Acute Effects of Antiglomerular Basement Membrane Antibody on the Process of Glomerular Filtration in the Rat

ROLAND C. BLANTZ and CURTIS B. WILSON

From the Departments of Medicine, University of California, San Diego, School of Medicine and Veterans Administration Hospital, San Diego, California 92161 and Department of Immunopathology Scripps Clinic and Research Foundation La Jolla, California 92037

ABSTRACT Nephron filtration rate (sngfr) and the factors controlling filtration were examined before and within 60 min of the intravenous infusion of 225–450 μg of antiglomerular basement membrane antibody (AGBM Ab) (> 50% antigenic saturation) in plasma-expanded (2.5% body wt) Munich-Wistar rats. Pressures in glomerular capillaries (P_G) and Bowman's space (P_t) were measured with a servo-nulling device, systemic (π_A) and efferent arteriolar oncotic pressures (π_E) were measured by microprotein methods, and nephron plasma flow (rpf) and sngfr were measured by micropuncture techniques in both control and post-AGBM Ab conditions in each rat. The sngfr fell from 52.7±2.9 to 24.1±1.9 nl/min per g kidney wt ($n = 7$, $P < 0.001$). Both afferent and efferent arteriolar resistances increased and rpf fell from 221±25 to 90±9 nl/min per g kidney wt ($P < 0.001$) but the hydrostatic pressure gradient across the glomerular membrane ($\Delta P = P_G - P_t$) increased from 37±1 to 50±2 mm Hg ($P < 0.001$). The increase in ΔP and a numerical decrease in π_A both acted to maintain sngfr after AGBM Ab and effectively nullified the influence of decreased rpf upon sngfr. The mean effective filtration pressure ($EFP = \overline{\Delta P} - \overline{\pi}$) increased from 14±2 to 30±3 mm Hg ($P < 0.001$) while sngfr decreased. The major and critical reason for this reduction in sngfr was a decrease in the glomerular permeability coefficient from 0.077±0.017 to 0.014±0.001 nl/s per g kidney wt per mm Hg ($P < 0.001$) where $sngfr = \overline{EFP} \cdot L_pA$.

Histologic sections revealed no proliferative changes, and capillary lumens were patent. From examination of light and electron microscopy we conclude that the loss of capillary conduits and surface area was not adequate to explain the large reduction in L_pA observed. Therefore, reduction in L_pA must result from an acute decrease in local glomerular hydraulic permeability (L_p) probably as a result of swelling and separation of endothelial cells from the glomerular basement membrane observed by electron microscopy.

INTRODUCTION

Acute diffuse glomerulonephritis is a clinical syndrome characterized by NaCl retention, proteinuria, varying reductions in glomerular filtration rate, hypertension secondary to volume retention, and abnormalities of the urinary sediment (1–5). The NaCl retention could be either the result of increased tubular NaCl and water reabsorption or due to a reduction in filtration rate in all nephrons. Recent studies have suggested that increased NaCl and water reabsorption is not the only or even primary cause for volume retention, which leaves only immune induced reductions in glomerular filtration to explain the findings (6–9).

The acute reduction in glomerular filtration rate with immune injury could be the result of several factors. The mean effective filtration pressure could be reduced due to (*a*) reductions in nephron plasma flow secondary to vasoconstriction and plugging of capillary lumens with inflammatory cells, (*b*) decreased hydrostatic pressure gradient (ΔP) due to reduced glomerular capillary hydrostatic pressure (P_G), and (*c*) decreased ΔP as a result of increased tubular pressure. Alternatively, the filtration rate may decrease as a result of reductions in the glomerular permeability coefficient (L_pA), which

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could result from either loss of capillary surface area (A) secondary to inflammatory changes or local reductions in capillary membrane hydraulic permeability (L_p), in spite of the observed increase in protein permeability of the glomerulus (1, 9). It is likely that several of the above mechanisms are operative in the early phases of reduced glomerular filtration, but data on this issue have not been available.

Studies were performed to examine the filtration process and the forces determining filtration before and immediately after the infusion of large quantities of complement-fixing antiglomerular basement membrane antibody (AGBM Ab). This experimental protocol will permit discrimination of the acute effects of antibody fixation and immune injury from the later effects of an established inflammatory process and the resulting NaCl and water retention. All measurements were completed within 60 min of administration of antibody before the established cellular inflammatory response within the capillary.

GLOSSARY OF SYMBOLS

A	Glomerular capillary surface area.
AGBM Ab	Antiglomerular basement membrane antibody.
AR	Afferent arteriolar resistance ($\times 10^9$ dynes·s/cm^5).
C	Protein concentration.
CFA	Complete Freund's adjuvant.
EFP	Effective filtration pressure ($EFP = \Delta P - \pi$) (mm Hg).
ER	Efferent arteriolar resistance ($\times 10^9$ dynes·s/cm^5).
FE_{Na}	Fractional sodium excretion (%).
GBM	Glomerular basement membrane.
GFR	Kidney filtration rate (ml/min per g kidney wt).
HP_E	Mean efferent peritubular capillary pressure (mm Hg).
L_pA	Total glomerular permeability (60 s/min·L_pA =sngfr/$\overline{EFP}$) (nl/s per g kidney wt per mm Hg).
MAP	Mean arterial pressure (mm Hg).
p	Plasma count rate.
P_G	Glomerular capillary hydrostatic pressure (mm Hg).
P_t	Bowman's space or proximal tubular hydrostatic pressure (mm Hg).
ΔP	Hydrostatic pressure gradient across glomerular capillary ($\Delta P = P_G - P_t$) (mm Hg).
π	Oncotic pressure (mm Hg).
π_A	Systemic oncotic pressure (mm Hg).
π_E	Efferent oncotic pressure (mm Hg).
rbf	Nephron blood flow (nl/min per g kidney wt).
rpf	Nephron plasma flow (nl/min per g kidney wt).
snff	Nephron filtration fraction.
sngfr	Nephron filtration rate (nl/min per g kidney wt).
$U_{Na}V$	Sodium excretion (μeq/min).
$U_{prot}V$	Protein excretion (μg/min).
UV	Urine volume (μl/min).
x^*	Normalized glomerular capillary length (dimensionless parameter).
—	Bar (superscript) designates mean value.

METHODS

Studies were performed in Munich-Wistar rats (190–250 g body wt), bred and maintained in a colony housed at the Veterans Administration Hospital, San Diego, Calif.

Preparation of AGBM Ab. AGBM Ab was produced by immunizing rabbits repeatedly with 10–20 mg of rat glomerular basement membrane (GBM) in complete Freund's adjuvant (CFA) prepared by modification of the Krakower and Greenspon method (8). When nephrotoxic amounts of AGBM developed, as monitored by induction of acute proteinuria after intravenous injection in rats, serum was collected, pooled, absorbed with rat plasma and peripheral blood cells, and the gamma globulin fraction separated and concentrated by precipitation at a final concentration of 50% saturated ammonium sulfate. The gamma globulin fraction thus obtained and a normal rabbit gamma globulin fraction were pair-labeled with ^{131}I and ^{125}I radioactive iodine, and the amount of kidney-fixing antibody was quantitated using the paired label technique (9, 10).

Micropuncture studies on glomerular dynamics before and after AGBM Ab. Surgical preparation for micropuncture was as previously described in recent studies from this laboratory (11–15). All micropuncture studies were paired. The control condition was isoncotic plasma expansion; the plasma was obtained from donor Munich-Wistar rats on the morning of the study (13, 14). A volume of plasma equal to 2.5% body wt was infused over a 60-min period. A separate infusion of [^{14}C]inulin in isotonic NaCl-$NaHCO_3$ (0.5% body wt/h) was begun at this same time and delivered at approximately 40 μCi/h. At the end of the 60-min plasma infusion period, urine flow increased to several times the hydropenic excretion rates, and the rate of an intravenous infusion of isotonic NaCl-$NaHCO_3$ was increased to equal the rate of total urine output and maintain the degree of volume expansion (13, 14).

Glomerular capillary and Bowman's space hydrostatic pressure were measured in all available surface glomeruli utilizing a servo-nulling device with 1-μm tip pipettes. Methods for pressure measurement and the mechanics of the pressure monitoring device were as described previously from this laboratory (11, 15). At least three samples of efferent peritubular blood were obtained from "star" vessels on the kidney surface (11–14). Samples of femoral artery blood were also obtained concurrently in triplicate for protein determinations.

Details of the micro-adaptation of the Lowry protein method (16) have been described in previous publications from this laboratory (11–14).

After completion of control period measurements, 225 or 450 μg of AGBM Ab in a volume of either 0.25 ml (rats 1 and 2) or 0.50 ml (rats 5–8 and 10) was injected intravenously over a period of approximately 5 min. The maintaining infusion was discontinued during AGBM Ab injection to prevent acute volume expansion. 15 min after the infusion of AGBM Ab measurements of glomerular capillary and Bowman's space pressures, femoral artery and efferent peritubular capillary blood samples for protein concentration, nephron filtration rates, and total kidney GFR were repeated within a 45-min period. At the end of this period, a sample of renal venous blood was obtained with a heparinized 25–35-μm tip glass pipette. From femoral arterial and renal venous concentrations of [^{14}C]inulin, the total filtration fraction was determined.

Validity of inulin clearances after immune injury. Both [^{14}C]mannitol and [^{3}H]inulin were infused at rates sufficient to perform clearances before and after AGBM Ab infusion

in the same rat to examine the possibility of inulin sieving across the glomerulus.

Control studies. Control studies were performed using similar quantities of gamma globulin fractions from rabbits injected with CFA alone. The protocol and time course of these studies were identical to those described for AGBM Ab.

Control studies were also conducted to examine the effects of immunologic and biologically active materials released by an Arthus reaction in skeletal muscle, induced by an antirat IgG antibody. The quantities of antirat IgG antibody utilized produced a maximum cellular response in muscle when evaluated by light microscopy and were approximately 80% of that which produced systemic hypotension. After intramuscular injection, a 15-min period elapsed and then measurements were repeated.

Analytic methods. Protein electrophoresis of plasma samples were performed as previously described (11, 12, 17). Urine protein concentration was determined by the microadaptation of the Lowry protein method (16, 11–14). Urine and plasma sodium and potassium concentrations were determined on an Instrumentation Laboratory flame photometer (Instrumentation Laboratory, Inc., Lexington, Mass.). Total filtration rates, renal plasma flow, and renal blood flow were calculated as previously described (11–14, 17). ^{14}C counts in plasma, urine, and tubular fluid were monitored on a model 2425 Packard liquid scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.). Nephron filtration rate (sngfr = UV/P) was calculated from the total count rate of urine collected per minute (UV) divided by the plasma count rate (p) (corrected for plasma water).

Preparation of tissues for histologic, immunofluorescence, and electron microscopic studies. Tissue for histologic study was obtained from both kidneys at the termination of the experiment, fixed in Bouin's solution for 24 h and postfixed in 50% ethyl alcohol before embedding for routine paraffin sections. Hematolylin and eosin, as well as periodic acid Schiff-stained sections, were evaluated as previously described (18). Tissue for immunofluorescence study was snap-frozen in liquid nitrogen and cryostat sections obtained from it were fixed and stained for the presence of rabbit IgG and rat C3 with appropriate controls as previously described (19). Tissue for electron microscopy was obtained by superficial wedge biopsy before disruption of the renal circulation. The small wedge was immediately diced in chilled 2% phosphate-buffered (pH 7.3) glutaraldehyde for fixation. Tissue was postfixed for 1 h in 1% phosphate-buffered osmic acid. It was then dehydrated through a series of alcohols, washed clear with propylene oxide and embedded in Epon 812 (Ladd Research Industries, Inc., Burlington, Vt.). The sections (500–700 Å) were stained with uranyl acetate and lead citrate and examined between 5,000 and 17,000 magnifications with a Hitachi 11A electron microscope (Perkin-Elmer Corp., Hitachi-Perkin Elmer Instruments, Mountain View, Calif.). Portions of two glomeruli from each kidney from each rat studied were evaluated.

Albumin disappearance studies. The rate of radioactive albumin disappearance in both control animals and in animals after injection of AGBM Ab was measured to determine if the antibody produced an acceleration in the rate of loss of protein from plasma volume. Approximately 10 μCi of ^{125}I-labeled rat albumin was injected into control rats and rats that had received 225 μg of AGBM Ab 15 min earlier. Femoral artery blood samples were then obtained at 15-min intervals for a total period of 90 min after [^{125}I]albumin infusion. Disappearance rates were expressed as a percentage of the initial peak [^{125}I]albumin count rate per 10 μl plasma over the 90-min period.

Calculations. The calculation of superficial nephron filtration fraction (snff), nephron plasma flow (rpf), nephron blood flow (rbf), afferent arteriolar resistance (AR), and efferent arteriolar resistance (ER) was as described in previous publications from this laboratory (11–15).

The relation between protein concentration and oncotic pressure (π) was described by Landis and Pappenheimer (20). The specific modifications to the equations describing these relationships have been described in previous publications from this laboratory (11–15).

Previous studies from this laboratory (11–14, 17) and the laboratory of Brenner and co-workers (21–23) have demonstrated that sngfr is determined by and controlled by changes in four factors: (*a*) The hydrostatic pressure gradient acting across the glomerular capillary (ΔP), (*b*) the systemic oncotic pressure (π_A), (*c*) the glomerular permeability coefficient (L_pA), and (*d*) the rate of nephron plasma flow (rpf). The relationship of these four factors is as follows:

$$\Delta P = P_G - P_t,$$

where P_G = directly measured glomerular capillary hydrostatic pressure and P_t = Bowman's space hydrostatic pressure.

The effective filtration pressure (EFP) can then be defined as follows:

$$EFP = \Delta P - \pi.$$

π rises along the length of the glomerular capillary (x^*) as a result of the formation of glomerular ultrafiltrate and the resultant increase in protein concentration (C). The mean EFP ($\overline{EFP}$) is defined as follows:

$$\overline{EFP} = \int_0^1 (\overline{\Delta P} - \bar{\pi}) dx^*,$$

where x^* = normalized unit glomerular capillary length, L_p = local capillary hydraulic permeability, and A = glomerular capillary surface area. Changes in rpf modify the EFP profile by affecting the rate of concentration of protein and π along x^*.

Utilizing mathematical models of glomerular filtration as previously described (11, 17) and applying data to the computer, a profile for EFP and the integrated value for $\overline{EFP}$ can be generated. A value for the glomerular permeability coefficient (L_pA) is also generated by this method (sngfr = $L_pA \cdot \overline{EFP}$). At filtration pressure equilibrium ($\pi_E \approx \Delta P$) as has been demonstrated for the hydropenic Munich-Wistar rat (11, 17, 24), a specific numerical value for L_pA and $\overline{EFP}$ cannot be defined, but rather only a minimal and maximal possible value, respectively. However, at higher rates of rpf, produced by isoncotic plasma volume expansion in this study, the efferent EFP becomes significantly positive, or ΔP greatly exceeds π_E. Under these conditions specific values may be defined for both L_pA and $\overline{EFP}$ (11, 17). The data required for such determinations include the systemic and efferent peritubular capillary C, rpf, and ΔP.

Statistical analysis. Significance of data between control and experimental conditions was determined by analysis of variance and Student's *t* test (25, 26).

TABLE I
The Effect of AGBM Ab on Urine, Sodium, and Protein Excretion

	Control* (Plasma expanded	AGBM Ab*	*P* value
UV, $\mu l/min$	28±4‡	1.6±0.3	<0.001
$U_{Na}V$, $\mu eq/min$	3.3±1.0	0.10±0.03	<0.02
FE_{Na}, %	1.85±0.30	0.10±0.01	<0.01
$U_{prot}V$, $\mu g/min$	2.9±0.6	2.6±0.8	>0.7

* n = seven.
‡ ±SEM.

RESULTS

The effects of AGBM Ab. After the infusion of AGBM Ab there was no change in color of the cortical surface of the kidney, but the capsule was somewhat less "tight" than in the control plasma-expanded condition before antibody infusion.

UV fell from 28±4 μl/min in the control condition to 1.6±0.3 μl min ($P < 0.001$) (Table I). Sodium excretion ($U_{Na}V$) also fell from 3.3±1.0 μeq/min to 0.10 ±0.03 μeq/min after AGBM Ab ($P < 0.02$). Fractional sodium excretion (FE_{Na}) fell from 1.85±0.3% to 0.10 ±0.01% ($P < 0.01$). Urine protein excretion ($U_{prot}V$) was 2.9±0.6 μg/min before AGBM and 2.6±0.8 μg/min after the infusion ($P > 0.7$). There was a large and significant increase in urinary C, but due to the marked decrease in urine flow, total protein excretion was not increased acutely. In cage studies, injection of 225 μg of AGBM Ab increased protein excretion within the first 24 h after antibody infusion.

The mean sngfr was 52.7±2.9 nl/min/g kidney wt in the control plasma-expanded condition and fell to 24.4±1.9 nl/min g kidney wt ($n = 7$) ($P < 0.001$) after infusion of AGBM Ab (Fig. 1 and Table II). When all sngfr data were analyzed to determine if there was more heterogeneity in sngfr within each animal after AGBM Ab, we find that the standard deviation averaged 15% of the mean in control rats compared to 27% after AGBM Ab, suggesting a modest increase in heterogeneity of sngfr. Total kidney filtration rate (GFR) decreased from 1.5±0.1 to 1.1±0.1 ml/min per g kidney wt ($P < 0.01$).

Studies were performed in two animals to determine if sngfr was reduced as early as 15 min after completion of the infusion of AGBM Ab. In the two paired studies sngfr decreased in one animal from 56.4±2.2 to 42.6 nl/min per g kidney wt but was not statistically different in the second animal studied.

The mean rpf decreased from 221±25 in control to 90±9 nl/min per g kidney wt ($P < 0.001$) after AGBM Ab (Fig. 2 and Table II). The mean rbf decreased from 399±47 to 168±20 nl/min per g kidney wt ($P < 0.01$) (Table II). The mean snff was 0.25± 0.02 in the control state and 0.29±0.04 ($P > 0.3$) after infusion of AGBM Ab. Individual and overall values from these studies are shown in Table II.

The mean P_t fell from 20.3±1.7 mm Hg to 11.8±1.1 mm Hg ($P < 0.001$). The mean P_G was 59.1±2.6 mm Hg in the control state and 61.9±2.2 mm Hg after AGBM Ab ($n = 7$, $P > 0.3$). These values were based upon 14 direct observations in control and 12 after AGBM Ab (Table II). As a result, the mean ΔP rose from 37.2±1.2 to 49.5±2.1 mm Hg ($P < 0.001$, $n = 7$) (Fig. 2). Mean efferent peritubular capillary ("star") pressure (HP_E) was 24.5±1.7 in the control condition and 19.0±1.5 mm Hg after AGBM Ab ($P < 0.02$).

The reduction in rpf and rbf were the result of specific changes in renal vascular resistance. Mean AR increased from 15.1±2.7 × 10^9 to 33.7±5.7 × 10^9 dynes/s per cm^5 ($P < 0.001$). The increase in mean ER was even greater, 8.8±1.0 × 10^9 to 26.6±3.4 × 10^9 dynes/s per cm^5 ($P < 0.001$) which accounts for the small numerical increase in P_G (Table II).

The plasma C was numerically lower after AGBM Ab in five of seven rats (5.5±0.2 in control to 4.9±0.3 g/100 ml) ($P > 0.1$). The mean systemic oncotic pressure (π_A) was 18.4±1.1 in control to 15.3±1.6 mm Hg after AGBM Ab ($P > 0.1$). This numerically lower systemic

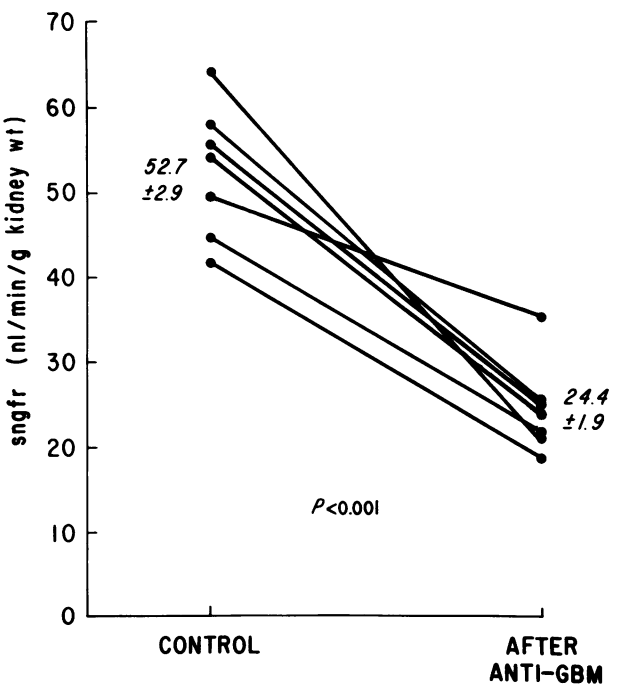

FIGURE 1 The sngfr in control rats and after the infusion of AGBM Ab. The individual animal mean sngfr is depicted on the left for the seven control plasma-expanded rats and the resulting sngfr in each rat after AGBM Ab. The sngfr fell consistently in each animal.

TABLE II

Pressures, Flows, Vascular Resistances, and Glomerular Permeability before and after the Infusion of AGBM Ab

Rat no.	MAP	P_G	P_t	ΔP	sngfr	snff	rpf	rbf	AR	ER	HP_E	π_A	π_E	$\overline{EFP}$	L_pA	EFP_E
	mm Hg	*mm Hg*	*mm Hg*	*mm Hg*	*nl/min/g kidney wt*		*nl/min/g kidney wt*	*nl/min/g kidney wt*	*×10⁹ dynes·s/cm⁵*	*×10⁹ dynes·s/cm⁵*	*mm Hg*	*mm Hg*	*mm Hg*	*mm Hg*	*nl/s·g/mm Hg*	*mm Hg kidney wt*
Control plasma expansion																
1	108	70.9 ±1.7* $n=2$	27.3 ±0.8 $n=6$	41.6 ±0.1 $n=2$	41.9 ±3.0 $n=5$	0.21	200 ±14 $n=5$	339 ±24 $n=5$	8.8	12.0	26.8 ±1.5 $n=3$	17.6	24.8	20.7	0.033 ±0.002	16.8
2	119	62.5 ±1.6 $n=2$	17.6 ±0.4 $n=6$	41.2 ±1.6 $n=2$	58.1 ±2.2 $n=5$	0.19	306 ±12 $n=5$	556 ±21 $n=5$	8.0	7.2	20.1 ±1.7 $n=3$	19.5	27.0	18.1	0.055 ±0.003	14.2
5	143	57.4 ±7.8 $n=2$	21.8 ±1.0 $n=6$	33.8 ±3.9 $n=2$	44.8 ±3.2 $n=5$	0.36	124 ±9 $n=5$	230 ±16 $n=5$	29.5	12.0	30.0 ±1.6 $n=3$	15.4	29.4	11.4	0.065 ±0.020	4.4
6	128	61.2 ±3.9 $n=3$	24.3 ±0.9 $n=6$	36.4 ±4.6 $n=3$	49.6 ±2.3 $n=5$	0.28	180 ±8 $n=5$	314 ±14 $n=5$	16.7	10.4	28.1 ±2.2 $n=3$	19.5	31.8	10.5	0.079 ±0.069	4.6
7	131	59.0 ±6.1 $n=2$	99.6 ±0.4 $n=6$	37.0 ±7.8 $n=2$	54.4 ±1.6 $n=5$	0.26	209 ±6 $n=5$	373 ±11 $n=5$	15.2	8.0	26.4 ±1.0 $n=3$	14.8	23.1	18.4	0.050 ±0.023	13.9
8	145	51.8 ±1.0 $n=2$	16.5 ±0.2 $n=6$	33.8 ±3.4 $n=2$	55.8 ±8.0 $n=5$	0.18	310 ±45 $n=5$	563 ±81 $n=5$	13.6	4.8	22.7 ±1.8 $n=3$	23.7	31.8	5.4	0.167 ±0.229	2.0
10	121	51.1 $n=1$	15.1 ±0.3 $n=7$	36.6 $n=1$	64.1 ±3.8 $n=5$	0.29	221 ±13 $n=5$	417 ±25 $n=5$	13.6	7.6	17.7 ±0.5 $n=3$	18.0	30.6	12.3	0.089 ±0.004	6.0
Overall mean	128 ±5	59.1 ±2.6	20.3 ±1.7	37.2 ±1.2	52.7 ±2.9	0.25 ±0.02	221 ±25	399 ±47	15.1 ±2.7	8.8 ±1.0	24.5 ±1.7	18.4 ±1.1	28.4 ±1.3	13.8 ±2.1	0.077 ±0.017	8.8 ±2.2
AGBM Ab																
1	111	68.0 ±0.8* $n=2$	11.3 ±1.8 $n=6$	52.1 ±2.0 $n=2$	18.7 ±3.2 $n=5$	0.18	104 ±18 $n=5$	193 ±32 $n=5$	17.6	22.3	19.7 ±1.4 $n=3$	18.5	24.8	30.7	0.010 ±0.001	27.3
2	110	69.1 $n=1$	14.1 ±0.8 $n=6$	53.8 $n=1$	25.2 ±2.4 $n=5$	0.20	126 ±12 $n=5$	225 ±21 $n=5$	14.4	18.4	22.8 ±3.4 $n=3$	14.8	20.5	36.5	0.011 ±0.001	33.3
5	131	62.6 ±4.0 $n=2$	17.2 ±0.3 $n=6$	46.0 ±4.6 $n=2$	21.9 ±1.9 $n=5$	0.34	64 ±6 $n=5$	117 ±10 $n=5$	46.3	34.3	21.8 ±7.8 $n=3$	13.1	24.2	28.1	0.013 ±0.001	21.8
6	130	58.2 ±1.2 $n=2$	10.3 ±1.1 $n=5$	47.6 ±2.0 $n=2$	35.1 ±4.1 $n=5$	0.45	78 ±9 $n=5$	134 ±15 $n=5$	43.1	31.9	18.3 ±1.4 $n=3$	11.4	27.3	29.7	0.020 ±0.002	20.3
7	128	54.4 ±3.0 $n=2$	11.7 ±0.7 $n=6$	42.1 ±2.3 $n=2$	23.7 ±2.7 $n=5$	0.37	64 ±7 $n=5$	109 ±13 $n=5$	54.3	33.5	18.2 ±1.1 $n=3$	11.8	23.1	25.5	0.016 ±0.001	19.0
8	134	65.4 ±0.2 $n=2$	8.8 ±1.6 $n=6$	58.4 ±1.0 $n=2$	24.9 ±5.5 $n=5$	0.29	86 ±19 $n=5$	154 ±34 $n=5$	35.9	33.5	11.0 ±0.6 $n=3$	14.4	23.7	40.0	0.010 ±0.002	34.7
10	132	55.6 $n=1$	9.0 ±0.5 $n=6$	46.6 $n=1$	21.0 ±1.3 $n=5$	0.20	105 ±7 $n=5$	245 ±16 $n=5$	24.7	12.0	21.2 ±5.5 $n=2$	23.1	32.5	19.0	0.018 ±0.001	14.1
Overall mean	125 ±4	61.9 ±2.2	11.8 ±1.1	49.5 ±2.1	24.4 ±1.9	0.29 ±0.04	90 ±9	168 ±20	33.7 ±5.7	26.6 ±3.4	19.0 ±1.5	15.3 ±1.6	25.2 ±1.4	29.9 ±2.6	0.014 ±0.001	24.4 ±2.9
P value‡	>0.3	>0.3	<0.001	<0.001	<0.001	>0.3	<0.001	<0.001	<0.001	<0.001	<0.02	>0.1	>0.05	<0.001	<0.001	<0.001

* ±SEM.

‡ Compared to control group.

oncotic pressure modified the sngfr by providing a positive influence upon filtration in most rats (Table II). The hematocrit was unchanged by AGBM Ab, 44±1% vs. 45±2% ($P > 0.5$), suggesting that fluid transfer from interstitium to plasma was not the reason for the numerically lower C and π_A. Also protein electrophoreses of plasma samples in each animal after AGBM Ab revealed that 46.6±1.0% of total protein was albumin, values not different from the control condition. A diffuse modest increase in protein permeability of all capillaries should have resulted in a reduction in the percentage of total protein that was albumin, since the molecular

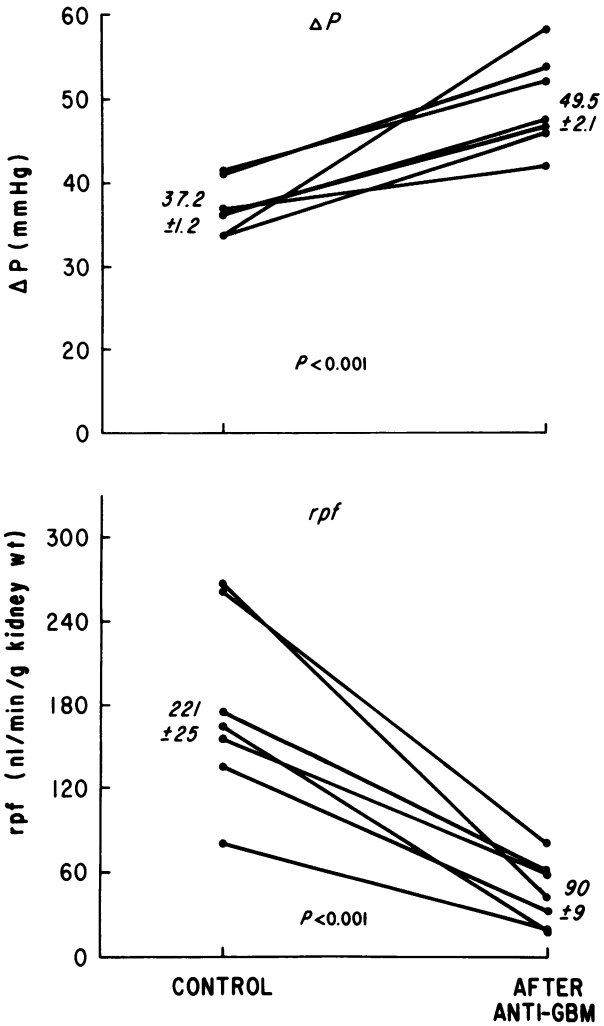

FIGURE 2 The ΔP and rpf in each rat in the control condition (left) and after AGBM Ab (right). ΔP (upper panel) increased in each rat, and the overall mean increased from 37.2±1.2 to 49.5±2.1 mm Hg. This finding was primarily the result of a large decrease in tubular pressure. On the lower panel, rpf also decreased in each rat from a mean of 221±25 to 90±9 nl/min per g kidney wt after anti-GBM. This decrease in rpf was the consequence of increases in both AR and ER.

weight of albumin in the smallest of the major protein subclasses.

During control plasma expansion, before AGBM Ab, the EFP at the afferent end of the glomerular capillary (EFP_A) was 18.8±1.7 mm Mg and rose to 34.2±2.5 mm Hg after AGBM Ab ($P < 0.001$) both as a result of the greater ΔP and decreased π_A (Fig. 4). The EFP fell to 8.8±2.2 mm Hg at the efferent end of the capillary (EFP_B) during the control condition, a value significantly greater than zero. After AGBM Ab the EFP_B remained significantly positive at 24.4±2.9 and was greater than the control value ($P < 0.001$). The mean effective filtration pressure ($\overline{EFP}$) rose from 13.8±2.1 to 29.9±2.6 mm Hg ($P < 0.001$) (Figs. 3 and 4). Since the sngfr decreased to 50% of control while $\overline{EFP}$ increased by more than 100%, the glomerular permeability coefficient (L_pA) must have decreased markedly and this reduction was the major factor resulting in decreased sngfr. The persistence of a markedly positive EFP_B and the increase in this value in spite of a large reduction in rpf to hydropenic levels can only result from a major decrease in L_pA.

The mean L_pA in the control plasma-expanded condition was 0.077±0.017 nl/s per g kidney wt mm Hg

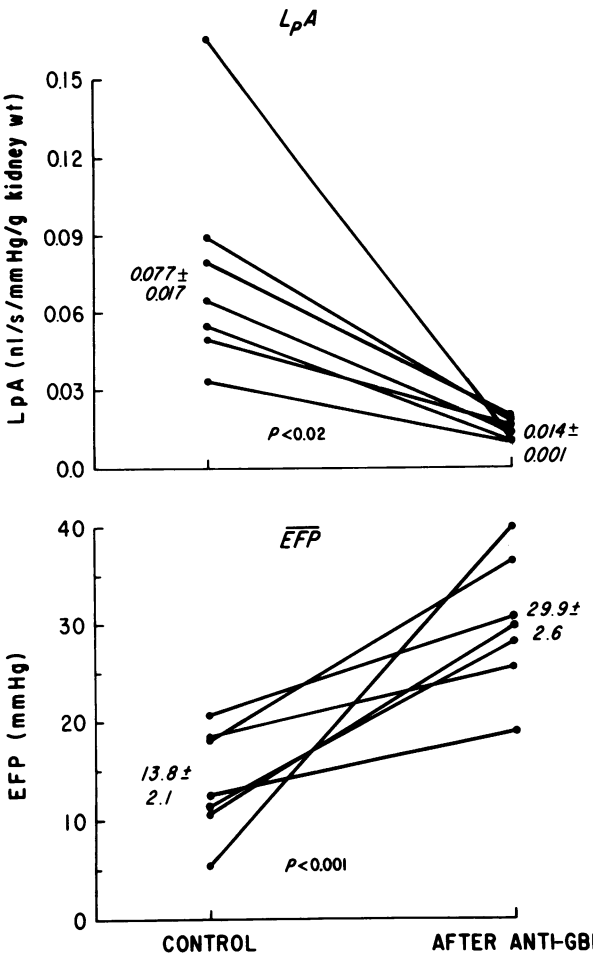

FIGURE 3 The L_pA and $\overline{EFP}$ in rats during control and after AGBM Ab ($sngfr = L_pA \cdot \overline{EFP}$). In the upper panel L_pA fell in each rat from a mean of 0.077±0.017 to 0.014 ±0.001 nl/s per mm Hg per g kidney wt. Although sngfr fell, the $\overline{EFP}$ rose in each animal and the mean value increased from 13.8±2.1 to 29.9±2.6 mm Hg. This increase was primarily the consequence of the increase in ΔP and the decrease in L_pA. Since $\overline{EFP}$ increased the decrease in sngfr was primarily the result of the large decrease in L_pA.

and fell markedly to 0.014±0.001 nl/s per g kidney wt mm Hg ($P < 0.001$) within 60 min of the infusion of AGBM Ab (Fig. 3).

The effect of control CFA-stimulated rabbit gamma globulin fractions upon glomerular filtration. The sngfr after CFA gamma globulin fraction was not different from control; 60.0±4.5 nl/min per g kidney wt before rabbit gamma globulin and 55.9±3.1 nl/min per g kidney wt after the infusion ($n = 4$, $P > 0.4$) (Table III). The rpf was 272±52 during the control condition and 232±13 after gamma globulin infusion ($P > 0.4$). P_G was 58.8±2.5 mm Hg and P_t 21.6±1.9 mm Hg in the control state and 59.2±4.7 and 21.1±1.4 mm Hg, respectively, after the gamma globulin infusion ($P > 0.9$ and $P > 0.8$). ΔP was unchanged at 37.3±3.2 mm Hg before and 38.0±3.8 mm Hg after the gamma globulin fraction ($P > 0.9$). Systemic oncotic pressure (π_A) was unaltered at 19.3±0.6 and 18.1±1.2 mm Hg, respectively ($P > 0.4$). Therefore all directly measured parameters were not significantly different after similar quantities of rabbit gamma globulin, demonstrating that the dramatic changes after AGBM Ab were the result of immune mediated injury. L_pA was also not significantly different at 0.094±0.026 and 0.076±0.022 nl/s per g kidney wt per mm Hg, respectively ($P > 0.5$).

The effect of other immunologic events upon the filtration process. To further demonstrate that the results were specific for the AGBM Ab and not just a reaction to any immunologic event, we have measured sngfr before and after an intramuscular Arthus reaction in three rats. The sngfr was unchanged with this immunologic event at 48.9±2.0 nl/min per g kidney wt in the control and 49.2±4.0 nl/min per g kidney wt during the Arthus reaction in muscle.

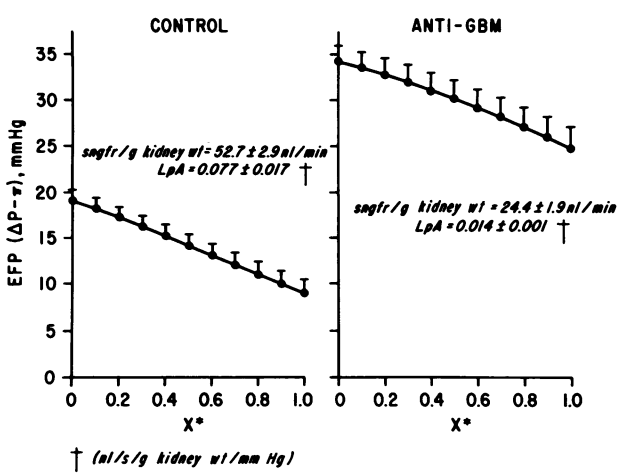

FIGURE 4 The effective filtration pressure (EFP = ΔP − π) along a normalized glomerular capillary length (x^*) during control plasma expansion (left) and AGBM Ab (right). Both afferent EFP and efferent EFP increased after antibody infusion such that $\overline{EFP}$ more than doubled. The EFP remained significantly disequilibrated at the efferent end of the capillary ($\Delta P >> \pi_E$) in spite of the large reduction in rpf. This finding and the fall in sngfr were the consequence of the large decrease in L_pA.

TABLE III
The Effects of CFA Gamma Globulin Fraction on Glomerular Microcirculation

	Control* (Plasma expanded)	CFA*	*P* value
sngfr, *nl/min per g kidney wt*	60.0±4.5‡	55.9±3.1	>0.40
ΔP, *mm Hg*	37.3±3.2	38.0±3.8	>0.90
π_A, *mm Hg*	19.3±0.6	18.1±1.2	>0.40
rpf, *nl/min per g kidney wt*	272±52	232±13	>0.40
L_pA, *nl/s per mm Hg per g kidney wt*	0.094±0.026	0.076±0.022	>0.50

* n = four paired.
‡ ±SEM.

Albumin disappearance studies. Studies in four rats revealed that 72±6, 65±4, and 64±6% of initial counts of ^{125}I-albumin remained in the plasma at 45, 60, and 75 min, respectively, after the infusion of CFA-stimulated rabbit globulin (control). After AGBM Ab, 80±1, 74±1, and 70±2% of initial counts remained at 45, 60, and 75 min. These studies suggested that administration of AGBM Ab did not increase the rate at which albumin leaves the plasma volume.

Comparison of inulin and mannitol clearances before and after AGBM Ab. The ratio of clearance of inulin to that of mannitol in the control plasma-expanded state was 0.94±0.02 in simultaneous clearance periods. In paired studies (12 control and 12 experimental periods in three rats) the ratio of inulin to mannitol clearances was not changed at 0.94±0.01 ($P > 0.8$) by AGBM Ab. Since inulin is at least 25 times the size of mannitol and the relative clearances were not altered, significant sieving of inulin was highly unlikely. In view of the constancy of the inulin to mannitol ratio, significant sieving of inulin at the glomerular capillary membrane would have required a highly improbable and specific increase in mannitol reabsorption by the tubules after AGBM Ab. Histologic and immunofluorescent studies revealed no changes in the tubules, such that damage sufficient to produce reabsorption of mannitol across the epithelium was not observed. Inulin, therefore, remained a valid indicator of glomerular filtration after glomerular injury due to AGBM Ab.

Morphologic changes after AGBM Ab infusion. Light and electron microscopic studies were done to judge if a loss in A could explain the large decrease in L_pA detected after AGBM Ab infusion or whether

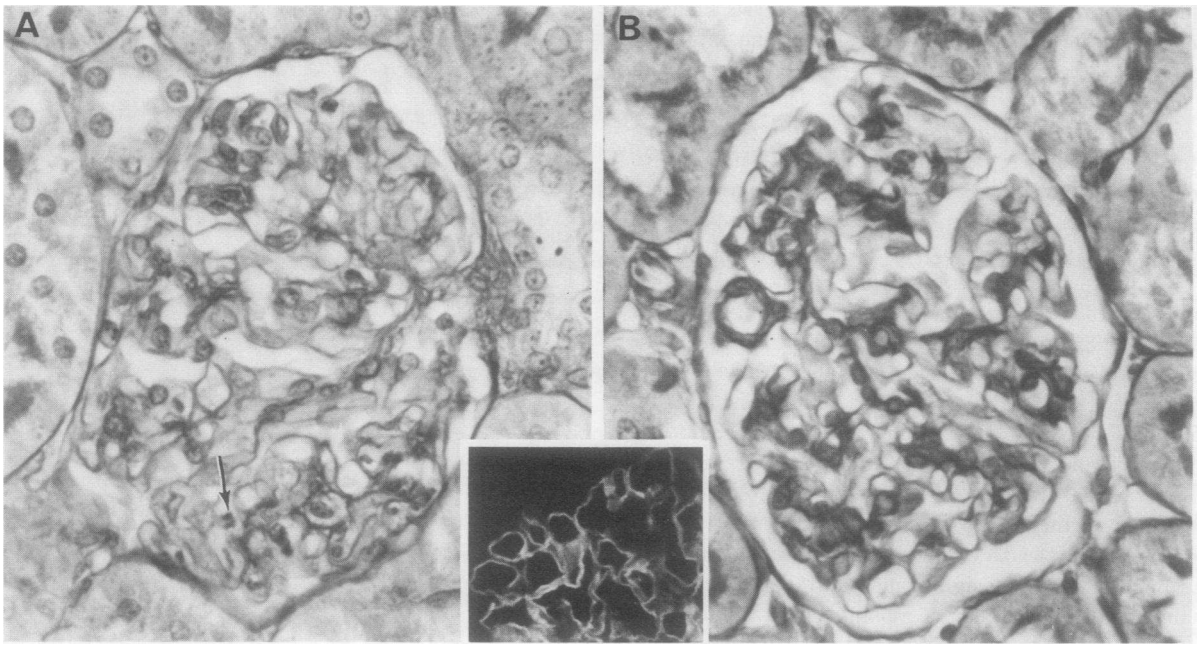

FIGURE 5 (A) The glomerulus from a rat 60 min after infusion of AGBM Ab is shown. Glomerular architecture is well preserved with an increase in neutrophils (arrow) and slight irregularity of the glomerular capillary wall. (B) The normal architecture of a control glomerulus from a rat 60 min after infusion of CFA-stimulated gamma globulin is shown for comparison. In the insert the typical linear deposits of rabbit IgG denoting the presence of AGBM Ab from a rat similar to that shown in panel A are seen. Original magnification A and B (× 400) and insert (× 250).

if in the absence of surface area loss the changes were attributable to a reduced glomerular hydraulic permeability (L_p). Histologic sections revealed no proliferative changes after AGBM Ab (Fig. 5). Glomerular capillary lumens were patent. A modest increase in neutrophils was found in the glomerular capillary lumens of the rats infused with AGBM Ab as early as 15 min after cessation of the infusion. The neutrophils present were observed more frequently in the outer cortical glomeruli, which may relate to the apparently greater reduction in sngfr than GFR after AGBM Ab. Even at 1 h, less than 10% of the loops had visible neutrophils. The rats infused with the CFA-stimulated control gamma globulin fraction remained normal.

Glomeruli taken from 15 to 75 min after AGBM Ab infusion revealed abnormalities when studied by electron microscopy. The endothelium appeared swollen and was variably separated from the GBM of some glomerular capillary loops (Fig. 6). Occasionally, loops had widely separated endothelium involving most of the circumference (Fig. 7); however, even in rats with the most severe changes less than 50% of the endothelium was clearly separated from the underlying GBM. In addition to separation of the endothelium, the endothelial aspect of the GBM had a frayed irregular appearance assumed to represent the attachment of AGBM Ab (Figs. 6 and 7).

After the infusion of AGBM Ab, neutrophils were observed in the glomerular capillary lumens (Figs. 7 and 8). At 15 min projections of neutrophil cytoplasm could be seen in the areas of the endothelial fenestrae, apparently attracted there by the AGBM Ab and complement present on the GBM. With time the neutrophils displaced the endothelium and approximated themselves along the GBM with morphologic evidence of degranulation (Fig. 8). The epithelial aspect of the GBM remained relatively free of change, aside from slight and variable fusion of epithelial foot processes (Figs. 6–8). No changes were observed in the control rats infused with CFA-stimulated rabbit gamma globulin.

Significantly the decrease in L_p and architectural changes with AGBM Ab were not associated with significant increased sieving of inulin.

DISCUSSION

Significant alterations in renal function occur within 1 h after the infusion of large quantities of AGBM Ab. In spite of the volume expansion produced by prior infusion of 2.5% body wt isoncotic plasma, the administration of AGBM Ab led to a reduction in both urine

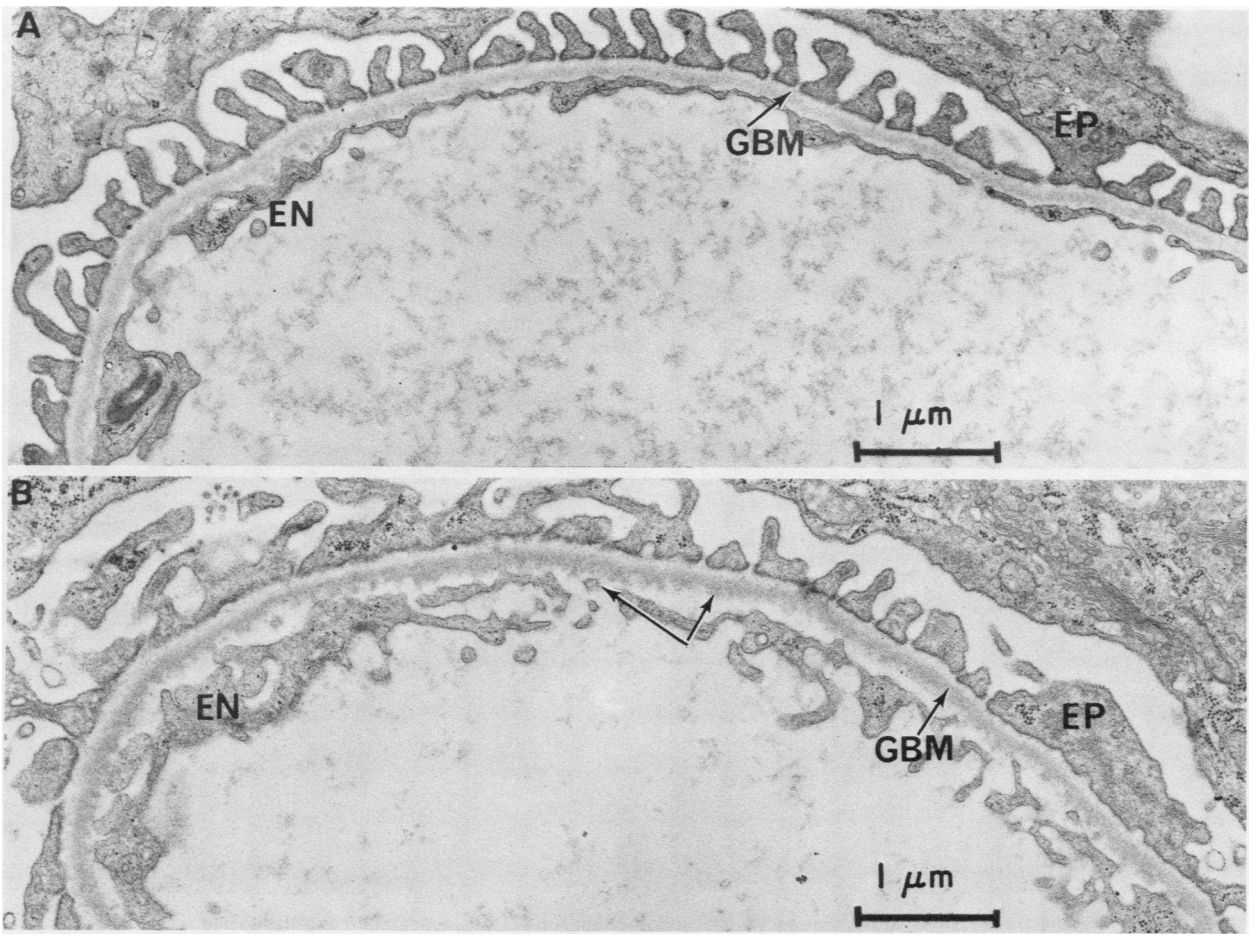

FIGURE 6 (A) The structure of the glomerular capillary wall from a rat that had received control CFA-stimulated rabbit gamma globulin is normal when examined at 60 min. (B) Several changes are apparent in the glomerular capillary wall of a rat 60 min after AGBM Ab. The epithelial cells (EP) foot process show focal areas of fusion. The endothelial cell (EN) cytoplasm is swollen and separated in areas from the underlying GBM. The subendothelial aspect of the GBM has an irregular appearance consistent with the fixation of AGBM Ab (joined arrows). Original magnification A (× 17,370) and B (× 17,250).

flow and sodium excretion to less than 5% of control, values near or below that of the hydropenic rat. This acute sodium and water retention was not the nonspecific result of the administration of rabbit gamma globulin, since rats which received gamma globulin maintained both urine flow and $U_{Na}V$ similar to the control condition. The major reason for reduced excretion with immunologic injury was the acute reduction in GFR and the filtered load of sodium and water. No change in either sngfr or factors controlling filtration was observed in control animals receiving nonantibody gamma globulin fractions. Because of the large reduction in sngfr, absolute tubular reabsorption of both sodium and water was actually lower than in the period before AGBM Ab administration.

Changes in any of four factors (*a*) rpf, (*b*) ΔP, (*c*) π_A, and (*d*) L_pA can potentially reduce the sngfr. In the present observations, all four factors changed, but only two factors contributed to a fall in sngfr, the others acting to maintain or increase filtration rate. Let us examine the pathogenetic mechanisms which have acted acutely to alter these factors and the final interaction of factors leading to the acute reduction in sngfr.

A consistent finding was the large reduction in rpf, secondary to increases in both AR and ER. As Brenner et al. have observed, the sngfr is normally highly plasma flow-dependent in the Munich-Wistar rat (21). The major reduction in rpf should have been an adequate single explanation for the fall in sngfr. However, the present examination was conducted in the plasma-expanded rat where filtration pressure equilibrium did not occur (13, 14), and under these experimental conditions sngfr will not change directly in proportion to rpf. If rpf reduction had been the sole or major mediator of

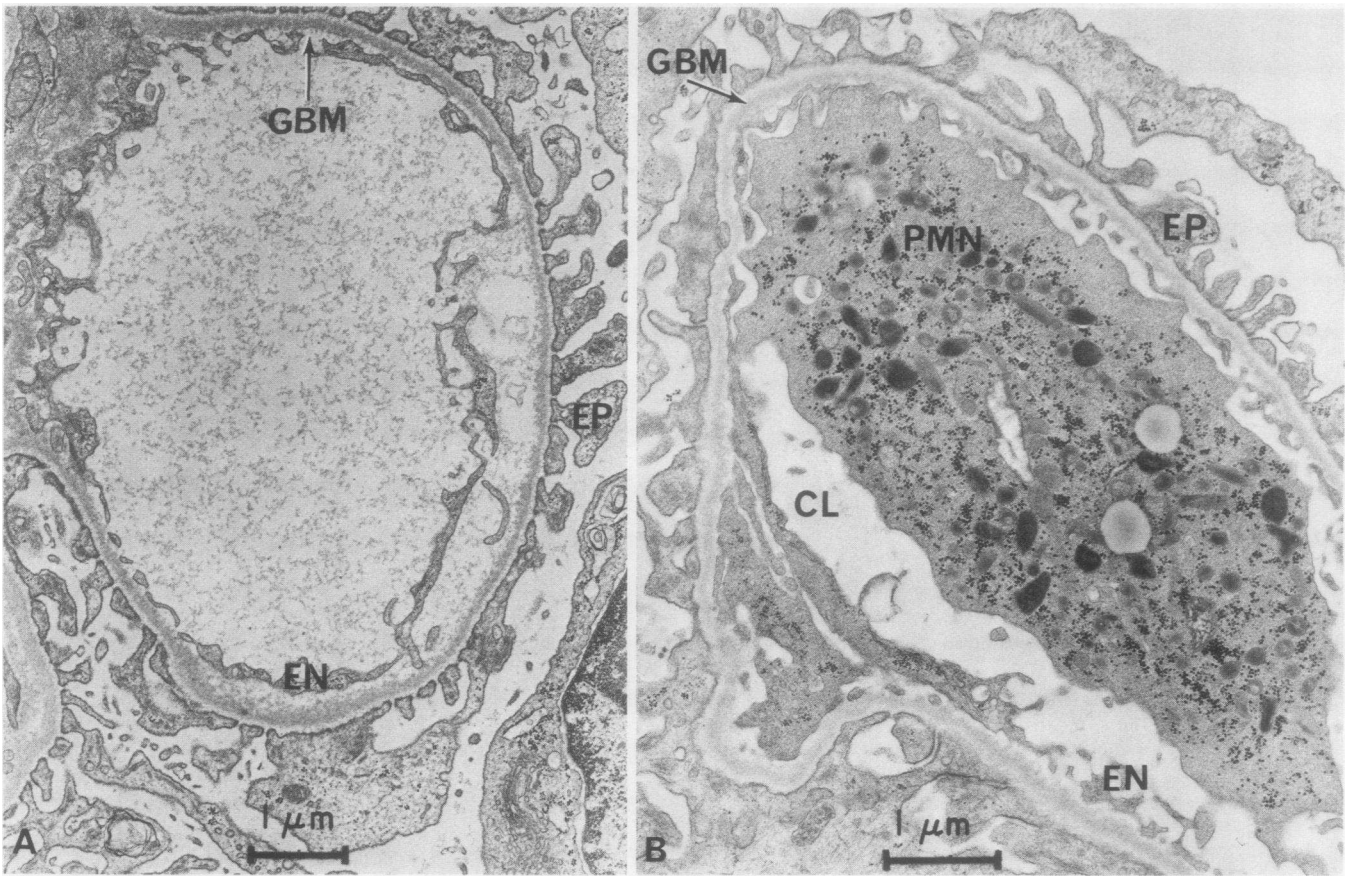

FIGURE 7 (A) A large portion of the endothelium (EN) is separated from the GBM in a glomerular capillary from a rat 60 min after AGBM Ab was given. The subendothelial aspect of the GBM is irregular with the apparent accumulation of electron dense material. The epithelial cell (EP) foot processes are generally well preserved with only questionable focal fusion. (B) The cytoplasm of a neutrophil (PMN) is seen within the lumen (CL) of a glomerular capillary 15 min after infusion of AGBM Ab. Projections of the cytoplasm are already entering the fenestrae of the endothelial cell (EN), apparently attracted by the combination of AGBM Ab and complement which is present on the GBM. The epithelial cell foot processes show some evidence of focal fusion. Magnification A (× 11,600) and B (× 13,880).

the decrease in sngfr, filtration pressure equilibrium should have occurred as a result of the decrease in rpf after AGBM Ab. The experimental values for rpf was approximately equal to that of hydropenic, nonexpanded rats (11, 12, 17). Since the effective filtration pressure remained significantly disequilibrated after AGBM Ab, other factors influencing sngfr must have also changed.

An important modifying factor on the resultant sngfr was the change in ΔP. A very large increase in ΔP was observed after AGBM Ab to values of 50 mm Hg, the highest direct measurement that has been observed in any condition in this species (11–14, 17, 21–23). Bowman's space pressures fell to values typical of the hydropenic state (11–13, 17). The fall in sngfr was probably the major reason for the reduction in P_t. The smaller numerical rise in P_G was the result of greater increases in ER. The finding of increasing ΔP while P_t decreases is unusual in the Munich-Wistar rat; the pressures usually move in the same direction with experimental maneuvers (11–13, 17). The only previous condition in which we have observed this finding was the transition from control plasma expansion to that after the infusion of angiotensin II (14). The changes in ΔP with this vasoconstrictor were usually of lesser magnitude than observed with AGBM Ab in this study.

The increase in ΔP was of sufficient magnitude that the effects of reduced rpf upon sngfr was nearly nullified. Had only changes in rpf and ΔP resulted from AGBM Ab, then sngfr would have decreased less than 20% from the control value. The reduction in rpf resulted from increases in resistance within both the pre- and postcapillary arterioles. The exact mechanism for this vasoconstriction is not known. Examination of light

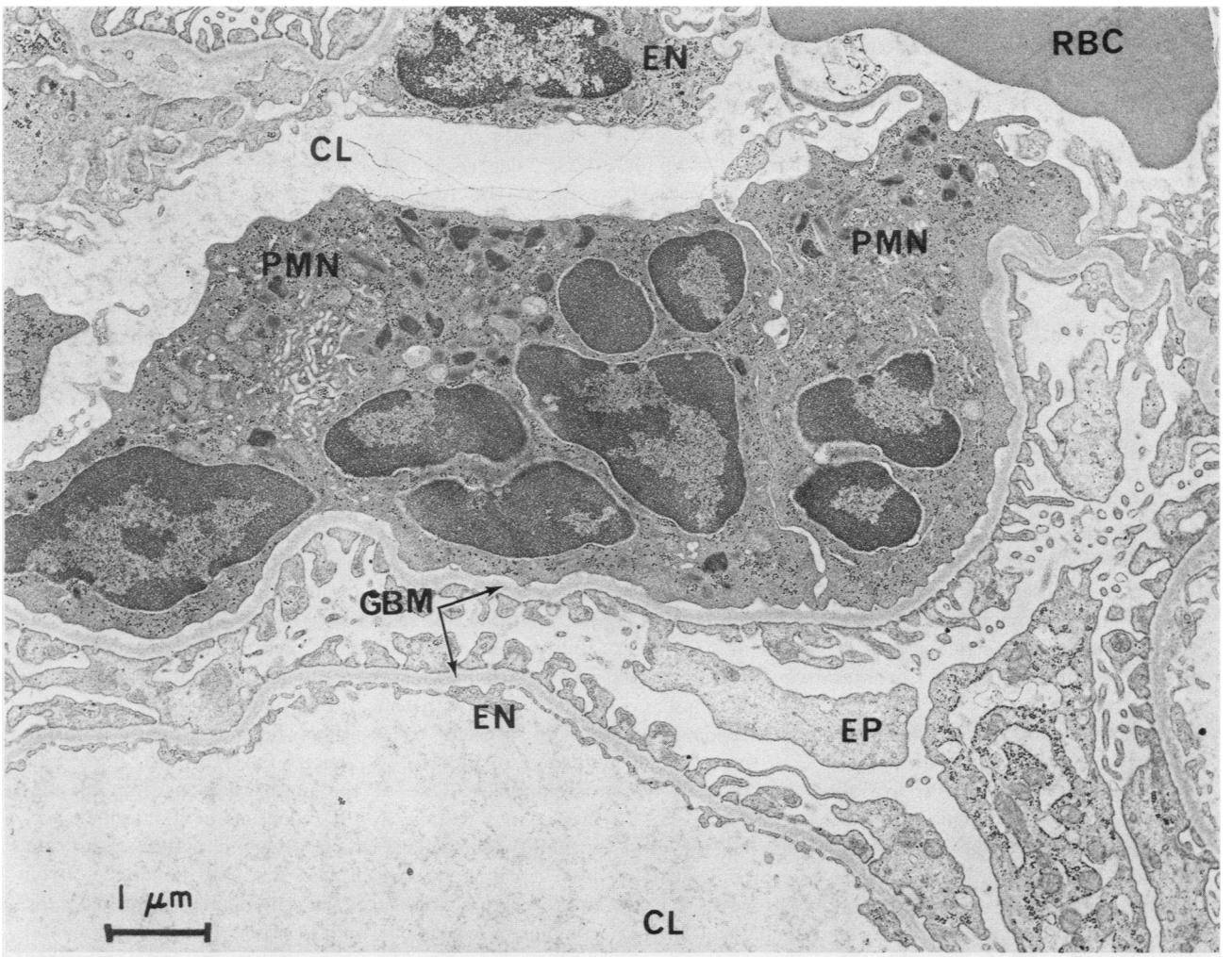

FIGURE 8 60-min after infusion of AGBM Ab, at least two neutrophils (PMN) can be seen approximated along the subendothelial aspect of the GBM of a glomerular capillary. The endothelial cytoplasm (EN) is completely lacking between the PMN and the GBM. In an adjacent capillary loop in the lower portion of the picture, the endothelium is well preserved. The epithelial cell (EP) foot processes are reasonably well preserved throughout. An erythrocyte (RBC) is visible within the capillary lumen (CL) in the upper right. (Magnification × 12,500).

microscopic sections did not reveal pathologic changes in the arterioles, which imply a functional cause, such as local release of vasoconstrictor substances. Preliminary observations with other nephritogenic AGBM Ab, performed since completion of this study, suggest that the magnitude of change in vascular resistance and rpf vary both among AGBM Ab and with the quantity of AGBM Ab, whereas a reduction in L_pA remains a characteristic early finding with all nephritogenic AGBM Ab studied.

Another modifying effect upon the sngfr after immune injury was a reduction in systemic C and π in five of seven rats. We have shown in previous studies that after mannitol infusion large reductions in π_A are a major positive influence upon sngfr. Had the systemic C not fallen after AGBM Ab it is reasonable to assume that sngfr would have decreased to much lower values in most animals. If rpf, ΔP, and π_A had changed as observed but L_pA remained constant after AGBM Ab the sngfr would have been approximately equal to the control value.

The reduction in C was associated with a constancy of the hematocrit, which is evidence against entry of fluid into the plasma and dilution of protein. Although C decreased in a majority of rats, the distribution of albumin to globulin was unchanged from the control state. Disappearance studies demonstrated that albumin exitted the vascular volume at the same rate in rats infused with AGBM Ab and control animals.

The critical factor leading to the decrease in sngfr after AGBM Ab was the large reduction in L_pA. The L_pA, or surface area permeability product, was reduced to approximately 20% of the normal value for this strain of rat (13, 14, 17, 27). The absolute value for L_pA after AGBM Ab was lower than we have observed in any other condition in which pathophysiologic reductions in L_pA have been observed (12, 13). As previously noted, there were not sufficient inflammatory changes during the 1 h after AGBM Ab to account for the decrease in L_pA solely as a result of loss of capillary conduits and A. By exclusion, the reduced L_pA must have resulted from decreases in glomerular hydraulic permeability (L_p).

The recent studies by Maddox et al. have also demonstrated a reduction in the glomerular permeability coefficient (L_pA or K_f) in the autologous phase of AGBM Ab-induced nephritis, 2–3 wk after the injection of 40 μg of nephrotoxic Ab (28, 29). In spite of the reduction in L_pA, the filtration rate was unchanged from control due to compensating increases in rpf and ΔP. These investigators concluded that the fall in L_pA was primarily the result of proliferative inflammatory changes leading to reductions in filtering capillary surface area. Recent observations (unpublished) from this laboratory suggest that with lower doses of other AGBM Ab similar findings are noted within 1 h; decreased L_pA and no change in rpf.

In the present study, changes were noted in the glomerular capillary wall by electron microscopy which probably influenced the ultrafiltration process. There was a definite separation of the endothelial cell cytoplasm from the GBM. Irregular material was also noted projecting from the endothelial surface of the GBM. The epithelial cell foot processes and the diameter of channels between podocytes appeared largely unchanged and similar to CFA control rats. The rate-limiting barrier for water movement across the glomerulus has not been defined with certainty. Antibody fixation to the GBM may occupy large but not readily quantifiable percentages of both the effective surface area within fenestrae and the GBM under the endothelium. Part of the explanation for the reduced L_p may be the separation of cells from the GBM which could provide another barrier to filtration through interposition of an "unstirred" compartment. This compartment would contain plasma which would neither be exposed to the normal rate of shear forces of blood flow nor the mixing action of erythrocytes, permitting a greater degree of concentration polarization of proteins and a diminished rate of ultrafiltration at the GBM. The deposition of antibody and complement upon the GBM may have also contributed to the reduction in L_p by imposing a different type of barrier to fluid movement.

The effects observed after the infusion of AGBM were qualitatively similar to those observed acutely after angiotensin II in the plasma-expanded rats (14). The rpf fell, ΔP rose, and L_pA was reduced but to a lesser degree in all respects than has been observed with AGBM Ab. Although the morphologic changes and antibody deposition may fully explain the changes in L_pA, it remains possible that the acute changes in AR and ER may be due, in part, to secondary, local angiotensin II release within the glomerulus.

Recent studies by Allison et al. have characterized nephron function in an established, chronic augmented form of glomerulonephritis after AGBM Ab (18). The nephron effects observed acutely in our study were much more homogeneous than those which occurred at the later time of observation in the study of Allison et al. The present examination has provided evidence that alterations in glomerular function begins within minutes of the infusion of AGBM Ab. The findings also suggest that the continued reductions in sngfr are in part due to reductions in the glomerular permeability coefficient. Further fixed inflammatory changes and continuing loss of capillary conduits may further reduce L_pA with time through decreases in A.

The present observations demonstrate that within minutes of the acute infusion of AGBM Ab, sngfr falls precipitously. One of the major reasons for this decrease in filtration was a reduction in glomerular permeability coefficient which occurred before significant loss of capillary surface area but with definite electron microscope changes of the endothelial aspect of the complex glomerular capillary wall. These experimental findings may provide a quantitative method for further examination of the mechanisms leading to reduced glomerular permeability and sngfr after antibody fixation.

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REFERENCES

1. Bradley, S. E., G. P. Bradley, C. J. Tyson, J. J. Curry, and W. D. Blake. 1950. Renal function in renal diseases. *Am. J. Med.* **9**: 766–798.
2. Cardozo, E. L. 1946. Hydremia in acute nephritis. *Acta Med. Scand.* **125**: 333–338.
3. Earle, D. P., S. J. Farber, J. D. Alexander, and E. D. Pellegrino. 1951. Renal function and electrolyte metabolism in acute glomerulonephritis. *J. Clin. Invest.* **30**: 421–433.
4. Farber, S. J. 1957. Physiologic aspects of glomerulonephritis. *J. Chronic Dis.* **5**: 87–107.

5. Eisenberg, S. 1959. Blood volume in patients with acute glomerulonephritis as determined by radioactive chromium tagged red cells. *Am. J. Med.* **27**: 241–245.
6. Rocha, A., M. Marcondes, and G. Malnic. 1973. Micropuncture study in rats with experimental glomerulonephritis. *Kidney Int.* **3**: 14–23.
7. Wagnild, J. P., F. D. Gutmann, and R. E. Rieselbach. 1974. Functional characterization of chronic unilateral glomerulonephritis in the dog. *Kidney Int.* **5**: 422–428.
8. Krakower, C. A., and S. A. Greenspon. 1951. Localization of the nephrotoxic antigen within the isolated renal glomerulus. *Arch. Pathol.* **51**: 629–639.
9. Unanue, E. R., and F. J. Dixon. 1965. Experimental glomerulonephritis. V. Studies on the interaction of nephrotoxic antibodies with tissues of the rat. *J. Exp. Med.* **121**: 697–714.
10. Pressman, D., E. D. Day, and M. Blau. 1957. The use of paired labeling in the determination of tumor-localizing antibodies. *Cancer Res.* **17**: 845–850.
11. Blantz, R. C. 1974. Effect of mannitol on glomerular ultrafiltration in the hydropenic rat. *J. Clin. Invest.* **54**: 1135–1143.
12. Blantz, R. C. 1975. The mechanism of acute renal failure after uranyl nitrate. *J. Clin. Invest.* **55**: 621–635.
13. Blantz, R. C., K. S. Konnen, and B. J. Tucker. 1975. Glomerular filtration response to elevated ureteral pressure in both the hydropenic and plasma-expanded rat. *Circ. Res.* **37**: 819–829.
14. Blantz, R. C., K. S. Konnen, and B. J. Tucker. 1976. Angiotensin II effects upon the glomerular microcirculation and ultrafiltration coefficient of the rat. *J. Clin. Invest.* **57**: 419–434.
15. Blantz, R. C., A. H. Israelit, F. C. Rector, Jr., and D. W. Seldin. 1972. Relation of distal tubular NaCl delivery and glomerular hydrostatic pressure. *Kidney Int.* **2**: 22–32.
16. Lowry, O. H., N. J. Rosebrough, A. I. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265–275.
17. Blantz, R. C., F. C. Rector, Jr., and D. W. Seldin. 1974. Effect of hyperoncotic albumin expansion upon glomerular ultrafiltration in the rat. *Kidney Int.* **6**: 209–221.
18. Allison, M. E. M., C. B. Wilson, and C. W. Gottschalk. 1974. Pathophysiology of experimental glomerulonephritis in rats. *J. Clin. Invest.* **53**: 1402–1423.
19. Wilson, C. B. 1976. Immunohistopathology of the kidney. *In* Manual of Clinical Immunology. N. R. Rose and H. Friedman, editors. American Society of Microbiology, Washington, D. C. 692–700.
20. Landis, E. M., and Pappenheimer, J. R. 1963. Exchange of substances through the capillary walls. *Handb. Physiol.* Section 2. Circulation. **2**: 961–1034.
21. Brenner, B. M., J. L. Troy, T. M. Daugharty, W. M. Deen, and C. R. Robertson. 1972. Dynamics of glomerular ultrafiltration in the rat. II. Plasma-flow dependence of GFR. *Am. J. Physiol.* **223**: 1184–1190.
22. Deen, W. M., C. R. Robertson, and B. M. Brenner. 1972. A model of glomerular ultrafiltration in the rat. *Am. J. Physiol.* **223**: 1178–1183.
23. Robertson, C. R., W. M. Deen, J. L. Troy, and B. M. Brenner. 1972. Dynamics of glomerular ultrafiltration in the rat. III. Hemodynamics and autoregulation. *Am. J. Physiol.* **223**: 1191–1200.
24. Brenner, B. M., J. L. Troy, and T. M. Daugharty. 1971. The dynamics of glomerular ultrafiltration in the rat. *J. Clin. Invest.* **50**: 1776–1780.
25. Snedecor, G. W. 1956. Statistical Methods. Iowa State College Press, Ames, Iowa. 5th edition. 534 pp.
26. Batson, H. C. 1956. An Introduction to Statistics in the Medical Sciences. Department of Public Health, University of Illinois College of Medicine, Chicago, Ill. Burgess Publishing Co., Minneapolis. 22–37.
27. Deen, W. M., J. L. Troy, C. R. Robertson, and B. M. Brenner. 1973. Dynamics of glomerular ultrafiltration in the rat. IV. Determination of the ultrafiltration coefficient. *J. Clin. Invest.* **52**: 1500–1508.
28. Maddox, D. A., C. M. Bennett, W. M. Deen, R. J. Glassock, D. Knutson, T. M. Daugharty, and B. M. Brenner. 1975. Determinants of glomerular filtration in experimental glomerulonephritis in the rat. *J. Clin. Invest.* **55**: 305–318.
29. Maddox, D. A., C. M. Bennett, W. M. Deen, R. J. Glassock, D. Knutson, and B. M. Brenner. 1975. Control of proximal tubule fluid reabsorption in experimental glomerulonephritis. *J. Clin. Invest.* **55**: 1315–1325.