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# THE IMMUNOPATHOLOGICAL SIGNIFICANCE OF THE HETEROGENEITY OF ANTIBODY AFFINITY

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SUMMARY

Mice of two inbred strains, prone to nephritis following neonatal lymphocytic choriomeningitis virus infection, produced antibody of lower affinity to two soluble protein antigens, human serum albumin and transferrin, than did two nephritis-resistant strains. Antigen in adjuvant elicited higher affinity antibody than did antigen in saline, in the nephritis-prone mice. The significance of these findings in the pathogenesis and treatment of nephritis, arthritis and other possible soluble complex disease is discussed.

## INTRODUCTION

Much progressive nephritis is due to deposition of soluble antigen–antibody complexes in the kidney (Unanue & Dixon, 1967). An excellent model of this disease occurs in a minority of rabbits injected repeatedly with soluble protein antigens; these chronic nephritis-prone rabbits are identifiable by the nature of their antibody response (Dixon, Feldman & Vazquez, 1961; Pincus, Haberkern & Christian, 1968). Lymphocytic choriomeningitis virus (LCM) infection in newborn mice leads to chronic release of virus antigen, and circulating antivirus antibodies. Oldstone & Dixon (1969) have shown that some inbred strains of mice get nephritis and other features of soluble complex disease when infected in this way, but others do not. Presumably this difference of immunopathogenic effect of infection is due to differences in the immune responses in the different strains, which are likely to be genetically determined. One possible explanation of this difference is heterogeneity of affinity of the antibody produced by these different strains of mice; this may be an antigen-non-specific phenomenon. We have tested this by measuring the average equilibrium constants ($K$) for antibodies to human serum albumin and transferrin, produced in two strains of mice (SWR/J and B10D2) shown by Oldstone & Dixon (1969) to be prone to nephritis on LCM infection, and two nephritis resistant strains (AJAX and $C_3H$).

## MATERIALS AND METHODS

*Animals.* Breeding pairs of the four inbred mouse strains were obtained originally from the Jackson Memorial Laboratories, Bar Harbor, Maine, U.S.A. SWR/J and B10D2 new

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line were obtained directly from them. Breeding pairs of $C_3HHe$ were kindly supplied by the Imperial Cancer Research Fund Laboratories, Mill Hill, London, and the AJAX mice by the London Hospital Medical College. Mice of either sex were first immunized at 2–3 months of age. Inter-strain weight differences were small and weights ranged between 22 and 28 g.

*Antigens.* Human serum albumin (HSA) was generously supplied by Dr W. d'A. Maycock of the Lister Institute of Preventive Medicine. Iron-free human serum transferrin (HST) was obtained from Sigma Chemical Co. Ltd.

*Immunization procedures.* Mice were injected intraperitoneally with 0·5 or 1·0 mg antigen in 0·1 ml sterile saline once a week for 4 weeks. HSA (0·5 mg) was also injected in Freund's complete adjuvant subcutaneously once a week for 4 weeks. Two weeks after the last injection, animals were exsanguinated under anaesthesia.

*Determination of antibody equilibrium constants.* The antigens were labelled with $^{125}I$ by the method of McFarlane (1958). After incubation of $^{125}I$-labelled antigen at five concentrations with sera from immunized mice, globulin bound and free antigen concentrations were determined by a micro-modification of the ammonium sulphate globulin precipitation method of Farr (1958). The amount of antibody (Abt = moles of antibody binding sites) was determined by extrapolation of the binding curves, using the equation:

$$\frac{1}{b} = \frac{1}{K} \times \frac{1}{c} \times \frac{1}{\text{Abt}} + \frac{1}{\text{Abt}}$$

(where $b$ = bound antigen, $K$ = equilibrium constant, and $c$ = free antigen). Equilibrium constants were calculated from the logarithmic transformation of the Sips function (Sips, 1948; Karush, 1962).

$$\log \frac{b}{\text{Abt}-b} = a \log K + a \log c$$

(where $a$ = index of heterogeneity).

Antibody concentrations ($\mu$g/ml) were calculated from Abt assuming two binding sites per molecule and a molecular weight of 160,000. These assumptions are valid for IgG antibody and are probably appropriate for the majority of antibody produced by animals immunized in this way.

Sera from unimmunized animals of the corresponding strains were used as controls for the non-antibody bound precipitable antigen at each antigen concentration in the assay. This was always less than 3% of the antigen added. A positive control was also included in each assay; this was a serum pool from mice immunized with antigen in Freund's complete adjuvant.

## RESULTS

### *Antibody affinity*

The equilibrium constants, $K$, of antibody to HSA and HST in the four inbred mouse strains tested are plotted in Figs 1 and 2 and Table 1. In each case the distribution appears to be logarithmic, and log transformed data is used for all calculations. The nephritis-resistant strains, $C_3H$ and AJAX, have consistently higher $K$ values than the nephritis-prone strains, SWR/J and B10D2, and there is little or no overlap. Antibody was not

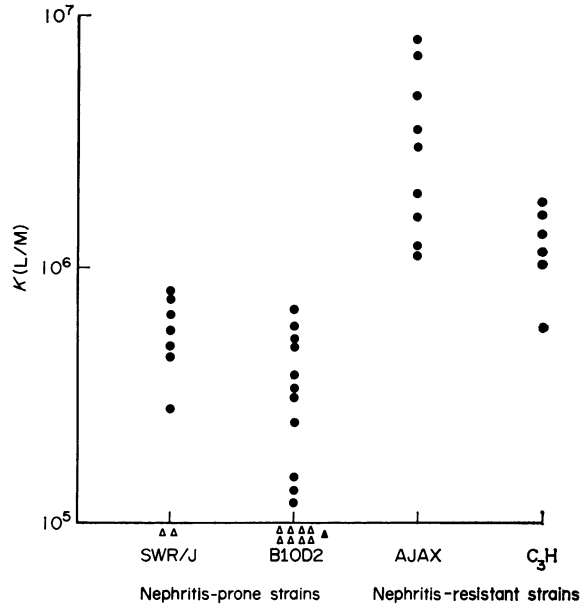


FIG. 1. The affinity ($K$) of anti-HSA antibodies in inbred mouse strains. △, Mice not producing detectable antibody; ▲, mice producing antibody but data not calculable.

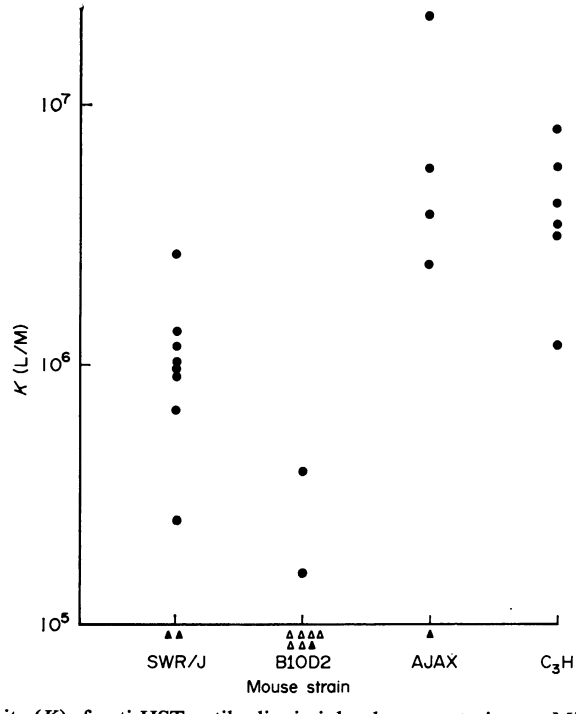


FIG. 2. The affinity ($K$) of anti-HST antibodies in inbred mouse strains. △, Mice not producing detectable antibody; ▲, mice producing antibody but data not calculable.

detected in some of the nephritis-prone mice (two SWR/J mice and fourteen B10D2 mice). The data derived from five mice were not calculable. All these mice have been omitted from further calculations and no statistical calculations have been made for anti-HST from B10D2 mice. With this exception, the differences between each of the nephritis-prone and each of the nephritis-resistant strains were highly significant (Student's *t*-test, see Table 2).

TABLE 1. Affinity and concentration of antibodies to HSA and HST in inbred mouse strains; numbers in parentheses refer to mice which produced antibody but the data was not calculable

| Strain | Antigen | No. of mice | No. of responders | Antibody ($\mu$g/ml) (log mean) | Affinity L/M (log mean) |
|---|---|---|---|---|---|
| SWR/J | HSA | 10 | 8 | 91 | $5{\cdot}7\times10^5$ |
| | HST | 10 | 8 (+2) | 68 | $9{\cdot}5\times10^5$ |
| B10D2 | HSA | 20 | 11 (+1) | 20 | $3{\cdot}1\times10^5$ |
| | HST | 9 | 2 (+1) | 23 | $2{\cdot}5\times10^5$ |
| AJAX | HSA | 9 | 9 | 36 | $2{\cdot}9\times10^6$ |
| | HST | 5 | 4 (+1) | 77 | $5{\cdot}8\times10^6$ |
| $C_3H$ | HSA | 6 | 6 | 25 | $1{\cdot}2\times10^5$ |
| | HST | 6 | 6 | 123 | $3{\cdot}7\times10^5$ |

TABLE 2. The statistical significance (Student's *t*-test) of the interstrain differences in affinity of antibodies to HSA and HST

| Strain | Antigen | Strain | | | |
|---|---|---|---|---|---|
| | | SWR/J | | B10/D2 | |
| | | *t* | *P* | *t* | *P* |
| AJAX | HSA | 5·644 | $<0{\cdot}001$ | 7·406 | $<<0{\cdot}001$ |
| | HST | 3·874 | $<0{\cdot}005$ | — | — |
| $C_3H$ | HSA | 3·732 | $<0{\cdot}005$ | 4·908 | $<<0{\cdot}001$ |
| | HST | 3·761 | $<0{\cdot}005$ | — | — |

*Antibody concentration*

Log mean values for antibody concentration for each strain and each antigen are given in Table 1. There was no suggestion of a relationship between amount of antibody produced, and either the affinity of the antibody, or of susceptibility to nephritis.

*Effect of adjuvant*

The effect of administration of 0·5 mg of HSA, either intraperitoneally in saline or subcutaneously in Freund's complete adjuvant, on affinity of antibody is shown in Fig. 3. The results with 0·5 mg in saline were similar to those obtained with 1 mg in Fig. 1, but

antibody of higher affinity, comparable to that of the nephritis-resistant strains, was produced by the nephritis-prone strains when the antigen was given in adjuvant. The affinity of antibody produced by AJAX mice was not altered by the use of adjuvant. All strains produced higher concentrations of antibody when adjuvant was used.

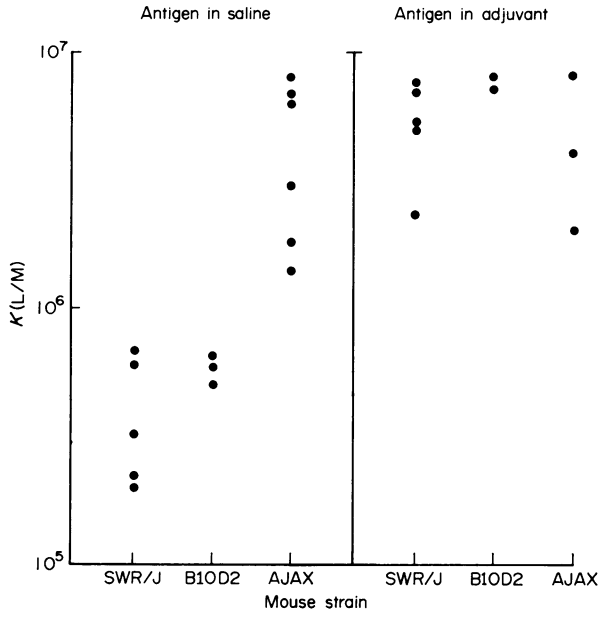


FIG. 3. The affinity of anti-HSA antibodies in inbred strains of mice when antigen is adminis tered in either saline or Freund's complete adjuvant.

## DISCUSSION

All antigen–antibody reactions are reversible, and the binding energy or affinity of the antibody can be described by an association constant. The affinity of all antibody is heterogeneous, but the affinity of antibody in different sera for the same antigen can be described by the average equilibrium constant, *K*. Most work in this field has concentrated on purified antibody of high affinity to single, relatively simple antigens, usually haptens (Eisen, 1966) and the biological significance of the marked heterogeneity of antibody in whole antisera, which is known to exist, has had only limited recognition (Hughes-Jones, 1967). Also, possible immunopathological significance of antigen-non-specific individual variation of antibody affinity has not been considered.

Our results show significant differences in the affinity of antibody produced by selected inbred strains of mice, to two soluble protein antigens, human serum albumin and transferrin, as expressed by their equilibrium constants. The strains studied were selected as a result of their susceptibility to nephritis when infected at birth with lymphocytic choriomeningitis (LCM) virus.

There are two basic mechanisms for the pathogenesis of nephritis: auto-allergic anti-kidney antibodies, and the deposition in the kidney of circulating soluble antigen–antibody

complexes (Unanue & Dixon, 1967). The latter probably predominates in progressive human nephritis, the best experimental model of which is the chronic antigen administration experiment of Dixon *et al.* (1961). They gave soluble protein antigens (bovine serum albumin, human serum albumin or bovine $\gamma$-globulin) intravenously to a very large number of rabbits, daily for many weeks, and showed that about one in twenty developed progressive nephritis. These rabbits were identifiable at the start of the experiment by the nature of their antibody response; they had no antigen or antibody detectable in the serum by a simple precipitation technique, 24 hr after the last injection. Animals which had free antigen did not develop nephritis, and those with free antibody developed acute nephritis and recovered. The authors interpreted the result as being related to the amount or rate of production of antibody, but Christian (1970) showed that these animals produced non-precipitating antibody; in any case, the liability subsequently to develop progressive nephritis appears to be related to the pattern of antibody response of the animal, and so to a heterogeneity of immunological response within a species (outbred), which is possibly genetically determined. Oldstone & Dixon (1969) studied the incidence of nephritis, and other evidence of chronic serum sickness (soluble complex induced vasculitis) in different inbred strains of mice, infected neonatally with LCM virus. All became chronically infected with the virus, and all developed antivirus antibodies, but the strains differed in the incidence of vascular disease. The SWR/J strain had 100% incidence, and the incidence fell away in the order B10D2, AJAX, $C_3H$; the latter had no disease. The authors discussed this in terms of the amount of circulating virus antigen, but, since the infecting dose was the same in all, any such differences would presumably depend on differences of immunity function of the host, specific or non-specific. One abnormality of function, possibly capable of producing this effect, might be the production of antibody which is poor at inducing immune elimination of antigen, perhaps a result of low affinity. Such antibody might itself fix complement in soluble complexes, and so produce complement mediated damage to the kidney or the small amount of high affinity antibody produced by such animals may be in a continuing state of antigen excess, because of the failure of immune elimination of antigen, so that it continuously forms antigen-excess complement-fixing soluble complexes. If such heterogeneity is indeed genetically determined, one individual in an outbred species (e.g. a patient with nephritis) will differ from another member of the same species in the same way as one inbred strain differs from another.

Since we started this study, one report of measurements of this type has appeared in which little or no inter-strain heterogeneity of affinity was noted (Paul, Yoshida & Benacerraf, 1970). These workers did not study strains known to be nephritis-prone and they used adjuvants in their immunization procedure, so their results cannot be regarded as inconsistent with ours.

There are at least four possible mechanisms for this phenomenon:

(1) Defective antigen processing by macrophages or helper-cells.
(2) Low energy threshold for stimulation of immunologically competent cells by antigen.
(3) Low energy threshold for induction of tolerance.
(4) Subclass-like deficiency, perhaps of light chain, leading to lack of heterogeneity of precise fit of antibody within broad ranges of specificity.

There are other possibilities, but these are put forward to indicate that this novel and unorthodox concept is not inexplicable. The lupus-like illness occurring in some mothers of patients with chronic granulomatous disease (Thompson & Soothill, 1970) may be a pointer

in favour of hypothesis (1). The finding (Nussenzweig & Benacerraf, 1967) that the light chains of guinea-pig high affinity antibodies to DNP are mainly $\kappa$, and those of low affinity are mainly $\lambda$ is possibly in favour of hypothesis (4).

If this does underlie the liability of some individuals to soluble complex disease if they get an infection which results in continuous release of large quantities of antigen into the circulation, the possibility of modifying this response is important if antigen-specific treatment is to be possible (Soothill, 1967). The finding that when antigen was given in adjuvant the affinity of antibody produced by nephritis-prone mice was similar to that of the nephritis-resistant mice points to the view that this may be possible. Clinical application of this idea could cover the whole range of soluble complex diseases, including nephritis, arthritis and various skin and gut diseases. More detailed studies are in hand of the response of these mice to haptens, of their *in vivo* handling of antigens, and of the genetics of this phenomenon (Steward, Petty & Soothill, 1971).

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