Glomerular immune injury in the rat: The influence of angiotensin II and α-adrenergic inhibitors

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Glomerular immune injury in the rat: The influence of angiotensin II and α-adrenergic inhibitors. Nephron filtration rate (SNGFR) decreases significantly after the administration of large doses of antiglomerular basement membrane antibody (anti-GBM) as a result of reductions in both nephron (renal) plasma flow (RPF) and the glomerular permeability coefficient (LpA). We have examined the participation of angiotensin II (AII) and α-adrenergic activity in this process in paired studies in three groups of Munich-Wistar rats: group 1, control and untreated; group 2, rats receiving continuous infusion of sar'-ala8-AII (1 µg · kg of body wt⁻¹ · min⁻¹), an AII receptor antagonist; and group 3, rats receiving continuous infusion of phentolamine (27 µg · kg of body wt⁻¹ · min⁻¹), a dose sufficient to block α-adrenergic responses. In group 1, SNGFR decreased from 58 ± 4 to 35 ± 6 nI · min⁻¹ · g of kidney wt⁻¹ (P < 0.001) after anti-GBM administration due to reductions in RPF (272 ± 35 to 170 ± 52 nI · min⁻¹ · g of kidney wt⁻¹, P < 0.0001) and LpA (0.13 ± 0.03 to 0.04 ± 0.01 nI · sec⁻¹ · g of kidney wt⁻¹ · mm Hg⁻¹, P < 0.02). In group 2, the sar'-ala8-AII-infused rats, SNGFR decreased to a greater extent than it did in group 1 (P < 0.01) (55 ± 2 to 18 ± 6 nI · min⁻¹ · g of kidney wt⁻¹, P < 0.005) due to a greater reduction in RPF and a similar decrease in LpA. In group 3, phentolamine infusion prevented the decrease in SNGFR (52 ± 3 to 52 ± 4 nI · min⁻¹ · g of kidney wt⁻¹, NS) due primarily to elimination of vasoconstriction and a significantly lesser reduction in LpA (0.10 ± 0.02 to 0.07 ± 0.01 nI · sec⁻¹ · g of kidney wt⁻¹ · mm Hg⁻¹, P < 0.001). There were no morphologic differences after anti-GBM administration that were unique to group 3. Blockade of AII activity does not prevent immune induced vasoconstriction or the reduction in LpA. α-Adrenergic blockade (1) prevents acute immune induced vasoconstriction and (2) partially prevents the immune induced reduction in LpA.

Lésion glomérulaire immunologique chez le rat. Influence des inhibiteurs de l'angiotensine II et α-adrénergique. Le débit de filtration glomérulaire individuel (SNGFR) diminue significativement après l'administration de fortes doses d'anticorps antémembrane basale (anti-GBM) glomérulaire en raison de la diminution du débit plasmatique (RPF) et du coefficient de perméabilité glomérulaire (LpA). Nous avons étudié la participation de l'angiotensine II (AII) et de l'activité α-adrénergique dans ce processus chez trois groupes de rats Munich-Wistar: groupe 1, contrôles et non traités; groupe 2, des rats recevant une perfusion continue de sar'-ala8-AII (1 µg · kg de poids corporel · min⁻¹), un antagoniste des récepteurs de l'AII; et groupe 3, des rats recevant une perfusion continue de phentolamine (27 µg · kg de poids corporel · min⁻¹), une dose suffisante pour bloquer les réponses α-adrénergiques. Dans le groupe 1, SNGFR diminué de 58 ± 4 à 35 ± 6 nI · min⁻¹ · g de poids renal, (P < 0.001) après anticorps anti-GBM du fait de la diminution de RPF (272 ± 35 à 170 ± 52 nI · min⁻¹ · g de poids renal, P < 0.0001) et de LpA (0.13 ± 0.03 à 0.04 ± 0.01 nI · sec⁻¹ · g de poids renal · mm Hg⁻¹, P < 0.02). Dans le groupe 2, chez les rats perfusés avec sar'-ala8-AII, SNGFR a diminué plus que dans le groupe 1 (P < 0.01) (55 ± 2 à 18 ± 6 nI · min⁻¹ · g de poids renal, P < 0.005) du fait d'une plus grande diminution de RPF et d'une diminution semblable de LpA. Dans le groupe 3, la perfusion de phentolamine a empêché la diminution de SNGFR (52 ± 3 à 52 ± 4 nI · min⁻¹ · g de poids renal, NS) du fait de l'élimination de la vasoconstriction et de la diminution significative ment moindre de LpA (0.10 ± 0.02 à 0.07 ± 0.01 nI · sec⁻¹ · g de poids renal · mm Hg⁻¹). Il n'a pas été observé de différences morphologiques particulières en groupe 3 après anticorps anti-GBM. Le blocage de l'activité de AII n'empêche pas la vasoconstriction à déterminisme immunitaire ou la réduction de LpA. Le blocage α-adrénergique (1) empêche la vasodilatation aiguë à déterminisme immunitaire et (2) empêche partiellement la réduction de LpA à déterminisme immunitaire.

We have previously demonstrated that within 1 hour of the administration of large quantities of antiglomerular basement membrane antibody (anti-GBM) to rats, the single nephron filtration rate (SNGFR) decreases significantly, due to major reductions in both nephron plasma flow (RPF) and the glomerular permeability coefficient (LpA) [1, 2]. More recent studies have demonstrated that the vasoconstriction leading to reductions in RPF was almost totally complement dependent [2]. A major unresolved issue remains—the mechanism whereby this complement dependent vasoconstriction is mediated. There are very few candidates for the vasoconstrictive mediator of this reduction in nephron plasma flow. Candidates include the major renal vasoconstrictor systems, the renin-angiotensin system, and the adrenergic system. Other less well defined vasoconstrictors such as adenine nucleotides and certain vasoconstrictor prostaglandins could also participate. An alternative possibility, is that no intermediary vasoconstrictor systems participate in the reduction in RPF, but rather certain complement components directly produce the vasoconstriction.

The present studies were designed to examine the influence of inhibition of the two major vasoconstrictor systems upon the mechanisms leading to glomerular immune injury following anti-GBM administration. Continuous infusions of the angiotensin II (AII) receptor blocker sar'-ala8-AII and the α-adrenergic antagonist phenolamine permitted evaluation of the potential role of these respective vasoconstrictor substances in the process of glomerular immune injury. Delineation of the respec-
tive pathogenetic roles of the two vasoconstrictor systems has been accomplished and the results suggest that (1) the alpha adrenergic system mediates acute vasoconstriction and that (2) blockade of α-adrenergic activity at least partially prevents a reduction in \( L_\text{pA} \) following glomerular immune injury. But infusion of the AII receptor blocker sar\(^1\)-ala\(^8\)-AII appears to augment the vasoconstriction associated with the infusion of anti-GBM antibody.

**Methods**

*Experiment animals.* The current studies were performed on Munich-Wistar rats (190 to 250 g of body wt), bred and maintained in a colony housed at the Animal Research Facility at the San Diego Veterans Administration Medical Center, San Diego, California.

**Preparation of anti-GBM antibody.** This was produced by immunizing rabbits repeatedly with 10 to 20 mg of rat 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 anti-GBM was recognized when the i.v. 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 rat gamma globulin fractions were pair-labeled with \(^{125}\text{I}\) and \(^{123}\text{I}\) radioactive iodine, and the amount of kidney-fixing antibody was quantitated by the paired-label isotope technique [4, 5]. This technique was also used to quantitate any effect of the inhibitors on the binding of antibody. The pool of anti-GBM used in this study was the same as that used in a previous study [2].

**Micro puncture studies evaluating glomerular ultrafiltration before and after anti-GBM.** The micro puncture protocol used in this study was nearly identical to that described in previous studies on glomerular immune injury [1, 2]. Surgical preparation was as previously described, and all studies were paired with iso-oncotic plasma expansion (2.5% body wt administered over 60 min) as the control condition [1]. A separate infusion of \(^{14}\text{C}\)-inulin dissolved in isotonic sodium chloride and sodium bicarbonate (0.5% body wt per hour) was begun at the time of plasma expansion and was delivered at approximately 40 μCi/hr.

**Control, group 1.** After the equilibration of radioactive inulin, initial measurements of glomerular capillary and Bowman’s space hydrostatic pressure (utilizing a servonulling device with 1- to 2-μm tip pipettes) [1, 6] and of SNGFR (\( N = 5 \)) were obtained, and at least three samples of efferent peritubular capillary blood from “star” vessels were obtained [6].

After completion of the first period measurements, a dose of 1.4 μg/g of body wt of anti-GBM was administered i.v. over 5 min in a volume of 400 μl of isotonic sodium chloride and sodium bicarbonate. Fifteen minutes after termination of anti-GBM infusion, all pressure, filtration rate, and efferent protein concentration measurements were repeated and completed within 45 min. In previous studies on the mechanism of glomerular immune injury with anti-GBM, we determined that inulin remains a valid marker of glomerular ultrafiltration after the antibody is administered [1].

**Infusion of sar\(^1\)-ala\(^8\)-AII, group 2.** Surgical preparation, inulin equilibration, and iso-oncotic plasma expansion were identical to the protocol for the control group. An infusion of sar\(^1\)-ala\(^8\)-AII, an AII antagonist (1 μg/kg of body wt/min Beckman Inst., Palo Alto, California), was initiated 30 min prior to the start of the first period of measurements and continued throughout the course of the experiment. It was infused in an isotonic sodium chloride and sodium bicarbonate solution (1 mg per 10 ml). After completion of the first period measurements, anti-GBM was administered using the same protocol as in the control group, followed by a second period during which all pressure, filtration rate, and efferent protein measurements were repeated and completed within 45 min.

**Phentolamine infusion, group 3.** Surgical preparation, inulin equilibration, and iso-oncotic plasma expansion were identical to the protocol for the control group. The infusion of phentolamine mesylate, an α-adrenergic blocker (27 μg/kg of body wt/min, Regitine, CIBA Pharmaceuticals, Summit, New Jersey), was initiated 30 min prior to the start of the first period of measurements and continued throughout the course of the experiment. The phentolamine was infused in a small amount of water (1.0 mg/ml). After completion of the first period measurements, anti-GBM was administered using the same protocol as in the control group, followed by a second period of measurements. At the completion of the second period, the efficacy of phentolamine infusion was evaluated with a bolus infusion of phenylephrine (6 μg/min, Neo-Synephrine hydrochloride®, Winthrop Laboratories, Div. Sterling Drug, New York, New York) for 5 min to verify that α-blockade was complete. This dose of phenylephrine was previously determined to raise the mean arterial pressure 30 to 40 mm Hg in this model without phentolamine.

**Analytic methods.** Protein concentration in systemic and efferent peritubular blood samples was measured by a microadaptation of the Lowry protein method [6–9]. SNGFR, GFR, RPF, and RBF, and urine and plasma sodium and potassium concentrations were determined as described in previous studies [1, 2, 6–8].

**Morphologic 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, 2, 10, 11]. The mean polymorphonuclear leukocytes (PMN) per glomerulus was determined by light microscopy by counting the PMN in 20 glomeruli from each kidney of each rat. Morphologic grading was based on a 0 to 4+ scale [2]. On light microscope examination, the glomerular capillary lumens appeared irregular with variable degrees of encroachment, which is presumably related to the endothelial abnormalities seen by electron microscopy as well as to PMN infiltration. Electron micrographs were evaluated as to the degree of endothelial separation, foot process fusion, and PMN approximation to the GBM.

**Studies on the effects of sar\(^1\)-ala\(^8\)-AII and phentolamine upon anti-GBM fixation, complement activity, and peripheral blood cell counts.** Measurements were made to determine that the blockers did not influence the process of glomerular immune injury indirectly by altering anti-GBM fixation (by altering the basic mechanism or renal load of anti-GBM delivered), complement activity (as measured by CH50), or peripheral white blood cell counts. With the paired labeled technique [4, 5], quantita-
tive renal fixation of anti-GBM was evaluated in 3 rats in each of the three groups: controls, rats receiving sar'-ala8-AII, and those receiving phenolamine. The doses of anti-AII and phenolamine used and the duration of drug infusion were identical to those used in the physiologic studies. Complement activity was measured by CH50 in 15 rats (3 control, 6 receiving anti-AII and 6 receiving phenolamine) in the final period. Blood samples were drawn before plasma volume expansion, after plasma volume expansion, and finally 45 min after drug infusion to determine if these agents might have mediated their effects by influencing complement activity [2]. Peripheral white blood cell counts were also measured and compared in 15 rats following the protocol used for CH50 sampling (3 control, 6 with sar'-ala8-AII, and 6 with phenolamine). Previous studies have suggested an important role for PMN in mediating a reduction in $L_{pA}$ with glomerular immune injury [1, 2].

Calculations. Superficial nephron filtration fraction (SNFF), single nephron renal plasma flow (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, 6–8, 12–14].

The four factors that define the SNGFR—hydrostatic pressure gradient across the glomerular membrane ($\Delta P$), systemic oncotic pressure ($\pi_a$), glomerular permeability coefficient ($L_{pA}$), and rate of RPF—interrelate in the following manner.

Because $\Delta P = P_G - P_{BS}$, where $P_G$ is the directly measured glomerular capillary hydrostatic pressure and $P_{BS}$ is the hydrostatic pressure in Bowman’s space, the effective filtration pressure (EFP) can be defined as follows:

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

where $\pi$ is the oncotic pressure. Oncotic pressures of systemic protein samples ($C_A$) and efferent arteriolar samples ($C_E$) were determined by the following relationship

$$\pi = 1.74C + 0.28C^2$$

which is a simplification of the empirical relationship defined by Landis and Pappenheimer [15] $\pi = 2.1C + 0.16C^2 + 0.0009C^3$.

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 (EFP) is defined as follows:

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

Changes in RPF modify the $\overline{EFP}$ 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:

$$SNGFR = L_{pA} \times EFP$$

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 states before anti-GBM and after anti-GBM administration in all three groups was determined by two-way analysis of variance and by Student’s $t$ test where appropriate [16]. Linear regression analysis was applied to compare PMN with $\Delta$SNGFR between periods for all rats measured ($N = 17$) [17].

Results

Measurements were obtained both before and after anti-GBM administration in the three groups studied—group 1, control and anti-GBM only; group 2, sar'-ala8-AII infusion, which was maintained before and after anti-GBM administration; and group 3, phenolamine infusion, which was maintained before and after anti-GBM administration. The anti-GBM dose used in all three groups was 1.4 $\mu$g/g of body wt.

As previously noted [1, 2] there were no gross morphologic changes on the kidney surface after anti-GBM administration, although a slight decrease in firmness of the kidney was evident in some animals.

Group 1: Control anti-GBM administration. The control studies were performed in such a fashion as to be interspersed in time sequence throughout the other two groups of studies. The anti-GBM administration produced a significant decrease in SNGFR (58 ± 4 to 35 ± 6 ml·min$^{-1}$·g of kidney wt$^{-1}$, $P < 0.0001$) (Table 1, Fig. 1). The mean RPF also significantly decreased from 272 ± 35 to 170 ± 52 ml·min$^{-1}$·g of kidney wt$^{-1}$ ($P < 0.0001$) (Fig. 1). The superficial nephron filtration fraction increased numerically, but the change was not statistically significant.

The hydrostatic pressure in Bowman’s space ($P_{BS}$) decreased significantly from 20 ± 1 to 13 ± 2 mm Hg ($P < 0.05$). The $P_G$ remained unchanged after anti-GBM administration. The glomerular hydrostatic pressure gradient ($\Delta P$) increased (36 ± 1 to 42 ± 1 mm Hg, $P < 0.01$) (Fig. 2), such that the overall effect of hydrostatic forces on SNGFR was positive. The mean efferent peritubular capillary pressure ($HP_E$) decreased from 24 ± 1 to 19 ± 1 mm Hg ($P < 0.01$).

The afferent (AR) and efferent (ER) arteriolar resistances increased after the anti-GBM administration (AR, 8 ± 1 to 19 ± 4 $\times$ 10$^8$ dynes·sec·cm$^{-5}$, $P < 0.05$; ER, 6 ± 1 to 14 ± 2 $\times$ 10$^8$ dynes·sec·cm$^{-5}$, $P < 0.02$), which is similar to the effects produced by the higher anti-GBM dose (2.4 $\mu$g/g of body wt) in an earlier study [2].

Afferent plasma protein concentration ($C_A$) was identical in both periods (6.1 ± 0.3 vs. 6.0 ± 0.3 g/dl), as was the efferent plasma protein concentration ($C_E$, 7.7 ± 0.6 vs. 7.9 ± 0.4 g/dl). Both the mean systemic oncotic pressure ($\pi_a$) and the mean efferent oncotic pressure ($\pi_E$) were unchanged between periods (Table 1). The hematocrit, however, was increased significantly by the anti-GBM dose (44 ± 1 vs. 48 ± 1, $P < 0.05$) a change not observed in the previous two studies [1, 2].

The EFP did not increase significantly although there was a numerical increase (9 ± 2 vs. 15 ± 3, $P > 0.1$). This contrasts with previous studies on anti-GBM where $\overline{EFP}$ significantly increased [1,2]. $EFP_{pA}$ did not increase significantly, but was significantly greater than zero (4 ± 1 mm Hg, $N = 18$) before the anti-GBM administration in all three groups. The SNGFR decreased significantly and the $\overline{EFP}$ was similar after anti-GBM administration. This finding suggests that a reduction in $L_{pA}$ (Table 1) was the essential factor in decreasing SNGFR. This finding is supported by two previous studies from this laboratory [1, 2]. The $L_{pA}$ was reduced from 0.13 ± 0.03 before to
the control group and the sar'-ala8-AII infusion group. A recent
is supported by the similarity of the pre-anti-GBM condition in
of nephron filtration in the pre-anti-GBM state. This conclusion
0.04
SNGFR.
whereas the EFP remained the same, indicating that a decrease
But, the EFPF was numerically lower during sar-a1a8-AII
change in the EFPE or EFPA after anti-GBM administration.
This finding is similar to the increase that was observed in
hematocrit,
unchanged between measurement periods and were quite simi-
strated in the control group.
The hydrostatic pressure of Bowman’s space (P_{BS}) was
significantly reduced (20 \pm 1 to 11 \pm 1 mm Hg, P < 0.01) after
anti-GBM administration. This reduction in pressure during
sar'-ala8-AII infusion was statistically the same as that observed
in the control group. The glomerular hydrostatic pressure
(P_{G}) and the glomerular hydrostatic pressure gradient (\Delta P)
were unchanged by administration of anti-GBM (Table I) (Fig.
2). HP_{G} was reduced from 23 \pm 2 to 17 \pm 2 mm Hg (P < 0.05)
after antibody administration, similar to the decrease demonstrated
in the control group.
The afferent (AR) and efferent (ER) arteriolar resistances
were numerically different between the two periods, but due to
a large standard error, the differences were not statistically
significant (Table I). In certain animals, nephron and renal
blood flow were negligible after anti-GBM during sar'-ala8-AII
infusion. If afferent conductances were compared (1/AR) the
difference between periods was significant (P < 0.01), but the
efferent conductance was not significantly different.
The protein concentration during sar'-ala8-AII infusion was
unchanged by antibody administration (C_{A}, 6.2 \pm 0.3 to 6.3 \pm
0.2 g/dl, NS; C_{E}, 8.1 \pm 0.2 to 8.6 \pm 0.2 g/dl, NS). The mean
systemic afferent (\sigma_{A}) and efferent (\sigma_{E}) oncotic pressures were
unchanged between measurement periods and were quite simi-
lar to the values for the control group (Table I). The hematocrit,
however, did change. It increased from 43 \pm 1 to 50 \pm 1\% (P <
0.01). This finding is similar to the increase that was observed in
the control group 1.
The mean effective filtration pressure (EFP) did not change
between periods (9 \pm 3 to 10 \pm 2, NS). There was no significant
change in the EFPF or EFP_{A} after anti-GBM administration. But,
the EFP_{E} was numerically lower during sar'-ala8-AII
infusion and anti-GBM (4 \pm 2 mm Hg) than was observed in
group 1 (10 \pm 3 mm Hg).
During sar'-ala8-AII infusion, L_{P}A decreased 0.17 \pm 0.05 to
0.04 \pm 0.01 nl \cdot sec^{-1} \cdot g kidney wt^{-1} \cdot mm Hg^{-1} (P < 0.05) (Fig.
2). As in group 1, the SNGFR was significantly reduced whereas the EFP remained the same, indicating that a decrease in
L_{P}A was the essential contributing factor to the reduction in
SNGFR.
The sar'-ala8-AII infusion had no effect on the determinants
of nephron filtration in the pre-anti-GBM state. This conclusion
is supported by the similarity of the pre-anti-GBM condition in
the control group and the sar'-ala8-AII infusion group. A recent
study from this laboratory has demonstrated that sar'-ala8-AII
produces no effects in a rat receiving a normal sodium chloride
intake [14]. Sar'-ala8-AII infusion enhanced the effect of the
increased vasoconstriction that was induced by anti-GBM, as
the dramatic reduction in SNGFR, RPF, and RBF (432 \pm 50 to
103 \pm 45 nl \cdot min^{-1} \cdot g kidney wt^{-1}, P < 0.0001) clearly
demonstrate.
Group 3: Continuous phentolamine infusion with anti-GBM
administration. Qualitatively, phentolamine had no obvious
effect on the appearance of the kidney prior to antibody
administration.
During anti-GBM administration (5 min), the kidney
appeared to pulsate, and there was a transient decrease in blood
pressure only in the phentolamine infusion group. The mean
arterial pressure returned to the preinfusion values, and the
arterial pulsations diminished after anti-GBM administration
was completed. A potential explanation for these events is that
phentolamine might unmask the concurrent release of a vasodi-
lator substance associated with glomerular immune injury.
The results during phentolamine infusion with anti-GBM
were quite different from those observed in the previous two
groups. The SNGFR did not change significantly after the anti-
GBM administration (52 \pm 3 to 52 + 4 nl \cdot min^{-1} \cdot g kidney wt^{-1},
NS) (Fig. 3). This result was significantly different from the
changes observed in control group 1 (\Delta SNGFR of Group 1 vs.
\Delta SNGFR of Group 3, P < 0.01) and the sar'-ala8-AII infused
group 2 (\Delta SNGFR of group 2 vs. \Delta SNGFR of group 3, P <
0.01).
The pre-anti-GBM values in the phentolamine group were
statistically not different from those in the control group 1 or in
the group 2. The values for RPF and RBF for phentolamine did
not differ significantly in the pre-anti-GBM state from the other
two groups. Although the numerical value for the RPF in the
first measurement period of phentolamine was lower (206 nl/
min) than that of the other groups (272 nl/min, control; 247 nl/
min, group 2), it was statistically not different. This value for
RPF (206 nl/min) is in fact quite consistent with values (range,
190 to 280 nl/min for group means) reported for the pre-anti-
GBM period in two previous studies from this laboratory [1, 2]
using identical plasma expansion protocols. The RPF as earlier
noted did not change significantly after anti-GBM administra-
tion during phentolamine infusion (206 + 16 to 222 \pm 15 nl \cdot min^{-1}
\cdot g kidney wt^{-1}) (Fig. 3). The directional changes in RPF (\Delta RPF)
after anti-GBM in groups 1 and 2 were both significantly different
(P < 0.01) from the directional change during phentolamine.
The SNFF did not change after anti-GBM in group 3.
The hydrostatic pressure of Bowman’s space (P_{BS}) was
not altered by the administration of anti-GBM Ab in the phentol-
amine-infused rat (Table 1), a finding considerably different
from the other two groups. The SNGFR did not change significantly after the anti-
GBM administration (52 \pm 3 to 52 \pm 4 nl \cdot min^{-1} \cdot g kidney wt^{-1},
NS) (Fig. 3). This result was significantly different from the
changes observed in control group 1 (\Delta SNGFR of Group 1 vs.
\Delta SNGFR of Group 3, P < 0.01) and the sar'-ala8-AII infused
group 2 (\Delta SNGFR of group 2 vs. \Delta SNGFR of group 3, P <
0.01).
Vascular resistances were similar before and after anti-GBM
infusion. Afferent arteriolar resistance (AR) was 10 \pm 1 in the
control period and 9 \pm 1 \times 10^{9} \text{ dynes} \cdot \text{sec} \cdot \text{cm}^{-2} \text{ after anti-
GBM}. Efferent arteriolar resistance was not altered by infusion
of anti-GBM (Table 1). This lack of change in critical parameters
after anti-GBM was quite different from the changes in
resistances observed in groups 1 and 2.
Afferent plasma protein concentration (C_{A}) did not change
after anti-GBM, but efferent protein concentration (C_{E}) did
decline significantly (8.1 \pm 0.3 to 7.1 \pm 0.3 g/dl, P < 0.05).
All and \alpha-adrenergic inhibitors and anti-GBM injury
Fig. 1. Effects of anti-GBM administration upon SNGFR and RPF in control group 1 (○) rats and in group 2 receiving continuous infusion of sar′-alaII (△), the All antagonist. The reductions in SNGFR and RPF were significant (*) in both groups but significantly greater in group 2.

Systemic oncotic pressure (πA) did not change significantly, although there was a numerical reduction. The decrease in πA was statistically significant (32 ± 2 to 27 ± 2 mm Hg, P < 0.02). The hematocrit remained identical in both periods, which was not the case in groups 1 and 2.

Phentolamine effects on EFP and Lm. The EFP increased numerically from 10 ± 1 mm Hg before antibody administration to 15 ± 2 mm Hg (N = 6) after anti-GBM was administered, but these values were not statistically different. The EFP values after anti-GBM administration were statistically higher than they were before its administration (9 ± 1, N = 18, vs. 15 ± 2, N = 6, P < 0.05). The same comparison was made for both groups 1 and 2 after anti-GBM administration. There were no statistical differences with the combined pre-anti-GBM Ab values (N = 18).

The Lm was decreased numerically from 0.10 ± 0.02 to 0.07 ± 0.01 nl · sec⁻¹ · g kidney wt⁻¹ · mm Hg⁻¹, N = 6, after anti-GBM administration. This was not a statistically significant change. When the experimental (post-anti-GBM) period is compared with a composite of pre-anti-GBM values from all

Table 1. Effect of sar′-alaII-angiotensin II infusion and phentolamine infusion on pressures, flows, permeabilities, and resistances before and after antglomerular basement membrane antibody administration

<table>
<thead>
<tr>
<th></th>
<th>MAP mm Hg</th>
<th>GFR ml/min/g kidney</th>
<th>Pgs mm Hg</th>
<th>Phs mm Hg</th>
<th>ΔP mm Hg</th>
<th>HPg mm Hg</th>
<th>SNGFR nl · min⁻¹ · g kidney⁻¹</th>
<th>RPF nl · min⁻¹ · g kidney⁻¹</th>
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<td>Group 1: Control</td>
<td>105</td>
<td>106</td>
<td>1.4 ± 1.0</td>
<td>1.0 ± 1.0</td>
<td>56 ± 2</td>
<td>54 ± 2</td>
<td>20 ± 1.3</td>
<td>19 ± 1.3</td>
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<td>±0.1 ±0.2</td>
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<td>±1 ±1</td>
<td>±4 ±2</td>
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</tr>
<tr>
<td>Group 2: Sar′-alaII infusion (N = 6)</td>
<td>97 ±3</td>
<td>98 ±2</td>
<td>1.4 ±0.4b</td>
<td>0.4b ±0.1</td>
<td>57 ±2</td>
<td>50 ±2</td>
<td>20 ±11b</td>
<td>17 ±18b</td>
</tr>
<tr>
<td>Group 3: Phentolamine infusion (N = 6)</td>
<td>99 ±2</td>
<td>97 ±3</td>
<td>1.2 ±1.2</td>
<td>1.2 ±1.2</td>
<td>55 ±4</td>
<td>54 ±4</td>
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* Values are the means ± SEM. Pre and post refer to pre-anti-GBM and post-anti-GBM administration. Abbreviations are defined in text.

b Post-anti-GBM period significantly different from pre-anti-GBM period, P < 0.05
Table 1. (Continued)

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Fig. 3. Effects of anti-GBM administration upon SNGFR and RPF in control group 1 (□) rats and in group 3 receiving continuous infusion of phenolamine (□), the $\alpha$-adrenergic blocker. Phenolamine infusion in group 3 rats prevented the reduction in both SNGFR and RPF observed in control group 1 after anti-GBM administration.

Fig. 4. Effects of anti-GBM administration upon $L_\alpha$ and $\Delta P$ in control group 1 (□) rats and in group 3 receiving continuous infusion of phenolamine (□), the $\alpha$-adrenergic blocker. Phenolamine infusion in group 3 rats prevented the reduction in $L_\alpha$ that was observed in control group 1 after anti-GBM administration. Also, $\Delta P$ did not change in group 3 rats.

three groups, there is a statistically significant decrease (0.13 ± 0.02, $N = 18$, vs. 0.07 ± 0.01 $nl\cdot sec^{-1}\cdot g$ kidney wt$^{-1}\cdot mm$ Hg$^{-1}$, $N = 6$, $P < 0.02$). This comparison is valid due to the statistical similarity of all three groups during the pre-anti-GBM period. Although the $L_\alpha$ in the phenolamine group decreases after anti-GBM administration, the decrease is less than that of the other two groups, indicating a partial beneficial effect of phenolamine on $L_\alpha$.

Immunopathologic studies. Histologic studies in all groups demonstrated changes similar to those previously noted [1, 2] and consisted predominantly of PMN accumulation and endothelial separation with only mild and focal fusion of epithelial foot processes (Fig. 5). Occasional monocytes were also seen in glomerular capillary lumens. The changes in the phenolamine group were perhaps slightly less, but consistent differences between the groups were not apparent. The moderate increase of PMN was observed within the glomerular capillary lumen and was quantitated. A mean of 7.7 ± 1.2 PMN ($N = 6$) was found in the sar$^4$-ala$^8$-All group, 4.8 ± 0.9 PMN ($n = 6$) in the phenolamine group, and 4.9 ± 1.2 PMN ($N = 6$) in the control group (Table 2). Due to individual variations, the differences in PMN counts between groups did not reach statistical significance. There was a relationship between the mean PMN count and the depression of SNGFR ($\Delta$SNGFR) for all groups (Table 2) ($N = 17$), but not the decrease in $L_\alpha$. The inhibitors did not cause any detected difference in antibody binding, complement
Fig. 5. Polymorphonuclear leukocytes (PMN). A PMN (arrows) are seen in the glomerulus of a rat from the sar-ala8-AII group. B Linear rabbit IgG deposits of anti-GBM antibody are present in a similar animal. C A PMN is seen displacing the endothelium (EN) of another rat in this group. EP is epithelial cell. (Original magnification for A and B, ×250; for C, ×12,300).

activity, circulating WBC counts, platelets, or percentage cell types. The antibody binding was measured in a separate group of rats, by the pair-label isotope technique [4, 5].

There was no statistical difference in the specific percent binding of 100 μg of radiolabeled anti-GBM globulin in the three groups (group 1, 1.86 ± 0.05, N = 2; group 2, 1.88 ± 0.03, N = 3; group 3, 1.84 ± 0.04, N = 3).

The effects of sar'-ala8-AII and phentolamine on the complement level was determined in another group of rats. The CH50 was determined in paired experiments after plasma expansion, 45 min after infusion of drugs (sar'-ala8-AII, N = 6, and phentolamine, N = 6) or isotonic sodium chloride and sodium bicarbonate solution (control, N = 3). There was no detectable difference between periods or between experimental groups. The preplasma expansion measurements made in two groups (sar'-ala8-AII, N = 3; phentolamine, N = 3) were not different from the postexpansion measurements. The circulating white blood cells, platelets, and percentage cell types were measured in the rats used for the CH50 determinations following the same protocol. The WBC counts were elevated after the plasma expansion in all groups (5800 ± 200 in hydrophilin vs. 10,500 ± 100 with plasma expansion, N = 15, P < 0.01). The WBC counts were not statistically different from the plasma expanded values after the sodium chloride and sodium bicarbonate infusion (9800 ± 1200, N = 2), the sar'-ala8-AII infusion (12,000 ± 1,900, N = 6), or the phenolamine infusion (8000 ± 1000, N = 6). The platelet counts decreased significantly after plasma expansion (7.97 × 10⁵ ± 0.20 × 10⁵ in hydropenia vs. 7.25 × 10⁵ ± 0.24 × 10⁵ in plasma expansion, N = 15, P < 0.05). The counts were not altered from the plasma expansion values by the infusion of sodium chloride and sodium bicarbonate solution (7.63 × 10⁵ ± 0.23 × 10⁵, N = 3), sar'-ala8-AII (7.04 × 10⁵ ± 0.30 × 10⁵, N = 6), or phentolamine (7.31 × 10⁵ ± 0.38 × 10⁵, N = 6). There was also no significant difference in the percentage of PMN's or lymphocytes after infusion of the inhibitors or in the control rats.

Discussion

The participation of the renin angiotensin and adrenergic nervous systems in the process of glomerular immune injury has been examined in the present study by using the blockers of AII and α-adrenergic activity (sar'-ala8-AII and phentolamine). It is clear from the present data that inhibition of the intrarenal action of AII in no manner prevents either the reduction in nephron plasma flow or the decrease in the glomerular permeability coefficient, which has been shown to follow the administration of large doses of anti-GBM. Examination of these data actually reveals that the concomitant infusion of sar'-ala8-AII results in a greater reduction in both SNGFR and RPF following antibody administration than was observed in the untreated control group. Phenolamine infusion, however, completely
prevented a reduction in RPF after anti-GBM and partially diminished the effects of the antibody upon LpA.

A full explanation of the specific mechanisms that produce the reduction in RPF after anti-GBM is not feasible from the present data. Previous studies have demonstrated that the vasoconstriction observed is complement dependent because complement depletion almost totally prevented the reduction in RPF at both low and very high doses of anti-GBM [2]. Administration of phentolamine in this study also completely prevented the reduction in RPF associated with glomerular immune injury. This response certainly suggests that some expression of α-adrenergic activity is functioning in this inflammatory mechanism. The complement cascade or some specific component thereof could either activate α-adrenergic receptors indirectly or some complement component could interact directly with an α-adrenergic receptor. Regardless of the specific mechanism, it is clear from the present and previous data that the complement system, probably acting via α-adrenergic vascular receptors, is involved in the reduction in RPF after anti-GBM administration and that this aspect of glomerular immune injury can be wholly prevented by α-adrenergic blockade.

The effect of sarLala8-AII that was observed is somewhat more difficult to explain in detail. The reduction in RPF after anti-GBM during sarLala8-AII infusion was definitely greater than that observed in the untreated control group. We have not observed a true agonist effect of sarLala8-AII in the plasma expanded rat, a conclusion supported by the comparison of the RPF, AR, and ER values prior to anti-GBM administration in all groups.

SarLala8-AII infusion did not, however, affect the reduction in LA following anti-GBM. Quantitatively, the decrease in LA consequent to anti-GBM was quite similar in both sarLala8-AII and control groups. These data then suggest that although sarLala8-AII infusion enhanced the immune-induced vasoconstriction, this agent apparently had no influence on the decrease in LA [181. Although the data provided in the study do not permit specific conclusions about the mechanism, we would speculate that two possibilities are not unreasonable. First, sarLala8-AII like All may have a significant effect on catecholamine release and reuptake [19, 20] and could magnify α-adrenergic-mediated vasoconstriction, or second, sarLala8-AII may somehow diminish the role of some vasodilator system normally activated during glomerular immune injury.

In previous studies from this laboratory, we have associated the reduction in LpA after anti-GBM with two morphologic findings: (1) the separation of the endothelial cell from the

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### Table 2. Correlation of changes in SNGFR with polymorphonuclear leukocyte (PMN) accumulation in the glomerulus

<table>
<thead>
<tr>
<th>Group 1: Control</th>
<th>Pre-anti-GBM SNGFR</th>
<th>Post-anti-GBM SNGFR</th>
<th>Change in SNGFR</th>
<th>PMN/glomerulus</th>
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</thead>
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<td></td>
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<th>Post-anti-GBM SNGFR</th>
<th>Change in SNGFR</th>
<th>PMN/glomerulus</th>
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<tr>
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<th>Post-anti-GBM SNGFR</th>
<th>Change in SNGFR</th>
<th>PMN/glomerulus</th>
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<td>± SEM</td>
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<td>Adjusted overall mean</td>
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* Percent determined as ΔSNGFR/pre-anti-GBM SNGFR.

* ND denotes no data available for PMN/glomerulus, experiment #13, hence N = 17 used for correlations.

* P value is for correlation with PMN/glomerulus by linear regression analysis.

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A full explanation of the specific mechanisms that produce the reduction in RPF after anti-GBM is not feasible from the present data. Previous studies have demonstrated that the vasoconstriction observed is complement dependent because complement depletion almost totally prevented the reduction in RPF at both low and very high doses of anti-GBM [2]. Administration of phentolamine in this study also completely prevented the reduction in RPF associated with glomerular immune injury. This response certainly suggests that some expression of α-adrenergic activity is functioning in this inflammatory mechanism. The complement cascade or some specific component thereof could either activate α-adrenergic receptors indirectly or some complement component could interact directly with an α-adrenergic receptor. Regardless of the specific mechanism, it is clear from the present and previous data that the complement system, probably acting via α-adrenergic vascular receptors, is involved in the reduction in RPF after anti-GBM administration and that this aspect of glomerular immune injury can be wholly prevented by α-adrenergic blockade.

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SarLala8-AII infusion did not, however, affect the reduction in LpA following anti-GBM. Quantitatively, the decrease in LpA consequent to anti-GBM was quite similar in both sarLala8-AII and control groups. These data then suggest that although sarLala8-AII infusion enhanced the immune-induced vasoconstriction, this agent apparently had no influence on the decrease in LpA [18]. Although the data provided in the study do not permit specific conclusions about the mechanism, we would speculate that two possibilities are not unreasonable. First, sarLala8-AII like All may have a significant effect on catecholamine release and reuptake [19, 20] and could magnify α-adrenergic-mediated vasoconstriction, or second, sarLala8-AII may somehow diminish the role of some vasodilator system normally activated during glomerular immune injury.

In previous studies from this laboratory, we have associated the reduction in LpA after anti-GBM with two morphologic findings: (1) the separation of the endothelial cell from the...
underlying GBM and (2) the migration of polymorphonuclear leukocytes (PMN) into the glomerular capillary and their attachment to the underlying GBM after removal of the endothelial cell [1, 2].

Based on our previous study with complement depletion, we can say that the elimination of the complement cascade prevented accumulation and attachment of PMN’s, but did not influence separation of the endothelial cell, a finding that appeared to relate primarily to fixation of anti-GBM and not to activation of complement [2]. An unexpected finding provided by the data of this study is that phentolamine infusion (group 3) also appears to have partially prevented much of the reduction in LpA observed in groups 1 and 2 after anti-GBM administration.

The LpA values in a paired determination in the phentolamine group before and after anti-GBM exhibited no statistically significant change. But, when the pre-anti-GBM LpA values for all three groups were combined (0.13 ± 0.02, N = 18), the LpA decreased significantly. Although the LpA was less affected by anti-GBM in the phentolamine group, the results indicate only a partial beneficial effect of α-adrenergic blockade upon the reduction in LpA with glomerular immune injury. No definite differences in the degree of endothelial cell separation were detected among the groups, and this may explain the modest reduction in LpA that was observed after anti-GBM in group 3. In addition, there was no morphologic finding unique to group 3 or prevented by phentolamine infusion. The only association observed was that the number of PMN’s accumulated within glomeruli after anti-GBM correlated reasonably well with the magnitude of reduction in SNGFR when all experimental animals were analyzed (N = 17).

It is tempting to speculate that any drug effects on the degree to which LpA decreases after anti-GBM administration might be also mediated by effects on the PMN [21, 25], but the morphologic finding of decreased PMN’s was not unique to the phentolamine-treated group.

The numerically lower but not statistically different value for RPF in the control condition in group 3 also does not explain this effect of phentolamine on LpA. Specific studies examining the binding of anti-GBM demonstrated that phentolamine infusion did not decrease the quantity of anti-GBM bound to the renal tissue. More recent data from our laboratory have also shown that if control RPF is decreased to values (208 nl/min) nearly identical to that observed with phentolamine via the infusion of cimetidine, a histamine-2 (H-2) receptor blocker, the magnitude of reduction in LpA after anti-GBM administration is in no way diminished and antibody binding is unaltered [26]. Activity of the complement system, as evaluated by blood CH50 and glomerular deposits of C3 as determined by immunofluorescence, was also unaffected by phentolamine infusion.

Obviously, further more specific studies are required to fully delineate the details of phentolamine effects on this early phase of immune injury in the glomerulus. It will also be of interest to determine if α-adrenergic blockade provides a significant and pronounced beneficial effect at later stages of this model of glomerular immune injury.

We have also recently observed that infusion of the H-2 receptor antagonist cimetidine also prevents changes in afferent and efferent arteriolar resistance following anti-GBM in this same experimental model, but this agent did not alter the magnitude of reduction in LpA [26]. How these expressions of α-adrenergic activity and H-2 receptor activity might interact in this model and whether this interaction is specific or nonspecific is not known.

The present studies suggest that concurrent infusion of sar'ala8-AII and phentolamine, inhibitors of AII and α-adrenergic activity, respectively, have reasonably profound, but differing influences on the multiple effects resulting from the acute infusion of anti-GBM. Sar’ala8-AII infusion magnifies, and phentolamine infusion prevents much of the immune induced vasocostriction. These drug effects seem to be mediated via their respective influences on α-adrenergic activity. A surprising and interesting, but possibly less specific, finding is the beneficial influence of phentolamine infusion in preventing in major part the immune-induced reduction in the glomerular permeability coefficient (LpA). It is apparent that multiple biologic systems contribute to the complex mechanisms producing glomerular immune injury.

Acknowledgments

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Reprint requests to Dr. R. C. Blantz, Department of Medicine, Veterans Administration Medical Center (111H), 3350 La Jolla Village Drive, San Diego, California 92161, USA.

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