Virchows Archiv A Pathol Anat (1988) 413:551–553

Virchows Archiv A
Pathological Anatomy and Histopathology

Chronic serum sickness glomerulonephritis: Passive immunisation inhibits the removal of glomerular antigen and electron dense deposits

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Summary. Chronic serum sickness glomerulonephritis was induced in 20 Wistar rats, using radiolabelled, chemically cationised bovine serum albumin (BSA) as antigen. Four days after the last injection of antigen, when relocation of antigen within the rat had effectively ceased, the rats were given a single large intraperitoneal dose of either non-immune rat gamma globulin or anti-BSA rat gamma globulin. Ten days later the rats were killed. The rats which had received the anti-BSA globulin had significantly more antigen in renal cortex and in isolated glomeruli than the control group. They also had larger mesangial deposits as assessed by morphometry at electron microscope level; assessment of subepithelial deposits provided equivocal results. These findings provide direct confirmation that circulating antibody which is directed against an antigen which is trapped within deposits in the glomerulus will inhibit the removal of the antigen and deposits from the mesangium.

Key words: Glomerulonephritis – Chronic serum sickness – Immune complexes – Passive immunisation

Introduction

In most forms of human glomerulonephritis there is an accumulation of immune complexes within the glomerulus over a period of time. These are detectable by electron microscopy as electron dense deposits, and can be found in several locations, the commonest being in the mesangium or between the podocytes and the glomerular basement membrane ('subepithelial'). There have been important advances in recent years in our understanding of the various mechanisms by which immune complexes may be deposited in the glomerulus, but most of these insights have come from relatively acute animal experiments. Human glomerulonephritis is usually a chronic disease, so factors which influence the rate of removal of electron dense deposits could be as important as factors which influence their initial accumulation. Much less is known about the mechanisms by which electron dense deposits are removed.

To investigate this area we have established and characterised a form of cationic bovine serum albumin (BSA) chronic serum sickness glomerulonephritis in the rat, which is unusually convenient and reproducible. (Furness and Turner 1987a) We have shown that manoeuvres undertaken during recovery from glomerulonephritis which increase the levels of circulating anti-BSA antibody will inhibit the rate of antigen and deposit removal, whereas manoeuvres which decrease antibody levels will enhance removal. (Furness and Turner 1987b) In the present experiment we have sought direct confirmation of the hypothesis that circulating antibody which is directed against an antigen bound in the glomerulus will inhibit the removal of that antigen.

Materials and methods

Preparation of antigen. Crystalline BSA (Sigma) was rendered cationic by a modification of the ethylenediamine – carbodiimide method of Hoare and Koshland (1967), as previously described (Furness and Turner 1987). The pI was found by isoelectric focussing to be between 8 and 9.5.

Preparation of antibody. Seven adult male Wistar rats were immunised with a subcutaneous injection of 1 mg of cationic BSA in saline and Freund's complete adjuvant (Sigma), total volume 0.5 ml. A booster immunisation of 1 mg of antigen in Freund's

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incomplete adjuvant was given three weeks later. Two weeks after that the rats were killed by exsanguination under anaesthetic (Hypnorm and Diazepam) and the blood collected. Seven age-matched non-immune rats were killed at the same time to provide control serum.

Immune and non-immune rat gamma globulin fractions were prepared by standard ammonium sulphate precipitation of these sera. The precipitates were dissolved, dialysed, centrifuged and passed through a 0.2 μ filter to remove particulate matter, and lyophilized. A simple ELISA assay was used to assess the ability of the immune serum to bind to BSA: compared to the control globulin, activity was still present at a dilution of 1×10^5.

Experimental animals. Chronic serum sickness glomerulonephritis was induced as previously described (Furness and Turner 1987) in 20 male Wistar rats, initial weight 110–125 g. Briefly, the rats were immunised twice using cationic BSA in Freund's incomplete adjuvant, then given seven intramuscular injections of 10 mg each of iodine-125 labelled cationic BSA, over two weeks. This regimen reliably produces nephrotic syndrome; glomeruli contain subepithelial and mesangial electron dense deposits without a detectable increase in cell numbers.

We have previously shown that relocation of antigen within the rat is most unlikely to the occurring at significant levels beyond four days after the last antigen injection (Furness and Turner 1987b). At this point therefore the rats were divided into two groups, and each animal was given an intraperitoneal injection of 75 mg of immune or non-immune rat gamma globulin, in 0.75 ml of saline. Ten days later the rats were anaesthetised and the abdomen opened. A small sample of kidney was taken into glutaraldehyde for electron microscopy. The aorta was then clamped below the diaphragm and cannulated at the bifurcation. The inferior vena cava was opened and the abdominal organs were flushed of blood with at least 20 ml of saline. Samples were taken of renal cortex, liver, spleen and lung, weighed, and submitted to a gamma counter for assessment of the antigen load. The rest of the kidneys was taken for isolation of glomeruli. The rats then died by exsanguination under anaesthetic.

Isolation of glomeruli. Renal tissue was mashed and passed through sieves down to 100 mesh. Glomeruli were collected on a 200 mesh sieve and washed several times in saline. Each sample was concentrated to 0.6 ml by sedimentation. A few drops of a strong solution of BSA were added, to minimize adhesion of glomeruli to glassware. The suspension was agitated and 10 μl removed to a glass slide for direct counting of numbers of glomeruli. 500 μl was placed in a tube for submission to the gamma counter. The preparations were in excess of 95% glomeruli, and contained on average 10000 glomeruli each.

Morphometry. Tissue was fixed in 2.5% glutaraldehyde, postfixed in osmium tetroxide and processed into Epon resin. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in a JEOL 1200 EX transmission electron microscope at 80 KV. Four photographs of glomeruli were taken per rat, at the interactions of the grid bars, to provide a random selection; magnification was ×5000, and no glomerulus was photographed more than once. Photographs were assigned a code number to eliminate observer bias. Clear acetate sheets were laid over each photograph in turn; first subepithelial then mesangial deposits were outlined by hand, and volume fraction was estimated by submitting the acetate sheets to a Seescan image analyser.

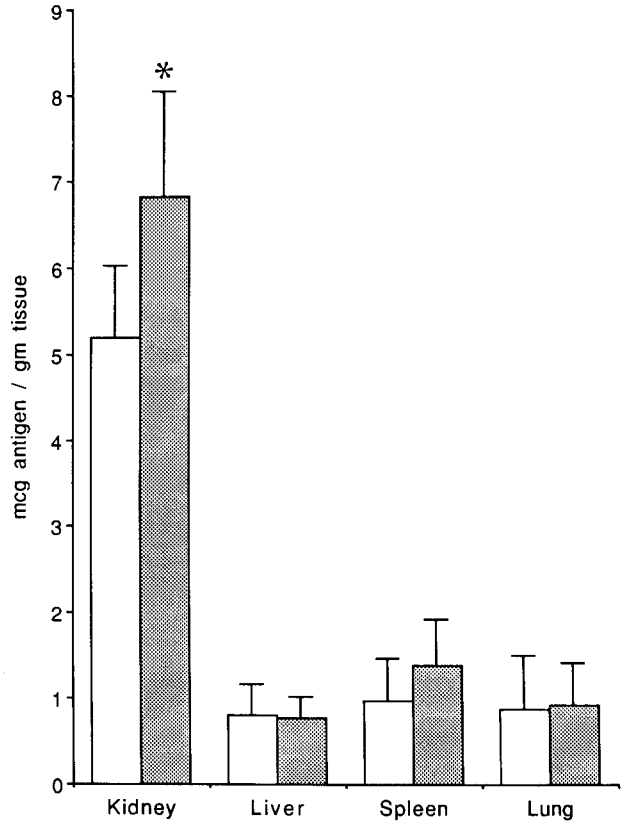

Fig. 1. The amount of antigen remaining in tissues after two weeks of recovery from chronic serum sickness. Open bars represent the results from rats given non-immune rat gamma globulin ten days before sacrifice. Solid bars represent rats given an equal amount of anti-BSA rat gamma globulin ten days before sacrifice. Error bars indicate 95% confidence limits of the mean. $*p < 0.05$, by Student's t test

Results

Assessment of the mean amount of antigen per glomerulus at the end of the experiment showed that the control group had a mean of 76 pg of antigen per glomerulus (95% limits ±10 pg), whereas the group which had received immune globulin had a mean of 107 pg per glomerulus (±17 pg). The administration of anti-BSA antibody had therefore inhibited the removal of cationic BSA from the glomeruli ($p < 0.005$).

The amount of antigen remaining in whole tissues is shown in Fig. 1. Passive immunisation is shown to result in a larger amount of antigen being left in the renal cortex ($p < 0.05$), though a significant effect was not found in any of the other tissues studied.

Morphometric evaluation of the volume fraction of glomerular deposits showed that the rats which had received anti-BSA globulin had significantly more mesangial deposits (Table 1: $p <$

Table 1. Volume fraction of subepithelial and mesangial electron dense deposits in rats after two weeks of recovery from chronic serum sickness glomerulonephritis, with administration of gamma globulin fraction during recovery. Mean of 10 rats ±95% confidence limits of mean

	Mesangial (%)	Subepithelial (%)
Non-immune globulin	0.268±0.07	0.267±0.066
Anti-BSA globulin	0.514±0.098	0.358±0.102

0.001). A similar smaller effect is suggested for the subepithelial deposits, but this did not reach statistical significance ($p=0.104$).

Discussion

The results of this experiment provide direct confirmation of the hypothesis that circulating antibody which is directed against an antigen which is bound in the glomerulus will inhibit the removal of that antigen. We had proposed this theory as an explanation of the unexpected finding that administration of antigen and Freund's complete adjuvant to an animal recovering from chronic serum sickness glomerulonephritis inhibits the removal of antigen from the glomerulus (Furness and Turner 1987b). We had also found that administration of large doses of native BSA (which is not nephritogenic in this model) enhanced antigen removal; this could be brought about by interference with the lattice of the immune complexes as has been suggested previously (Haakenstad et al. 1983; Mannik and Striker 1980), but our present findings support the contention that the removal of circulating antibody caused by native BSA administration could also facilitate antigen removal.

The results of the morphometric assessment of deposit volume fraction indicate that the major effect of circulating immunoglobulin is on the deposits located in the mesangium. Our results suggest that there was a smaller effect on the subepithelial deposits which did not reach statistical significance ($p=0.104$). A recent report studying the solubilization of 'planted' (i.e. acute) deposits by excess antigen provided evidence that, not surprisingly, access of plasma proteins to subepithelial deposits is limited (Agodoa and Mannik 1987), whereas mesangial deposits are subjected to a constant flow of plasma proteins (Latta and Fligiel 1985; Goode et al. 1985). We would suggest that our inability to confirm a significant effect on subepithelial deposits reflects this problem of access, even in proteinuric rats, rather than a fundamental difference in the properties of the deposits at the two sites.

We have demonstrated that antibody inhibits the removal of glomerular electron dense deposits in an animal model of nephrotic syndrome which does not show cellular proliferation, and in which glomerular phagocytes do not seem to be directly involved in the removal of deposits (Furness and Turner 1988). The relevance of this finding to human glomerulonephritis has yet to be confirmed, and other mechanisms of deposit removal may be utilised in proliferative forms of glomerulonephritis; nevertheless, it seems likely that inhibition of antigen removal by antibody is a common phenomenon, which may have therapeutic implications.

Acknowledgements. We are grateful for the skilled technical assistance of Mr S. Turner. This work was supported by grants from Trent Regional Health Authority and the National Kidney Research Fund.

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Accepted July 12, 1988