

## The acute effects of antiglomerular basement membrane antibody upon glomerular filtration in the rat. The influence of dose and complement depletion.

R C Blantz, ... , B J Tucker, C B Wilson

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### Research Article

Recent studies from this laboratory have revealed that single nephron filtration rate (sngfr) decreases significantly within 1 h of the administration of large doses of complement-fixing antiglomerular basement membrane antibody (AGBM Ab) in plasma-expanded Munich-Wistar rats. This reduction in sngfr was due to decreases in nephron plasma flow (rf) and the glomerular permeability coefficient (LpA) utilizing direct evaluation of all pertinent pressures, flows, and permeabilities. With identical micropuncture techniques, we have determined (a) the respective influences of rpf and LpA upon sngfr by examining the effects of differing doses of AGBM Ab, and (b) the specific effect of complement fixation upon the reduction in sngfr. In normal rats, low dose (1.4 microgram/g body wt) AGBM Ab decreased sngfr from 57.9 +/- 3.4 to 50.8 +/- 3.9 nl/min per g kidney wt (kw) (P less than 0.001), and this was due to a 10% reduction in rpf and a decrease in LpA FROM 0.069 +/- 0.014 in control to 0.041 +/- 0.007 nl/s per g kw per mm Hg (P less than 0.02). At the high dose (2.3 microgram/g body wt), sngfr fell dramatically from 58.4 +/- 4.0 to 7.6 +/- 3.8 nl/min per g kw (P less than 0.001), and this effect upon filtration was the result of an 86% reduction in rpf and a decrease in LpA from 0.092 +/- [...]

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# The Acute Effects of Antiglomerular Basement Membrane Antibody upon Glomerular Filtration in the Rat

## THE INFLUENCE OF DOSE AND COMPLEMENT DEPLETION

ROLAND C. BLANTZ, *Department of Medicine, University of California, San Diego School of Medicine, and the Veterans Administration Hospital, San Diego, California 92161*

BRYAN J. TUCKER, *Department of Medicine, University of California, San Diego School of Medicine, San Diego, California 92093*

CURTIS B. WILSON, *Department of Immunopathology, Scripps Clinic and Research Foundation, La Jolla, California 92037*

**ABSTRACT** Recent studies from this laboratory have revealed that single nephron filtration rate (sngfr) decreases significantly within 1 h of the administration of large doses of complement-fixing antiglomerular basement membrane antibody (AGBM Ab) in plasma-expanded Munich-Wistar rats. This reduction in sngfr was due to decreases in nephron plasma flow (rpf) and the glomerular permeability coefficient ( $L_pA$ ) utilizing direct evaluation of all pertinent pressures, flows, and permeabilities. With identical micro-puncture techniques, we have determined (a) the respective influences of rpf and  $L_pA$  upon sngfr by examining the effects of differing doses of AGBM Ab, and (b) the specific effect of complement fixation upon the reduction in sngfr. In normal rats, low dose (1.4  $\mu\text{g/g}$  body wt) AGBM Ab decreased sngfr from  $57.9 \pm 3.4$  to  $50.8 \pm 3.9$  nl/min per g kidney wt (kw) ( $P < 0.001$ ), and this was due to a 10% reduction in rpf and a decrease in  $L_pA$  from  $0.069 \pm 0.014$  in control to  $0.041 \pm 0.007$  nl/s per g kw per mm Hg ( $P < 0.02$ ). At the high dose (2.3  $\mu\text{g/g}$  body wt), sngfr fell dramatically from  $58.4 \pm 4.0$  to  $7.6 \pm 3.8$  nl/min per g kw ( $P < 0.001$ ), and this effect upon filtration was

the result of an 86% reduction in rpf and a decrease in  $L_pA$  from  $0.092 \pm 0.020$  to  $0.007 \pm 0.004$  nl/s per g kw mm Hg ( $P < 0.001$ ). Therefore, at lower doses sngfr fell primarily as a result of a 40% reduction in  $L_pA$  and a 10% decrease in rpf; however, at the high dose massive reductions in both rpf and  $L_pA$  led to the large decrease in sngfr.

In complement-depleted rats, receiving identical doses, low-dose AGBM Ab no longer reduced the sngfr, but a reduction in  $L_pA$  persisted (other factors compensating to maintain sngfr). At the high dose, complement depletion ameliorated the reduction in sngfr ( $55.1 \pm 2.4$  to  $37.2 \pm 3.4$  nl/min per g kw mm Hg) by nearly eliminating the vasoconstriction but only partially diminished the reduction in  $L_pA$  ( $0.097 \pm 0.020$  to  $0.032 \pm 0.004$  nl/s per g kw mm Hg,  $P < 0.05$ ).

Complement depletion prevented the migration of polymorphonuclear leukocytes (present in larger numbers after the high dose of AGBM Ab) into the capillary and eliminated vasoconstriction. Complement depletion resulted in a lesser effect of high-dose AGBM Ab upon  $L_pA$  than in normal rats, and this is likely due to lesser polymorphonuclear leukocyte effects upon capillary surface area. The persistent reduction in  $L_pA$  observed in complement-depleted rats correlated with separation of the endothelial cell from the glomerular basement membrane after AGBM Ab. AGBM Ab diminishes glomerular ultrafiltration by decreasing  $L_pA$  and altering the endothelial surface of the glomerular membrane, and this effect is not totally dependent upon the fixation of complement.

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Dr. Blantz is a Clinical Investigator of the Veterans Administration. B. J. Tucker is a graduate student in Applied Mechanics and Engineering Sciences, Univ. of Calif., San Diego.

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## INTRODUCTION

Recently (1), we demonstrated that nephron filtration rate (sngfr)<sup>1</sup> in rats decreased within 60 min after the administration of a large dose of complement-fixing anti-glomerular basement membrane antibody (AGBM Ab). The various determinants of nephron filtration were measured utilizing micropuncture techniques that allow all pertinent pressures, flows, and permeabilities to be determined directly in the control condition and after antibody administration. The study revealed that sngfr decreased because of reductions in both the glomerular permeability coefficient ( $L_pA$ ) and nephron plasma flow (rpf). This experimental approach to the assessment of glomerular immune injury provides certain significant advantages. With micropuncture techniques not only can changes in sngfr that follow immune injury be evaluated accurately, but also factors that affect the filtration rate can be assessed individually both before and after the immune insult.

Two questions relevant to the specific mechanisms leading to reduced nephron filtration have been posed in this study. Firstly can we separate the respective influences of the factors that lead to reduction in sngfr: decreased rpf and reduced  $L_pA$ ? Secondly, what role does activation of the complement system play in reducing the filtration rate after glomerular immune injury? We have examined these two issues by studying the changes that occur in the factors influencing glomerular filtration when (a) high and low doses of AGBM Ab are administered and (b) when complement activity is depleted with cobra venom factor (2).

## METHODS

**Experiment animals.** The current studies were performed on Munich-Wistar rats (180–240 g body wt), bred and maintained in a colony housed at the Animal Research Facility 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. Rat GBM was prepared by a modification of the method of Krakower and Greenspon (3). The attainment of nephrotoxic levels of AGBM Ab in rabbits was recognized when intravenous injection of rabbit serum induced acute

proteinuria in rats. Rabbit serum was then 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 fractions obtained by this procedure and normal rabbit gamma globulin fractions were pair labeled with  $I^{131}$  and  $I^{125}$  radioactive iodine, and the amount of kidney-fixing antibody was quantitated using the paired label isotope technique (4, 5). The pool of AGBM Ab utilized in this study was obtained from a different group of rabbits than that used in the previous study (1).

**Methods utilized for de complementation.** Cobra venom factor (CVF; kindly prepared by Dr. Richard J. Ulevitch of Scripps Clinic and Research Foundation, La Jolla, Calif.) (6) was injected intraperitoneally into one group of rats. Each rat received 85 U/kg body wt of CVF at 48, 44, 40, 28, and 24 h before micropuncture for a total dose of 425 U/kg body wt (7). Blood was drawn from the tail vein approximately 16 h before micropuncture surgery, and the serum assayed for C3 levels by double immunodiffusion in gel. Rats pretreated with CVF had <5% of the C3 immunoreactant normally present in the Munich-Wistar rat. The effectiveness of complement depletion in all rats studied was also assessed by immunofluorescence of the glomeruli for C3.

In separate studies, CVF at the doses used had no quantitative effect upon the binding of AGBM Ab to renal tissue as shown by the quantitative paired label radioisotope technique (4, 5).

**Micropuncture studies evaluating glomerular ultrafiltration before and after AGBM Ab.** The micropuncture protocol utilized in this study was nearly identical to that described in our previous study on glomerular immune injury (1). Surgical preparation was as previously described, and all studies were paired with iso-oncotic plasma expansion (2.5% body wt administered over 60 min) which was chosen as the control condition (1). A separate infusion of [ $^{14}C$ ]inulin dissolved in isotonic NaCl-NaHCO<sub>3</sub> (0.5% body wt/h) was begun at the time of plasma expansion and was delivered at approximately 40  $\mu$ Ci/h.

After equilibration of radioactive inulin, control measurements of glomerular capillary and Bowman's space hydrostatic pressure (utilizing a servo-nulling device with 1- to 2- $\mu$ m tip pipettes; 1, 8) and of sngfr ( $n = 5$ ) were obtained and at least three samples of efferent peritubular capillary blood from "star" vessels were obtained (8).

After completion of the control measurements, either low or high dose (1.4 or 2.3  $\mu$ g/g kidney wt [kw]) AGBM Ab was administered intravenously over 5 min in a volume of 400  $\mu$ l isotonic NaCl-NaHCO<sub>3</sub>. Urine flow decreased acutely with infusion of high doses of AGBM Ab but was changed little by the lower dose. 15 min after initiation of AGBM Ab infusion, all pressure, filtration rate, and efferent protein concentration measurements were repeated and completed within 45 min. In a previous study on the mechanism of glomerular immune injury with AGBM Ab, we have determined that inulin remains a valid marker of glomerular ultrafiltration even after antibody is administered (1).

**Analytic methods.** Protein concentration in systemic and efferent peritubular blood samples was measured by a microadaptation of the Lowry protein method (8–10). Sngfr, glomerular filtration rate, renal plasma flow and renal blood flow, urine and plasma sodium and potassium concentrations were determined as described in the previous study (1).

**Morphological studies.** Tissue for histologic, immunofluorescent, and electron microscope studies was obtained from both kidneys at the termination of the study and processed as previously described (1, 11, 12). The range of

<sup>1</sup> *Abbreviations used in this paper:* AGBM, Ab, anti-glomerular basement membrane antibody; AR, afferent arteriolar resistance; C, protein concentration; CVF, cobra venom factor;  $\Delta P$ , hydrostatic pressure gradient across glomerular membrane; EFP, effective filtration pressure;  $\bar{EFP}$ , mean effective filtration pressure; ER, efferent arteriolar resistance; GBM, glomerular basement membrane; kw, kidney weight;  $L_pA$ , glomerular permeability coefficient;  $\pi_A$ , systemic oncotic pressure;  $\pi_E$ , efferent arteriolar oncotic pressure;  $P_G$ , glomerular capillary hydrostatic pressure; PMN, polymorphonuclear leukocytes;  $P_b$ , Bowman's space hydrostatic pressure; rpf, nephron plasma flow; sngfr, single nephron filtration rate;  $x^*$ , normalized unit glomerular capillary length.

**TABLE I**  
*The Effect of AGBM Ab upon Various Clearances Indices at Both Doses in Normal and Complement-Depleted Rats*

	$U_{Na}V^*$		$U_KV\dagger$		$UV\ddagger$	
	C	E	C	E	C	E
	<i>μeq/min</i>		<i>μeq/min</i>		<i>μl/min</i>	
Normal rats						
Low-dose group (n = 6) <sup>  </sup>	2.6 ±0.6	1.1 <sup>¶</sup> ±0.4	1.2 ±0.2	0.6 <sup>¶</sup> ±0.2	24 ±7	8.0 <sup>¶</sup> ±2.7
High-dose group (n = 6)**	5.1 ±1.7	0.10 <sup>¶</sup> ±0.03	1.4 ±0.3	0.20 <sup>¶</sup> ±0.07	40 ±6	1.0 <sup>¶</sup> ±0.2
Complement-depleted rats						
Low-dose group (n = 9) <sup>  </sup>	1.8 ±0.4	1.4 ±0.3	1.0 ±0.3	0.8 ±0.1	17.1 ±5.4	15.0 ±4.0
High-dose group (n = 6)**	2.3 ±0.5	0.5 <sup>¶</sup> ±0.2	1.7 ±0.3	0.3 <sup>¶</sup> ±0.1	21 ±3	2.6 <sup>¶</sup> ±1.0

\* Sodium excretion.

† Potassium excretion.

‡ Urine volume.

<sup>||</sup> 1.4 μg/g body wt of AGBM Ab.

<sup>¶</sup> Experiment (E) significantly different from control (C), *P* < 0.05 or less.

\*\* 2.3 μg/g body wt of AGBM Ab.

polymorphonuclear leukocytes (PMN) per glomerulus was recorded by light microscopy. Morphologic grading was based on a 0–4+ scale. On light microscope examination, the glomerular capillary lumens appeared irregular with vari-

able degrees of encroachment, which is presumably related to the endothelial abnormalities seen by an electron microscope as well as to PMN infiltration. This change was graded according to the approximate number of capillary

**TABLE II**  
*Effect of Dose and Complement Depletion upon Pressures, Flows, Permeabilities, and sngfr before and after the Administration of AGBM Ab*

	MAP*		$P_G$		$P_I$		$\Delta P$		sngfr		$\pi_A$	
	C	E	C	E	C	E	C	E	C	E	C	E
	<i>mm Hg</i>		<i>mm Hg</i>		<i>mm Hg</i>		<i>mm Hg</i>		<i>nl/min/g kw</i>		<i>mm Hg</i>	
Normal rats												
Low dose (1.4 μg/g body wt)	115 ±6	119 ±4 <sup>  </sup>	61 ±3	61 ±2	19 ±2	13 ±2	42 ±2	48 ±2	58 ±3	51 ±4	19 ±1	19 ±1
<i>P</i>	>0.1		>0.8		<0.01		<0.02		<0.001		>0.9	
High dose (2.3 μg/g body wt)	108 ±8	116 ±5	60 ±2	55 ±2	19 ±1	10 ±1	41 ±1	45 ±2	58 ±4	8 ±4	22 ±1	20 ±1
<i>P</i>	>0.1		>0.1		<0.001		<0.05		<0.001		>0.5	
Complement-depleted rats												
Low dose (1.4 μg/g body wt)	117 ±4	118 ±4	57 ±2	54 ±2	18 ±1	12 ±2	39 ±2	42 ±2	50 ±4	47 ±5	20 ±1	18 ±1
<i>P</i>	>0.5		>0.3		<0.01		>0.3		>0.3		<0.05	
High dose (2.3 μg/g body wt)	109 ±6	114 ±6	58 ±3	57 ±2	18 ±2	8 ±2	40 ±2	48 ±1	55 ±2	37 ±3	22 ±1	23 ±2
<i>P</i>	>0.1		>0.6		<0.01		<0.01		<0.005		>0.1	

\* Mean arterial pressure.

†  $\pi_E$ , efferent arteriolar oncotic pressure.

‡ Superficial nephron filtration fraction.

<sup>||</sup> SEM.

loops involved with 1 and 2+ changes denoting 25 and 50%, respectively. By electron microscopy, the degree of endothelial separation was graded 1+ when scattered areas or segments of separation were noted, 2+ when separation involved the entire circumference of capillary loops of some capillaries, and 3+ when roughly half of the endothelium was partially or completely separated. Foot process fusion was graded largely by the area involved with 1 and 2+ corresponding roughly to 25 and 50%, respectively. PMN approximated to the GBM were scored simply according to ease of identification, i.e., rare, occasional, or many.

**Calculations.** Superficial nephron filtration fraction, rpf, afferent arteriolar resistance (AR), and efferent arteriolar resistance (ER) and oncotic pressure ( $\pi$ ) from protein concentration (C) were calculated as described in our previous publications (1, 8, 9, 13-15).

The four factors that define the sngfr-hydrostatic pressure gradient acting across the glomerular membrane ( $\Delta P$ ), systemic oncotic pressure ( $\pi_A$ ), glomerular permeability coefficient ( $L_pA$ ), and rate of rpf (6, 7) interrelate in the following manner.

Because  $\Delta P = P_G - P_t$ , where  $P_G$  = directly measured glomerular capillary hydrostatic pressure and  $P_t$  = Bowman's space hydrostatic pressure, the effective filtration pressure (EFP) can be defined as follows:  $EFP = \Delta P - \pi$ , where  $\pi$  = oncotic pressure.

As a consequence of glomerular ultrafiltration,  $\pi$  rises along the length of the glomerular capillary ( $x^*$ ) as a result of the increase in protein concentration (C). The mean EFP ( $\overline{EFP}$ ) is defined as follows:

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

where  $x^*$  = normalized unit glomerular capillary length.

Changes in rpf modify the EFP profile along  $x^*$  by affecting the rate at which protein is concentrated and the rate of rise in  $\pi$  along  $x^*$ .

The sngfr can therefore be defined as follows:  $sngfr = L_pA \times \overline{EFP}$ , where  $L_pA$  is the glomerular permeability coefficient and which in turn is a product of the hydraulic conductivity ( $L_p$ ) of the glomerular membrane and (A) the total filtering surface area of the glomerular capillary.

**Statistical analysis.** The significance of data between control and experimental states among all four groups in control and experimental states was determined by two- and three-way analysis of variance and by Student's *t* test where appropriate (16, 17).

## RESULTS

Measurements were made both before and after AGBM Ab infusion in four separate groups—low- and high-dose normal rats and low- and high-dose complement-depleted rats.

Studies utilizing either low-dose (1.4  $\mu\text{g/g}$  body wt) or high-dose (2.3  $\mu\text{g/g}$  body wt) AGBM Ab were performed in alternating sequence with normal and complement-depleted rats. The changes in urine volume, sodium excretion, and potassium excretion after AGBM Ab infusion at both doses are summarized in Table I. All groups except the low dose of AGBM Ab in complement-depleted rats showed uniform reductions in excretion. Administration of AGBM Ab did not alter mean arterial blood pressure in any of the four respective experimental groups ( $P > 0.1$ ).

TABLE II (Continued)

$\pi_E \ddagger$		snff §		rpf		$L_pA$		$\overline{EFP}$		$EFP_E$	
C	E	C	E	C	E	C	E	C	E	C	E
mm Hg				nl/min/g kw		nl/s/g kw mm Hg		mm Hg		mm Hg	
32	31	0.28	0.27	220	200	0.069	0.041	16	23	10	17
$\pm 2$	$\pm 2$	$\pm 0.03$	$\pm 0.02$	$\pm 30$	$\pm 30$	$\pm 0.014$	$\pm 0.007$	$\pm 3$	$\pm 3$	$\pm 3$	$\pm 4$
>0.8		>0.9		<0.01		<0.02		<0.05		>0.05	
32	28	0.21	0.19	280	40	0.092	0.007	14	21	9	17
$\pm 2$	$\pm 2$	$\pm 0.02$	$\pm 0.02$	$\pm 20$	$\pm 10$	$\pm 0.020$	$\pm 0.004$	$\pm 3$	$\pm 1$	$\pm 3$	$\pm 2$
<0.02		>0.4		<0.001		<0.001		<0.02		<0.02	
34	29	0.27	0.28	190	180	0.106	0.047	12	18	6	12
$\pm 2$	$\pm 1$	$\pm 0.02$	$\pm 0.03$	$\pm 20$	$\pm 20$	$\pm 0.027$	$\pm 0.006$	$\pm 2$	$\pm 2$	$\pm 3$	$\pm 2$
>0.05		>0.8		>0.5		<0.05		<0.05		>0.1	
35	34	0.28	0.22	270	180	0.097	0.032	12	20	5	15
$\pm 2$	$\pm 2$	$\pm 0.02$	$\pm 0.03$	$\pm 20$	$\pm 30$	$\pm 0.020$	$\pm 0.004$	$\pm 2$	$\pm 3$	$\pm 3$	$\pm 3$
>0.4		>0.1		<0.01		<0.05		<0.01		<0.01	

*Effect of dose of AGBM Ab.* No changes were observed on the kidney surface after low dose AGBM Ab infusion. With the higher dose, the kidney became less firm, and some decrease in color intensity of the surface was noted, possibly reflecting the reduced nephron blood flow. As shown in Table II, the mean  $\text{sngr}$  fell somewhat ( $58 \pm 3$  vs.  $51 \pm 4$  nl/min per g kw) after a low dose of AGBM Ab. This reduction was significantly greater at the higher dose  $58 \pm 4$  as compared to  $8 \pm 4$  nl/min per g kw). The mean  $\text{rpf}$  decreased minimally but significantly after the lower dose of AGBM Ab and to a significantly greater extent after the higher dose. The mean superficial nephron filtration fraction remained unchanged at both low and high doses of AGBM Ab.

Bowman's space hydrostatic pressure ( $P_i$ ) fell from  $19 \pm 2$  to  $13 \pm 2$  mm Hg after low-dose AGBM Ab infusion ( $P < 0.01$ ; Table II). At the higher dose  $P_i$  fell from  $19 \pm 1$  to  $10 \pm 1$  mm Hg ( $P < 0.001$ ). The  $P_G$ , however, was not changed by AGBM Ab at either low dose ( $61 \pm 3$  vs.  $61 \pm 2$  mm Hg;  $P < 0.8$ ) or high dose ( $60 \pm 2$  vs.  $55 \pm 2$ ;  $P < 0.1$ ). The net hydrostatic pressure gradient ( $\Delta P$ ) increased with both low-dose AGBM Ab ( $42 \pm 2$  vs.  $48 \pm 2$  mm Hg;  $P < 0.02$ ) and high-dose infusion ( $41 \pm 1$  vs.  $45 \pm 2$  mm Hg;  $P < 0.05$ ), so that the net effect on  $\text{sngr}$  was positive rather than negative.

The afferent (AR) and efferent (ER) arteriolar resistances were unchanged ( $11 \pm 1$  vs.  $14 \pm 3 \times 10^9$  dyn·s/cm<sup>5</sup>;  $P > 0.3$ ) and  $10 \pm 1$  vs.  $12 \pm 2 \times 10^9$  dyn·s/cm<sup>5</sup>;  $P > 0.4$ ), respectively, after low-dose AGBM Ab infusion. However, at the higher dose AR increased from  $8 \pm 1$  to  $161 \pm 70 \times 10^9$  dyn·s/cm<sup>5</sup> ( $P < 0.001$ ) and ER from  $7.0 \pm 0.5$  to  $148 \pm 70 \times 10^9$  dyn·s/cm<sup>5</sup> ( $P < 0.001$ ).

Plasma C was identical at both the low-dose ( $5.8 \pm 0.2$  vs.  $5.8 \pm 0.1$  g/100 ml) and high-dose AGBM Ab ( $6.3 \pm 0.2$  vs.  $5.9 \pm 0.3$  g/100 ml;  $P > 0.05$ ). The mean systemic oncotic pressure ( $\pi_A$ ) was not altered by low and high doses of AGBM Ab (Table II). The hematocrits were also unchanged at either dose of AGBM Ab ( $P > 0.1$ ).

The EFP at the afferent end of the glomerular capillary ( $\text{EFP}_A$ ) increased after administration of low-dose AGBM Ab from  $23 \pm 2$  to  $29 \pm 2$  mm Hg ( $P < 0.01$ ) and after high-dose infusion  $19 \pm 2$  vs.  $25 \pm 1$  mm Hg ( $P < 0.05$ ). The EFP at the efferent end of the capillary ( $\text{EFP}_E$ ) increased from  $10 \pm 3$  to  $17 \pm 4$  mm Hg ( $P < 0.05$ ) after low-dose infusion and from  $9 \pm 3$  to  $17 \pm 2$  mm Hg ( $P < 0.02$ ) after the higher dose. The  $\overline{\text{EFP}}$  rose from  $16 \pm 3$  to  $23 \pm 3$  mm Hg ( $P < 0.05$ ) after low-dose AGBM Ab administration and from  $14 \pm 3$  to  $21 \pm 1$  mm Hg ( $P < 0.02$ ) after the higher dose. Finding that  $\text{sngr}$  decreased at both doses of AGBM Ab in spite of increased  $\overline{\text{EFP}}$  was also observed in our previous study on glomerular immune

injury. This suggests that reductions in  $L_pA$  were critical to the decrease in  $\text{sngr}$  observed at both doses of AGBM Ab (Table II).

The mean  $L_pA$  fell after low-dose AGBM Ab infusion and decreased to a significantly greater extent ( $P < 0.01$ ), as did  $\text{sngr}$ , with the higher dose of AGBM Ab (Table II;  $0.092 \pm 0.020$  vs.  $0.007 \pm 0.004$  nl/s per g kw mm Hg) ( $P < 0.001$ ). Thus, the influence of  $\text{rpf}$  can be separated from that of  $L_pA$  by varying the dose of AGBM Ab.

*Effect of prior complement depletion upon low- and high-dose AGBM Ab.* The appearance of the kidney surface was basically unaltered in complement-depleted rats by both low and high doses of AGBM Ab which suggests that high doses of AGBM Ab have a lesser influence after complement depletion.

After complement depletion, low-dose AGBM Ab no longer significantly reduced  $\text{sngr}$ :  $50 \pm 4$  vs.  $47 \pm 5$  nl/min per g kw;  $P > 0.3$ ;  $n = 9$  (Table II). At the higher dose, prior complement depletion provided an even more impressive protection effect:  $\text{sngr}$  decreased from  $55 \pm 2$  to  $37 \pm 3$  nl/min per g kw;  $P < 0.005$ ;  $n = 6$  (Table II), a much lesser reduction than observed in rats with an intact complement system ( $P < 0.005$ ).

The beneficial effects of complement depletion were similarly observed when  $\text{rpf}$  was evaluated. The  $\text{rpf}$  was  $189 \pm 17$  in control and  $183 \pm 21$  nl/min per g kw ( $P > 0.5$ ) after low-dose AGBM Ab (Table II), signifying that complement depletion totally prevented the vasoconstriction observed in rats with intact complement given low-dose AGBM Ab. Also, with high-dose AGBM Ab, a reduction in  $\text{rpf}$  persisted, but to a much lesser extent ( $P < 0.005$ ), at  $209 \pm 23$  and  $184 \pm 30$  nl/min per g kw ( $P < 0.01$ ), respectively.

However,  $P_i$  also decreased in the low-dose, complement-depleted group ( $P < 0.01$ ; Table II) and in the high-dose complement-depleted group.  $P_G$  was unchanged by low-dose AGBM Ab infusion in the complement-depleted group as well. Similarly, high-dose AGBM Ab did not alter  $P_G$ . As a result,  $\Delta P$  was now unchanged by the lower dose but increased after the high-dose Ab infusion.

AR was unchanged in the low-dose group ( $17 \pm 2$  vs.  $23 \pm 4 \times 10^9$  dyn·s/cm<sup>5</sup>;  $P > 0.3$ ) and at the higher dose ( $12 \pm 2$  vs.  $16 \pm 2 \times 10^9$  dyn·s/cm<sup>5</sup>;  $P > 0.1$ ). ER was also unchanged at the lower dose ( $15 \pm 3$  vs.  $18 \pm 5 \times 10^9$  dyn·s/cm<sup>5</sup>) and higher dose ( $11 \pm 2$  vs.  $14 \pm 2 \times 10^9$  dyn·s/cm<sup>5</sup>;  $P > 0.1$ ).

Plasma C ( $C_A$ ) was reduced slightly at the lower dose ( $6.0 \pm 0.2$  vs.  $5.5 \pm 0.3$  g/100 ml;  $P < 0.05$ ) but was unchanged at the higher dose ( $6.2 \pm 0.3$  vs.  $6.5 \pm 0.3$  g/100 ml;  $P > 0.2$ ). Similarly, the  $\pi_A$  was reduced slightly in the low-dose group (Table II) but was unchanged at the higher dose.

The initial driving force for ultrafiltration was increased slightly at the lower dose ( $19 \pm 2$  vs.  $23 \pm 1$  mm

Hg;  $P < 0.05$ ) and to a somewhat greater extent at the higher dose ( $19 \pm 2$  vs.  $25 \pm 3$  mm Hg;  $P < 0.01$ ). The EFP at the efferent end of the capillary was  $6 \pm 3$  mm Hg in control and  $12 \pm 2$  mm Hg ( $P > 0.1$ ) after low-dose infusion; it increased from  $5 \pm 3$  to  $15 \pm 3$  mm Hg ( $P < 0.01$ ) after the higher dose of antibody. Although sngfr remained constant, the  $\overline{\text{EFP}}$  increased at the lower dose in the complement-depleted group ( $12 \pm 2$  vs.  $18 \pm 2$  mm Hg;  $P < 0.05$ ). At the higher dose, the EFP, again increased from  $12 \pm 2$  to  $20 \pm 3$  mm Hg ( $P < 0.01$ ).

Although sngfr remained constant, the reduction in  $L_pA$  persisted with lower dose AGBM Ab infusion ( $0.106 \pm 0.027$  vs.  $0.047 \pm 0.006$  nl/s per g kw mm Hg;  $P < 0.05$ ) (Table II). The persistent decrease in  $L_pA$  with no change in sngfr suggests that the beneficial effects that complement depletion exerted upon the low-dose group was mediated by preventing the vasoconstriction observed with AGBM Ab in the group with an intact complement system. The increase in  $\overline{\text{EFP}}$  which permitted constant sngfr resulted from reduced  $\pi_A$ , decreased  $L_pA$ , and a numerical increase in  $\Delta P$ . At the higher dose, complement depletion significantly diminished the extent to which  $L_pA$  was reduced ( $0.097 \pm 0.020$  vs.  $0.032 \pm 0.004$  nl/s per g kw · mm Hg;  $P < 0.05$ ). In fact, the  $L_pA$  after high-dose AGBM Ab infusion in the complement-depleted group was not different from the experimental  $L_pA$  in both low-dose groups ( $P > 0.05$ ) (Table II).

In summary, complement depletion prevented the reduction in sngfr in the low-dose AGBM Ab group primarily by preventing a decrease in rpf, but did not affect the antibody-mediated reduction in  $L_pA$ . At

the higher dose, the decrease in sngfr persisted in spite of complement depletion, but to a much lesser degree. This beneficial effect of complement depletion at the higher dose resulted from a lesser degree of vasoconstriction and a decrease in the magnitude of reduction in  $L_pA$ . However, at both doses in normal and complement-depleted rats, there remains a persistent reduction in  $L_pA$  after AGBM Ab administration that appears independent of the complement system.

**Immunofluorescence and morphologic findings.** Immunofluorescence study revealed that all rats had typical heavy, linear deposits of rabbit IgG along their GBM (Fig. 1). The deposits of rabbit IgG were perhaps more prominent in the high-dose group, but the differences could not be clearly distinguished among dosage groups by immunofluorescence. Rat C3 accompanied the rabbit IgG in the normal complementemic animals (Fig. 1). Glomerular C3 deposits were virtually absent in the complement-depleted rats (Fig. 1).

Morphologic changes that accompanied AGBM Ab infusion were similar but quantitatively different for the four groups of rats, and there was only minor intragroup variation (Table III). The high-dose normal complementemic group was the most severely affected. With light microscopy, PMNs could be detected easily within the glomerular capillary lumina, where as many as 15 were present per glomerulus (Fig. 2). The endothelial cytoplasm appeared irregular which, combined with the PMNs, appeared to compromise the glomerular capillary lumina; however, the extent of compromise was dif-

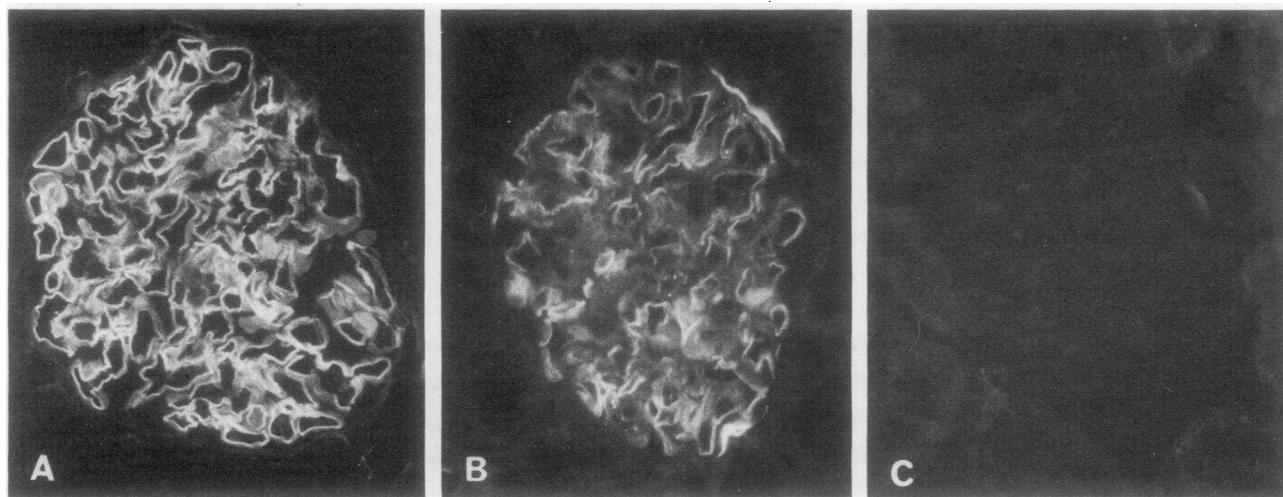


FIGURE 1 The typical linear deposits of rabbit AGBM Ab IgG are shown along the GBM of a rat from a low-dose CVF-treated group in A. In B, the irregular linear deposits of C3 that accompanied the IgG in normal complementemic rats are seen. The virtual absence of C3 found in the complement-depleted CVF-treated rats is visualized in C. (Fluorescein isothiocyanate conjugated anti-rabbit IgG in A, and anti-rat C3 in B and C.  $\times 312$ .)

**TABLE III**  
*Individual Correlations of Physiologic and Morphologic Changes with AGBM Ab Infusion at Low and High Doses in Normal and Complement-Depleted Rats*

	Physiologic changes						Morphologic changes				
	Rat	sngfr		rpf		L <sub>p</sub> A change	Light microscopy		Electron microscopy		
		control	change	control	change		PMN glomerulus	Capillary irregularity and narrowing*	Endothelial separation†	PMN on GBM	Focal foot process fusion‡
		number	nl/min/g kw	±%	nl/min/g kw						
<b>Normal rats</b>											
Low-dose group	1	47	0	143	+10	-40	0-3	tr- + <sup>  </sup>	1-2+	Occasional	tr- +
	2	65	-23	251	-20	-44	1-5	tr- +	1-2+	Moderate	tr- +
	6	68	-15	275	-4	-41	0-4	tr- +	2+	Occasional	tr- +
	9	62	+7	158	+91	-62	2-5	tr- +	1-2+	Many	+
	11	55	-26	305	-61	-26	2-8	tr	2+	Many	+
	15	50	-12	194	-21	0	1-6	tr- +	1-2+	Moderate	+
High-dose group	3	49	-23	262	-61	-86	ND <sup>¶</sup>	ND	1-2+	Many	tr- +
	4	54	-49	220	-86	-94	3-8	1-2+	1-2+	Many	+
	5	75	-70	277	-91	-95	3-10	2+	1-2+	Many	+
	7	51	-46	337	-85	-89	3-10	2+	1-2+	Many	tr- +
	10	63	-60	299	-96	-96	5-15	2+	2-3+	Many	1-2+
	13	58	-57	261	-97	-99	5-15	2+	2-3+	Many	1-2+
<b>Complement-depleted rats</b>											
Low-dose group	1	49	-30	182	+11	-83	0-1	tr	1-2+	0	tr
	4	34	-38	140	-69	-21	0-4	0	1-2+	Occasional	tr- +
	6	43	+17	135	+30	+13	0-2	0	tr- +	0	tr
	8	39	+26	205	-4	+8	0-2	0	tr- +	Rare	tr
	9	76	-19	304	-16	-72	0-2	0	1-2+	0	tr- +
	12	54	+19	163	+22	+20	0-2	0	tr	0	tr
	14	42	-11	201	-30	-38	0-2	0	1+	0	tr
	15	57	-6	204	-10	-42	0-2	0	1-2+	Rare	tr- +
	16	54	0	164	+57	-76	0-3	0	1-2+	Rare	+
High-dose group	3	59	-27	236	-16	-49	0-3	tr	1-2+	0	tr
	5	64	-48	227	+33	-77	0-4	tr	1-2+	Occasional	tr- +
	7	55	-54	211	-37	-75	0-3	tr	ND	ND	ND
	10	55	-38	161	-28	-77	0-3	tr	2+	Rare	tr- +
	11	53	-7	288	-21	-26	0-4	tr	1-2+	0	tr- +
	13	46	-16	132	-2	-43	0-3	tr	1-2+	Occasional	tr- +

\* Graded according to the approximate number of capillary loops involved with 1 and 2+ changes denoting 25 and 50%, respectively.

† Graded 1+ when scattered areas or segmental separation were noted, 2+ when separation involved the entire circumference of some capillary loops, and 3+ when partial or complete involvement was present in roughly half of the capillary lumens.

‡ Graded according to extent with 1 and 2+ corresponding roughly to 25 and 50%, respectively.

<sup>||</sup> tr, trace.

<sup>¶</sup> ND, not done.

ficult to estimate. Similar but less extensive changes were observed in the low-dose normal complementemic group (Fig. 2). The complement-depleted rats were virtually free of PMN accumulation in comparison with their complementemic counterparts, and had less noticeable endothelial irregularity and little compromise in their capillary lumens (Fig. 2 B and D).

No extraglomerular or vascular histologic alterations were noted.

Endothelial abnormalities, approximation of PMN along the GBM, and focal foot process fusion were observed in various combinations by electron microscopy (Table III). The GBM itself appeared somewhat irregular, particularly on its subendothelial



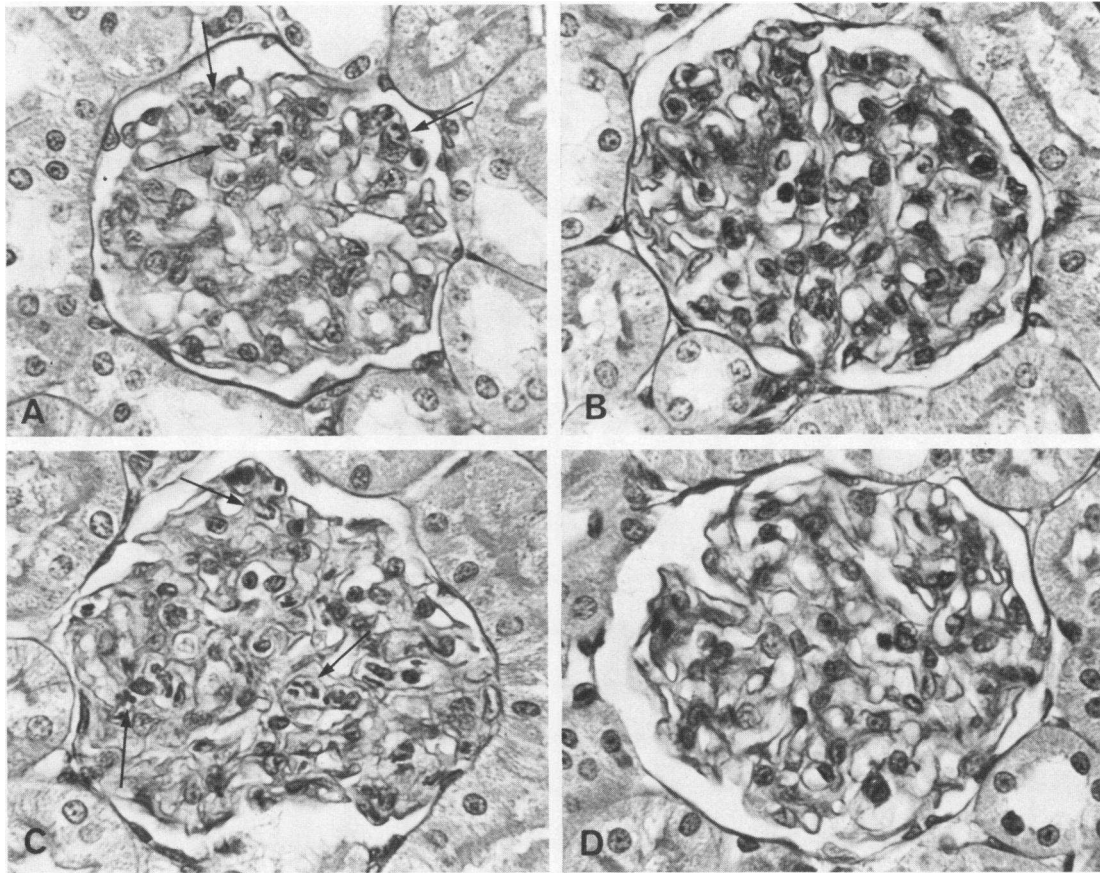


FIGURE 2 Glomerular histologic alterations in the four groups of rats are shown. (A) Portion is from a low-dose normal complementemic rat and has scattered PMN infiltrates (arrows). More extensive PMN infiltration (arrows) and greater glomerular capillary lumen irregularity are seen in a glomerulus from a high-dose normal complementemic rat in (C). The PMN accumulation was inhibited and the glomerular capillary lumen irregularity less noticeable in the complement-depleted CVF-treated low-dose (B) and high-dose (D) rats, respectively. (Periodic acid-Schiff stain,  $\times 400$ .)

aspect which is consistent with the fixation of AGBM Ab. Endothelial cell swelling, irregularity, and separation were most pronounced in the high-dose normal complementemic group and involved segments of the endothelium—in some glomerular capillaries the entire circumference of the endothelial lining. However,  $<50\%$  of the endothelium was involved in all but two of the most severely affected rats. The endothelial changes were only slightly less pronounced in the other groups. In the normal complementemic rats, the endothelial separation often accomplished accumulation and approximation of PMNs along the denuded GBM, with PMN cytoplasm actually displacing endothelial cell cytoplasm from the underlying GBM (Fig. 3). The number of capillary lumens containing PMNs varied in proportion to the number of PMNs identified by light microscopy; however,  $<20\%$  of capillary lumens on any given section

were affected, even in the most severely involved rats. When present, the PMNs rarely filled the lumen completely. The PMN approximation to the GBM was strikingly inhibited in the decompensated animals. Rare or occasional PMNs were identified in three of the five high-dose complement-depleted rats and in three of the nine low-dose complement-depleted rats studied (Table III). When present, the PMNs in the complement-depleted group that were attached to the GBM had roughly the same appearance as those in the normal complementemic rats.

Focal epithelial cell foot process fusion was observed in all groups (Fig. 3). The focal areas of fusion were slightly more extensive in the high than the low-dose normal complementemic animals; somewhat fewer areas appeared in their complement-depleted counterparts (Table III). The foot process fusion did not involve  $>20\%$  of the epithelial cell foot processes,

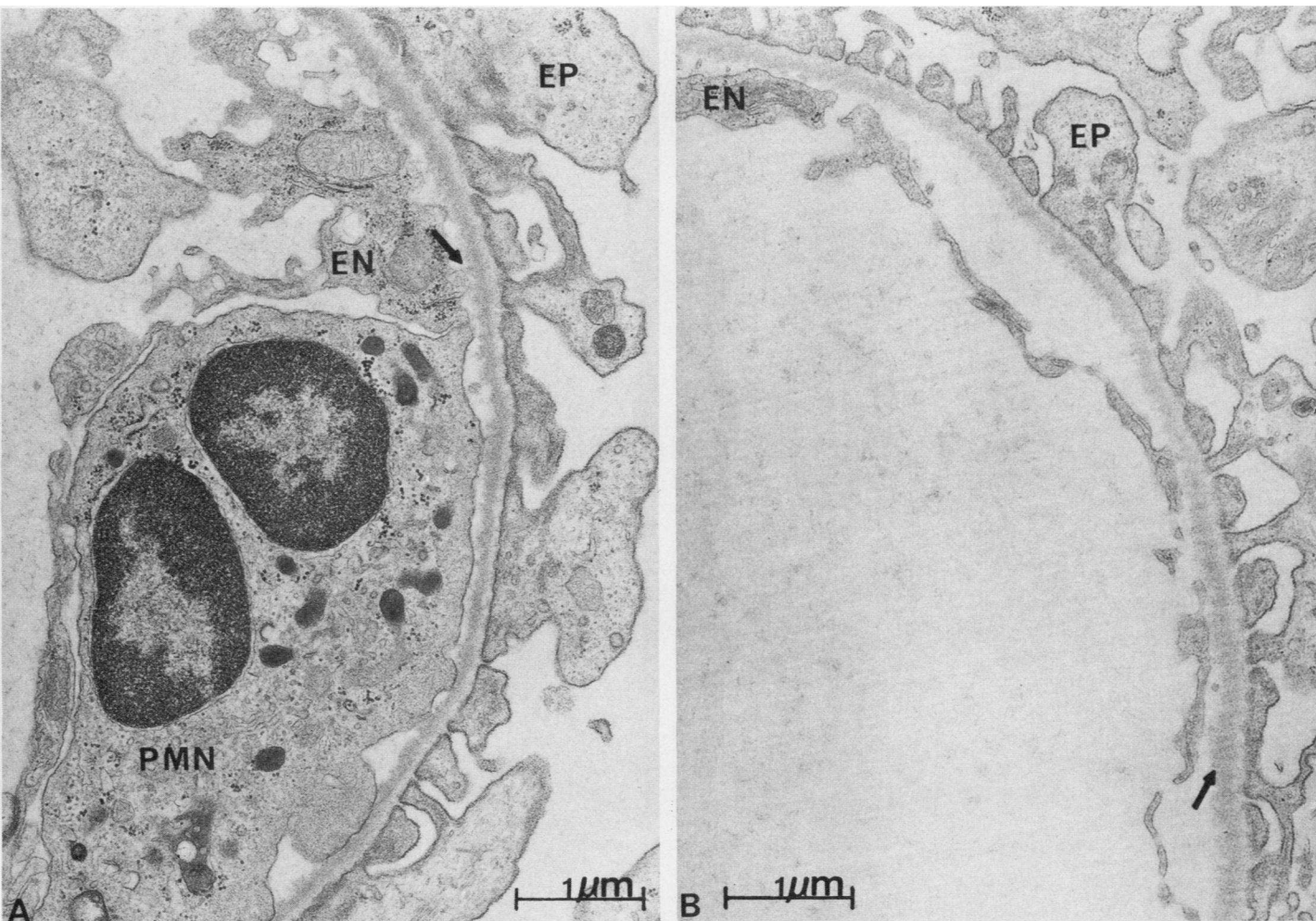


FIGURE 3 Electron microscope studies of a high-dose normal complementemic rat (A) and a complement-depleted C566-treated rat (B) are shown. The subendothelial aspect of the GBM (arrows) appeared irregular, presumably representing the fixation of anti-GBM antibody. A PMN was found approximated along the GBM displacing the endothelium (EN) in the normal complementemic rat in A. Endothelial separation occurred in the complement-depleted rat seen in B; however, PMN infiltration was almost completely lacking. Focal areas of epithelial cell (EP) foot process fusion were found and were particularly evident in A. ( $\times 17,750$ .)

except in the two most severely affected high-dose rats. This finding was not clearly related to the presence of PMNs.

Electron microscope studies revealed minor individual variations within animals of each group. Even though portions of four glomeruli were studied in each rat, the possibility of sampling error was felt to preclude meaningful, detailed animal to animal correlation. It is interesting, however, that the three rats in which  $L_pA$  did not decrease had the least striking endothelial change in the low-dose complement-depleted group.

## DISCUSSION

Micropuncture techniques permit direct evaluation of glomerular fluxes, flows, pressures, and permeability coefficients and can be usefully applied as quantitative assay tools to examine the series of factors and events that lead to the altered glomerular filtration that follows immune injury (1). Previous studies utilizing these tools have demonstrated that within 1 h after immune insult, the glomerular filtration rate falls because of both alterations in the  $L_pA$  and increases in renal vascular resistance (1). To gain

further insights into the exact mechanisms involved in this early phase of immune injury, we have determined whether these two events leading to reduced sngfr are intimately linked or whether vasoconstriction and permeability effects are separable mechanisms. Utilizing differing doses of AGBM Ab, we have been able to separate quantitatively these two mechanisms and their respective influence upon filtration. At higher doses of AGBM Ab, massive reductions in both  $L_pA$  and rpf occurred, and both factors contributed to the large decrease in sngfr. However, at the lower dose, although a minimal vasoconstrictive effect occurred, a significant reduction in  $L_pA$  was largely responsible for the decrease in filtration rate.

Although the qualitative conclusions that decreases in both rpf and  $L_pA$  contribute to reduced filtration rate in the early phase of immune injury are common to this and our previous study on this issue (1), there were significant quantitative differences observed between the antibody preparations utilized. Doses of 225 and 450  $\mu g$  of AGBM Ab in our previous study produced similar quantitative effects on rpf,  $L_pA$ , and sngfr. However, the current microtechniques have permitted us to determine that these same low and high doses resulted in quantitative differences after the antibody utilized in this study. The specific reasons for the quantitative differences between antibody preparations generated to the same antigen is not known, but may relate to secondary effects such as the respective capacities of antibody preparations to fix complement. Also, similar high doses of antibody preparations led to different degrees of PMN accumulation, also possibly related to the complement activity generated by the respective preparations at identical doses administered.

The fixation of complement is critical in the process of immune injury. The studies of Cochrane et al. (2), and reviews of Muller-Eberhard (18), and Cochrane and Janoff (19) have stressed the role of complement in the pathogenesis of experimental glomerulonephritis. Activation of the complement cascade has multiple effects, including the attraction of PMN, the enhancement of cellular adherence, and the activation and release of a variety of biologically active substances. Judgments as to the role of complement fixation have been based primarily upon evaluating the renal morphology and the proteinuria that follow immune challenge (2, 18). No studies have evaluated the possible beneficial effects upon glomerular ultrafiltration and the respective determinants of filtration that might result from preventing complement fixation.

The present studies in the complement-depleted rat and ultrastructural analysis of all experimental groups have provided information on the mechanisms

leading to vasoconstriction and reduced  $L_pA$  and the specific role of the complement system in the process of immune injury. CVF, which appears to be cobra C3B (20), interacts with factors B and D of the alternative complement pathway to form a C3 cleaving enzyme (7), thereby causing C3 depletion. Complement depletion largely prevented the acute vasoconstriction that occurred at both doses of AGBM Ab. Inasmuch as the vasoconstriction was probably functional, results from studies on complement depletion suggest that vasoconstriction was not due to acute fixation of antibody alone. It is possible that activation of the complement system causes the local release of intrarenal substances capable of mediating constriction of resistance vessels. It is also possible that certain components of the complement cascade may have vasoconstrictor activity (18). Complement depletion also prevented migration of PMN into the glomerular capillary, but this complement-mediated event is unlikely to be the direct cause of increased vascular resistance, since the cells accumulated only within the capillary, normally a region of very low resistance in the renal vasculature (15). Our previous study on glomerular immune injury also provided evidence that the decrease in rpf and migration of PMNs were separate effects of complement activation. With a different AGBM Ab, significant vasoconstriction occurred but fewer PMNs were noted within the capillary 1 h after the infusion of the complement-fixing AGBM Ab than was noted in the high dose group in this study (1). Although vasoconstriction and migration of PMNs are greatly dependent upon complement fixation, the two events are presumably the unrelated effects of complement activation. Where individual rats were studied, the degree of infiltration of the capillaries with PMNs correlated strongly with the degree of rpf reduction (both presumably effects of complement fixation; Table III). We cannot exclude the possibility that PMNs somehow contribute directly to the increase in vascular resistance.

Prevention of complement activation also had a major effect upon  $L_pA$ . With the higher dose of AGBM Ab, complement depletion greatly decreased the effect of antibody upon  $L_pA$ . In the normal rat,  $L_pA$  was reduced 92% after high-dose AGBM Ab; this effect was diminished to 67% in the complement-depleted, high-dose group. This beneficial effect of complement depletion correlated with the prevention of PMNs migrating into the capillary. Although the effects of complement activation upon PMNs and vasoconstriction appear to be unrelated events, we believe that there is reasonable evidence that PMNs may mediate in part the larger  $L_pA$  reduction observed when normal rats were injected with high doses of

AGBM Ab. At high doses, PMNs reduced capillary surface to a small extent in normal complementemic rats by adherence, but in addition may have had a greater effect by partially obstructing certain capillary conduits within the glomerulus, diverting glomerular blood flow through fewer channels. This latter effect is impossible to evaluate quantitatively but should reduce effective filtering surface area without contributing measurably to increased vascular resistance because the capillary normally contributes little to total renal vascular resistance. At lower doses of antibody, there was no beneficial effect of complement depletion upon the reduction in  $L_pA$ , but there were also fewer PMNs within the capillary at this dose. In addition, at the higher dose,  $L_pA$  remained low in spite of complement depletion. We therefore conclude that complement depletion partially ameliorates the effect of a high dose of antibody upon  $L_pA$  by preventing any loss of capillary surface area resulting from migration of PMNs into the capillary.

AGBM Ab fixation exerts a significant and persistent effect upon  $L_pA$  that appears independent of the complement system. The quantitative reductions in  $L_pA$  after antibody infusion in low-dose, in low-dose complement-depleted, and in high-dose complement-depleted groups were not significantly different (Table II). This persistent reduction in  $L_pA$  did not result from loss of surface area secondary to the adherence of PMNs but was associated with structural alterations upon the endothelial surface of the glomerular membrane and, to a lesser extent, with focal fusion of epithelial cell foot processes. The detachment of endothelial cells, an effect noted in all groups (and in a previous study; 1), appears to be the logical cause of this persistent decrease in  $L_pA$  within 1 h of AGBM Ab infusion. The causes of reduction in  $L_pA$  are additive. The first is complement dependent and related to loss of capillary surface area ( $A$ ) from PMN accumulation; the second is independent of the complement system and due to structural alterations upon the endothelial surface of the capillary membrane which leads to reductions in local capillary permeability ( $L_p$ ).

In a previous study on glomerular immune injury, we have postulated that fixation of antibody globulin to the GBM may have produced separation of the endothelial cell from the underlying basement membrane by interfering with the potentially charge-dependent attachment of these structures (1). This endothelial separation may lead to a relatively less "well-stirred" compartment in which serum proteins may become concentrated from the lack of "stirring" effect of capillary blood flow. Although we can be certain that much of the alteration in capillary permeability ( $L_p$ ) is independent of complement activation, the data do not permit us to conclude that endothelial changes result from antibody fixation alone. It remains

possible that other mediators of immune injury and the steric effects of AGBM Ab fixation are independent of the complement cascade and may have a part in producing the endothelial injury.

There may be other mechanisms which further decrease  $L_pA$  and affect glomerular filtration at longer intervals after immune injury. Ultrastructural and proliferative cellular changes (21) could contribute to further reductions in  $L_pA$  at a later time, and the role of the complement system in this process is less well defined.

It is difficult to extrapolate the results of the current study on the effects of differing doses of AGBM Ab to the clinical state associated with AGBM Ab-induced nephritis. Although large amounts of AGBM Ab can be demonstrated within the glomeruli of patients with this form of nephritis (22, 23), it is not likely that such a large quantity of AGBM Ab as was utilized in this study (with respect to weight of the animal) could be generated into the circulation in such a short time period in the clinical condition. It is, therefore, not likely that vasoconstriction is the dominant mode of the initial glomerular filtration rate reduction in the analogous clinical condition but rather the reduction in  $L_pA$ . Also the present studies have not defined that specific substances that mediate vasoconstriction. Histologic and immunofluorescent examination of renal tissue revealed neither structural alterations nor immunoglobulin deposition within resistance vessels, suggesting that vasoconstriction resulted from functional causes rather than structural alterations.

The application of micropuncture techniques to the evaluation of glomerular immune injury provides a quantitative assessment of substances that mediate decreased sngfr and the extent of their influence. The current study demonstrates that the vasoconstriction observed is a phenomenon associated with large doses of antibody and, in the acute condition, requires an intact complement system. Mechanisms leading to reduced filtration rate secondary to immune injury can be further separated into complement-dependent effects and into noncomplement-dependent mechanisms related to altered capillary hydraulic permeability and to changes in the ultrastructure of the glomerular membrane.

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