Relationship of antibody affinity to onset of immune complex disease in New Zealand mice

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SUMMARY Affinity (K_R) of antibody to human serum transferrin in New Zealand mice was measured by a globulin precipitation technique. K_R was low in mice immunized early in life, increased with age of immunization up to 16–26 weeks, and then fell to low levels. K_R in young and old NZB mice was increased by immunization with antigen in Freund's complete adjuvant. The correlation between low affinity antibody production and susceptibility to immune complex disease is discussed.

The New Zealand Black (NZB) mouse, New Zealand White (NZW) mouse, and the first generation offspring (NZW/W F_1) of the cross between NZB and NZW mice have been extensively studied because of their susceptibility to a disease similar to systemic lupus erythematosus (Helyer and Howie, 1963; Mellors, 1966; Howie and Helyer, 1968; East, 1970). The demonstration of immunoglobulin and complement components in the renal glomeruli (Aarons, 1964; Mellors, 1965; Lambert and Dixon, 1968) and the presence of circulating antibody to nuclear constituents and to other antigens (Howie and Helyer, 1968; McGiven and Ghose, 1968; Siegel *et al.*, 1972) suggest that the glomerulonephritis of the NZ mice, like that of systemic lupus erythematosus in man, is mediated by immune complexes. Immune complex nephritis can be induced in some strains of mice by neonatal infection with lymphocytic choriomeningitis (LCM) virus. Oldstone and Dixon (1969) have shown that while some strains (B10D2 new and SWR) are susceptible to such nephritis, others (C3H and A/jax) are resistant. In previous studies (Soothill and Steward, 1971; Petty *et al.*, 1972), we showed that in addition to quantitative differences in antibody produced by these two groups of mice, there are demonstrable qualitative differences; that is, there are differences in the relative affinity (K_R) of the antibody produced to protein and hapten antigens. Thus, those strains which are resistant to LCM-induced nephritis made

antibody of higher affinity to human serum albumin, human serum transferrin (HST), bovine serum albumin, dinitrophenol-human serum albumin, and nitrophenylacetic acid-rabbit serum albumin than did those strains which were susceptible to the LCM-induced disease.

Because of the possible relevance of antibody affinity to immune complex disease in NZ mice, previous studies (Petty and Steward, 1972) were conducted in these mice using HST as the antigen. Three observations were made. First, the relative affinity of anti-HST antibody in NZB and NZB/W F_1 mice immunized at 8–12 weeks of age was lower than that seen in any of the 12 other strains tested. Second, the K_R rose with age at immunization through the age range tested (up to 26 weeks). This was a unique observation since none of the other strains previously tested showed this age-related variation. Third, immunization with antigen in Freund's complete adjuvant (FCA) in young mice significantly increased both levels and affinity of antibody.

This study is a continuation of earlier studies and examines the relative affinity of antibody produced to HST injected in saline into NZ mice at 26–52 weeks of age, and the ability of old NZ mice to produce high affinity antibody in response to immunization with antigen in FCA.

Methods

Mice were raised by brother-sister mating in a colony originally kept at the Institute of Child Health and subsequently at the Kennedy Institute.

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Hybrid (NZB/W F₁) mice were raised by reciprocal crosses between NZB and NZW mice. Groups of 8 male and female NZB, NZW, and NZB/W F₁ mice were immunized by intraperitoneal injection of 0.5 mg HST (Sigma Chemical) at weekly intervals for 4 weeks and blood was obtained by cardiac puncture 2 weeks after the last injection.

Groups of 7- to 12-week-old NZB males and 48- to 52-week-old NZB males were also immunized intraperitoneally with 0.5 mg HST in FCA (Difco, Detroit; 1:1 FCA:saline emulsion) at weekly intervals for 4 weeks and bled 2 weeks after the last injection. Determination of antibody affinity (K_R) and amount (Ab_t pmol/10 μ l) was performed as previously described using an ammonium sulphate globulin precipitation method (Steward and Petty, 1972a, b). Negative and positive control sera were included with each assay. Nonspecific binding (seen in the negative control) was subtracted from the test values.

Results

NZB MICE

The mean values for K_R and Ab_t of anti-HST antibody in NZB mice immunized at various ages are shown in Table 1, and the individual values for K_R are shown in Fig. 1. The rise in K_R with increasing age of immunization up to 26 weeks previously reported (Petty and Steward, 1972) was confirmed with larger groups of mice. The mean K_R was highest in NZB adult males immunized at 16 weeks, but fell thereafter. The K_R of antibody from mice immunized at 52 weeks of age (mean $K_R=1.37 \times 10^5$) was not significantly different from that produced by mice immunized at 8-12 weeks of age (mean $K_R=2.09 \times 10^5$). A similar pattern was seen with NZB females. In NZB females immunized at 26 weeks of age, mean $K_R=5.5 \times 10^5$ l/mol but fell to a low of 1.37×10^5 l/mol in those immunized at 52 weeks,

Table 1 Antibody response in NZB mice

Age at immunization (w)	No. immunized	No. responding	Ab_t (pmol/10 μ l serum) (mean)	K_R (l/mol $\times 10^5$) (mean)
Male				
8-12	14	7	11.4	2.09
16	5	5	12.4	8.26
26	13	11	9.0	6.52
40	10	10	14.3	3.76
52	7	7	18.9	1.37
Female				
8-12	21	14	11.2	2.5
16	8	7	7.6	3.10
26	9	9	10.2	5.52
40	6	6	6.6	1.88
52	8	7	10.16	1.37

Fig. 1 K_R of anti-HST antibody in NZB mice immunized at 8-52 weeks of age. Each $\bullet$ represents a single mouse. $\triangle \triangle$ represent mice with no detectable antibody.

a slightly lower value than that obtained in mice immunized at 8-12 weeks (mean $K_R=2.5 \times 10^5$ l/mol). In both groups the incidence of nonresponders (that is, those mice making no detectable antibody) was greater in the younger mice than in older mice. Although Ab_t did show some variation, particularly in females, there was no clear association between K_R and Ab_t .

NZW MICE

In the NZW mice, although the K_R was low, the high proportion of nonresponders at young and very old ages made assessment difficult. In NZW males the mean K_R was lower than that in NZW females but there was no demonstrable age dependence (Table 2).

Table 2 Antibody response in NZW mice

Age at immunization (w)	No. immunized	No. responding	Ab_t (pmol/10 μ l serum) (mean)	K_R (l/mol $\times 10^5$) (mean)
Male				
8	12	4	8.0	2.13
26	9	9	4.2	2.17
52	9	1	(11.0)	(1.40)
Female				
8	12	6	3.50	4.82
26	4	1	(7.0)	(3.6)
40	5	5	6.86	4.7

NZB/W F₁ MICE (TABLE 3)

In the NZB/W F₁ males there was an age-dependent decline in K_R similar to that in the NZB males (Table 3, Fig. 2). After reaching a maximal mean K_R of 8.96×10^5 in mice immunized at 26 weeks of age, the values in mice immunized at a later age progressively declined to a mean of 0.93×10^5 for those immunized at 52 weeks of age. The rarity of

females of this type surviving until 40 to 52 weeks of age prevented their study. Unlike the NZB mice, the B/W F_1 mice tended to make higher levels of antibody with increasing age at immunization.

Table 3 Antibody response in NZB/W F_1 mice

Age at immunization (w)	No. immunized	No. responding	Ab _t (pmol/10 μ l serum) (mean)	K _R (l/mol $\times 10^5$) (mean)
Male				
8-12	28	11	13.4	3.15
26	13	13	15.9	8.96
40	5	5	18.0	1.83
52	7	7	40.0	0.93
Female				
8-12	25	18	14.0	1.50
16	9	7	6.0	2.90
26	14	7	18.5	5.66

Fig. 2 K_R of anti-HST antibody in NZB/W F_1 mice immunized at 8-52 weeks of age. Each $\bullet$ represents a single mouse. $\triangle\triangle$ represent mice with no detectable antibody.

Fig. 3 Effect of Freund's complete adjuvant (FCA) on K_R of anti-HST antibody in young and old NZB male mice. Each $\bullet$ represents a single mouse.

EFFECT OF FCA ON K_R IN OLD NZB MICE

As the data in Table 1 indicate, the K_R of antibody in old NZB mice was very low when antigen was administered in saline. When antigen was administered in FCA, however, the K_R of antibody in old mice was even higher than that made in the young mice similarly immunized (Fig. 3). Mean Ab_t of 8 to 12-week-old male NZB mice immunized with antigen in saline was 12.5 pmol/10 μ l and in FCA 34 pmol/10 μ l. For 52-week-old NZB males the mean Ab_t of those immunized with antigen in saline was 18.9 pmol, and in FCA 180 pmol/10 μ l. Thus, the effect of adjuvant on Ab_t appeared to be much greater in the old mice than in the young mice, although both responded about equally with respect to K_R .

Discussion

The previously reported increase in K_R of antibody to HST with increasing age in NZ mice is followed by a decline in mice immunized beyond 16-26 weeks of age. This is accompanied by small changes in Ab_t. Extensive studies (Mellors, 1966; Howie and Helyer, 1968; Lambert and Dixon, 1968; East, 1970) have shown the age-dependence of many of the autoimmune phenomena, proteinuria, and renal glomerular histopathological changes in NZ mice. Recently a similar age-dependent variation in avidity of antibody to native DNA was shown in these mice (Steward *et al.*, 1975), with maximum avidity occurring at 20 weeks in both male and female NZB/W F_1 mice. As with K_R of antibody to HST, the avidity of antibody to DNA falls with age. The striking sex differences in avidity of anti-DNA antibody to NZB/W F_1 mice, males being higher than females, was not seen with anti-HST antibody. Other studies (Elkerbout and Hijmans, 1974a, b) give indirect evidence for production of low affinity antibodies in old NZB mice.

In the case of antibodies to DNA, the K_R of anti-DNA present in the serum of older animals may be different from the K_R of anti-DNA antibody deposited as immune complex in the renal glomeruli. Thus the reported fall in serum anti-DNA K_R could reflect selective deposition of high K_R anti-DNA antibody in glomeruli. The fall in K_R of serum anti-HST could reflect the same phenomenon although HST or other heterologous proteins are not known to participate in the NZ mouse immune complex nephritis. While this explanation cannot at present be refuted, the alternative explanation is that the mean K_R of anti-HST antibody synthesized varies with age at immunization.

The mechanisms which determine K_R are not known, although it is apparent from the work of

Andersson (1972) and others that the affinity of circulating antibody is representative of the affinity of the B-cell surface immunoglobulins. The responsiveness of old NZ mice to immunization with antigen in adjuvant suggests that the low K_R antibody elicited by antigen in saline is not an absolute defect in B-cells. It is important to consider whether age-dependent alterations in T-cells or macrophages could result in the observed age-dependent alterations in K_R . Reports have noted the decline in T lymphocyte responses with increasing age in NZ mice (Talal, 1970; Rodey *et al.*, 1971; Bach *et al.*, 1973) and the efficacy of thymosin in correcting this abnormality (Dauphinee *et al.*, 1974; Gershwin *et al.*, 1974). Although deficient T-cell function might explain failure of T-B cell co-operation and resultant low affinity antibody in the old mouse, it could not, on the basis of present data, explain the low K_R values seen in the very young mouse.

Less attention has been paid to the function of macrophages in NZ mice. Thomas and Weir (1972) showed that peritoneal exudate cells of old NZB mice take up more bovine serum albumin than do such cells from young mice but that old NZB mice failed to degrade such protein as efficiently as the younger mice. Using the clearance of ^{125} I-labelled polyvinylpyrrolidone as a measure of macrophage function, it had been shown that macrophage function in NZB/W F₁ mice declines with increasing age (Morgan and Steward, 1976). Such reticuloendothelial inefficiencies could contribute to less efficient processing of antigen and thus to lower antibody affinity.

If, as we have postulated, antibody of low affinity is important in determining predisposition of an animal to chronic soluble complex disease, it might be expected that production of low affinity antibody would occur either at the time at which immunization with the immunopathogenic antigen takes place, or at the time of onset of immune complex disease. The demonstration within the first week of life of antibody to DNA in NZ mice (Siegel *et al.*, 1972) suggested that transplacental or neonatal infection with virus could be the initial event leading to immune complex disease. The data of Steward *et al.* (1975) with respect to anti-DNA and the data presented here with respect to HST support the suggestion that both at the time of initial (neonatal) immunization and at the time of occurrence of severe disease (after 6 months of age) the mean K_R of serum antibody is low.

It is clear that demonstration of low affinity antibody to HST does not prove the production of or the pathogenic potential of low affinity antibody to viral agents. An assumption must be made that, within limits, NZ mice make low affinity antibody to

many, if not all antigens, and that the relatively low affinity of anti-HST antibody accurately reflects the potential of that strain for making antibody of any given affinity. Studies of other strains have shown that 'high affinity strains' (A, C3H) make antibody of high affinity to all antigens tested (HST, DNA, human and bovine serum albumin, dinitrophenol, human serum albumin, nitrophenylacetic acid), whereas 'low affinity strains' make antibody of low affinity to the same antigens. Therefore, the affinity of the antibody response may be central to the disease process. The demonstrated effect of adjuvant substances on such a response may be of potential therapeutic importance in preventing or treating such disease.

The age-dependent variations in affinity of antibody to HST and to DNA (Steward *et al.*, 1975) are difficult to ascribe to a single mechanism. It is likely that such variations arise as a result of the interaction of the several age-related deficiencies already clearly described for these mice.

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