

THE GLOMERULAR MESANGIUM

III. ACUTE IMMUNE MESANGIAL INJURY: A NEW MODEL OF GLOMERULONEPHRITIS*

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A number of experimental immunologic models of glomerulonephritis have been devised in animals in order to better understand mechanisms of immune glomerular injury. From these studies two general pathogenic categories have been defined (1). The first, immune complex injury, involves the interaction of circulating host or foreign antigen with antibody resulting in the formation of soluble complexes that localize in glomeruli, producing injury (2). The second involves the fixation of circulating antibody to host glomerular basement membrane (GBM)¹ after the passive administration of heterologous anti-GBM antibody (nephrotoxic nephritis) (3), or after the immunization of the host with heterologous or homologous GBM resulting in the production of autologous anti-GBM antibodies (4). Recent evidence has implicated or proved one or the other of these two basic mechanisms to be operative in a variety of human diseases including acute poststreptococcal glomerulonephritis (5), lupus nephropathy (6), glomerulonephritis associated with chronic infection (7), membranous glomerulopathy (8), and Goodpasture's disease (9). However, in many forms of glomerulonephritis seen clinically the role of these mechanisms is uncertain. In at least three diseases, anaphylactoid purpura nephritis, focal glomerulonephritis associated with the syndrome of "benign" recurrent hematuria, and membranoproliferative glomerulonephritis, the primary glomerular pathology occurs in the glomerular mesangium (10).

The mesangium is capable of taking up a large variety of circulating macromolecular substances (10) and has been demonstrated, in this regard, to function quantitatively in a manner analogous to that of the general reticuloendo-

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¹ *Abbreviations used in this paper:* Ab, antibody; Ag, antigen; AHIgG, aggregated human IgG; AHSA, aggregated human serum albumin; AV, arteriovenous; C, complement; DK, donor kidney; FITC, fluorescein isothiocyanate; GBM, glomerular basement membrane; H and E, hematoxylin and eosin; PAS, periodic acid-Schiff; PMN, polymorphonuclear cell; RES, reticuloendothelial system; RK, recipient's kidney.

thelial system (RES) (11). This paper describes a new experimental model of immune glomerular injury in which circulating antibody fixes to a foreign macromolecular antigen that has localized in the mesangium. The resulting glomerular injury bears striking similarity to certain forms of glomerulonephritis seen in animals and man.

Materials and Methods

Animals.—The animals used in these experiments were from an outbred strain of New Zealand white rabbits weighing 2–3 kg.

Aggregated Proteins.—Aggregated human IgG (AHlgG) was prepared from human immune serum globulin (Cohn fraction II), and aggregated human serum albumin (AHSA) was prepared from normal serum albumin (salt poor), both kindly provided by the American National Red Cross. The aggregation methods used have been reported in detail (11, 12).

Antisera.—Monospecific rabbit antisera against human IgG, albumin, C3, and properdin were prepared as previously described (13, 14). Antisera against rabbit IgG and IgM were prepared in goats and antisera against rabbit C3 and fibrin were prepared in guinea pigs by methods previously reported (15). These latter antisera did not react on immunoelectrophoresis with normal human serum. After absorption with human IgG the goat anti-rabbit IgG antibody did not fix to human IgG present in tissue sections; the guinea pig anti-rabbit C3 did not fix to human C3 present in tissue sections. The IgG fractions from all the above antisera were isolated by DEAE-cellulose chromatography and were tagged with fluorescein-isothiocyanate (FITC) by the method of Wood, Thompson, and Goldstein (16) as modified in our laboratory (15).

IgG fractions from the rabbit antisera against human IgG and human albumin were prepared as outlined above and were administered to rabbits as passive antibody (Ab) during the course of the experiments. Specific antibody content of these IgG preparations was determined by quantitative precipitin curve analysis.

Surgical Techniques.—The technique of renal transplantation is similar to that of Holter et al. (17) but with certain modifications. A few minutes before surgery the donor received furosemide (5 mg, intravenous [i.v.]) and both the donor and recipient received heparin (500 U, i.v.). Blood samples obtained from recipient rabbits within 2 h after transplantation did not clot. Surgery was performed under halothane-oxygen anesthesia; 4% halothane was used for induction and 1% for maintenance.

The donor and recipient operations were performed simultaneously through midline abdominal incisions. In the donor the left kidney, entire ureter, renal artery, and renal vein with a segment of vena cava were mobilized. The kidney was perfused via the renal artery with 12 cc of ice-cold Collins' solution resulting, usually, in complete cortical blanching. The renal artery was severed near its aortic origin. The vena cava was divided with a 10 mm cuff left attached to the renal vein. Finally the ureter was cut at the bladder and the kidney removed and immediately cooled in a saline ice bath.

The recipient's vena cava was isolated just below the left renal vein and a 15–20 mm segment was occluded by tension applied to two 2-0 silk strands looped around the vena cava. A 10 mm longitudinal slit was made in the occluded portion of the recipient's cava and anastomosis was accomplished to the donor caval patch with two running 6-0 silk sutures.

After occlusion of the recipient's left renal artery with a small vascular clip, the recipient's left kidney was removed and the donor and the recipient renal arteries were anastomosed end-to-end with two running 8-0 nylon sutures. The clip was then removed and blood flow reestablished. During the anastomosis procedures, the donor kidney was kept wrapped in cold saline sponges. Total ischemia time averaged less than 30 min.

Donor-to-recipient uretero-ureterostomy or ureteral-neocystostomy was then accomplished:

the former by tying the free ends of both donor and recipient ureters over a short length of plastic tubing; the latter by pulling the donor ureter through the recipient's ureteral orifice and suturing the open end of the donor ureter to the recipient's bladder mucosa with two 6-0 chromic catgut sutures. Both of these methods were equally satisfactory, and the choice of method depended upon the length of donor ureter available.

The transplanted kidney usually made urine immediately. Control, bilaterally nephrectomized, untreated recipient rabbits maintained a normal blood urea nitrogen for approximately 6 days. The overall technical success rate of the transplantation procedure was approximately 70%.

Experimental Design.—Animals serving as kidney donors in these renal transplantation experiments received AHIgG or AHSA as the antigen (Ag) in a dose of 500 mg (20 cc of a 25 mg/ml solution) by slow (20 min) intravenous infusion. 10 h later the Ag by immunofluorescent microscopy was present in the donor kidney (DK) only in the glomerular mesangium. At this time the DK with the Ag in the mesangium (referred to as DK + Ag) was removed, perfused, and transplanted into a normal recipient rabbit. One of the recipient's own kidneys (RK) was left *in situ*. $\frac{1}{2}$ h after good perfusion of the DK + Ag had been established the recipient rabbit received, intravenously, 10.6 mg of rabbit antibody protein (Ab) against the Ag previously administered to the donor animal. Biopsies of the DK + Ag and RK were obtained before and at various time periods, after Ab administration.

Control studies consisted of renal transplantation alone, transplantation of a DK + Ag without subsequent Ab administration, and transplantation of a DK that did not contain the mesangial Ag followed by Ab administration.

Immunofluorescent-Histopathologic Techniques.—Part of a slice of renal cortical tissue was immediately frozen in isopentane precooled in liquid nitrogen, sectioned at 4 μ m in a Lipshaw cryostat (Lipshaw Mfg. Co., Detroit, Mich.), and processed for immunofluorescent microscopy according to methods previously described (13-15). The tissue was stained for human IgG (with AHIgG as the Ag), human serum albumin (with AHSA as the Ag), and for rabbit IgG, IgM, C3, and fibrin.

In addition, selected tissues were studied for their ability to fix, *in vitro*, heterologous (human) complement and properdin according to methods previously reported (15). The amount of immunofluorescence was arbitrarily graded as negative, trace, 1+, 2+, 3+, and 4+; photomicrographs were taken as previously described (14).

A portion of the biopsy was placed in formalin for paraffin embedding and subsequent staining with hematoxylin and eosin (H and E) and periodic acid-Schiff (PAS). Selected tissues were processed for ultrathin (0.5 μ m) section microscopy and electron microscopy by methods previously described (18). Glomerular polymorphonuclear cell (PMN) infiltration was graded as follows: 0-4 PMN per glomerulus, normal; 5-8, minimal; 9-20, moderate; more than 20, marked. Descriptive terminology as to the distribution of glomerulonephritic morphologic changes was taken from Muehrcke et al. (19) and may be summarized as follows: (a) *local*: lesions involving one or more areas within a given glomerulus; (b) *diffuse*: lesions evenly distributed throughout a given glomerulus; (c) *focal*: lesions affecting some, but not all, glomeruli; and (d) *generalized*: lesions involving essentially all glomeruli.

Biopsies of the nontransplanted kidney from the donor rabbit and the normal kidney removed from the recipient animal served as the preantibody specimens. Tissue was also obtained from the transplanted DK and the RK at varying time intervals after Ab administration: 5 min; 1, 3, 5, and 24 h; and 2, 3, 4, and 8 days. However, biopsies were not obtained in every experiment at all of the time periods mentioned.

Complement Studies.—Serum obtained from the recipient rabbits before surgery, after transplantation, and at various time intervals after Ab administration were assayed for total hemolytic complement (CH₅₀ units) by the method of Fong and Good (20). The C1 component of complement was assayed by the hemolytic method of Lindorfer and Fong (21).

RESULTS

Light and Electron Microscopy.—Results of all light microscope studies are summarized in Table I. Biopsy of DK's + Ag (AHIgG) obtained 10 h after AHIgG administration and processed for regular light microscopy were normal

TABLE I
Results of Light Microscope Studies in Transplanted Kidneys

Pathological findings	Minimal glomerular PMN infiltration	Moderate glomerular PMN infiltration	Marked glomerular PMN infiltration and glomerular cell swelling	Minimal-to-moderate glomerular PMN infiltration. Local or diffuse increase in mesangial cells and matrix	Local or diffuse increase in mesangial cells and matrix	Increased mesangial cells and matrix. Focal and local tuft sclerosis		
	Time after antibody administration or transplantation							
	Pretransplantation	5 min	1 h	5 h	24 h	3 days	4 days	8 days
DK + Ag (AHIgG). Ab administered	±* 19/19‡	+	+	+	+	+	+	+
DK + Ag (AHSA). Ab administered	— 2/2		+	+	+	+	+	
DK + Ag (AHIgG). without Ab	± 5/5	—	—	—		3/5		
DK without mesangial Ag. Ab administered	— 3/3	—	—	—	—	—	—	
Normal kidney trans- plant (DK without mesangial Ag and without Ab)								— 8/8§

* Symbols: +, present in all biopsies; —, absent from all biopsies; ±, present in some of the biopsies.

‡ Numerator, no. of animals biopsied at any given time period; denominator, total no. of animals in a given group.

§ Biopsies obtained 6–10 days after transplantation.

or had a minimal increase in PMN's in glomerular capillary lumina (Fig. 1 A). Two donor rabbit kidneys obtained 10 h after AHSA infusion were completely normal. Examination of some of the specimens with 0.5 μ m thin sectioning revealed a slight-to-moderate expansion of the mesangial space and a denser, more homogenous mesangial matrix when compared with normal rabbit glomeruli. Glomeruli of DK's + Ag (AHIgG) did not change in light micro-

scope appearance after transplantation alone in the absence of Ab administration.

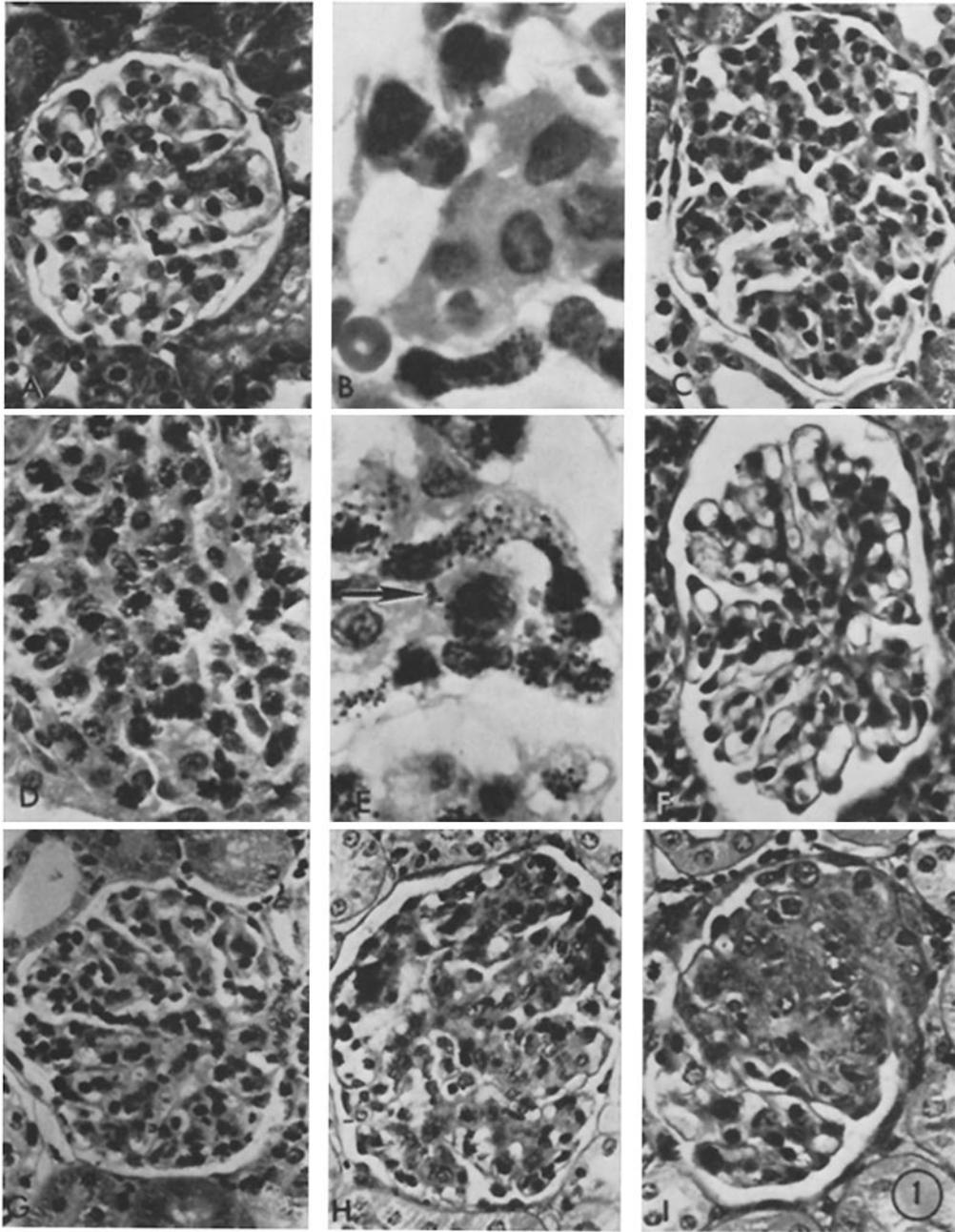
Preliminary studies by electron microscopy of four experiments including observations of DK's + Ag 10 h after Ag and after transplantation and Ab administration have been done. These revealed expansion of the mesangial matrix area by granular material of increased density. No subendothelial, intramembranous, or subepithelial dense deposits were observed along the capillary loops. The epithelial cell foot processes were essentially intact. These observations will be expanded upon in detail in a future report.²

Six DK's + Ab (AHlgG) were biopsied 5 min after Ab infusion and demonstrated moderate glomerular capillary luminal PMN infiltration (Fig. 1 B). Some of these cells appeared to be entering the area of the glomerular mesangium. 1 h after Ab in DK's + Ag (AHlgG or AHSA) there was marked glomerular PMN accumulation (Fig. 1 C). Frequently, 50-60 PMN's were counted in a single glomerulus. This was accompanied by endothelial cell cytoplasmic swelling producing obliteration of the capillary space and a paucity of red blood cells within capillary lumina. At 3 and 5 h after Ab infusion DK's + Ag showed some minimal reduction in the number of PMN's when compared with the 1-h specimens, but cellular swelling, with the loss of fine detail in glomerular architecture, and luminal narrowing were more prominent (Fig. 1 D).

In the first few hours after Ab infusion many PMN's appeared to be leaving capillary lumina and entering the mesangial area. Further, these migrating PMN's had become irregular in shape and were noted to have undergone marked degranulation; granules that were tightly packed within the PMN cytoplasm at 5 min after Ab (Fig. 1 B) were, by 1 h, loosely arranged within the cell. Further, at 1 and 5 h these granules often appeared to be lying free outside the PMN, in the mesangial and subendothelial areas, and, to a lesser extent, in capillary lumina (Fig. 1 E).

At 24 h the DK's + Ag demonstrated remarkable recovery from the acute injury described above. PMN infiltration was moderate, but many of these cells were located in the mesangium. Cellular swelling and capillary luminal obliteration was minimal. There was, however, a definite increase in mesangial matrix and occasional focal and local areas of mesangial cell proliferation. Biopsies taken at 3 and 4 days from DK's + Ag showed a marked increase in PAS positive mesangial matrix (Fig. 1 H). In addition, most glomeruli showed frank evidence of glomerulonephritis. There was a diffuse or local proliferation of mesangial and endothelial cells and, in the areas of proliferation, obliteration of the capillary space and adhesions to Bowman's membrane (Fig. 1 G, H). In some areas necrosis of entire glomerular tufts had occurred. Kidney tissue obtained 8 days after antibody administration showed interstitial changes typical of trans-

² Mauer, S. M., D. E. R. Sutherland, R. J. Howard, and R. L. Vernier. Work in progress.



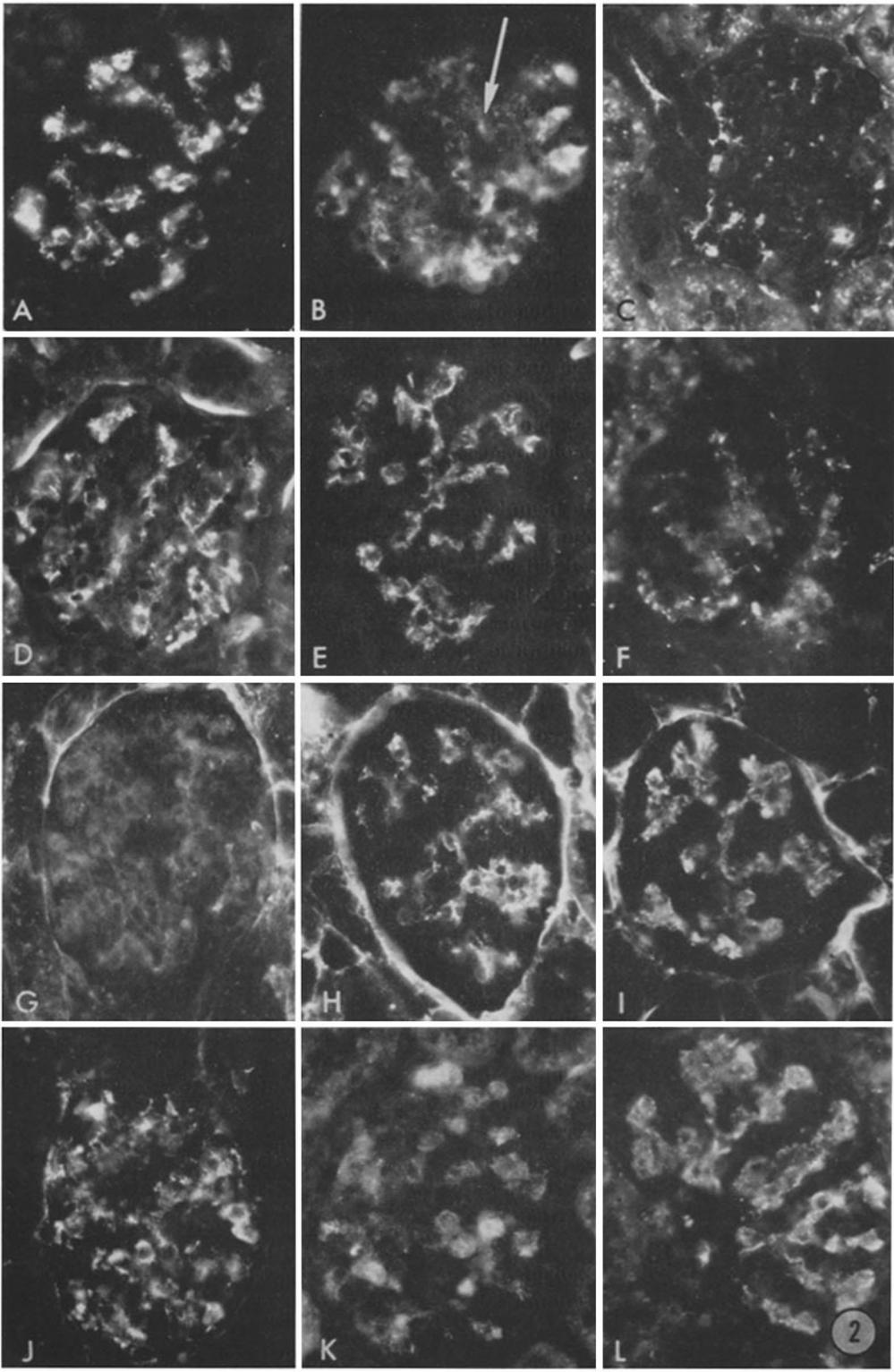
plantation rejection. The glomeruli, however, showed progression of the proliferative glomerulonephritic lesions with increased mesangial cell proliferation, lobulation, mesangial matrix accumulation, and now, focal tuft sclerosis (Fig. 1 I).

DK's + Ag (AHIgG) transplanted into rabbits that did not receive Ab were normal or had minimal glomerular PMN infiltration at 1 h. Glomeruli were normal at 1 and 3 days after transplantation. Glomeruli of DK's without mesangial Ag remained normal throughout whether or not the recipient rabbit received Ab. Eight normal transplants were studied from 6 to 10 days after transplantation and, although evidence of rejection was prominent, the glomeruli showed no pathologic changes (Fig. 1 F). The recipient's own kidney remained normal in all experiments.

Immunofluorescent Microscopy.—Animals given AHIgG (Fig. 2 A) or AHSA (Fig. 2 J) demonstrated localization of these antigens in the area of the glomerular mesangium at 10 h. Glomeruli containing AHIgG demonstrated, in addition, small quantities (trace to 1+) of C3 in a distribution identical with that of the antigen (Fig. 2 C) while the AHSA containing glomeruli were negative for C3. Rabbit IgG, fibrin, and IgM were not seen in the mesangium at this point.

After transplantation and Ab administration, several changes were seen. Within 5 min of Ab infusion rabbit IgG (1+) and C3 (2 to 3+) were deposited in a mesangial pattern (Fig. 2 D). Fibrin was seen in most DK's + Ag in the same distribution in 1+ to 2+ intensity (Fig. 2 H). However, some DK's + Ag showed no glomerular fibrin deposition at this time. At 1–5 h after Ab, increased quantities of rabbit IgG (2+) (Fig. 2 B, K) and C3 (3 to 4+) (Fig. 2 E, L) were present in the glomerular mesangium. Fibrin deposition was variable; some DK's + Ag demonstrated marked (3 to 4+) mesangial deposition (Fig. 2 I), others showed less marked localization, and others were completely normal. At these times staining for the antigen was reduced markedly, presumably because antigenic sites available to the FITC-labeled antibodies were occupied by the identical antibody administered intravenously.

FIG. 1. (A) Normal glomerulus in a DK + Ag (AHIgG), pretransplantation. H and E. $\times 400$. (B) PMN's with tightly packed granules in glomerular capillary lumina in a DK + Ag (AHIgG) 5 min after Ab. H and E. $\times 1,200$. (C) PMN infiltration, cellular swelling, and capillary luminal narrowing in a glomerulus from a DK + Ag (AHSA) 1 h after Ab. H and E. $\times 400$. (D) Changes as in Fig. 2 C but with extreme cellular swelling and loss of architectural detail in a glomerulus from a DK + Ag (AHIgG) 5 h after Ab. H and E. $\times 400$. (E) PMN's in capillary lumina and (arrow) in the mesangium in a DK + Ag (AHIgG) 5 h after Ab. Granules loosely packed in PMN's or lying free in tissue. H and E. $\times 1,000$. (F) Normal glomerulus from a normal transplant 9 days after transplantation. H and E. $\times 400$. (G) Glomerulus from a DK + Ag (AHIgG) 3 days after Ab with mesangial cell proliferation. H and E. $\times 320$. (H) Glomerulus from a DK + Ag (AHIgG) 3 days after Ab with mesangial cell proliferation and increased matrix. PAS. $\times 300$. (I) Glomerulus from a DK + Ag (AHIgG) 8 days after Ab with local tuft sclerosis. PAS. $\times 400$.



24-h DK + Ag specimens demonstrated marked reduction in glomerular localization of antigen, rabbit IgG, C3 (Fig. 2 F), and fibrin. Although rabbit C3 and fibrin tended to persist the longest, by 3 days staining for these proteins was negative or present in trace to 1+ quantities. At 4 days when by light microscopy, glomerulonephritic changes were well established, glomerular fluorescence for all components studied was normal. Staining for rabbit IgM was negative at all times before and after Ab administration.

DK's + Ag (AHlgG) transplanted into recipients not receiving Ab showed at 5 min and at 1 and 5 h mesangial antigen in 2 to 3+ intensity. No discernible decrease in intensity of fluorescence was found at these times as was seen after Ab infusion. The small quantities of C3 (trace to 1+) in the mesangium before transplantation remained constant after transplantation. In these kidneys, mesangial fibrin deposition was never seen. By 24 h the intensity of staining for human IgG and rabbit C3 had markedly diminished and by 3 days had disappeared. DK's without mesangial Ag whether or not Ab was administered, and all of the RK's from the experimental and control rabbits were normal when stained for Ag (AHlgG or AHSA) and rabbit C3, IgG, fibrin, and IgM.

Heterologous C3 and Properdin Fixation.—DK's + Ag (AHlgG), before Ab, fixed human C3 in the glomerular mesangium in 2 to 3+ intensity with staining maximal at a 1:5 dilution of the human serum. 5 h after Ab, when rabbit C3 staining was most intense, little or no human C3 fixation could be demonstrated. Similarly DK's + Ag (AHlgG), before Ab, fixed human properdin in a mesangial distribution in 1 to 2+ intensity using undiluted human serum; 5 h after Ab this fixation could no longer be detected. DK's + Ag (AHSA) did not fix human C3 or properdin before Ab and fixed little or no C3 and properdin at 1–5 h after Ab.

Complement Studies.— CH_{50} titers fell in most recipients with transplantation alone, whether or not AHlgG or AHSA had been given to the donor rabbit. In the 10 instances in which this was measured, the CH_{50} titer fell from a mean pretransplant value of 48.5 ± 20 (mean \pm SD) to a mean posttransplant titer of 33.0 ± 9 ($0.05 > P > 0.02$). In animals receiving a DK without mesangial

FIG. 2. (A) Human IgG (3+) in a DK + Ag (AHlgG) pretransplantation. $\times 480$. (B) Rabbit IgG (2+) in a glomerulus from a DK + Ag (AHlgG) 5 h post-Ab. The mesangial deposition (arrow) is obscured by the rabbit IgG normally found in the rabbit glomerulus. $\times 480$. (C) Rabbit C3 (trace to 1+) in a glomerulus from a DK + Ag (AHlgG) pretransplantation. $\times 450$. (D) Rabbit C3 (2+) in a glomerulus from a DK + Ag (AHlgG) 5 min after Ab. $\times 450$. (E) Rabbit C3 (3+ to 4+) in a glomerulus from a DK + Ag (AHlgG) 5 h after Ab. $\times 500$. (F) Rabbit C3 (1+) in a glomerulus from a DK + Ag (AHlgG) 24 h after Ab. $\times 530$. (G) Glomerulus from a DK + Ag (AHlgG) pretransplantation demonstrating the normal background staining for rabbit fibrin. $\times 390$. (H) Rabbit fibrin (1+) in a glomerulus from a DK + Ag (AHlgG) 5 min after Ab. $\times 440$. (I) Rabbit fibrin (3+) in a glomerulus from a DK + Ag (AHlgG) 5 h after Ab. $\times 440$. (J) Human serum albumin (3+) in a glomerulus from a DK + Ag (AHSA) pretransplantation. $\times 430$. (K) Rabbit IgG (2+) in a DK + Ag (AHSA) 1 h after Ab. $\times 480$. (L) Rabbit C3 (3+) in a DK + Ag (AHSA) 5 h after Ab. $\times 460$.

TABLE II
Serial Total Hemolytic Complement Titers in an Experimental (DK + Ag) and a Control (DK Without Mesangial Ag) Recipient Rabbit

Time	DK + Ag* CH ₅₀ titers	DK without mesangial Ag CH ₅₀ titers
Pretransplantation	56	51
Posttransplantation; pre-Ab‡	37	21
1 min post-Ab§	<10	22
3 min post-Ab	<10	25
8 min post-Ab	<10	31
30 min post-Ab	<10	25
3 h post-Ab	<10	—

* Each animal chosen as representative of its respective group.

‡ DK functioning in recipient rabbit but Ab not yet administered.

§ 1 min after Ab administration.

TABLE III
Serial Total Hemolytic Complement Titers in Recipient Rabbits with DK + Ag

Time	CH ₅₀ titers*								
Pre-Ab‡	50	34	45	37	29	47	34	84	42
15 min post-Ab§			<10	<10	<10		14	<10	<10
1 h post-Ab	12	<10	<10	<10	<10	<10	<10	<10	<10
5 h post-Ab			11	16	<10	17	15		
24 h post-Ab				40	<10	28			32
3-4 days post-Ab				48	90	70			49

* Values in each vertical column represent results obtained in a single recipient rabbit.

‡ DK functioning in recipient rabbit but Ab not yet administered.

§ 15 min after Ab administration.

Ag (Table II), Ab administration produced no further drop in CH₅₀ titer. In these animals and in the recipients given a DK + Ag but not given Ab, the CH₅₀ titers returned to pretransplantation values by 5 h and to above normal titers by 24-48 h. In recipient animals transplanted with DK's + Ag, Ab administration produced a rapid and profound fall in CH₅₀ titer. The mean of the pre-Ab titers was 44.6 ± 16 (Table III). 15 min after Ab five of six animals had titers of less than 10, while one animal's titer was 14. The titers remained low at 1 h after Ab and, although still low, appeared to be increasing at 5 h. By 24 h titers in most animals had returned to normal levels and by 3-4 days titers were generally higher than pretransplantation levels. In two of the DK + Ag recipients, serial samples were obtained during the first 30 min after Ab infusion and the CH₅₀ titers fell to less than 10 within 1 min. The results of one of these experiments are presented in Table II. Additional experiments were performed to determine whether this very rapid fall in CH₅₀ titer was due to complement

TABLE IV
Serial Total Hemolytic Complement Titers in Two Recipient Rabbits with DK's + Ag: Effect of Renal Artery Clamping on the Fall in CH₅₀ After Ab Administration

Time	CH ₅₀ titers*	
Pretransplantation	84	42
Posttransplantation; pre-Ab‡	52	34
1 min post-Ab; renal artery clamped§	48	32
3 min post-Ab; renal artery clamped	51	36
8 min post-Ab; renal artery clamped	46	33
1 min; renal artery unclamped	40	26
3 min; renal artery unclamped	18	<10
8 min; renal artery unclamped	<10	<10
15 min; renal artery unclamped	<10	<10
30 min; renal artery unclamped	<10	<10
60 min; renal artery unclamped	<10	<10

* Values in each vertical column represent results obtained in a single recipient rabbit.

‡ DK functioning in recipient rabbit but Ab not yet administered.

§ DK renal artery clamped before Ab infusion; sample obtained 1 min after Ab administration.

|| DK renal artery unclamped and kidney exposed to circulating Ab for 1 min.

TABLE V
Serial Arteriovenous Differences in Hemolytic C1 Titer Across the DK + Ag Before and After Ab Administration

Time	Sample source		
	DK renal vein	Aorta	Femoral vein
Posttransplant; pre-Ab‡	640*	590	600
1 min post-Ab§	<10	11	13
3 min post-Ab	16	70	74
8 min post-Ab	17	160	160
30 min post-Ab	190	230	210
5 h post-Ab	—	—	700

* Hemolytic C1 titer.

‡ DK functioning in recipient rabbit but Ab not yet administered.

§ 1 min after Ab administration.

fixation in the DK + Ag rather than to Ab interaction with Ag that may have left the mesangium and entered the circulation before Ab administration. In two animals after transplantation of DK + Ag (AHIgG) and the establishment of good renal blood flow for ½ h, the renal artery was clamped and Ab infused. In the 8 min after Ab administration while circulation to the transplanted kidney was interrupted, the CH₅₀ titers did not change significantly (Table IV). Within 1–3 min after release of the arterial clamp, a fall in CH₅₀ titer was observed. In another rabbit, after transplantation, catheters were placed in the

aorta at the level of the renal arteries, in the renal vein of the transplanted DK + Ag, and in the femoral vein. Serial blood samples were obtained from these three sites before and at various times after Ab injection. Within 1 min after Ab, the CH_{50} titer fell to less than 10 in blood samples obtained from all three sites. Thus, arteriovenous (AV) differences in CH_{50} titers could not be determined. However, by measuring C1 titers, a definite AV difference could be demonstrated across the kidney after Ab infusion. This AV difference did not occur across the leg (Table V).

DISCUSSION

These studies were initially designed to determine whether the glomerular mesangium was an immunologically "sequestered" site inaccessible to circulating antibody. Although in experimental animals AHlgG had been demonstrated by immunofluorescent microscopy to localize in the mesangium, unaggregated human IgG administered in similar quantities has not been seen in this area of the glomerulus (11, 12). The kidney transplantation system was employed in the present studies to avoid the reaction of circulating antibody with antigen distributed throughout the RES, and to eliminate the possible development of circulating antigen-antibody complexes. In these experiments AHlgG was given to donor rabbits 10 h before transplantation of one of their kidneys into a normal uninephrectomized recipient rabbit that then received rabbit antihuman IgG antibody. The rapid appearance of rabbit IgG, complement, and fibrin in the mesangium of the donor kidney after antibody infusion demonstrated the accessibility of an antigen in this locus to circulating antibody and to other serum proteins. Since AHlgG is biologically active, having many of the properties of antigen-antibody complexes including the ability to fix complement components and produce tissue injury (22, 23), it was possible that the demonstrated penetration of the mesangium by circulating antibody was secondary to mesangial injury consequent to AHlgG localization. However, similar results were obtained using AHSA, a biologically inert macromolecular aggregate as the antigen.

The early consequences of the interaction of circulating antibody with antigen localized in the mesangium include the rapid mesangial deposition of large quantities of complement and fibrin, and associated with this, marked glomerular PMN infiltration, evidence of glomerular injury with mesangial and endothelial cell swelling, and capillary luminal narrowing.

In nephrotoxic nephritis, chemotactic components of the complement system are presumed to be responsible for the glomerular PMN infiltration (24). These PMN's are postulated to produce injury through the release of lysosomal enzymes that are capable of digesting the GBM. Neutrophil lysosomal enzymes and GBM constituents have been found in the urine in this model (25). In vitro studies have shown that antigen-antibody complex precipitates (26), including rheumatoid factor-AHlgG precipitates (27), are readily phagocytosed

by PMN's and result in the release of lysosomal enzymes (26, 27). In our studies the *in vivo* interaction of antibody with the macromolecular antigens localized in the glomerular mesangium probably resulted in immune complexes capable of the release of large amounts of PMN lysosomal enzymes. Our observations of PMN degranulation suggest that these cellular events are involved in the immune glomerular injury that was produced. The light microscope appearance of large numbers of PMN granules found in glomeruli free of the parent cell testifies to the great intensity of this reaction. Recently Schumacher and Agudelo (28) demonstrated, by electron microscopy, intraluminal degranulation of PMN's in synovial biopsies from canine joint spaces previously injected with urate crystals; they suggested that release of PMN mediators in this intravascular locus may be an important mechanism of tissue injury.

The acute glomerular inflammatory events after antibody administration subsided within 24 h with return of glomerular morphology towards normal; however, by 3-4 days mesangial and endothelial cell proliferation and increased mesangial matrix were evident. At these later times the immune reactants that had been demonstrated by immunofluorescent microscopy had either disappeared or were barely detectable and the serum complement levels had returned to normal. Thus it appears that the early acute injury was capable of initiating a proliferative response and increased mesangial matrix deposition that could be sustained in the absence of the inciting stimuli. It may be that this mesangial proliferative change was programmed by the initial acute inflammatory events as PMN lysosomal granules can result in cellular hyperplasia *in vivo* (29) (in synovial lining cells after intra-articular PMN granule injection) and *in vitro* (30).

Animals studied at 8 days showed further development of these glomerular lesions and, in addition, evidence of focal glomerular sclerosis. The onset of the transplantation rejection reaction by 8 days makes interpretation of these findings difficult, although studies of rejecting kidneys in control experiments failed to reveal glomerular alterations. The late phase of glomerular injury in this model is currently under investigation in our laboratory using a highly inbred strain of animals where the transplantation rejection reaction is avoided.

The interaction of circulating antibody with a foreign antigen fixed in the mesangium results in a rapid fall in total hemolytic complement and C1, suggesting the classical mechanism of complement component activation is involved (31). The fall consequent to antibody infusion does not occur when the renal artery is clamped but follows within 1-3 min of unclamping. This suggests that the complement depletion is secondary to fixation in the donor kidney. The demonstration of a fall in C1 across the donor kidney vessels supports this conclusion. Sequential studies demonstrated a profound fall in the C1 level 1 min after antibody infusion. Remarkably the level began to rise by 3 min probably indicating diffusion of C1 from the extravascular pool to the intravascular

space. The early trend towards recovery of C1 levels suggests that the rate of complement consumption was greatest in the 1st min after antibody infusion. In fact, by 30 min after antibody infusion arteriovenous differences of C1 across the donor kidney were barely detectable.

In vitro experiments demonstrated heterologous (human) C3 fixation to AHIgG in the donor kidney glomerular mesangium. Failure of heterologous complement to fix to glomeruli after antibody administration suggests that in vivo rabbit complement fixation had, by 5 h after antibody, used up all available complement binding sites on the immune reactants in the mesangium. Human properdin fixed in vitro to mesangial AHIgG before antibody infusion. It is tempting to speculate that the failure to detect in vitro properdin fixation after antibody infusion was due to in vivo utilization of available properdin binding sites. If true, this would indicate that the properdin system, and thus the alternate pathway of complement activation (31, 32), may be involved as a consequence of direct antigen-antibody interaction.

Glomerular fibrin(ogen) deposition is thought to be important in the pathogenesis of several forms of immune glomerular injury (33). Our findings of fibrin(ogen) in an identical (mesangial) distribution to Ag, Ab, and C3 support the in vitro evidence that immunological reactions may, through the complement system, activate coagulation mechanisms (34). Although AHIgG alone accelerates prothrombin consumption in vitro (35), mesangial fibrin was not seen in DK's + Ag (AHIgG) unless Ab was given. The variability noted in the amount of fibrin(ogen) deposited in the mesangium of DK's + Ag shortly after Ab administration may be due to the heparin which recipient animals received before surgery. That this deposition occurred at all in anticoagulated animals is surprising (36) and suggests great potency of this immune glomerular reaction in triggering the coagulation mechanism at the site of Ag-Ab interaction.

In a general sense, the localization of antigen to a tissue site (e.g. skin, synovium, and the glomerular mesangium) to which antibody has ready accessibility represents the basic conditions for the production of the classical Arthus reaction. It is known that PMN's play an essential role in the development of this reaction (37). Further these cells may be important in terminating the reaction by removing the antigen-antibody complexes (37). The PMN infiltrate and vessel wall damage of the Arthus reaction are known to depend upon an intact complement system (38). Finally, the glomerular cell proliferation noted in the present model may be analogous to the synovial cell hyperplasia and pannus formation consequent to the Arthus reaction model of immunologic arthritis (39).

The long-term persistence of immunoglobulins, complement, and fibrin in the mesangium in diseases such as anaphylactoid purpura nephritis in man (18) and Aleutian mink disease (40) may be due to a glomerular injury that results in an imbalance in the mesangial macromolecular uptake (afferent limb) and clearance mechanisms (efferent limb) (11). We have previously demonstrated

that animals with increased glomerular permeability to protein (aminonucleoside nephrosis [11] and nephrotoxic nephritis³) have a marked increase in mesangial uptake of circulating macromolecules (AHlgG) while maintaining a normal ability to clear the AHlgG from the mesangium with time. In the present study, despite evidence of severe mesangial injury, the rabbit mesangium demonstrated the ability to rapidly clear biologically active macromolecular proteins. Thus, it appears that the mesangial clearing mechanisms may be resistant to injury. The persistence of immune complexes in the mesangium may result from a continuous process in which localization is occurring in spite of effective clearance mechanism(s). For example, in chronic lymphocytic choriomeningitis infections of mice, a disease in which circulating virus-antibody complexes are present during the animals entire life-span, chronic glomerulonephritis develops and is associated with the progressive deposition of viral antigen, immunoglobulins, and complement in glomeruli (41). Furthermore, alteration in glomerular capillary permeability to protein could accelerate the glomerulonephritic process associated with circulating antigen-antibody complexes by markedly increasing the rate of mesangial localization of the circulating immune material (11).³

Finally, the present study offers another hypothesis to explain persistent mesangial immune material. We have demonstrated that circulating antibody can fix to a foreign antigen localized in the mesangium producing severe glomerular injury. If the foreign mesangial antigen was persistent or self-replicating (e.g. a virus), or indeed if some component of the glomerular mesangium was itself the antibody target, chronic immune mesangial injury and chronic progressive glomerulonephritis could result.

SUMMARY

A mechanism of immune glomerular injury is described based on the fixation of antibody (Ab) to an antigen (Ag) that has localized in the glomerular mesangium. Rabbits were given, intravenously (i.v.), aggregated human IgG (AHlgG) or albumin (AHSA) and 10 h later, when the Ag by immunofluorescent microscopy was present in the mesangium, a kidney was removed and transplanted into a normal rabbit. The recipient then received, i.v., rabbit anti-HlgG or anti-HSA.

Within minutes of Ab infusion, glomeruli of the donor kidney had polymorphonuclear (PMN) infiltration that over the next few hours became marked and was associated with glomerular cell swelling. At 24 h a decrease in PMN's and early mesangial proliferation was seen. By 3 days there was marked mesangial hypercellularity and increased mesangial matrix. Within minutes after Ab administration rabbit IgG, C3, and fibrin were seen in the glomerular

³ Mauer, S. M., A. J. Fish, N. K. Day, and A. F. Michael. The mesangial uptake of macromolecules in nephrotoxic nephritis in rats. Manuscript in preparation.

mesangium. There was a fall in complement titer by 1 min after Ab infusion that was due to complement consumption by the donor kidney. Complement then returned to normal levels by 48 h.

Significant glomerular injury did not occur (*a*) in the recipient's own kidney, (*b*) from Ag administration and transplantation without recipient Ab administration, or (*c*) from transplantation and Ab administration without prior Ag administration.

These studies demonstrated that Ag localized in the glomerular mesangium can react with circulating Ab and complement resulting in severe glomerular injury.

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