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Chronic serum sickness in the rat: influence of antigen dose, route of antigen administration and strain of rat on the development of disease

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SUMMARY

Chronic serum sickness (CSS) with systemic immune complex deposition has been produced in female Fischer (F344) rats by the daily intravenous (i.v.) administration of 2·0 mg bovine serum albumin (BSA) (Arisz *et al.*, 1979). In the experiments described below, daily i.v. doses ranging from 0·5 to 10·0 mg BSA were found to be effective in producing CSS in F344 strain rats. The severity of renal disease and the extent of extrarenal immune complex deposition were increased with higher daily doses of BSA. Daily administration of different doses of BSA by an intraperitoneal (i.p.) route resulted only in slight mesangial glomerular abnormalities and did not cause abnormal elevation of urinary protein excretion. At the same time, extrarenal accumulation of immune deposits similar to that observed in rats given BSA by the i.v. route was seen. Wistar and Lewis (LEW) strain rats were similar to F344 strain rats in susceptibility to the induction of CSS, but daily i.v. injection of 2·0 mg BSA failed to produce the disease in Brown Norway (BN) rats. The latter observation suggests that genetic differences may influence the expression of immune complex disease in this model.

INTRODUCTION

Immune complex glomerulonephritis can be produced in rabbits by chronic intravenous (i.v.) immunization with heterologous proteins (Dixon, Feldman & Vazquez, 1961; Germuth, 1953). Systemic deposition of immune complexes in organs besides the kidney has been described to occur in rabbits with a high antibody response which are given correspondingly high doses of bovine serum albumin (BSA) (Brentjens *et al.*, 1975). Several immunization protocols have been reported to lead to the development of immune complex glomerulonephritis in other animal species, including mice (Stilmant, Couser & Cotran, 1975; Steward, 1979; Hagstrom *et al.*, 1979; Noble *et al.*, 1980), rats (Fennell & Pardo, 1967; Arisz *et al.*, 1979) and chickens (Albini *et al.*, 1979b). The advantages of studying the pathogenesis of immune complex disease in these species are obvious. For example, because of the availability of inbred strains, the disease may be elicited with great uniformity of expression among individuals, the genetic basis of susceptibility to disease induction can be analysed and cell-transfer studies can be performed to examine the significance of the underlying cellular immune response.

We have shown that severe chronic serum sickness (CSS) glomerulonephritis associated with deposition of immune complexes in many organs besides the kidney can be produced in female F344

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rats (Arisz *et al.*, 1979). To characterize this model in more detail, different daily doses of BSA administered i.v. were compared for efficiency in producing glomerulonephritis and systemic immune complex deposition in rats of the F344 strain. Because intraperitoneal (i.p.) injections of BSA were effective in producing CSS in mice (Noble *et al.*, 1980), the i.p. route of administration of different doses of BSA was also tested in F344 rats. In addition, several different strains of rat were immunized with the same 2-mg dose of BSA to evaluate possible strain differences in susceptibility to the induction and expression of CSS.

MATERIALS AND METHODS

Animals

F344 and Wistar strain rats were obtained from Microbiological Associates (Walkersville, Maryland) and LEW rats from Charles River Breeding Laboratories (Wilmington, Massachusetts). BN rats were the gift of Dr C. J. Abeyounis, Department of Microbiology, State University of New York at Buffalo, Buffalo, New York. All rats used in these experiments were female, weighing approximately 125 g at the start of the immunization protocol. All were fed rat pellets (Charles River Rat Formula, Agway, Syracuse, New York) and water *ad libitum*.

Immunization protocols

All rats were immunized three times in footpads with 3·0 mg BSA in Freund's incomplete adjuvant (FIA), according to a scheme described previously (Arisz *et al.*, 1979). Three subcutaneous injections of BSA with FIA resulted in production of antibodies to BSA with titres (expressed as the $\log_2$ of the highest serum dilution giving an unequivocal positive reaction) of 4–5, as detected by immunodiffusion tests (Arisz *et al.*, 1979).

Intravenous injections. To avoid death from anaphylaxis, daily i.v. tail vein injections of BSA were introduced gradually, until the desired daily i.v. dose of 0·5, 2·0, 5·0 or 10·0 mg was achieved (Arisz *et al.*, 1979). Animals in all groups were killed when death appeared imminent. BN rats were an exception, and were killed after 10 weeks, in the absence of clinical signs of illness.

Intraperitoneal injections. Intraperitoneal injections of high BSA doses were also introduced gradually, increasing the daily dose to 5·0, 10·0 or 20·0 mg as desired. Rats in all groups were killed when free BSA was detectable in plasma samples taken 24 hr after the previous daily injection.

Control experiments. Twenty F344 rats given footpad immunizations of BSA in FIA served as controls. Ten received daily i.v. injections of 0·5 ml saline for 8 weeks; 10 received i.p. injections of 0·5 ml saline daily for 4 weeks. Twenty rats were given footpad injections of FIA alone. Of these, 10 received i.v. injections of 2·0 mg BSA for 8 weeks, the others, i.p. injections of 10 mg BSA for 4 weeks.

Other procedures

BSA (Cohn Fraction V) obtained from Miles Laboratories (Kankakee, Illinois) was either emulsified with FIA (DIFCO Laboratories, Detroit, Michigan) or dissolved in sterile isotonic saline at the desired concentration.

For the determination of urinary protein excretion, rats were kept overnight in metabolism cages for 16 hr without access to food. The protein content of the urine was measured by the biuret method. The mean ($\pm$s.d.) urinary protein excretion of normal rats was 15 ± 4 mg with this test. Creatinine in plasma samples was analysed after absorption and elution from Lloyd's reagent (Hare, 1950). Albumin concentration in urine was determined by micro-continuous-gradient gel electrophoresis (Feld *et al.*, 1977).

Blood samples for measurement of antibodies to BSA were collected in haematocrit tubes by tail vein puncture. Samples to be tested were obtained 24 hr after the last daily injection of BSA. Precipitating antibodies to BSA were detected in Ouchterlony plates containing 1·0% agarose as previously described (Arisz *et al.*, 1979). Free circulating BSA was detected with Ouchterlony immunodiffusion tests, using rabbit anti-BSA. In blood samples obtained at death by heart

puncture, serum albumin concentrations were measured by the method of Laurell with a monospecific rabbit antiserum to rat albumin, as previously described (Arisz *et al.*, 1979).

Examination of tissues by light, immunofluorescence and electron microscopy was done by methods described in detail before (Brentjens *et al.*, 1974, 1975). For immunofluorescence tests, FITC-conjugated rabbit antisera to BSA and rat immunoglobulin (Ig) and an FITC-conjugated goat anti-rat complement (C3) were obtained from Cappel Laboratories (Cochranville, Pennsylvania).

Tissues studied by means of direct immunofluorescence tests included kidney, spleen, ovary, heart, intestine, lung and liver. The extent of deposition of rat Ig in glomeruli of the kidney was evaluated and scored on a scale from 0 to 3·0 (0 = no detectable deposits, 1·0 = finely granular deposits, 2·0 = heavy granular deposits, 3·0 = granular to ribbon-like deposits). The extent of deposition of rat Ig in tissues besides the kidney was also scored on a scale from 0 to 3·0 (0 = no detectable deposits, 1·0 finely granular deposits of focal distribution, 2·0 = granular deposits of moderate density of distribution throughout the tissue, 3·0 = heavy deposits distributed diffusely in the tissue).

RESULTS

Daily intravenous injections of different doses of BSA

Antibody titres and urinary protein excretion. Fig. 1 shows antibody titres to BSA in F344 rats receiving different daily doses of BSA i.v. The rapidity with which the titre of circulating antibodies dropped to undetectable concentrations was correlated inversely with the daily dose of BSA.

Clinical observations. With the onset of proteinuria (Table 1), rats in all groups rapidly developed severe glomerulonephritis. The majority of animals given 10·0 mg BSA i.v. appeared to be seriously ill and were killed in the 3rd week of the experiment, those given 5·0 mg BSA i.v. in the 4th week, and those given 2·0 mg in the 5th week. Rats on an i.v. dose of 0·5 mg BSA did not become moribund for 15 weeks. The mean creatinine concentration measured in plasma samples taken at the time of death was 0·9 mg/dl, compared with a normal mean value of 0·4 mg/dl. Mean albumin excretion, which was < 1·0 mg/24 hr before i.v. injections began, rose to > 50 mg/24 hr at the time of death. Mean (± s.e.) serum albumin concentrations at the time of death in eight rats given 10·0 mg BSA i.v. were 15 ± 2·3% of the normal value, a decrease similar to that previously reported for rats given 2·0 mg BSA i.v. (Arisz *et al.*, 1979). Average serum albumin concentration in two rats given 0·5 mg was 25% of normal. At autopsy, lipaemic sera, ascites and oedema were observed in all rats. All kidneys were pale and enlarged. The clinical picture of nephrotic syndrome was comparable to that previously documented in rats given 2·0 mg BSA i.v. (Arisz *et al.*, 1979).

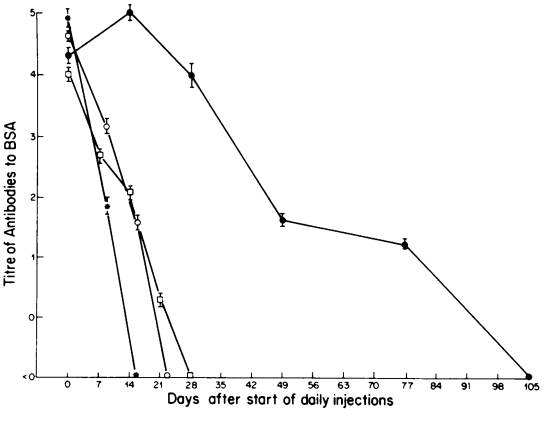

Fig. 1. Mean titres of antibodies to BSA in F344 rats receiving various doses of BSA i.v. (*——*) Titres in rats given 10·0 mg, (o——o) 5·0 mg, (□——□) 2·0 mg and (●——●) 0·5 mg. The standard error of each mean value is also indicated.

Table 1. Chronic immunization of F344 rats with bovine serum albumin

Daily dose	Injection route	*n*	Week of onset of proteinuria*	Maximum urinary protein excretion (mg/24 hr)	Extent of renal Ig deposits in:†	
					Glomerular capillary wall	Mesangium
0·5	i.v.	3	15	85±31‡	2·0±0‡	0·5±0·1‡
2·0	i.v.	12	4	160±22	3·0±0	1·5±0·2
5·0	i.v.	5	3	145±63	3·0±0	2·8±0·4
10·0	i.v.	24	2	195±80	3·0±0	3·0±0
5·0	i.p.	13	—	20±3	0·3±0·2	2·2±0·3
10·0	i.p.	22	—	17±5	0·3±0·1	2·3±0·3
20·0	i.p.	12	—	21±7	0·4±0·2	2·6±0·2

* First week after start of daily injections in which mean urinary protein excretion of the group was elevated significantly above normal values (> 20 mg/24 hr).

† As tested by direct immunofluorescence.

‡ Mean ± standard error.

Direct immunofluorescence tests of kidney and other organs. Heavy granular to ribbon-like deposits of BSA, Ig and C3 were found in the glomerular capillary wall of the kidneys of all rats given 10·0 mg BSA i.v.; the mesangium also contained heavy deposits (Table 1). Immune deposits of comparable extent were found in the glomerular capillary wall with i.v. doses of 2·0 and 5·0 mg. With a daily dose of 0·5 mg i.v., fine granular immune complex deposits were seen in the glomerular capillary wall with little or no accumulation observed in the mesangium.

Immune deposits were found to be distributed throughout all organs examined from rats receiving various doses of BSA i.v. The pattern of distribution of deposits was identical to that previously reported in F344 rats with CSS induced by 2·0 mg BSA i.v. (Arisz *et al.*, 1979). Spleen and ovary tissue were positive in all rats in all groups. An incidence of approximately 80% of positive immunofluorescence in heart and intestine was found. Liver (mainly periportal vessels) and lung were positive in approximately 50% of samples studied. More clearly dose-dependent than the absolute incidence of immune deposits in organs other than the kidney was the extent of deposits seen in tissues obtained from rats in different groups. Heavy granular deposits, judged to be 2·0–3·0 in extent in all cases, were present in all tissues taken from animals given 5·0 and 10·0 mg BSA i.v. daily, whereas deposits in animals given lower doses (2·0 and 0·5 mg) were finer, less diffusely distributed in the tissues, and judged to range from 0·5 to 3·0 in extent.

Histology of the kidney. Examination by light and electron microscopy of kidney tissue from rats receiving 5·0 and 10·0 mg BSA i.v. revealed the development of severe necrotizing proliferative glomerulonephritis in all cases (Fig. 2). Interstitial cellular infiltration and tubular atrophy were observed in most kidneys. A milder proliferative glomerulonephritis found in kidneys of animals given 0·5 and 2·0 mg BSA was comparable in severity to that previously described (Arisz *et al.*, 1979). With all doses of BSA, the glomeruli contained many lipid-laden monocytes (Fig. 2) which could be seen to phagocytose electron-dense deposits present at the endothelial side of the glomerular basement membrane (Fig. 3). The large monocytes were frequently seen to occlude the lumen of glomerular capillaries, either partially or completely.

Daily intraperitoneal injection of different doses of BSA

Antibody titres and urinary protein excretion. Titres of antibodies to BSA dropped rapidly in all groups, reaching undetectable concentrations within 14 days. Mean urinary protein excretion did not rise significantly above normal values in any group during the course of the immunization (Table 1).

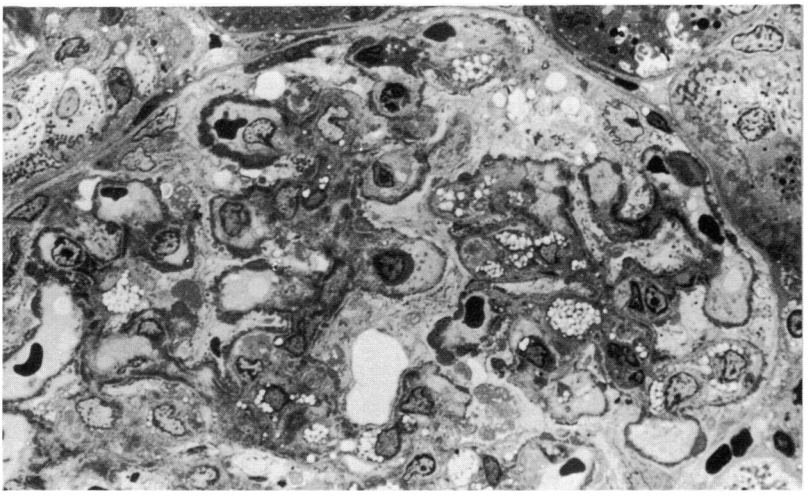

Fig. 2. Light micrograph of part of a glomerulus from the kidney of a rat given 10·0 mg BSA i.v. Destruction of normal glomerular architecture and proliferation are evident. Polymorphonuclear leucocytes and vacuolated macrophages have accumulated, causing partial or complete obstruction of many capillary lumina. Foreign protein deposits are visible along the walls of many capillaries. (Toluidine blue, × 1,000.)

Clinical observations. Rats in all groups appeared healthy and normal at the time of killing. Serum albumin concentrations in samples collected at the time of death were 44 ± 14% of the normal value in rats receiving 20·0 mg i.p., 53 ± 18% in those given 10 mg i.p. and 49 ± 8% in rats given 5·0 mg i.p.

Direct immunofluorescence tests of the kidney and other organs. Deposits of Ig, BSA and C3 were found in the kidneys of all rats. The predominant site of deposition in all cases was the mesangium (Table 1). Occasional deposits were observed in the glomerular capillary wall in some kidneys, but these were never judged to be greater than 1·0 in extent in individual kidneys. Granular deposits of immune complexes were detected in many organs besides the kidney of all rats given BSA i.p. The extent of extraglomerular deposits showed little dependence on antigen dose, and varied from 1·0 to 2·8. Deposits were least extensive in liver tissue (limited primarily to periportal vessels) and lung, and most extensive in intestine and spleen.

Histology of the kidney. Examination of kidney tissue by light microscopy revealed evidence of mild renal disease. Lobulation, segmental proliferation and increase in mesangial matrix were observed. Electron-dense deposits were observed only in mesangial areas. Polymorphonuclear leucocytes were present infrequently within glomeruli; the vast majority of capillaries remained patent; tubules and the renal interstitium appeared normal.

Daily intravenous injections of 2·0 mg BSA to rats of different strains

Antibody titres and urinary protein excretion. Fig. 4 shows antibody titres to BSA in LEW, Wistar and BN rats receiving a 2·0-mg dose of BSA i.v. daily. Titres dropped to undetectable concentrations in LEW rats after 6 weeks of daily injection and in Wistar rats within 8 weeks. In BN rats, the titre of circulating antibodies to BSA remained high throughout the observation period. Abnormal elevation of mean urinary protein excretion was first detected in LEW rats 4 weeks after the start of daily i.v. injection and after 5 weeks in Wistar rats (Table 2). Urinary protein excretion of BN rats remained within normal limits throughout the observation period.

Clinical observations. LEW and Wistar rats with glomerulonephritis developed ascites and lipaemia, and were found to have pale, enlarged kidneys at autopsy. BN rats appeared normal in all respects at the time of death.

Direct immunofluorescence tests of the kidney and other organs. Extensive granular to ribbon-like deposits of Ig, BSA and C3, comparable in extent to those in F344 rats, were observed in the

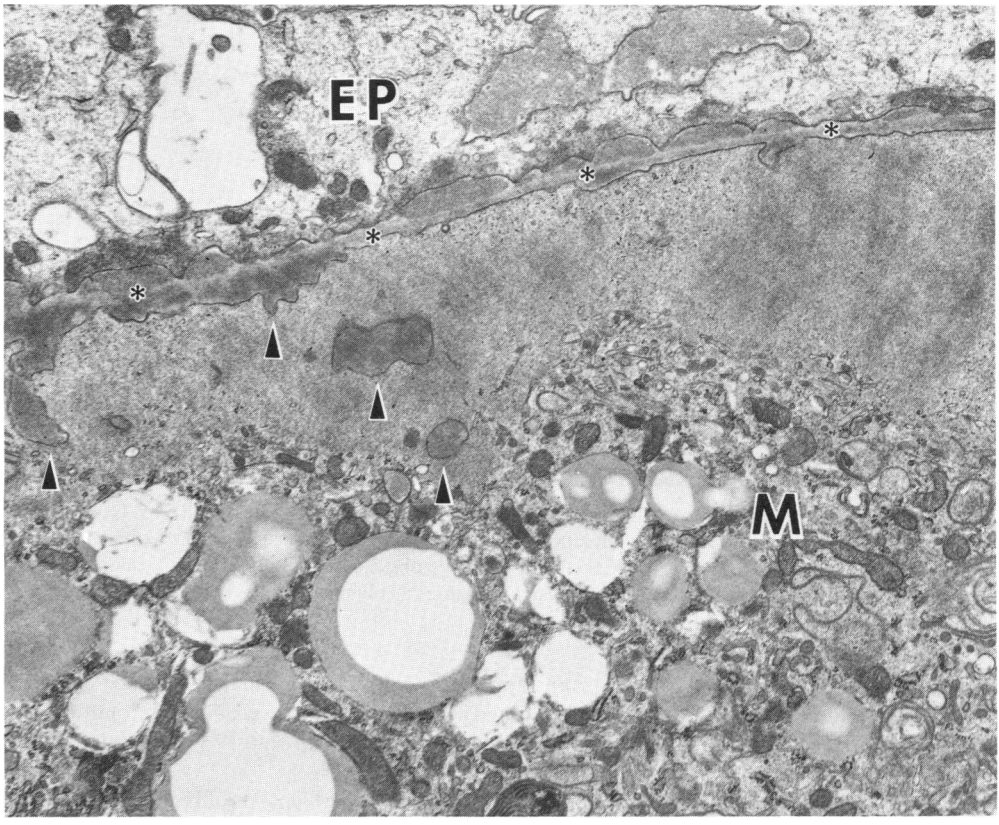

Fig. 3. Electron micrograph of part of a glomerulus from a rat given 10·0 mg BSA i.v. Part of a lipid-laden cell of the monocyte–macrophage series (M) is in direct contact with the glomerular basement membrane (*). Subepithelial electron-dense deposits are present. Deposits at the endothelial side of the glomerular basement membrane appear to be phagocytosed (*arrows*) by the monocyte. In the area of close contact between the phagocytic cell and the basement membrane, the cytoplasm of the phagocyte has a homogeneous appearance. The epithelial cell (EP) has lost foot processes. (× 17,000.)

glomerular capillary wall of all LEW and Wistar rats studied. In the kidneys of BN rats, a moderate accumulation of immune reactants, judged to be 1·0 in extent, was limited to mesangial areas of the glomerulus (Table 2).

Deposits of immune complexes were found in many organs besides the kidney of LEW and Wistar rats. The deposits detected in tissues of Wistar strain rats were among the heaviest and most extensive encountered in the course of these studies. In BN rats, all organs examined by direct immunofluorescence tests were found to be free of Ig (Table 2).

Histology of the kidney. The histology of kidneys of the LEW and Wistar rats did not differ from that of F344 rats receiving 2·0 mg of BSA i.v. In kidneys of the BN rats a mild mesangial pathology was observed.

Daily intravenous or intraperitoneal injections in control experiments

Twenty F344 rats immunized with BSA in FIA had a mean (±s.e.) titre of antibodies to BSA of 4·4±0·3 at the start of daily saline injections. The mean titre of antibody to BSA in 10 rats after 8 weeks of i.v. saline injections was 3·5±0·3. In 10 receiving saline i.p. daily for 4 weeks, the mean titre at the time of killing was 4·1±0·2. Antibodies to BSA were never detected in plasma of rats immunized subcutaneously three times with FIA followed by daily i.v. or i.p. injections of BSA. However, BSA was present in all plasma samples taken 24 hr after the previous daily injection of

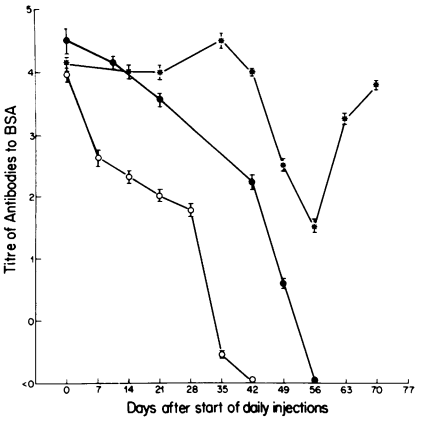

Fig. 4. Mean titres of antibodies to BSA in rats of different strains receiving 2·0 mg BSA i.v. daily. (o——o) Titres in LEW rats, (●——●) Wistar rats and (*——*) BN rats. The standard error of each mean value is also indicated.

Table 2. Chronic immunization of several rat strains with bovine serum albumin

Strain	*n*	Week of onset of proteinuria*	Maximum urinary protein excretion (mg/24 hr)	Ig deposits in glomerular capillary wall	Ig deposits in organs besides kidney†
LEW	16	4	242±53‡	Yes	Yes
Wistar	6	5	270±81	Yes	Yes
BN	5	—	22±4	No	No
F344	12	4	160±22	Yes	Yes

* First week after start of daily i.v. injection in which mean urinary protein excretion was >20 mg/24 hr.

† Of each strain, tissues taken from five animals were studied.

‡ Mean ± standard error.

BSA. Mean urinary protein excretion was never abnormally elevated in control groups. Kidneys of animals in all control groups were normal in every respect.

DISCUSSION

Daily i.v. administration of BSA is effective in producing glomerulonephritis as well as systemic immune complex disease in F344 rats, using a wide range of different doses. High doses of BSA (5·0 and 10·0 mg per day) resulted in more severe proliferative glomerulonephritis and produced disease more rapidly than lower doses (2·0 and 0·5 mg daily). An accumulation of fat-laden monocytes was observed in glomeruli of all rats receiving various doses of BSA i.v., but their appearance was most prominent in kidneys of rats given high doses. Monocytes are thought to play a pathogenic role in immunologically mediated kidney disease (Monga *et al.*, 1979; Hunsicker *et al.*, 1979). Their potential to accumulate fat has also been shown *in vitro* (Zucker-Franklin, Grusky & Marcus, 1978). In patients it has been noted that intraglomerular monocytes are most numerous in nephropathies characterized by subendothelial electron-dense deposits (Magil, Wadsworth &

Loewen, 1981). In CSS nephritis in the rat, monocytes with lipid inclusions appeared to phagocytose the deposits located in this position. Furthermore, the cytoplasm of phagocytic cells in areas adjacent to the glomerular basement membranes was homogeneous in appearance and did not contain clearly differentiated organelles. A similar appearance of cytoplasm has been described in giant cells seen to phagocytose basement membrane segments of tubules in guinea-pigs with autoimmune tubulointerstitial nephritis (Andres *et al.*, 1979).

With i.v. administration of antigen, systemic deposition of immune complexes was always associated with the development of severe renal disease. Tissues from animals killed before the onset of proteinuria or in the early stages of renal disease were never found to have significant accumulation of immune complex deposits when the glomerular capillary wall did not also contain extensive immune deposits (unpublished observations). In contrast, with i.p. injection of antigen, the deposition of immune complexes in many tissues was not accompanied by proteinuria. Kidneys of rats receiving BSA i.p. were found to have only mesangial accumulation of immune deposits with minimal changes in glomerular architecture limited primarily to the mesangium. The dissociation of systemic immune complex deposition from serious renal pathology, achieved by the protocol of i.p. immunization, may provide a model in which to study functional consequences of immune complex deposition in a number of organs. The reduced concentration of albumin found in sera of rats immunized chronically by an i.p. route may reflect immunologically mediated impaired function of other organs, e.g. the intestine, which could permit abnormal loss of serum components.

In systemic lupus erythematosus (SLE), a human disease of which experimental chronic serum sickness may be considered a laboratory model, intestinal manifestations associated with protein-losing enteropathy have been reported (Waldmann, Wochner & Strober, 1969; Pachas, Linscheer & Pinals, 1971; Trentham & Masi, 1976). The observation of granular deposits of Ig and C3 in the intestine of patients with severe SLE (Brentjens *et al.*, 1977) is consistent with the idea that immune complex deposits may play a role in the pathogenesis of increased permeability of the intestine for macromolecules in SLE.

Data reported here indicate that genetic differences which alter susceptibility to develop CSS can be studied in the rat model. Because possible intrinsic strain differences in primary immune responsiveness to BSA were circumvented by vigorous and repeated subcutaneous immunization with BSA in adjuvant at the outset, it seems likely that these results reflect strain differences in subsequent immune regulation or immune modulation of the response to BSA. Differences in the pattern of immune complex deposition between two lines of selectively bred mice immunized daily with human serum albumin have been attributed to genetic differences in antibody affinity (Steward, 1979).

A decrease in antibody titre during chronic immunization of rabbits with BSA has been described to coincide with the appearance of proteinuria (Albini, Brentjens & Andres, 1979a). A similar phenomenon occurred in CSS produced by i.v. immunization of rats with BSA. An association between decreases in the titre of circulating antibodies to Ro antigen and the development of fatal glomerulonephritis has also been noted in patients with SLE (Maddison & Reichlin, 1979). Similarly, it has been reported that C1q and C3 concentrations in sera of patients with SLE were inversely related to the concentrations of antibodies to dsDNA and to the development of renal disease. Clinical symptoms of nephritis in these patients were found to coincide with a sharp drop in antibody concentration and with an increase in measurable serum complement activity (Swaak *et al.*, 1979).

In conclusion, the observations presented here indicate that further study of rats with chronic serum sickness, rather than the rabbit model traditionally employed, may offer advantages for the analysis of the pathogenesis of immune-complex-mediated tissue injury.

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