity $>0{\cdot}9\times10^6$ L/M (60%) to Group 1 and those with affinity of $<0{\cdot}9\times10^6$ L/M to Group 2 (Fig. 1a). In Group 1, there was a sequential increase in antibody affinity with time (a maturing response) and a further increase was seen after a booster injection of HSA in saline given after the week 5 bleed. In Group 2, antibody affinity remained low (non-maturing) and did not increase even after the booster injection. The amount of antibody produced by animals in the two groups showed no significant differences (Fig. 1b).

Selection and breeding of the low affinity N/M mice

A further group of low affinity mice were injected i.p. with 1 mg HST in FCA and bled after 3·5 weeks. The 35% who produced low affinity antibody ($<0{\cdot}9\times10^6$ L/M) were interbred and their progeny termed low affinity N/M mice.

Induction of chronic ICD in low affinity N/M mice

Low affinity N/M mice (27 males and 39 females aged 4–5 months) were injected daily with HSA and the occurrence of proteinuria, death and diffuse immune complex deposition in the GBM recorded (Table 1). Mice were not considered to have ICD unless they had at least two of the above features. Low affinity N/M male mice had a significantly higher incidence of ICD than males and females of the original low and high affinity lines (data for the 70 mice in the latter two groups were from a separate study performed just

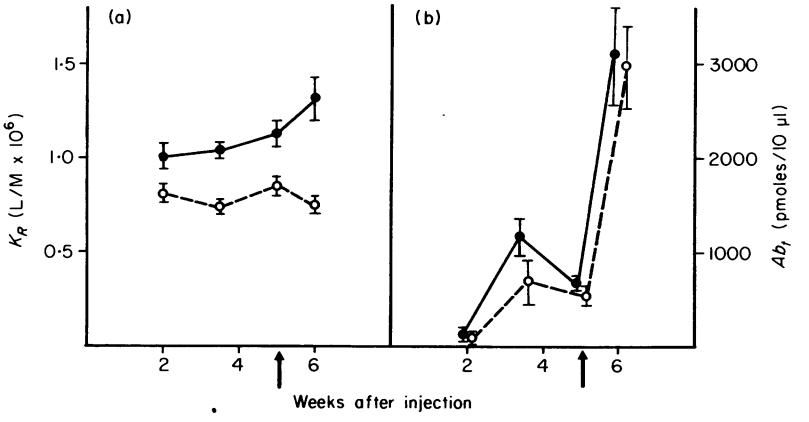

Figure 1. (a) Mean antibody affinity (K_R) and (b) mean amount (Ab_t) ± standard error in low affinity mice after injection of HSA in FCA. Group 1 (●) consisted of 26 mice and Group 2 (○) of 17 mice. A booster injection of HSA in saline (*arrow*) was given after the week 5 bleed.

Table 1. Immune complex disease in low affinity N/M mice and the original low affinity and high affinity lines*

Line	Sex	Number	Died	Proteinuria	GBM IC deposition	Total incidence of ICD†
Low affinity N/M	♂	27	14 (52%)	18 (67%)	22 (81%)	19 (70%)
	♀	39	12[a] (31%)	12[b] (31%)	13[c] (33%)	13[d] (33%)
Low affinity	♂	18	7[e] (39%)	5[f] (28%)	7[g] (39%)	7[h] (39%)
	♀	18	6 (33%)	4 (22%)	6 (33%)	5 (28%)
High affinity	♂	16	1 (6%)	1 (6%)	3 (19%)	2[k] (13%)
	♀	18	2 (11%)	3 (17%)	3 (17%)	2 (11%)

* Data from low N/M mice compared to data from low and high affinity mice from a separate experiment.

† Mice with two or more disease features.

Significance of differences from male low affinity N/M mice.

a: $\chi^2 = 2{\cdot}97$ (N.S.).
b: $\chi^2 = 8{\cdot}29$ ($P < 0{\cdot}005$).
c: $\chi^2 = 14{\cdot}85$ ($P < 0{\cdot}0005$).
d: $\chi^2 = 8{\cdot}76$ ($P < 0{\cdot}005$).
e: $\chi^2 = 0{\cdot}73$ (N.S.).
f: $\chi^2 = 6{\cdot}64$ ($P < 0{\cdot}025$).
g: $\chi^2 = 8{\cdot}55$ ($P < 0{\cdot}005$).
h: $\chi^2 = 4{\cdot}39$ ($P < 0{\cdot}05$).
k: $\chi^2 = 13{\cdot}47$ ($P < 0{\cdot}0005$).

before the present experiment and were consistent with previously reported data for the high and low affinity lines). Low N/M males also had a significantly higher incidence of disease than low N/M females, in contrast to our previously reported finding of a similar incidence of ICD in males and females of the original low affinity line. In addition, the onset of disease occurred more rapidly in low N/M male mice than in the original low and high affinity lines (Fig. 2).

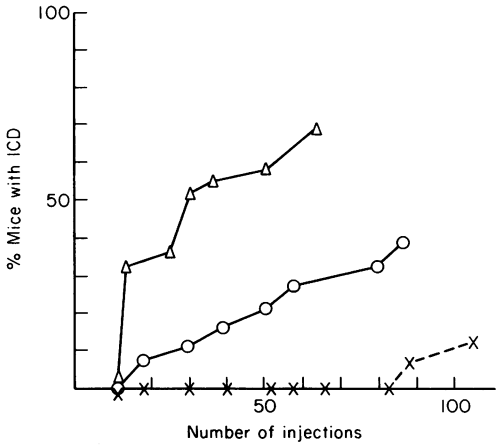

Figure 2. The incidence and onset of ICD in low N/M (△——△) low affinity (○——○) and high affinity (× - - - ×) male mice given daily injections of albumin in saline.

Antibody levels and antibody affinity in low affinity N/M mice

In both male and female mice there were significant differences between the levels and affinity of the free antibody measured in low affinity N/M mice with ICD compared to those without. Antibody affinity remained low ($< 1 \times 10^6$ L/M) in mice with ICD but increased with time in those without the disease (Fig.

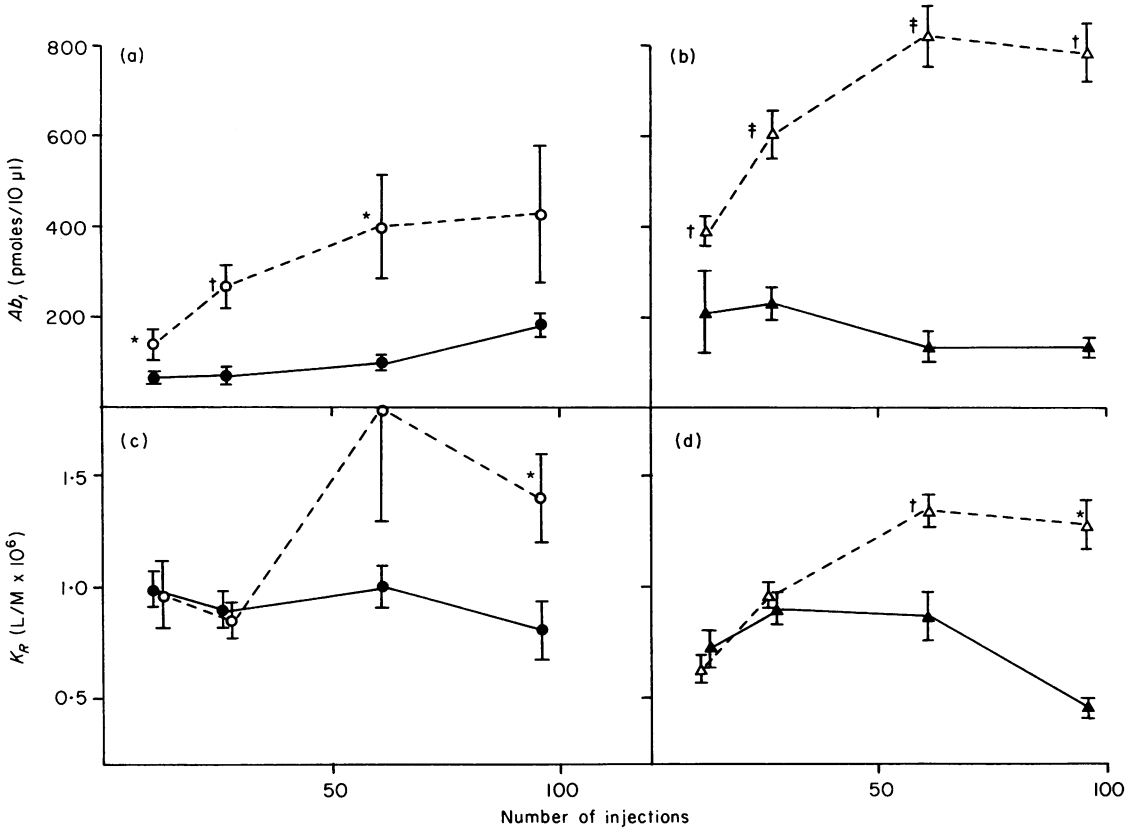

Figure 3. Mean antibody affinity (c, d) and amount (a, b) ± standard error in low N/M male mice (a, c) with ICD (●——●) and without ICD (○ - - - ○) and in low N/M female mice (b, d) with ICD (▲——▲) and without ICD (△- - -△) (*$P < 0{\cdot}05$; †$P < 0{\cdot}01$; ‡$P < 0{\cdot}001$).

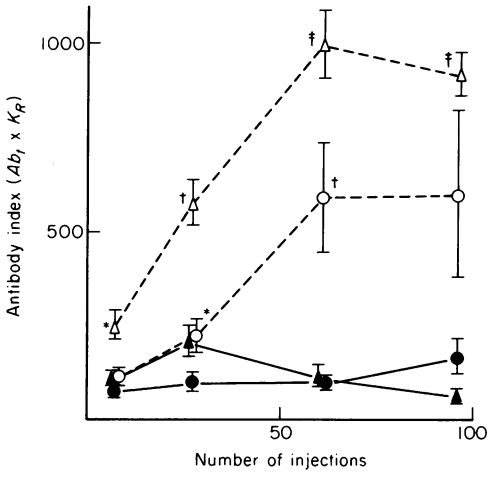

Figure 4. Mean antibody index ($K_R \times Ab_t$) ± standard error in low N/M male mice with ICD (●——●), low N/M male mice without ICD (○ - - - ○), low N/M female mice with ICD (▲——▲) and low N/M female mice without ICD (△- - -△) (*$P<0.05$; †$P<0.01$; ‡$P<0.001$).

3c, d). Animals with ICD also had significantly lower antibody levels than those without ICD (Fig. 3a, b), and antibody levels were similar in males and females with ICD. However, in mice without ICD, females had significantly higher antibody levels than males (Fig. 3a, b). The 'Antibody Index', obtained as the product of the Ab_t and K_R values, was significantly different between mice with ICD and mice without the disease (Fig. 4). Mice, mainly females, who did not develop ICD had either high levels ($Ab_t > 600$ pmoles/10 μl) of low affinity antibody or lower levels of high affinity antibody (Fig. 5).

DISCUSSION

The original basis for the selection of the low affinity line of mice was the affinity of the antibody produced after four once-weekly injections of antigen in saline (Katz & Steward, 1975). However, when these low affinity mice were given multiple daily antigen injections, about half showed an increase in the affinity of free antibody with time, to values comparable to that produced by the high affinity line (Devey *et al.*, 1982). This was perhaps not surprising in view of the finding that adjuvants (which probably give an antigenic stimulus comparable to that given by daily injections of small amounts of antigen) have been shown to increase antibody affinity in inbred strains of mice that usually produce low affinity antibody (Petty & Steward, 1977). The finding that low affinity mice who develop ICD did not produce this time associated increase in antibody affinity suggested that the line included two subpopulations. When low affinity mice were injected with antigen and adjuvant, 60% showed a maturation of their antibody response from low to high affinity whereas in the remaining 30% antibody

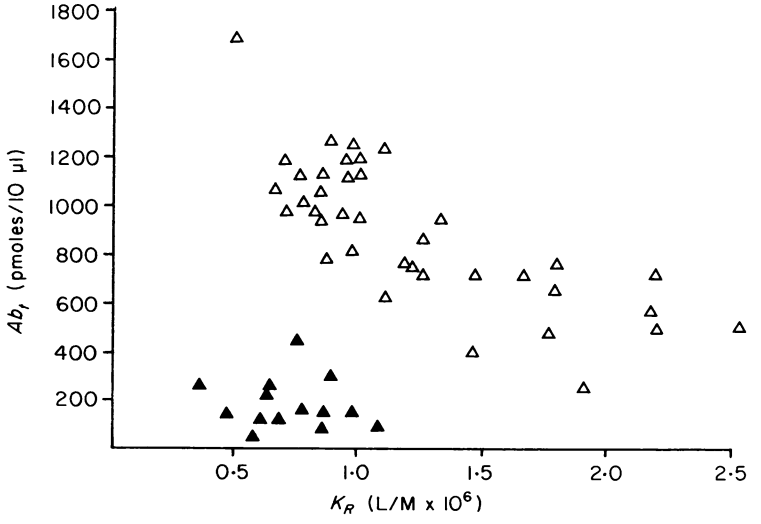

Figure 5. Antibody affinity (K_R) and amount (Ab_t) in low N/M female mice with ICD (▲) and without ICD (△).

affinity remained low although a comparable amount of antibody was produced. Low affinity mice which failed to produce high affinity antibody after injection of antigen in adjuvant were therefore interbred to produce low affinity N/M mice.

ICD, induced in low affinity N/M mice by daily injections of antigen in saline was characterized by proteinuria and death with extensive immune complex deposition in the glomerular basement membrane. In low affinity N/M males the incidence of ICD was much higher than that observed in the original low affinity line and the disease occurred more rapidly with over 50% mice affected after only 30 injections of antigen. However, in female low affinity N/M mice, the incidence of ICD was not significantly greater than that in the original low affinity line.

As we reported previously (Devey *et al.*, 1982), neither male nor female mice that developed ICD showed the maturational transition from low affinity to high affinity antibody during the course of the daily antigen injections. In addition, and contrary to our previous findings, mice with ICD had significantly lower levels of antibody than those without the disease. This finding should be treated with some caution, as it might be argued, particularly for mice with ICD, that free antigen and immune complexes may still be present in serum samples taken 24 hr after the previous antigen injection and that their presence may affect antibody measurements. However, even mice with severe ICD appear to clear both free and complexed antigen rapidly from their circulation with only very small amounts persisting 24 hr after injection (Devey & Bleasdale, 1984).

Antibody activity and the ability to form potentially pathogenic immune complexes depends on both the amount and affinity of the antibody response. We therefore calculated an 'Antibody Index' (obtained by the product of the Ab_t and K_R values) for each animal and found that this was significantly lower in both male and female mice with ICD, suggesting that the disease was most likely to occur when both antibody levels and antibody affinity were low. This may explain why, in this study, fewer female mice developed ICD as, in confirmation with our previous findings (Devey *et al.*, 1982), low affinity female mice produced significantly more antibody than male mice when given daily antigen injections. In addition, it appeared that those mice that produced a predominantly low affinity antibody response did not develop ICD if they had very high antibody levels, possibly because they had antibody excess *in vivo*. Furthermore, ICD did not occur in those mice that produced very small amounts of high affinity antibody. These findings are consistent with those of Alpers *et al.* (1972) who showed that inbred strains of mice that produce high affinity antibody eliminated antigen effectively regardless of the amount of antibody produced, whereas immune elimination in strains that produce low affinity antibody was generally poor but became more effective at high antibody–antigen ratios. The lattice structure of immune complexes is thought to be of importance in determining their fate and deposition in the renal glomeruli (Haakenstad, Striker & Mannik, 1982). In view of the present findings, it is suggested that a functionally poor antibody response, in terms of both affinity and amount, is likely to result in the formation of immune complexes with a lattice structure $> Ag_2Ab_2$ but a sedimentation coefficient $< 19S$ that will tend to deposit in the renal glomeruli and lead to immune complex glomerulonephritis.

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