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Chronic immune complex disease in mice: the role of antibody affinity

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SUMMARY

Two lines of mice selectively bred for producing high and low affinity antibody to protein antigens were repeatedly injected with human serum albumin and the severity and pattern of immune complex disease induced in this way was studied in the two lines. In low affinity mice, there was a greater intensity of deposits in the glomeruli shown by immunofluorescence, and more antibody was eluted from kidney homogenates compared to high affinity line mice. In the low affinity mice, complexes were mainly on the basement membrane whereas in high affinity mice, the localization of immune complexes was predominantly mesangial. However, no significant difference in glomerular filtration rates between the two lines was obtained. The immunopathological significance of antibody affinity is discussed in the light of these results.

INTRODUCTION

Much of our understanding of the role of immune complexes in inducing disease arises from information obtained from experiments in rabbits. The injection of a single large dose of heterologous serum protein into rabbits results in an acute disease with the deposition of immune complexes in the kidneys, joints and blood vessels. However, although this 'acute serum sickness' model has provided insight into the mechanisms of immune complex disease, it has limited value for the study of human immune complex diseases. Longcope (1913), demonstrated that the repeated injection of foreign serum proteins into experimental animals (mainly rabbits) leads to the persistence of circulating immune complexes and the development of chronic disease and it is widely accepted that there is a relationship between the presence of circulating immune complexes and the development of chronic glomerulonephritis (see Weigle, 1961; Unanue & Dixon, 1967; Cochrane & Koffler 1973 for reviews). It is clear that several factors are involved in the persistence of immune complexes in the circulation and their subsequent tissue localization, including the amount (Dixon, Feldman & Vazquez, 1961), quality (Pincus, Haberkern & Christian, 1968), affinity (Soothill & Steward, 1971), and class of antibody (Lambert & Dixon, 1968; Steward & Hay, 1976); size (Cochrane & Hawkins, 1968; Mannik & Arend, 1971) and antibody–antigen ratio of complexes (Weigle, 1958; Mannik, Arend, Hall & Gilliland, 1971; Mannik & Arend, 1971; Arend & Mannick, 1971); reticuloendothelial function (Haakenstad & Mannik, 1975; Passwell, Steward & Soothill, 1974) and vascular permeability factors (Cochrane, 1971).

In infections with high antigenic load, even large amounts of antibody will result in the formation of injurious complexes. Under conditions where antigen is limited, an immune response producing low levels of antibody or antibody of a particular quality or affinity will fail to eliminate the antigen and this favours the production, persistence and subsequent tissue localization of antigen-excess immune complexes. In inbred mice selected for their susceptibility or resistance to chronic immune complex disease

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following infection with lymphocytic choriomeningitis virus (Oldstone & Dixon, 1969) the production of low affinity antibody is associated with susceptibility to immune complex disease (Soothill & Steward, 1971; Petty, Steward & Soothill, 1972). The hypothesis has been proposed that low affinity antibody fails to form immune complexes with a stable lattice and this results in the failure of immune elimination of antigen (Alpers, Steward & Soothill, 1972) and favours the formation of antigen excess immune complexes. The antigen excess complexes which are subsequently deposited in the tissues may contain either low affinity antibody or more probably, that small proportion of high affinity antibody, presumably present even in a low average affinity antibody population.

There is considerable evidence from this and other laboratories that antibody affinity is an antigen non-specific parameter of the immune response genetically controlled independently of antibody levels (Katz & Steward, 1975; 1976; Steward & Petty, 1976; Kim & Siskind, 1978). Two lines of T.O. mice have been produced by selective breeding on the basis of antibody affinity—one producing high, and the second producing low affinity antibody to protein antigens injected with saline (Katz & Steward, 1975). The two lines are histocompatible (Steward, Reinhardt & Staines, 1979), have similar macrophage clearance function (M.C. Reinhardt & M.W. Steward, unpublished observations) and produce similar concentrations of antibody and are therefore appropriate for determining whether the characteristic of low affinity antibody production predisposes to the production of chronic immune complex disease, following chronic administration of protein antigen in saline. The results of such experiments are described in this paper.

MATERIALS AND METHODS

Mice. Groups of 12 age and sex matched high and low affinity mice at the tenth generation of selective breeding were used.

Injections. Mice received daily (five times a week) injections of human serum albumin (Miles Ltd.) intraperitoneally in saline, and periodically 100 μl blood was obtained from the retro-orbital venous plexus for the determination of free antibody levels in the serum (see below). In addition, ^{125}I-labelled HSA was injected i.p. to determine the proportion of the injected antigen which was antibody-bound (i.e. circulating immune complex) by ammonium sulphate precipitation. On the basis of the results of these tests, the amount of injected antigen was adjusted to maintain the animals in a state of antigen excess. The doses of antigen injected ranged from 0·25 mg/day at the start of the experiment to 2·0 mg/day for the last 2 weeks, and both lines of mice were injected with the same doses of antigen.

Assessment of renal function. Glomerular function was assessed by measurement of the clearance of [^{51}Cr]-EDTA (Knight, Adams & Purves, 1977).

Measurement of antibodies and immune complexes. The levels of free anti-HSA antibody in serum and in renal eluates was determined by a double-isotope ammonium sulphate precipitation method using [^{125}I]-HSA (Gaze, West & Steward, 1973). The major immunoglobulin class of antibody was determined in a similar way using a double-antibody technique (Steward & Hay, 1976). The relative affinity (K_R, litres/mole) of anti-HSA antibody in sera and renal eluates was determined by the method described by Gaze, West & Steward (1973). Antibody-bound [^{125}I]-HSA (i.e. immune complex) present in sera following injection of mice with [^{125}I]-HSA was determined by precipitation of the bound antigen by 50% saturated ammonium sulphate.

Ultracentrifugation. Serum was obtained 24 hr after injection of mice with [^{125}I]-HSA, layered onto a linear 10–40% sucrose gradient and centrifuged at 35,000 r.p.m. for 18 hr at 4°C in a Sorvall OTD-2 Ultracentrifuge. Fractions (0·2 ml) were collected through a hole pierced through the bottom of the centrifuge tube. Molecular weight markers were run in separate tubes centrifuged at the same time as the experimental samples.

Histology and immunofluorescence studies. Kidneys were snap-frozen in isopropyl-alcohol-CO_2. Cryostat sections from both high and low affinity mice were stained with haematoxylin and eosin or with FITC-conjugated anti-mouse immunoglobulin or with FITC-conjugated rabbit anti-HSA antiserum (Nordic). Fluorescence was scored blind on a 0–4+ scale by an independent observer, the sections from both lines of mice were stained on the same day with the same antiserum preparation. The pattern of localization of fluorescence staining, i.e. on the basement membrane or in the mesangium, was also determined blind by an independent observer.

Elution of kidneys. Kidneys were snap-frozen and stored at −70°C until required, when they were thawed, homogenized (× 2) in 5 volumes (w/v) of 0·1 M phosphate buffered saline, pH 7·5, (PBS) at 4°C. The homogenate was mixed (× 2) with 10 volumes (w/v) of 2·4 M KBr, pH 9·0 (Bartolotti, 1977) for 30 min. The supernatants from the KBr extraction were pooled and dialysed against 0·02 M glycine HCl buffer, pH 2·2, and the free anti-HSA antibody was precipitated by the addition of an equal volume of saturated ammonium sulphate. The precipitate was dissolved in PBS and dialysed against PBS and assayed for free antibody as described above.

TABLE 1. Levels and affinity of anti-HSA antibody in generation 10 high and low affinity line mice

Mouse line	Antibody characteristics of mouse line*		Free serum antibody at the end of the chronic experiment†	
	K_R ($\times 10^6$ litres/mole) $\pm$ s.d.	Ab_t (p moles/10 μl serum) $\pm$ s.d.	K_R ($\times 10^6$ litres/mole) $\pm$ s.d.	Ab_t (p moles/10 μl serum) $\pm$ s.d.
High affinity	2·59 $\pm$ 1·20	85·1 $\pm$ 25·0	1·71 $\pm$ 0·58	178 $\pm$ 109
Low affinity	0·53 $\pm$ 0·38	61·7 $\pm$ 33·0	0·55 $\pm$ 0·35	163 $\pm$ 85·0

* Values from thirty-five mice of high line and thirty-nine mice from the low line from which the experimental animals were drawn. Differences in affinity between the two lines were highly significant ($P = 0{\cdot}005$) by the Student's t-test.

† Values from four mice of each line alive at the end of the chronic experiment. Differences in affinity between the two lines were significant ($P = 0{\cdot}025$) by the Student's t-test.

TABLE 2. Mortality in low and high affinity line mice during chronic antigen administration

Mouse line		Weeks of daily injections						
		2	4	6	8	10	12	15
Low affinity mice	Number of animals alive	11/12	11/12	11/12	11/12	9/12	7/12	7/12
	Total deaths due to renal disease*	0	0	0	0	2	4	4
High affinity mice	Number of animals alive	12/12	12/12	12/12	11/12	11/12	11/12	10/12
	Deaths due to renal disease	0	0	0	0	0	0	1

* As evidenced by a prior decrease in renal function assessed by increased $t_{\frac{1}{2}}$ for (^{51}Cr)-EDTA clearance.

RESULTS

Affinity characteristics of serum antibody

The relative affinity values (K_R) of anti-HSA antibody produced by generation 10 high and low line mice following four once-weekly injections of HSA in saline are shown in Table 1.

The difference in affinity between the two lines was highly significant when analysed by the Student's t-test ($P = 0{\cdot}005$). In addition, the K_R values of free serum antibody in mice of the same generation which had received repeated injections of HSA for 15 weeks are shown. The inter-line difference in affinity was still apparent and was significant by the student's t-test ($P = 0{\cdot}025$). Antibody (Ab_t) levels did not markedly differ between the two lines, although they were higher in the chronically-injected group than in the acutely-immunized animals.

Renal function and mortality during chronic antigen administration

The deaths of animals during the course of the experiment are shown in Table 2. One low affinity line mouse died after one week of injections whilst being handled. Four low line mice and one high line mouse died following a marked decrease in renal function as assessed by [^{51}Cr-EDTA] clearance (Fig. 1), presumably from renal failure. Histological and immunofluorescence studies on kidney section indicates severe renal disease (see below). A further high line mouse showed a marked decrease in renal

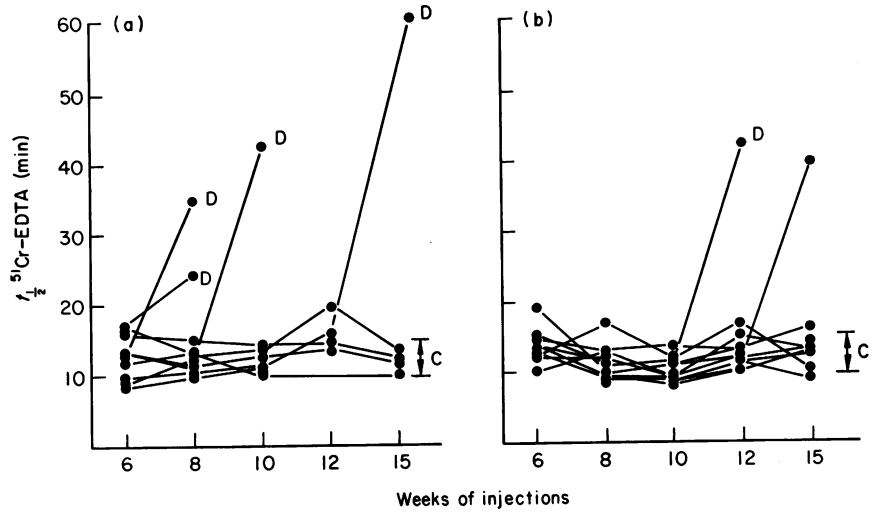

FIG. 1. [^{51}Cr]-EDTA clearance rates in (a) low and high (b) affinity mice. C, range of values for control animals. D, animals died within one week of measurement.

function after 15 weeks of injections but was still alive at the termination of the experiment. Thus chronic antigen administration did not result in statistically significant differences in renal function and mortality between the two lines.

Detection of circulating immune complexes

Antibody-bound [^{125}I] HSA in the serum of mice 24 hr after the i.p. injection of radiolabelled antigen was determined by precipitation with 50% saturated ammonium sulphate. The results are given in Table 3 together with the ratio of the labelled antigen remaining in the circulation of low and high affinity line mice. Complexed antigen was detected in the circulation of both low and high affinity mice, but no consistent pattern was observed. Sucrose density gradient ultracentrifugation (Fig. 2) of pools of sera from high and low affinity mice 24 hours after injection of [^{125}I]-HSA showed that the complexes in the sera of high affinity mice were slightly larger and less heterogeneous than those in low affinity mice. In molecular weight terms, the majority of the complexes in pools from high and low affinity mice were less than that of IgM (900,000) although a small proportion in both pools was of a molecular weight greater than this.

Immunofluorescence studies

The differences in intensity of fluorescence with FITC-conjugated anti-mouse immunoglobulin serum in high and low line kidneys (Fig. 3) were significant by both the chi-squared test ($\chi^2 = 5{\cdot}84$, $P = 0{\cdot}05$) and the Fisher's exact test ($P = 0{\cdot}02$). The differences between the two lines with FITC-anti-HSA staining were significant by the chi-squared test ($\chi^2 = 6{\cdot}66$, $P = 0{\cdot}05$) whilst the Fisher's exact test gave a probability of 0·06. The localization of fluorescence fell into three groups: (i) predominantly basement membrane (Fig. 4), (ii) predominantly mesangial (Fig. 5) and, (iii) both basement membrane and mesangial (Fig. 6).

Similar results were obtained with both anti-immunoglobulin and anti-HSA antisera. Kidneys from control mice of both lines showed very little fluorescence.

Most Low affinity mice (6/8) had predominantly basement membrane localization of fluorescence when stained with FITC-anti-mouse immunoglobulin whereas 3/7 high affinity mice had predominantly mesangial localization of fluorescence (Fig. 7). Statistical analysis of these small groups is difficult but chi-squared analysis of the differences between the two lines with both FITC-anti-HSA and FITC-anti-Ig staining gave χ^2 values of 5·30 and 5·54 respectively which just fail to achieve significance at the 5%

TABLE 3. Ammonium sulphate-precipitable ^{125}I-HSA in the serum of chronically immunized low and high affinity mice 24 hr after injection

Weeks of injections	Antigen dose (mg/day)	% serum [^{125}I]-HSA globulin bound* Low affinity mice	High affinity mice
2	0·25	14	11
8	1·0	33	55
12	1·0	74	87
13	2·0	51	61
15	2·0	74	56

* Mean values of groups of 7–12 mice.

TABLE 4. Anti-HSA antibody in pooled renal eluates from high and low affinity mice

	Renal eluate		
Mice	Antigen binding capacity (μg HSA)*	Ab_t (pmoles/30 μl)	Affinity (L/M)
Low affinity	33·3	25·4	$1{\cdot}7\times10^6$
High affinity	8·3	6·25	$1{\cdot}3\times10^6$

* Antigen binding capacity based on the binding by the eluate of 1 μg[^{125}I]-HSA expressed as μg HSA bound/g kidney.

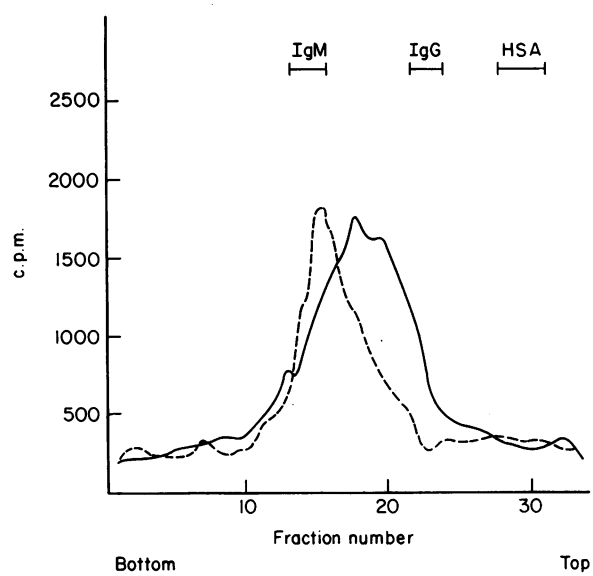

FIG. 2. Sucrose density gradient ultracentrifugation (10–40%) of pooled sera from low and high affinity line mice 24 hr after injection of[^{125}I]-HSA injected intraperitoneally. (– – –) High line mice. (———) Low line mice. Sedimentation positions of HSA, IgM and IgG are also shown.

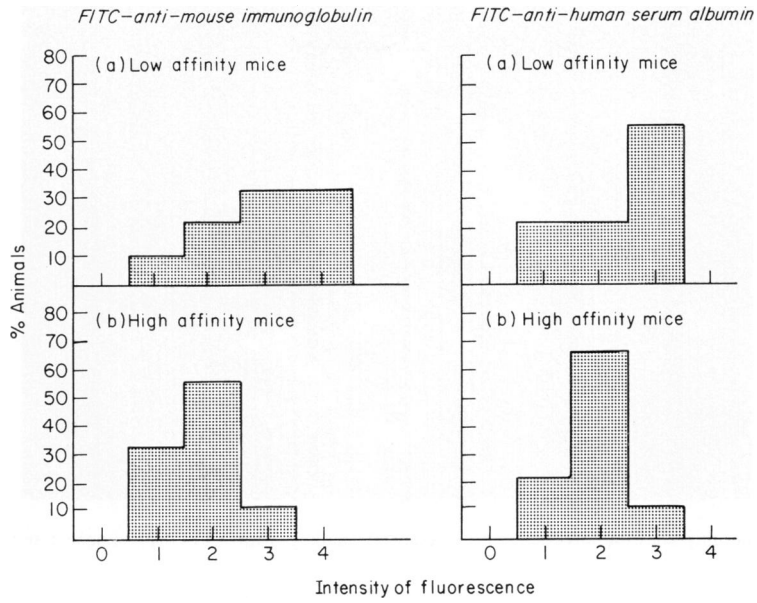

FIG. 3. Intensity of fluorescent deposits in glomeruli of chronically-immunized (a) low and (b) high affinity line mice. The sections were coded and scored blind by an independent observer. Stained with FITC-conjugated rabbit anti-mouse globulin and with FITC-conjugated anti-HSA.

level. However, using the Fisher's exact test, the localization of fluorescence with FITC-anti-Ig in high and low affinity mice was significant ($P = 0{\cdot}03$) when mice with basement membrane localization were compared with all mice with mesangial localization of fluorescence. In addition, significant differences between the two lines ($P = 0{\cdot}045$) were obtained when those mice showing mesangial localization were compared with all mice showing basement membrane localization of FITC-anti-HSA. Other comparisons did not show significant differences.

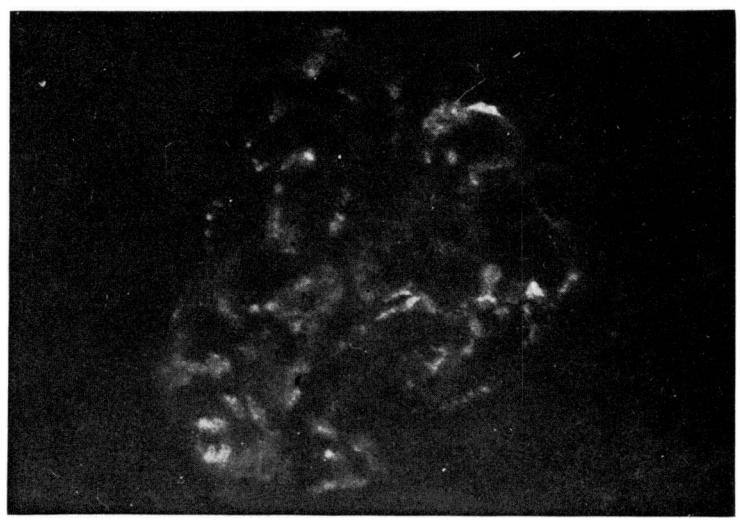

FIG. 4. Glomerulus from a mouse chronically injected with HSA stained with FITC-conjugated anti-mouse immunoglobulin showing predominantly basement-membrane localization of staining (× 300).

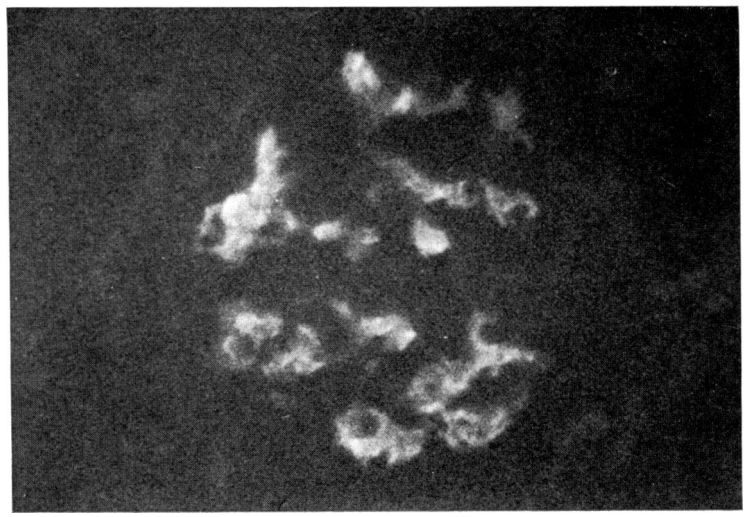

FIG. 5. Glomerulus showing predominantly mesangial staining with FITC-anti-mouse immunoglobulin (× 300).

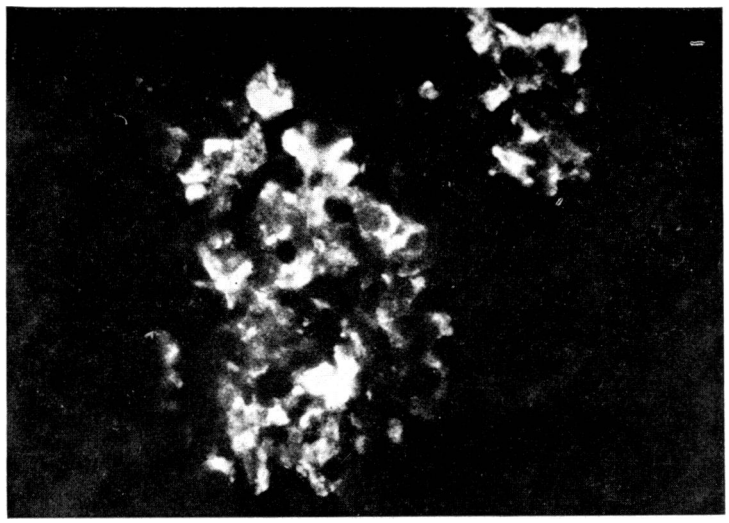

FIG. 6. Glomerulus showing basement membrane and mesangial staining with FITC-anti-mouse immunoglobulin (× 300).

The severity of the immune-complex mediated damage arising in mice following chronic antigen administration is illustrated by the light-microscopy picture of a glomerulus of a kidney from a low affinity line mouse showing epithelial crescent formation, cellular infiltration and necrosis (Fig. 8). The kidneys from control mice of both strains were normal.

Antibody levels in renal eluates

The renal eluates from both low and high affinity mice bound [^{125}I]-HSA, i.e. contained antibody and the class of antibody was predominantly IgG in both lines. However, the pooled eluates from low affinity kidneys bound four times more [^{125}I]-HSA than did that from high affinity mice (Fig. 4). This is consistent with the greater intensity of fluorescence in low affinity kidneys. The affinity of the antibody was high (i.e. greater than 10^6 L/M) in both renal eluate pools but the low line pool had a higher Ab_t value.

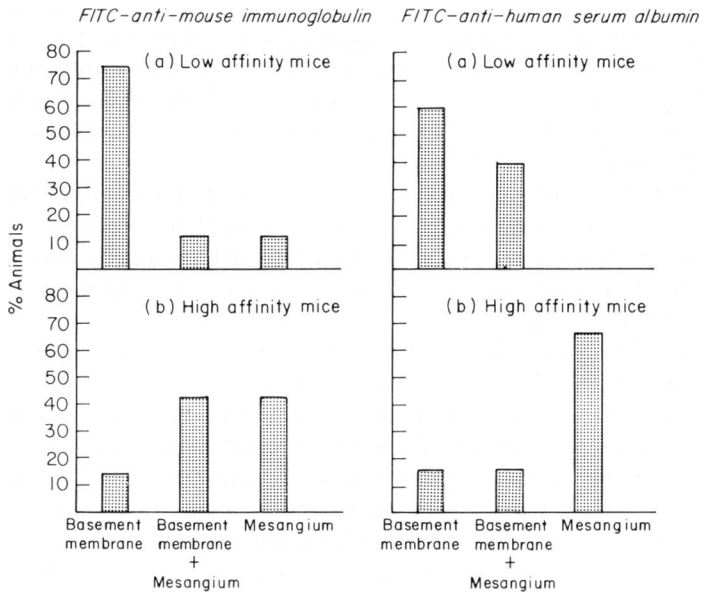

FIG. 7. Immunofluorescence staining patterns of glomeruli in low and high line affinity mice—proportion of animals with the various staining patterns Stained with FITC-conjugated anti-mouse Ig, and FITC-conjugated anti-HSA.

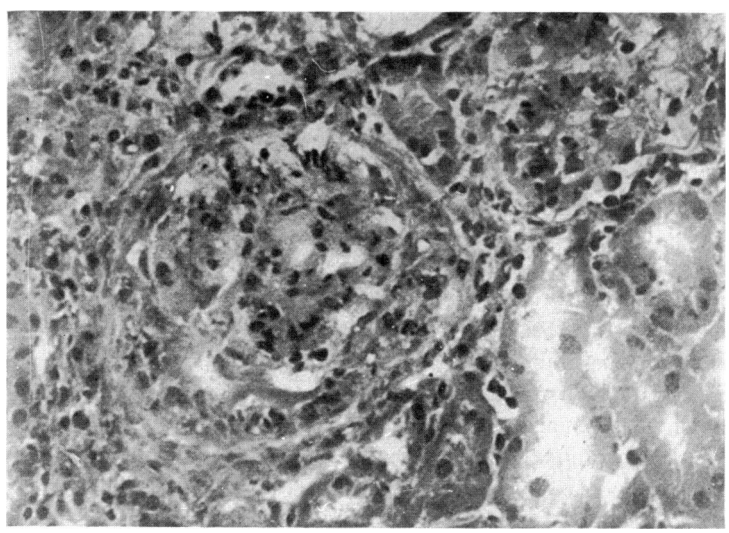

FIG. 8. Glomerulus from mouse chronically injected with HSA showing epithelial crescent formation, cellular infiltration and evidence of necrosis. Haematoxylin and eosin (× 225).

DISCUSSION

The work described here has demonstrated the induction of chronic immune complex disease in mice following repeated injections of human serum albumin. Studies of this kind have been carried out almost exclusively in rabbits but Wood & White (1956) have reported the induction of disease in mice following multiple injections of heat-killed *Proteus Mirabilis*. However, the present studies are the first to attempt to investigate genetically-determined differences in susceptibility to chronic serum sickness in mice. The mice used were obtained by a process of selective breeding based on the relative affinity of

antibody produced to protein antigens injected in saline (Katz & Steward, 1975) which resulted in the generation of two lines of mice—one producing high and the second producing low affinity antibody to HSA and HST. The two lines had similar macrophage clearance function, histocompatibility type and antibody levels and the object of the study was to determine whether differences in susceptibility or disease pattern following chronic protein antigen administration could be demonstrated and whether such differences were related to antibody affinity.

The amount of HSA given daily was calculated to maintain the animals in a state of slight antigen excess in a manner analogous to the approach used by Dixon *et al.* (1961) in rabbits. The results obtained showed that under these experimental conditions, both high and low affinity mice developed immune complex disease as indicated by the presence of circulating immune complexes and deposition of immunoglobulin and antigen in the glomeruli.

However, there were significant differences between the two lines in the intensity of immunofluorescent deposits and in the pattern of localization of fluorescence. Sections of kidneys from low affinity mice showed intense fluorescent staining localized predominantly on the basement membrane; kidneys from high affinity mice had less intense staining localized predominantly in the mesangium. Furthermore, four times as much anti-HSA antibody was recovered from renal eluates from low affinity mice than from high affinity mice. In spite of these differences in intensity and localization of fluorescence, the experiments described here did not result in a statistically significant difference in the incidence of renal function impairment between the two lines of mice in that four low affinity and two high affinity mice showed decreased glomerular filtration. In this context it is of interest that mesangial deposition of antigen-antibody complexes in human lupus nephritis is generally associated with a better prognosis than are proliferative and membranous glomerulonephritis (Baldwin *et al.*, 1977), but such mesangial lesions may also be associated with an impaired glomerular filtration rate (Appel *et al.*, 1978).

Recently, Koyama *et al.* (1978) have demonstrated differences in the renal localization of passively-transferred anti-DNP–DNP-protein complexes—comprised of antibodies of different average affinity. Complexes with high affinity anti-DNP antibody localized primarily in the mesangial region whereas complexes with lower affinity anti-DNP localized on the basement membrane. These observations in an acute nephritis model system are consistent with those presented in this paper in a chronic nephritis model and both can be interpreted in the context of the theory proposed by Germuth & Rodriguez (1973). These workers have suggested that small soluble immune complexes (Class 1) localize on the glomerular basement membrane to form sub-epithelial deposits and larger, slightly less soluble complexes (Class II) localize in the sub-endothelial-mesangial region. If high affinity antibody produces larger, more stable complexes than a lower affinity antibody, the predominantly basement membrane deposition seen in the low affinity line mice can be interpreted as resulting from the localization of small soluble immune complexes (Class 1) involving low affinity antibody. The predominantly mesangial deposition of immunoglobulin and antigen in the high affinity mice can be viewed as resulting from the localization of large (Class II) immune complexes comprised of antigen and higher affinity antibody. The results of the renal elution studies described here in which high affinity antibody was recovered in eluates from both high and low affinity mice are not totally in accord with this interpretation. The recovery of high affinity antibody from the kidneys of low affinity mice may have resulted from a loss of the low affinity antibody during the isolation procedure. It is more likely, however, that this antibody represents the high affinity antibody present in the heterogeneous low average affinity response of these mice, deposited as antigen-excess immune complexes. The presence of high-affinity antibody in the kidneys of high affinity mice presumably arises from the injection of such levels of antigen that even this high affinity strain, capable of eliminating lower levels of antigen effectively, would be in antigen excess. Experiments are in progress to fully define the affinity characteristics of antibody deposited in the glomeruli in antigen–antibody complex form at different stages of chronic serum sickness in these genetically-selected mice.

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