

## INHERITED VARIATIONS IN GLOMERULAR PROPERTIES

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### Summary

Inherited glomerular properties have been investigated in two strains of rats chosen because one, Lewis, is susceptible to Heymann's nephritis and the other, DA, is not. The negative charge in the glomerular capillary wall was less in Lewis than DA rats as judged by a reduced uptake of cationic IgG and the degree of proteinuria following infusion of a polycation. Mesangial flow or uptake of plasma proteins was greater in DA rats as judged by the uptake of aggregated IgG. There is considerable inherited variation in glomerular properties which influence the glomerular handling of antigen.

### Introduction

No adequate explanation has yet been found to account for individual susceptibility or resistance to a particular nephropathy. Immune complexes are thought to be common mediators of glomerular disease but are frequently detected in the sera of patients whose renal function is normal. The quality of the immune response to a given antigen may be important and subtle immune deficiencies may predispose to glomerular injury [1,2]. Many glomerulopathies are associated with particular DR types which suggests an inherited predisposition. We postulate that inherited glomerular properties may determine the response of the glomerulus to injury. In this study, we have examined some glomerular properties in two strains of rats to see their influence on renal handling of antigen.

### Methods

Healthy DA and Lewis rats weighing 200–250g were used. Human IgG (Sigma) was made cationic by the method of Danon et al [3], and its pI determined by isoelectric focusing. Human IgG was aggregated by alkalization. Cationic IgG

(IgG<sup>+</sup>), native IgG (IgG<sup>n</sup>) and aggregated IgG (agg IgG) were radiolabelled with <sup>125</sup>I. The final concentrations of these proteins were adjusted to 1mg/100μl. Rats were lightly anaesthetized with ether and the proteins given by intravenous bolus through a jugular vein. The animals were sacrificed at various times thereafter and the liver, spleen and kidneys removed. Sections of one kidney were snap frozen in liquid nitrogen. The other kidney, liver and spleen were weighed and whole organs counted in a Beckman Gamma counter.

Hexadimethrine was injected intravenously into two groups of six rats of either strain. Saline was infused for 40 minutes to produce a diuresis of approximately 500μl/10 minutes. Then hexadimethrine 70μg/min was infused for 60 minutes followed by saline for a further 60 minutes. Serial 10 minute aliquots of urine were collected throughout the experiment. Urine protein concentration was measured by the Lowry method.

Rat kidneys were examined for human IgG deposits using standard immunofluorescent techniques.

## Results

The pI of IgG<sup>+</sup> was always greater than nine. The molecular weight of agg IgG was approximately  $1 \times 10^6$  daltons.

1. *Variations in renal uptake of IgG<sup>+</sup> with time* Table I shows the uptake of IgG<sup>+</sup> per g of kidney in the two strains of rats at various times from 15 minutes to 24 hours.

TABLE I. Uptake of IgG<sup>+</sup>/g of kidney following IgG<sup>+</sup> 1mg intravenously

Time	Lewis			DA			Significance p<
	No	Mean (IgG <sup>+</sup> μg/g of kidneys)	SD	No	Mean (IgG <sup>+</sup> μg/g of kidneys)	SD	
15 min	5	8.6	2.5	5	19.2	2.5	0.0005
1 hr	5	11.6	1.1	5	17.8	2.3	0.005
3 hrs	5	9.5	1.4	5	18.1	3.1	0.0005
6 hrs	4	10.8	3.0	4	17.3	2.0	0.025
24 hrs	4	4.8	0.7	4	7.5	1.0	0.05

2. *Site of deposition* This was similar in both strains. At 15 minutes, IgG<sup>+</sup> was detected only on the GBM. By one hour, it was present both in the GBM and in the mesangium. By three hours, no IgG could be detected in Lewis rats, and it was only within the mesangium of DA rats. At six hours, no IgG could be found in either strain even though the total renal content of IgG<sup>+</sup> was unchanged. The density of staining was greater in DA rats.

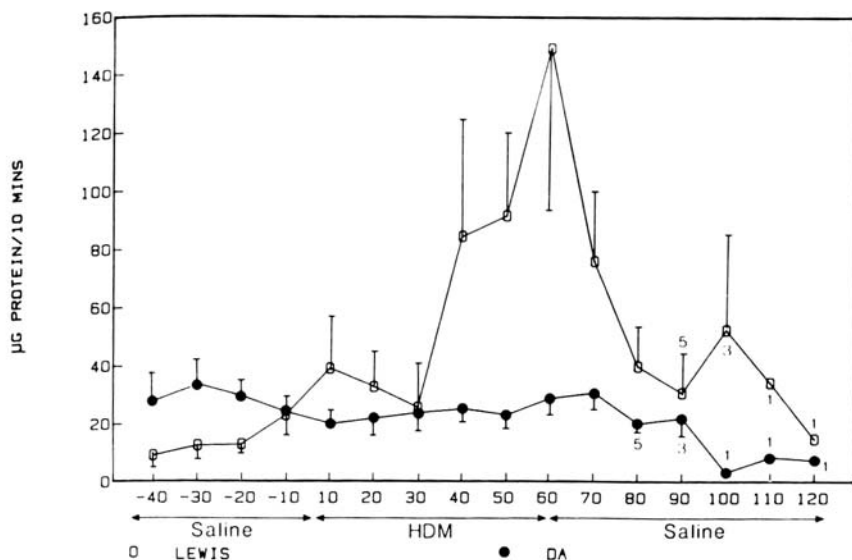


Figure 1. Urine protein excretion ( $\mu\text{g}/10 \text{ min}$ ) of DA and Lewis rats before, during and after an infusion of hexadimethrine. Each observation is the mean and standard error of the mean of six experiments, unless otherwise indicated

3. *Effect of infusion of hexadimethrine (see Figure 1)* Urine protein excretion was similar in both strains during the period of saline infusion. It remained unchanged in DA rats. Forty minutes after the start of infusion of HDM, the urine protein loss of Lewis rats increased sharply and continued to increase until the end of the infusion, when it quickly returned to basal values.

4. *Effect of dose of  $\text{IgG}^+$  on renal uptake*  $\text{IgG}^+$  was infused in three doses, 0.25mg, 1mg and 5mg, and the animals killed one hour later. The uptake in Lewis rats compared with DA rats was 43 per cent, 65 per cent and 90 per cent respectively (see Table II).

TABLE II. Effect of dose of  $\text{IgG}^+$  on renal uptake at one hour

Dose	No	Lewis ( $\mu\text{g IgG}^+/\text{g kidneys}$ )	DA ( $\mu\text{g IgG}^+/\text{g kidneys}$ )	% Lewis/DA
0.25mg	3	1.6	3.7	43.2
1.0mg	5	11.6	17.8	65.2
5.0mg	2	47.9	52.8	90.7

5. *Variation in uptake of agg IgG* Figures 2 and 3 show the renal uptake of agg IgG following an infusion of 1mg and 5mg respectively. Two rats were

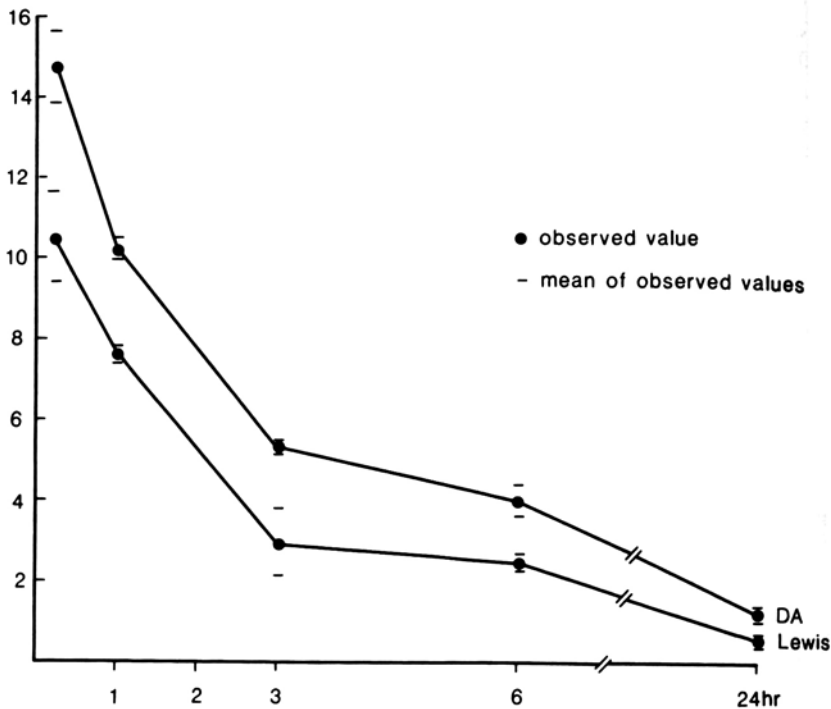


Figure 2. Renal uptake in  $\mu\text{g/g}$  of kidney of aggregated IgG following an intravenous bolus of 1mg

studied at each time point, and the value of each observation, and the mean of the two values are shown. The renal uptake was greater in DA rats at all times with both doses.

### Discussion

We have attempted to define the influence of inherited glomerular properties on the glomerular handling of antigens in order to see whether this could account for an individual's susceptibility to a particular nephropathy. Previous explanations have concentrated on variations in the immune or inflammatory responses and have assumed that glomeruli are passive receptors of systemic events. More recently, the physical properties of the antigen, and in particular its charge, have been shown to determine the type of nephropathy which results. Border et al [4] demonstrated that cationic BSA caused a membranous nephropathy, whereas anionic or native BSA caused a mesangial nephropathy in rabbits. They had previously performed the same experiments in rats, all of which had developed a mesangial nephropathy whether the antigen was cationic or not [5]. Therefore, it is possible that inherited glomerular properties may determine the type of nephropathy that a particular individual acquires after exposure to a given antigen.

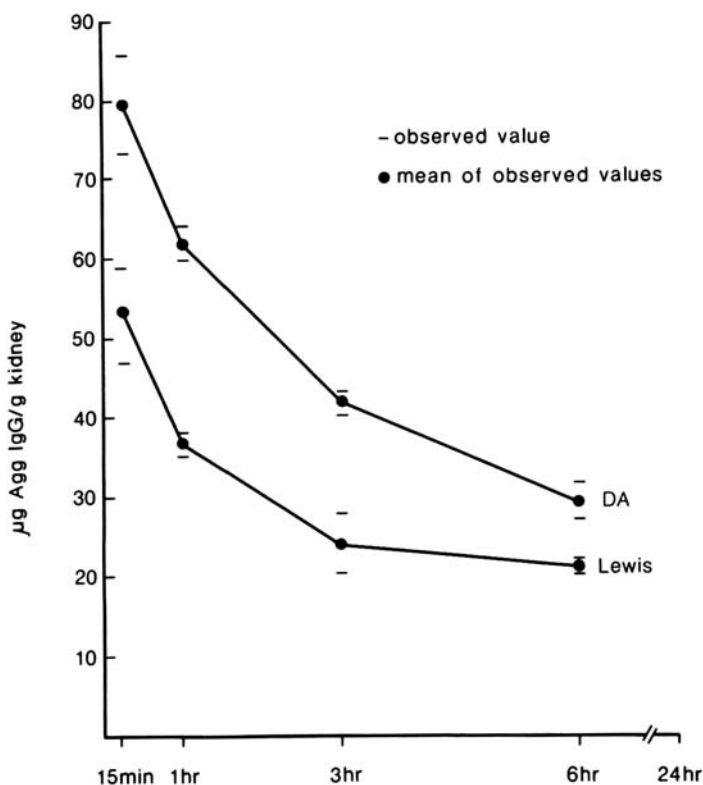


Figure 3. Renal uptake of agg IgG ( $\mu\text{g/g}$  of kidney) following an intravenous bolus of 5mg

We studied the negative charge on the glomerular capillary wall because it is crucial in maintaining the impermeability of the GBM to negatively charged molecules of the size of albumin. It is also a binding site for positively charged antigens or antibodies. We also studied the mesangial uptake of macromolecules because the greater the uptake, the more immune complexes will be found in the mesangium either as a result of in situ formation or deposition of intact complexes. Thus a higher rate of mesangial uptake could predispose to mesangial nephropathies. Lewis and DA rats were chosen for comparison because the former are susceptible to Heymann's nephritis whereas the latter are resistant [6].

Our results show that the negative charge on the GBM of Lewis rats was significantly less than that of DA rats for two reasons. Firstly, the quantity of  $\text{IgG}^+$  bound to the GBM 15 minutes after an intravenous bolus was only 50 per cent in Lewis rats compared with DA rats. It is interesting that the strain difference was greatest following the lowest  $\text{IgG}^+$  dose which is most likely to be clinically relevant. Secondly, infusion of a polycation led to proteinuria in Lewis but not DA rats. There are two possible explanations for the reduction

in charge. The content of heparan sulphate and other negatively charged glycoproteins may be reduced in the glomerular wall of Lewis rats or, the charge may be masked by positively charged molecules fixing to them. It is possible that this substance may be the Heymann's antigen. Lewis rats are, therefore, more likely to develop proteinuria as a result of any mechanism which leads to the abolition of charge. A proteinuric factor released by T cells of patients with the nephrotic syndrome has been postulated [7] and some experimental evidence exists [8]. Bakker et al [9] found that lymphocytes of patients with mesangial IgA disease and modest proteinuria released as much vascular permeability factor as did patients with minimal change nephropathy and gross proteinuria. They argued that it was therefore unlikely that the factor was of pathogenic importance. Our results provide an alternative explanation, namely, that there is an inherited quantitative difference in the glomerular response to the factor and that patients who have mesangial IgA disease may be relatively resistant to the effect of the proteinuric factor compared to patients with membranous or minimal change nephropathies.

Mesangial flow of traffic has been shown to include very large molecules such as keyhole limpet haemocyanin and aggregated IgG [10]. Antigen, unless it has an affinity for the GBM because of its charge or chemical properties, will enter the mesangium in an amount proportional to the mesangial flow. Our study examined the renal uptake of aggregated IgG and showed that the uptake in Lewis kidneys was only 65–75 per cent that of DA kidneys following either 1mg or 5mg intravenous boluses. Therefore, it is likely that DA rats would be more susceptible to mesangial glomerulopathies.

Thus DA rats may have some similarities to DR4 positive subjects who are prone to mesangial IgA disease but seldom develop heavy proteinuria, whereas Lewis rats are more like DR3 subjects who are susceptible to membranous nephropathy and heavy proteinuria. We postulate that this difference may result from inherited differences in glomerular properties rather than the immune response.

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