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Research Article

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The Glomerular Mesangium

II. STUDIES OF MACROMOLECULAR UPTAKE IN NEPHROTOXIC NEPHRITIS IN RATS

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ABSTRACT These studies were designed to explore the effects of nephrotoxic serum (NTS) in rats on the uptake and processing by the glomerular mesangium of intravenously administered protein macromolecules (radiolabeled aggregated human IgG, [^{125}I]AHIgG).

Measurements of [^{125}I]AHIgG levels in preparations of isolated glomeruli correlated well with qualitative estimates of glomerular IgG deposition seen by immunofluorescent microscopy. Rats given 2 ml NTS received 25 mg/100 g body wt [^{125}I]AHIgG 48 h later and were sacrificed at varying time intervals thereafter. NTS-treated animals demonstrated a marked increase in glomerular uptake of [^{125}I]AHIgG as compared with concurrent controls but a normal ability to clear [^{125}I]-AHIgG from the mesangium over 72 hr. Animals given 1.0, 0.5, and 0.25 ml NTS had neither proteinuria nor significant light microscopic changes, yet had increased uptake of [^{125}I]AHIgG of 4.4, 3.0, and 2.1 times control values, respectively at 8 h after the infusion of aggregates. Rats given 1 ml NTS and 12.5, 6.25, and 3.125 mg [^{125}I]AHIgG/100 g body wt had increased glomerular uptake at 8 h, but there was, within both NTS and control groups, a disproportionate decrease in uptake at lower [^{125}I]AHIgG doses.

Rats given cobra venom factor (CoF) followed by a NTS shown to be complement dependent (proteinuria reduced by prior complement depletion with CoF) had less mesangial [^{125}I]AHIgG uptake than NTS controls but greater uptake compared with normal controls. CoF itself had no effect on mesangial or reticuloendothelial system [^{125}I]AHIgG uptake.

These studies demonstrate a striking change in glomerular mesangial activity after fixation of nephrotoxic antibodies to the glomerular basement membrane, even in the absence of proteinuria and suggest that subtle alterations of the filtration surface can influence mesangial function. The effect of NTS on the mesangium is due, in large part, to the glomerular injury and proteinuria which nephrotoxic antibodies produce.

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INTRODUCTION

The glomerular mesangium has the capacity to take up a wide variety of macromolecular materials (1–5). Kinetic studies of the mesangial uptake and loss of radiolabeled aggregated human IgG [^{125}I]AHIgG[1] in normal rats have demonstrated that this glomerular cell system functions quantitatively in a manner analogous to that of the general reticuloendothelial system (RES) (6). In aminonucleoside nephrosis, a state characterized by increased glomerular permeability to protein, a marked increase in mesangial [^{125}I]AHIgG uptake has been demonstrated (6). Although it is known that proteinuria is due to a direct effect of aminonucleoside of puromycin (PA) on the kidney (7), detailed understanding of the pathogenesis of this model of nephrosis is lacking. Thus in our earlier studies, it was unclear as to whether the alterations in mesangial function observed were related to increased glomerular permeability

[1] *Abbreviations used in this paper:* CH^{50}, total hemolytic complement titer; CoF, cobra venom factor; GBM, glomerular basement membrane; [^{125}I]AHIgG, radiolabeled aggregated human IgG; NGS, normal goat serum; NTS, nephrotoxic serum; PA, aminonucleoside of puromycin; PMN, polymorphonuclear cell; RES, reticuloendothelial system; TCA, trichloroacetic acid.

to protein, to a direct effect of PA on the mesangium, or to other factors.

In nephrotoxic serum nephritis the glomerular injury results from the phlogistic consequences of the fixation of anti-glomerular basement membrane (GBM) antibody to antigenic sites in the GBM (8). Studies reported here were undertaken to determine if GBM injury is associated with functional alterations of the glomerular mesangium. The following observations were made: in nephrotoxic nephritis a marked increase in mesangial macromolecular uptake occurred; this increased uptake was seen in the absence of detectable proteinuria; significant mesangial uptake was detected with very small doses of macromolecules; and finally, proteinuria and the increased uptake of macromolecules could be decreased by prior complement depletion of the nephrotoxic nephritis rats with cobra venom factor (CoF).

METHODS

Materials

[^{125}I]AHIgG was prepared as previously detailed (6) except for one modification. The protein solution was heated to 63°C for 15 min instead of 30 min as this markedly increased the ability to resuspend the aggregates after ultracentrifugation. Nephrotoxic sera (NTS) were made by immunizing two goats with rat GBM prepared by sonication of intact glomeruli in 1 M sodium chloride and sedimentation and washing at 121 *g* (9). 2 mg of rat GBM suspended in 2 ml of complete Freund's adjuvant and 2 ml of isotonic saline were injected at three 2-wk intervals. The goat antisera were absorbed with washed rat erythrocytes, decomplemented by heating to 56°C for 30 min, and stored at −20°C until use. *Naja haje* CoF was prepared and assayed according to the methods of Ballow and Cochrane (10) except that Sephadex G-100 was employed in the second step of the purification process.

Experimental design

Kinetic studies of mesangial function in nephrotoxic (NTS) and normal rats. Animals in five groups of 10 rats each (male, Sprague-Dawley, 100 g) were given 2 ml of NTS by tail vein injection. Their concurrent controls were given 2 ml of normal goat serum (NGS). 48 h later the NTS and control animals were given [^{125}I]AHIgG i.v., in a dose of 25 mg/100 g body wt. Phenergan (Wyeth Laboratories, Philadelphia, Pa.) administered intraperitoneally 5 min before [^{125}I]AHIgG injection in a dose of 0.5 mg/100 g body wt eliminated the occurrence of death after aggregate injection. The NTS and control animals were sacrificed in five groups of 10 rats at 4, 8, 16, 36, and 72 h after [^{125}I]AHIgG injection.

Dose-response relationships: smaller doses of nephrotoxic serum. Three groups of 10 rats each were given 1 ml, 0.5 ml, and 0.25 ml, respectively, of NTS, while 30 concomitant controls received similar quantities of NGS. 48 h later the nephritic and control rats were given [^{125}I]AHIgG i.v. (25 mg/100 g). All animals were sacrificed at 8 h after [^{125}I]AHIgG injection.

Dose-response relationships: small doses of [^{125}I]AHIgG. Three groups of 10 rats each received 2 ml of NTS, followed 48 h later by [^{125}I]AHIgG in respective doses of 12.5, 6.25, and 3.13 mg/100 g body wt. Controls were given 2 ml of NGS and similar doses of [^{125}I]AHIgG. All animals were sacrificed at 8 h after [^{125}I]AHIgG injection.

Studies with CoF. To evaluate the effect of complement depletion on mesangial uptake in nephrotic and normal animals, four groups of rats were studied: in 10 rats a total of 28 μg protein/100 g body wt (0.5 ml) of CoF was administered intraperitoneally in four equal doses at 6-h intervals. 2 h after the final dose of CoF these animals were injected with 2 ml of NTS, and 48 h later they received 25 mg/100 g of [^{125}I]AHIgG. 10 rats were given identical doses of NTS and [^{125}I]AHIgG but no CoF. 10 rats received CoF, NGS, and [^{125}I]AHIgG. Finally, 10 rats were given NGS and [^{125}I]AHIgG but no CoF. All animals were sacrificed at 8 h after [^{125}I]AHIgG administration. These studies demonstrated that the NTS used was not complement dependent in that CoF did not reduce the quantity of proteinuria induced (see Results). The studies were then repeated using a second, complement-dependent NTS (see Results). The basic design of the experiments was as described above except that varying doses of this second NTS were used.

Immunofluorescent studies. Immediately after sacrifice, the kidneys were removed and a cortical slice placed in formalin for paraffin embedding and subsequent staining with hematoxylin and eosin and periodic acid-Schiff reaction. Part of the kidney was prepared for immunofluorescent microscopy and stained for human IgG (11), goat IgG, and rat B_1C (12). Goat IgG was isolated and purified using half-saturated ammonium sulfate precipitation of normal goat serum followed by DEAE cellulose chromatography in 0.01 M phosphate buffer, pH 8.0. Antiserum to goat IgG was prepared in rabbits and shown to have one line by immunoelectrophoresis against normal goat serum.

Preparation of fluoroscein isothyocyanate-labeled antisera in these studies was carried out using procedures standard in this laboratory (11). The amount of immunofluorescence was arbitrarily graded as negative, trace (tr) 1+, 2+, 3+, and 4+.

Glomerular isolation. Each glomerular isolate was prepared from a pool of 10 kidneys from five rats and prepared for ^{125}I counting as previously detailed (6, 9). In homogenized glomerular preparations, more than 98% of the counts were precipitatable by trichloroacetic acid (TCA).

Preparation of renal cortical slices for ^{125}I counting. Approximately uniform cortical slices were obtained using a Stadie-Riggs microtome. Samples consisting of three slices of renal cortex from each animal were washed four times in isotonic saline, dried overnight in a ventilated oven at 85°C and weighed. 4 ml of saline was added to each sample and the tissue was homogenized in a ground glass homogenizer with a loose fitting glass pestle. Each homogenate was precipitated with 4 ml of 10% TCA; the precipitate was washed four times in 5% TCA and counted for ^{125}I.

Preparation of lung, liver, spleen, and blood for ^{125}I counting. Samples of blood, lung, liver, and spleen were obtained at the time of sacrifice and prepared for ^{125}I counting by methods previously reported (6).

Urine studies. Urine was collected during the second 24 h after NTS or NGS administration and values for 24 h urinary excretion of protein determined by the biuret reaction on TCA-precipitable protein (13). In some ani-

mals urine specimens were also collected in the 24 h period following [^{125}I]AHIgG administration. The TCA precipitate of this urine was washed four times in 5% TCA and counted for ^{125}I.

Complement studies. Determinations of rat total hemolytic complement titers (CH^{50}) were carried out as has been reported (14) using buffers and sensitized sheep erythrocytes prepared as previously described (15).

Statistical methods. Where applicable, data is denoted by the mean of values ± the standard error (SE). Student's *t* test was applied to evaluate differences between means. Slopes (*b*) were calculated by the method of least squares. Differences between slopes were estimated according to the Student's *t* distribution.

RESULTS

Kinetic studies of mesangial function

Light and immunofluorescent microscopy. Animals given 2 ml of NTS demonstrated, 48–64 h later, swelling of glomerular cells, mild polymorphonuclear cell (PMN) infiltration with two to three PMN per glomerulus, striking amounts of eosinophilic material in the subendothelial space, and expansion of the mesangial matrix. By 84–110 h (5 days) after NTS, the acute glomerular injury appeared to be resolving. Glomeruli of control animals were normal at all time periods.

By immunofluorescent microscopy all NTS kidney tissues showed 3+ linear staining of the GBM for goat IgG (Fig. 1A). Rat B_1C was seen in a similar distribution but staining was less intense (1–2+) and was granular or interrupted linear in type and there were small amounts of rat B_1C (tr) in a mesangial pattern (Fig. 1B). Control tissues showed only B_1C (tr) in the mesangium and were negative for goat IgG. NTS kidneys 14 h after AHIgG infusion, had large quantities (4+) of human IgG in a diffuse distribution within glomeruli but the precise localization was difficult to characterize. At 8 h human IgG (4+) was in the mesangium and in a subendothelial capillary distribution (Fig. 1C). At 16, 36, and 72 h human IgG staining was present only in the mesangium, i.e. radiating in a branch-like pattern from the vascular pole towards the periphery of the glomerulus (Fig. 1D and E). Glomeruli in kidneys from control rats given NGS had much smaller quantities of human IgG at all time periods studied (Fig. 1F and Fig. 2). In control kidneys at 4 h human IgG was localized in the glomerular capilliary wall and in the mesangium, but at all subsequent times was found only in the mesangium.

Quantitative studies of glomercular [^{125}I]AHIgG. Levels of [^{125}I]AHIgG in glomeruli isolated from NTS rats were considerably higher than control levels at all time periods (Fig. 3). After 8 h glomerular [^{125}I]-AHIgG levels in nephritic animals are presumed to reflect the quantities of aggregates in the mesangium as seen by immunofluorescent microscopy. Thus at 16, 36, and 72 h there was, respectively, 22.2, 19.0, and 25.6 times as much [^{125}I]AHIgG in the mesangium of NTS animals as compared with NGS animals (Table I). After 4 h the fall-off slope derived with the data from NTS glomeruli ($b = 1.41 \pm 0.13$) was almost identical to that derived from control glomeruli ($b = 1.44 \pm 0.05$) ($P > 0.5$).

Studies of kidney slices (Table I) demonstrated that levels of [^{125}I]AHIgG were higher in the cortex of NTS kidneys than in controls. However, the ratios of NTS to control levels were lower with cortical slices as compared with isolated glomeruli (Table I).

Blood studies. Blood levels of [^{125}I]AHIgG decreased rapidly with time in both NTS and control animals. There were no significant differences between nephritic and control animals except at 4 h where NGS were slightly higher than NTS values.

Urine studies. The urinary protein excretion in the second 24 h after NTS administration was 55.7±5.7 mg ($N = 29$), significantly higher than control (NGS) values of 4.0±0.5 mg ($N = 10$) ($P = < 0.001$). However, in the first 24 h after aggregate administration, no differences were detected in quantities of [^{125}I]AHIgG in the urine of NTS (2.0±0.4 μg [$N = 10$]) as compared with NGS animals (1.7±0.2 μg [$N = 10$)] ($P > 0.2$).

Dose response relationships: smaller doses of nephrotoxic serum. Glomeruli of rats given 1.0 ml of NTS had minimal cellular swelling and mesangial expansion by light microscopy; 4 of 10 specimens could not be differentiated from controls. Glomeruli of rats given 0.5 and 0.25 ml NTS were normal by light microscopy. Human IgG in NTS kidneys was seen at 8 h only in the mesangium. The intensity of mesangial staining for human IgG was consistently greater in nephritic as compared with control kidneys and greater at higher as compared with lower doses of NTS (Figs. 1G, H, I). Despite the absence of proteinuria in the small-dose NTS animals; mesangial [^{125}I]AHIgG levels were higher in these rats than in controls (Table II). The magnitude of this increase in uptake was directly related to the dose of NTS.

Dose response relationships: smaller doses of [^{125}I]-AHIgG. All animals in this study received 1 ml NTS and were given varying doses of [^{125}I]AHIgG. In all instances, human IgG, where detectable, was found in the mesangial area. The intensity of human IgG staining was greater in nephritic versus control animals at each dose tested and was greater at higher doses within both nephritic and control groups (Table III). Quantitative measurements of mesangial levels of [^{125}I]AHIgG paralleled the qualitative immunofluorescent findings (Table III). Of interest a 50% reduction in [^{125}I]-AHIgG dose from 25 to 12.5 mg/100 g resulted in a

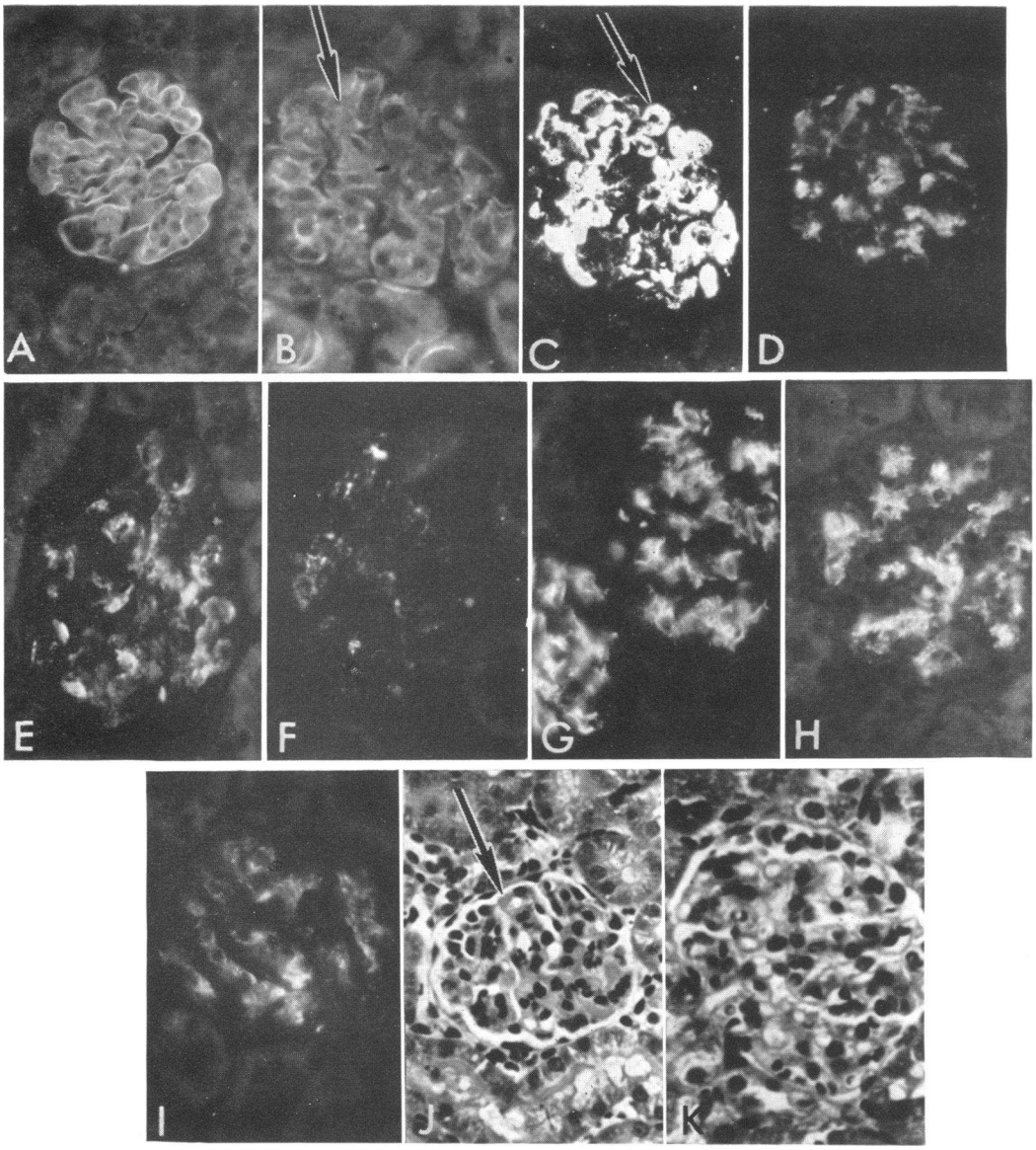

FIGURE 1 Immunofluorescent photomicrographs of glomeruli from: (*A*) a 2 ml NTS rat demonstrating goat IgG in a linear GBM distribution; (*B*) a 2 ml NTS rat with rat B_1C distributed along the GBM in a linear and granular pattern. Small amounts of B_1C are seen (arrow) in the mesangium; (*C*) a 2 ml NTS rat 8 h after [^{125}I]AHIgG administration demonstrating intense staining for human IgG in the mesangium and (arrow) in peripheral glomerular areas which seem to correspond to the subendothelial space; (*D*) a 2 ml NTS rat 16 h after [^{125}I]AHIgG administration with human IgG localized almost entirely in the mesangium; (*E*) a 2 ml NTS rat 36 h after [^{125}I]AHIgG infusion with mesangial human IgG localization of 3+ intensity; (*F*) a control (NGS) rat 36 h after [^{125}I]AHIgG infusion with less intense (1+) mesangial staining for human IgG as compared with Fig. 1E; (*G*) a 1 ml NTS rat 8 h after [^{125}I]AHIgG administration with marked (4+) staining of the mesangium for human IgG; (*H*) a 0.5 ml NTS rat 8 h after [^{125}I]AHIgG administration with more intense (3+) mesangial human IgG localization as compared with Fig. 1I; (*I*) a control (NGS) rat 8 h after [^{125}I]AHIgG administration showing localization of human IgG in the mesangium in 2+ intensity. Photomicrograph of glomeruli from: (*J*) a 0.5 ml NTS rat demonstrating glomerular cell swelling, PMN infiltration and (arrow) subendothelial collections of eosinophilic material (hematoxylin and eosin); (*K*) a CoF + 0.5 NTS rat demonstrating less glomerular injury as compared with Fig. 1*J* (hematoxylin and eosin).

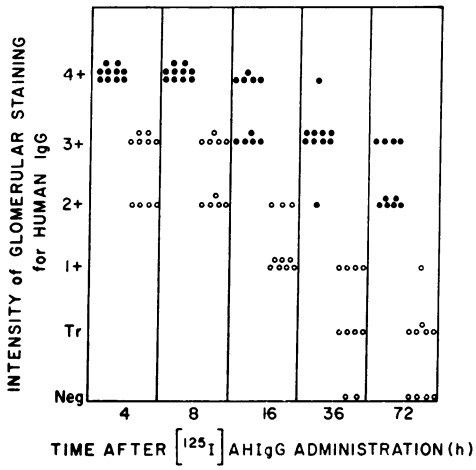

FIGURE 2 Results of immunofluorescent microscopy studies with staining for human IgG; ●, 2 ml NTS; ○, 2 ml NGS.

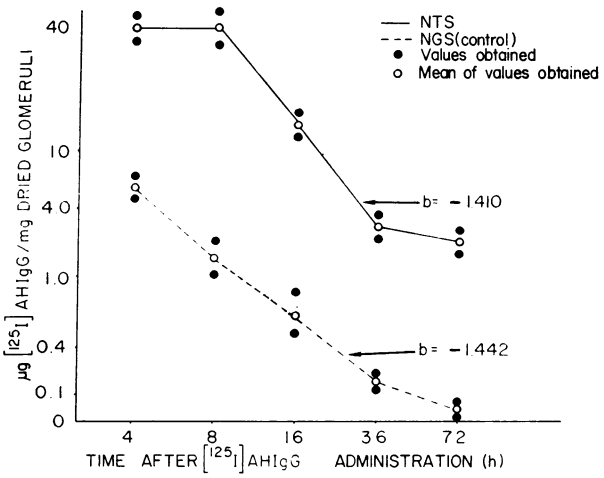

FIGURE 3 [125I]AHIgG in preparations of isolated glomeruli.

much greater than 50% reduction in uptake in both NTS and NGS glomeruli.

Studies with CoF. The mean CH^{50} titer of 19 normal rats was 159.3±15. After CoF, all animals had CH^{50} titer of < 10 except for four animals with a mean CH^{50} titer of 14. Despite this dramatic fall in CH^{50} titers, no effect of CoF was seen on the proteinuria produced by the first, apparently noncomplement dependent, NTS (Table IV). Inability of CoF to protect the glomerulus from injury as evidenced by proteinuria was confirmed by light microscopy studies demonstrating glomerular injury equal in CoF pretreated and untreated rats given NTS. The lack of protection occurred despite the fact that this NTS itself caused a fall in CH^{50} titer of < 10 in five animals so tested. CoF alone had no effect on the mesangial uptake of [125I]AHIgG (Table IV), or on the quantities of [125I]AHIgG found in blood, lung, liver, or spleen.

The second nephrotoxic serum, NTS, was much more potent than the first and was apparently complement dependent. 1 ml of the second NTS produced severe glomerular injury with marked cell swelling, large subendothelial collections of eosinophilic material, and moderate PMN infiltration (four to six per glomerulus). There was intense linear GBM staining for goat IgG (4+) and granular or interrupted linear staining for rat B_1C (2–3+). 1 ml of the second NTS resulted in marked proteinuria (Table V).

With each dose of the second NTS tested, pretreatment of rats with CoF resulted in a decrease in glomerular injury as seen by light microscopy (Figs. 1J and K), a decrease in the intensity of GBM staining for

TABLE I

Aggregated Human IgG ([125I] AHIgG) in Cortical Slices and Glomeruli from Nephritic and Control Rats at Various Time Intervals after [125I] AHIgG Administration

Time after [125I]AHIgG administration	μg [125I]AHIgG/mg dried renal cortex			Ratio of μg [125I]AHIgG in dried renal cortex NTS:NGS	Ratio of μg [125I]AHIgG in isolated glomeruli NTS:NGS*
	NTS	NGS (control)	*P*		
h					
4	1.75±0.23‡	0.51±0.04	<0.001	3.4:1	7.0:1
8	1.49±0.17	0.30±0.04	<0.001	5.0:1	22.4:1
16	0.62±0.08	0.20±0.02	<0.001	3.1:1	22.2:1
36	0.18±0.03	0.052±0.005	<0.001	3.4:1	19.0:1
72	0.063±0.001	0.027±0.004	<0.01	2.3:1	25.6:1

* See Fig. 3.

‡ N = 10 except for NTS 8 h, 16 h, 36 h, and NGS 16 h where N = 9. Number presented is mean of values plus or minus standard error.

TABLE II

Relationship of Mesangial Uptake of Aggregated Human IgG ([125I]AHIgG to Proteinuria in Rats after Various Doses of Nephrotoxic Serum (NTS)

Dose of NTS	μg [125I]AHIgG/mg dried glomeruli*	Urinary protein excretion	P‡
ml		*mg/24 h*	
	30.1§	55.7±5.7§ (n = 29)	<0.001§
2.0	47.2§		
1.0	8.1	4.8±0.9 (n = 6)	>0.2
	7.0		
0.5	4.9	4.7±0.7 (n = 5)	>0.2
	5.6		
0.25	4.2	2.8±0.4 (n = 5)	>0.2
	2.9		
0.0 (2 ml NGS)	1.1§	4.0±0.5§ (n = 10)	—
	2.3§		

* Each value represents the concentration of [125I]AHIgG in one glomerular isolation from a pool of kidneys of five animals. All animals were sacrificed 8 h after [125I]AHIgG administration.

§ Values obtained from kinetic studies.

‡ P values obtained by comparing urinary protein excretion data from each NTS dose group with control (2 ml NGS) data.

rat BIC and a significant diminution in proteinurea (Table V). Furthermore, at each dose of this NTS, CoF pretreatment produced a relative decrease in the glomerular uptake of [125I]AHIgG (Table V); this finding was confirmed by immunofluorescent microscopy.

TABLE III

Relationship of Mesangial Uptake of Aggregated Human IgG ([125I]AHIgG to Various Doses of [125I]AHIgG in Nephritic (NTS) and Control (NGS) Rats

Dose of [125I]AHIgG	μg [125I]AHIgG/mg dried glomeruli*		Intensity of mesangial staining for human IgG by immunofluorescent microscopy‡	
	NTS	NGS	NTS	NGS
mg/100 g body wt				
25	8.1§	1.1§	4+	2+
	7.0§	2.3§		
12.5	0.50	0.13	1+	Tr
	0.66	0.08		
6.25	0.14	0.03	Tr	Neg
	0.18	0.06		
3.125	0.07	0.00	Neg-tr	Neg
	0.08	0.00		

* Each value represents the concentration of [125I]AHIgG in one glomerular isolation from a pool of the kidneys of five animals. Each animal received 1 ml NTS and was sacrificed 8 h after [125I]AHIgG administration.

‡ Approximate mean of qualitative readings obtained in the 10 animals in each group.

§ Values obtained from studies of smaller doses of NTS.

Animals given 0.5 ml or more of the complement dependent NTS all had large quantities of human IgG localized in the mesangium and in the area of the subendothelial space, but lesser quantities of staining were seen in CoF-pretreated animals. Pretreatment of animals with CoF did not alter the pattern or intensity of the linear GBM staining for goat IgG seen with either the first or second NTS used but lesser quantities of BIC were seen.

TABLE IV

Effect of Cobra Venom (CoF) on the Increased Mesangial Uptake of Aggregated Human IgG ([125I]AHIgG) and on the Proteinuria Associated with Noncomplement Dependent Nephrotoxic Serum (NTS)

Condition	μg [125I]AHIgG/mg dried glomeruli*	Urinary protein excretion
		mg/24 h
2 ml NTS	10.7	26.4±5.6 (N = 10)‡
	17.4	
CoF + 2 ml NTS	13.2	34.2±6.0 (N = 10)‡
	16.6	
2 ml NGS	1.4	Not done
	1.7	
CoF + 2 ml NGS	1.3	Not done
	2.6	

* Each value represents the concentration of [125I]AHIgG in one glomerular isolation from a pool of the kidneys of five animals. All animals were sacrificed 8 h after [125I]AHIgG administration.

‡ $P > 0.2$ on comparing urinary protein excretion of the 2 ml NTS and CoF + 2 ml NTS groups.

TABLE V

Effect of Cobra Venom Factor (CoF) on the Increased Glomerular Uptake of Aggregated Human IgG ([^{125}I]AHIgG) and on the Proteinuria Associated with Varying Doses of Complement-Dependent Nephrotoxic Serum (NTS)

Condition	μg [^{125}I]AHIgG/mg dried glomeruli	Urinary protein excretion	P*
		mg/24 h	
1 ml NTS	70.9‡ 79.7	91.8±14.0 (N = 7)	<0.01
CoF + 1 ml NTS	33.9 42.3	36.3±6.8 (N = 7)	
0.5 ml NTS	45.5 52.1	78.1±10.2 (N = 8)	<0.001
CoF + 0.5 ml NTS	38.2 30.7	8.3±1.8 (N = 8)	
0.25 ml NTS	32.4 31.3	26.3±3.2 (N = 7)	
CoF + 0.25 ml NTS	7.1 10.5	12.6±2.0 (N = 8)	<0.01
1 ml NGS	1.8 1.1	—	

‡ All animals were sacrificed 8 h after [^{125}I]AHIgG administration. Each value represents the concentration of [^{125}I]AHIgG in one glomerular isolation from a pool of the kidneys of five animals.

* *P* values obtained by comparing urinary protein excretion data from each NTS dose group with the corresponding COF + NTS group.

DISCUSSION

Recent evidence derived from electron microscopic studies using various tracer molecules suggest that small proteins are rapidly and completely filtered into the urinary space while larger molecules are held up at the epithelial filtration slit pore or in the GBM (16). Very large molecules may go as far as the lamina rara interna but most are excluded by the GBM and, from the subendothelial space, enter the area of the glomerular mesangium (2, 16); thence they are removed by an unknown mechanism(s). Although anti-GBM antibodies appear to fix in the glomerulus discreetly and exclusively to the GBM (17) the phlogistic responses which they trigger cause damage to other glomerular structures, particularly the endothelial cell (18).

In animals with marked proteinuria after NTS administration, [^{125}I]AHIgG is seen in large quantities in the area of the subendothelial space in the first few hours after aggregate infusion. This may correspond to the light microscopic observations of subendothelial collections of eosinophilic material and may represent the loss of the integrity of the endothelial cell as a filtration barrier to macromolecules. From the subendothelial space aggregates are rapidly transported into the mesangium and from there are cleared from the glomerulus. The failure to detect aggregates by immunofluoresence within or upon the GBM in nephrotoxic rats suggests that even with injury to the filtering barrier(s) severe enough to produce massive and nonselective proteinuria (18), large macromolecules are excluded from filtration beyond the endothelial cell. This is supported by the failure to find increased quantities of [^{125}I]AHIgG in the urine of NTS animals.

Although a marked increase in the mesangial uptake of [^{125}I]AHIgG has been demonstrated in nephrotoxic nephritis the kinetic studies prove that this was not due to impairment of mesangial clearing mechanisms as the rate of disappearance of [^{125}I]AHIgG from the mesangium was the same in NTS and control glomeruli.

It is difficult to explain why the ratios of [^{125}I]AHIgG levels in isolated glomeruli of NTS animals compared with controls were higher than similar ratios derived from cortical slices. There were no differences in blood levels of [^{125}I]AHIgG between NTS and control rats at any time period after 4 h. Based on our unpublished observations in experiments on rats other than those reported here, the effect of blood contamination on glomerular levels of [^{125}I]AHIgG is negligible, and the effect on cortical slices is small. Only minute amounts of [^{125}I]AHIgG were found in the urine of NTS and con-

trol animals virtually ruling out urinary contamination as a cause of the lower ratios seen in the cortical slice studies. Other explanations include the possibility of significant amounts of [^{125}I]AHIgG in renal lymphatics; the release of large polypeptides labeled with ^{125}I from [^{125}I]AHIgG molecules in the RES which could be filtered by the glomerulus and reabsorbed into the renal tubular cells. Whatever the explanation, it is apparent that the measurement of the levels of radiolabeled protein in the glomerulus is best accomplished in preparations of isolated glomeruli as opposed to whole renal cortex even after correction for circulating ^{125}I protein-bound counts. This pertains even if the given protein is localized by immunofluorescent microscopy exclusively to the glomerulus.

Animals given smaller doses of NTS did not develop significant proteinuria and had little or no glomerular changes by light microscopy. Nevertheless, these animals demonstrated increased mesangial [^{125}I]AHIgG uptake proportionate to the dose of NTS administered. Similarly, we have previously shown that increased mesangial uptake was demonstrable with quantities of aminonucleoside of puromycin insufficient to produce overt proteinuria (6). These observations have led us to speculate that mesangial injury in conditions such as diabetic nephropathy may be secondary to subtle alterations in glomerular capillary permeability insufficient to produce significant proteinuria but sufficient to chronically expose the mesangium to an increased work load in the clearing of endogenous circulating and potentially noxious macromolecules (12).

Glomeruli of NTS animals given smaller doses of [^{125}I]AHIgG had higher levels of aggregates in the mesangium than did concurrent controls. In both groups, however, halving the [^{125}I]AHIgG dose from 25 to 12.5 mg/100 g body wt resulted in a 13-fold decrease in glomerular [^{125}I]AHIgG levels. Previous kinetic studies of glomerular, lung, liver, and spleen levels of aggregates in normal and aminonucleoside nephrotic rats given 25 mg/100 g of [^{125}I]AHIgG led us to suggest that the mesangium may function in a manner analogous to that of the general RES (6). However, the present studies indicate that at high dose levels the glomerular concentrations of [^{125}I]AHIgG may, in part, be due to overloading of the RES. Clearer definition of any functional relationships between the mesangium and the RES would require kinetic evaluations at various dosages of macromolecular substances. Glomerular levels of [^{125}I]AHIgG in control animals in these studies are significantly higher than in control animals in our previous work (6). This may reflect the alterations in the technique of preparing the [^{125}I]AHIgG which would modify the molecular weight distribution of the IgG aggregates. In studies of this type it is important to do concurrent controls as variations in [^{125}I]AHIgG from batch to batch may significantly alter the absolute result.

The studies with CoF were designed to answer the following question: Is the increased mesangial uptake of [^{125}I]AHIgG seen in NTS animals due to the fixation of anti-GBM antibody to the GBM or was this increase a consequence of the immunological glomerular injury which results from this fixation? Studies by Cochrane, Müller-Eberhard, and Aikin have shown that CoF does not influence the quantity of nephrotoxic globulin which fixes to glomeruli in rats, but does diminish glomerular injury and proteinuria which this antibody produces. In our studies CoF did not influence glomerular injury, urinary protein excretion or glomerular aggregate uptake in animals given the first, apparently noncomplement dependent NTS. Similar observations of noncomplement dependent proteinuria in nephrotoxic nephritis have been reported by Hawkins and Cochrane (20). However, with the second antiserum prior complement depletion with CoF did result in less glomerular pathology, less proteinuria, and diminished glomerular aggregate uptake. Thus the increased glomerular localization of [^{125}I]AHIgG was, at least in part, a consequence of complement induced glomerular injury which followed anti-GBM antibody administration.

These studies also suggest that complement may not be required for mesangial or RES uptake of circulating aggregates of IgG. This is consistent with studies demonstrating that antigen-antibody complexes can be eliminated from the circulation at a normal rate in complement-depleted animals (21). However, the removal of complexes from the circulation in vivo (21) and the uptake of AHIgG by peritoneal macrophages in vitro (22) are in part dependent on their ability to fix complement components.

The role of the mesangium in the uptake and processing of circulating potentially noxious macromolecules such as heavy molecular weight immune complexes may be protective of injury to the glomerular filtering surfaces (23). In performing these activities the mesangium may itself become damaged (6). Further understanding of the interplay between mesangial and capillary loop functioning may provide important insights into the pathogensis of glomerular disease.

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