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Immune complex effects on glomerular eicosanoid production and renal hemodynamics

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Immune complex effects on glomerular eicosanoid production and renal hemodynamics. We examined the effect of glomerular immune complex (IC) deposition on glomerular eicosanoid synthesis and the role of the eicosanoids in glomerular pathophysiology. Rats received daily 10 mg i.v. injections of native bovine gamma-globulin (NBGG) or cationic bovine gamma-globulin (CBGG) for 21 days; age-matched controls were maintained. Immunofluorescence and electron microscopy showed mesangial deposits of IC in the NBGG group and capillary wall deposits in the CBGG group, without light or electron microscopic evidence of leukocyte infiltration. One week after the last antigen dose, GFR was similar in all three groups, but RPF increased in the rats given CBGG; (8.37 ± 0.90 vs. control 5.54 ± 0.56 ml/min, $P < 0.05$). Glomerular synthesis of prostaglandin E_2 (PGE_2) and thromboxane B_2 (TxB_2) was normal in animals that received NBGG. Rats given CBGG had increased glomerular production of PGE_2, (2.23 ± 0.37 vs. control 1.03 ± 0.16 ng/mg glomerular dry wt, $P < 0.05$) and TxB_2 (3.12 ± 0.50 vs. control 0.48 ± 0.07 ng/mg glomerular dry wt, $P < 0.001$). Proteinuria only developed in the rats given CBGG, 86.6 ± 18 mg/24 hr, which correlated with glomerular TxA_2 synthesis, $r = 0.82$, $P = 0.01$. Acute administration of the TxA_2 synthesis inhibitor, UK-38,485, and a TxA_2 receptor antagonist, EP-092, to rats given CBGG did not affect GFR or RPF. The cyclo-oxygenase inhibitor, indomethacin, reduced both GFR and RPF by up to 40% in CBGG-immunized rats. Oral administration of UK-38,485 for six days to nephrotic rats did not result in a statistically significant reduction of proteinuria despite 85% inhibition of glomerular TxB_2. We conclude that cationic antigen induces a glomerular disease pathologically similar to membranous nephropathy. The increment of RPF is most probably due to increased glomerular PGE_2. The increased TxA_2 has no effect on glomerular hemodynamics and probably is not a component in the pathogenesis of proteinuria.

Glomerular synthesis of eicosanoids is increased in several models of immune and non-immune glomerular injury. Several studies have linked changes in glomerular synthetic patterns of eicosanoids with both toxic and immune injury to the kidney. In adriamycin nephrosis, a non-immune model of glomerular disease, a causal relationship between enhanced glomerular synthesis of thromboxane A_2 (TxA_2) and proteinuria has been noted [1]. Treatment of adriamycin nephrosis with a thromboxane synthetase inhibitor, UK-38,485, normalized glomerular TxA_2 synthesis and reduced but did not eliminate proteinuria [1]. In antibody-mediated glomerular injury (nephrotoxic serum nephritis; NSN), increments of glomerular TxA_2 are responsible for the acute reductions of renal plasma flow (RPF) and glomerular filtration rate (GFR) [2]; however, glomerular synthesis of PGE_2, a vasodilatory autacoid, increases RPF and GFR within 24 hours of immune injury [3]. Inhibition of glomerular TxA_2 synthesis in NSN did not prevent or reverse proteinuria [3]. The presence of infiltrating leukocytes in NSN obscures the cellular source of increased glomerular prostaglandin and thromboxane.

The goals of the present study were: first, to develop a non-infiltrative model of immune-mediated glomerular disease in which we could study the response of glomerular eicosanoid synthesis to immune complexes; and second, to examine the role of eicosanoids in the pathogenesis and pathophysiology of glomerular immune injury.

Several laboratories have established non-infiltrative models of glomerular disease utilizing differently charged antigens, which resulted in differential localization of immune deposits. It is well known that the glomerular capillary wall has a net negative charge due to both sialoglycoproteins and proteoglycans [4]. The negative charge creates a charge-selective barrier repelling anionic and attracting cationic molecules. Circulating antigens and/or antibodies may interact with this barrier, leading to a deposition of cationic particles in the capillary wall. Experimental serum sickness models, in the rabbit and mouse, have established a significant role for electrostatic charge [5–8]. In addition, antibody eluted from kidneys of a murine model of systemic lupus erythematosus was found to have a higher isoelectric point than those present within the circulation [9]. Also, in Heymann nephritis, cationic antibodies localized in the glomerulus better than anionic or neutral antibodies [10]. Finally, enzymatic removal of the proteoglycan which comprises the glomerular polyanion prevented the subsequent deposition of cationic immune complexes within the capillary wall [11].

In view of these observations on the role of electrostatic charge, we utilized cationic and neutrally charged bovine gamma globulin to develop a non-infiltrative model of glomerular disease in the rat. Our findings support the conclusion that in non-infiltrative immune complex glomerulonephritis, glomerular synthesis of PGE_2 and TxA_2 is increased, and glomerular PGE_2 can alter glomerular hemodynamics.

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Methods

Materials

Bovine gamma globulin (BGG), and fluoresceinated antisera to BGG, rat IgG and rat complement C3 were obtained from U.S. Biochemicals (Cleveland, Ohio, USA); alkaline phosphatase–conjugated goat anti-rat IgG was from Cappel Laboratories (Cochraneville, Pennsylvania, USA); the calcium ionophore, A23187, was from Calbiochem–Behring (San Diego, California, USA); *p*-nitrophenyl phosphate (PNP) and 1-ethyl–3-[3-dimethylaminopropyl]-carbodiimide hydrochloride (EDC) were from Sigma Chemical Co. (St. Louis, Missouri, USA) and ethylenediamine was from Fisher Scientific (Pittsburgh, Pennsylvania, USA). ^{3}H inulin, ^{14}C para-amino hippurate (PAH) and all radioactive ligands were obtained from New England Nuclear (Boston, Massachusetts, USA). The antiserum to TxB_2 was provided by Dr. William Campbell, Dallas, Texas, USA and the anti-PGE_2 was purchased from the Pasteur Institute, Paris, France. UK-38,485 (TxA_2 synthetase inhibitor), was from Pfizer Inc., Groton, Connecticut, USA. Indomethacin was from Merck, Sharp, and Dohme (West Point, Pennsylvania, USA) and EP-092 from Dr. R.L. Jones, Edinburg, Scotland.

Preparation of the cationic antigen

BGG was cationized as previously described [5, 12]. Two g BGG was dissolved in 20 ml 0.01 M NaCl, mixed with 40 ml ethylenediamine, and the pH adjusted to 7.0 with 2 N HCl. Four g EDC was added and the pH was maintained at 7.0. The mixture was left overnight at room temperature, then dialyzed against four changes of one liter of 0.15 M NaCl over 24 hours. Cationic BGG prepared by this procedure had an isoelectric point (pI) in excess of 9.5; the native BGG had a range of pI between 4.5 and 8.0, when measured by isoelectric focusing in a thin layer agarose gel with Pharmalytes^R 3-10.

Experimental protocol

Sprague–Dawley rats (Zivic Miller, Allison Park, Pennsylvania, USA), 150 to 200 g each, were divided into three groups. Each received daily intravenous injections for three weeks (21 injections). The first group (control, $N = 9$) received 1 ml phosphate buffered saline (PBS), the second group received 10 mg per day of native bovine gamma globulin (NBGG, $N = 6$), and the third group received 10 mg per day of cationic bovine gamma globulin (CBGG, $N = 9$). We wished to allow a period of equilibration after immunization before studying glomerular hemodynamics, and arbitrarily chose five to seven days after the last injection when all animals were studied.

Pre-immunized model

Twenty–two Sprague–Dawley rats were pre-immunized in the foot pads with 1 mg CBGG in complete Freund's adjuvant. Seven days later the rats received daily intravenous antigenic challenge with increasing doses of CBGG for six days (Day 1: 1 mg, Day 2: 2 mg, Day 3: 4 mg, Day 4: 6 mg, Day 5: 8 mg, Day 6: 10 mg).

Assessment of proteinuria

Rats were placed in metabolic cages, and urine was collected for 24 hours. Urinary protein was measured by sulfosalicylic acid–induced turbidimetry [13]. Proteinuria was assessed weekly during the 21 day protocol, and five to seven days after the last injection.

Hemodynamic studies

Rats were anesthetized with intraperitoneal pentobarbital (50 mg/kg) and surgery was performed on a thermocontrolled operating table to maintain a stable body temperature. After anesthesia, a tracheostomy was done, and the femoral and jugular veins were exposed and catheterized. Also, the bladder was exposed through a midline incision and catheterized for urine collection. ^{3}H inulin (sp. act. 250 μCi/mg) and ^{14}C PAH (sp. act. 230 μCi/mg) were mixed, diluted in normal saline, and infused at a rate of 1.5 ml/hr; the concentrations were adjusted to deliver 1.6 μCi/hr ^{3}H inulin and 1.2 μCi/hr ^{14}C-PAH. After an equilibration period of 60 minutes, clearance was measured during three periods. Each period consisted of a 20 minute urine collection with a 100 μl blood sample drawn from the tail artery at the midpoint of each collection. At the end of the clearance study a blood sample was drawn from the renal vein to calculate the percentage of PAH extracted.

Glomerular isolation and incubation

Following the clearance study, rats were killed by exsanguination. Kidneys were removed, and small portions of the cortex were taken for electron, light and immunofluorescence microscopy (*vide infra*). The remainder of the kidney tissue was placed in ice cold PBS (0.15 M NaCl, 0.01 M sodium phosphate, pH 7.6) and used for glomerular isolation. Glomeruli were isolated by a differential sieving technique as previously described [14]. The cortex was separated from the medulla and minced to a paste-like consistency. The minced cortical paste was pressed through a 106 μm metal sieve to remove large tubular fragments and suspended in PBS. This suspension was triturated and poured over a 75 μm sieve. The glomeruli were trapped on the 75 μm sieve, collected and suspended in PBS. The purity of the glomerular preparation was assessed by light microscopy and varied between 92 and 98%. The isolated glomeruli were transferred to a pre-weighed siliconized glass tube and suspended in Earle's Balanced Salt Solution (EBSS) with 20 mM HEPES and 3.0 μM calcium ionophore (A23187) at a total volume of 1 ml. This suspension was incubated for 30 minutes at 37°C with mild agitation. At the end of the incubation the glomeruli were placed on ice and centrifuged at 2,500 × g for 10 minutes. The supernate was collected and frozen for prostaglandin and thromboxane determination. The glass tube containing the glomerular pellet was placed in a 70°C oven for 24 hours and re-weighed to determine the glomerular dry weight.

Assay for prostaglandins and thromboxane

The radioimmunoassays for PGE_2 and TxB_2, the stable end product of TxA_2, were done using unextracted media. The sensitivity and cross reactivity of the antisera were previously described [15]. Incubation media without glomeruli were used as blank controls.

Immunofluorescence microscopy

The cortical tissue was snap–frozen in cold 2-methylbutane which was immersed in liquid nitrogen. Frozen tissue was

sectioned at 4 μm in a Spencer Cryostat (American Optical, Buffalo, New York, USA) and stored at −70°C. Staining was done as previously described [16]. Sections were layered with goat antiserum to rat IgG (rhodamine conjugated), bovine IgG (fluorescein conjugated) or rat complement C3 (fluorescein conjugated), all diluted at 1:20. In randomly selected animals, additional slides were layered with both anti-rat IgG and anti-rat C3, or with anti-rat IgG and anti-bovine IgG for double immunofluorescence to enable comparison of the distribution of these various immune reactants. Controls included sections of rat kidney from rats with the heterologous phase (day 5) and the autologous (day 14) phase of nephrotoxic serum nephritis [3] and rats which had been intravenously injected with a single dose of 3 mg CBGG. Rat C3, but not rat IgG or bovine IgG, was present in a linear pattern in glomerular capillary wall five days after injection of nephrotoxic serum; fourteen days after injection of nephrotoxic serum, both rat C3 and rat IgG were present in a linear capillary wall pattern; bovine IgG was absent. One hour after injection of CBGG into a normal rat, there was finely granular staining in capillary walls for bovine IgG; rat IgG and rat C3 were absent. Thus, by immunofluorescence on known positive and negative control tissue, the anti-sera used were specific, and cross reactivity was not a problem. Ouchterlony double immunodiffusion also confirmed appropriate binding without cross reactivity. Coded sections, to prevent observer bias, were examined microscopically (Ernst Leitz Inc., Wetzlar, West Germany).

Electron microscopy

Small, 1 mm portions of renal cortex were immersion-fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate adjusted to pH 7.3. The tissue was post-fixed in 1% OsO_4 and embedded in Spurr's epoxy. Ultrathin 50 nm sections were cut, picked up on uncoated nickel grids and stained with uranyl acetate and lead citrate. Coded specimens were evaluated in a Philips 201 C electron microscope (Philips Medical Electronics Div., Piscataway, New Jersey, USA) for the number and size of electron dense deposits in subendothelial, intramembranous, subepithelial and mesangial glomerular sites, in a manner identical to that previously described [16].

Light microscopy

Tissue was immersion–fixed in 10% formalin buffered to pH 7.5 with 0.01 M sodium phosphate for 24 hours. The tissue was then processed through graded alcohols and xylenes and infiltrated in molten Paraplast® (Fisher Scientific, Pittsburgh, Pennsylvania, USA). The tissue was embedded, sectioned at 4 μm and stained with periodic acid silver–methenamine or with hematoxylin and eosin. Coded sections were examined in a light microscope (American Optical, Buffalo, New York, USA). Cell nuclei were counted in 10 randomly–selected glomeruli from each rat, and cross–sectional glomerular area in the section measured, using morphometric software (Bioquant, Nashville, Tennessee, USA) in a microcomputer (Apple Computers, Sunnyvale, California, USA), coupled to a microscope–mounted video camera.

Determination of serum antibody

Prior to death, a blood sample was drawn from the aorta and antibody to bovine IgG was determined by an ELISA similar to that previously described [16], using modifications of the technique developed by Engvall [17] and characterized by Cantarero, Butler and Osborne [18]. Polystyrene microtiter plates (Cooke Engineering, Alexandria, Virginia, USA) were coated with bovine IgG (10 μg/ml) in 0.1 M sodium carbonate, pH 9.5; after coating, and between all subsequent steps, all wells were washed three times in PBS. The coated plates and uncoated control plates were "blocked" by incubation with 1% bovine serum albumin in PBS. Samples of rat serum or a standard pooled rat serum were incubated at room temperature for one hour (100 μl/well), serially diluted in PBS over a range from 1:10 to 1:270. One hundred μl of a 1:100 dilution of alkaline phosphatase–conjugated goat anti-rat IgG was added to each well for one hour at room temperature, and the plates were developed with 100 μl/well of 4 mg/ml PNP in glycine/NaOH buffer pH 10.5. The reaction was followed at 410 nm in a microplate reader (Dynatech, Inc., Alexandria, Virginia, USA) during the 60 minute linear phase of the reaction. Amount of antibody was determined by OD_{410} at 1:10 dilution interpolated into a semilog regression of a known standard for comparison of antibody level. The titer was computed by extrapolating OD_{410} for the serial dilutions against the number of dilutions. The titer of each sample was the reciprocal of the dilution at the point where the extrapolated line crossed a line 2 SD above the OD_{410} developed with normal rat serum as a negative control. Geometric mean titers were computed for each group. Coefficient of variation was less than 10% throughout.

In vivo cyclo-oxygenase inhibition

Experiments were done on a group of five rats after three weeks of CBGG injection (21-day model), and on a group of four rats after pre-immunization and six days of daily, intravenous antigenic challenge (pre-immunized model). The initial three clearance measurements were performed as described above, five to seven days after the last antigenic challenge. This was followed by intravenous infusion of indomethacin, 3.0 mg/kg, over 20 minutes. The clearances were then repeated. Indomethacin was dissolved in 0.5 M sodium carbonate, then neutralized with 0.5 M HCl and diluted with saline.

In vivo thromboxane inhibition and receptor blockade

Experiments were performed on four rats after the animals received three weeks of CBGG and became proteinuric. Initially, three clearance periods were done, then the rats received the TxA_2 synthetase inhibitor UK-38,485, 1.0 mg/kg i.v. over 20 minutes, followed by a 20 minute i.v. infusion of the TxA_2 receptor antagonist, EP-092, 2.0 mg/kg. After completion of both infusions a second set of clearances was determined. UK-38,485 was dissolved in a small volume of 0.1 N NaOH, then adjusted to pH 8.3 with 0.1 N HCl. Final dilution was made in saline. EP-092 was dissolved in ethanol and an equimolar amount of NaOH was added. The ethanol was then evaporated under nitrogen, and the residue was dissolved in saline.

Experimental protocol for thromboxane synthesis inhibition

Twenty–two rats were pre-immunized with CBGG as described above. After seven days the rats were divided into two groups of 11 rats each. The control group received daily i.v. CBGG for six days and 2 ml saline every 12 hours by oral gavage. The second group received daily i.v. CBGG for six

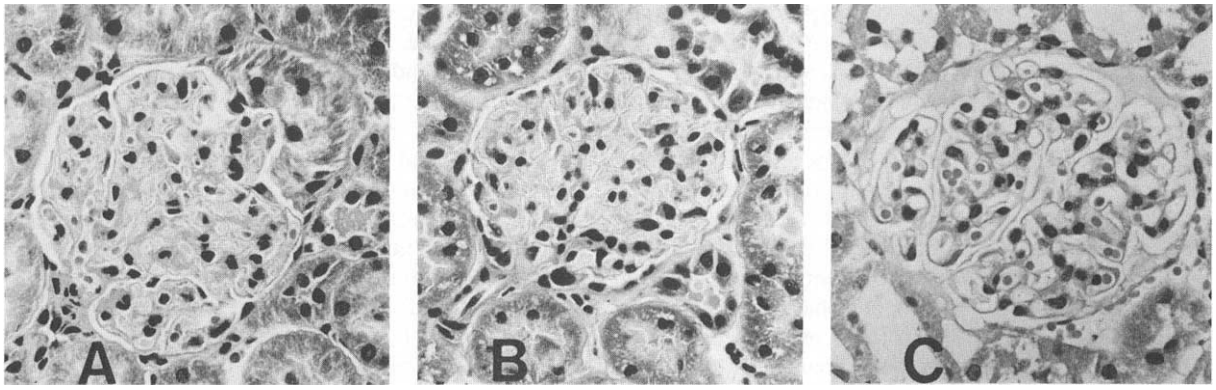

Fig. 1. *Light micrographs.* Representative glomeruli from age–matched control (A), NBGG-immunized (B) and CBGG-immunized (C) rats are shown. Note the absence of cellular infiltrate or proliferation, the widely patent capillary lumina, and lack of basement membrane changes in both rats with immune complex deposits without disease (B) and in nephrotic rats (C); both are indistinguishable from controls (A). (Hematoxylin and eosin stain, final magnification 333×).

Table 1. Morphometric observations in glomeruli from experimental rats

Treatment group (*N*)	Nuclei/glomerulus	Glomerular cross–sectional area $10^3\ \mu m^2$	Nuclei per glomerular area $cells/10^3\ \mu m^2$
NBG (6)	67.2 ± 10.13	10.0[a] ± 1.14	6.7[a] ± 0.43
CBGG (9)	67.3 ± 9.37	12.8 ± 0.89	5.2 ± 0.44
Control (9)	62.6 ± 9.11	12.9 ± 0.46	4.9 ± 0.64

[a] Different from both control and CMBGG, $P < 0.01$, by Fisher's protected *t*-test from ANOVA

Table 2. Individual 24-hour protein excretion from experimental rats

	Rats injected for 21 days		Pre-immunized rats[a]	
Controls	NBGG	CBGG	Treated with UK 38,485	Treated with saline
10.3	12	87	244	236
12	14	110	325	412
8	31	16	113	114
7.6	12	22	511	206
9	15	68	330	438
8.5	12	180	60	92
6.2		120		
10.4		90		
12.6		105		

[a] Data are 24-hour protein excretions of six rats of 11 in each group. These rats were maintained on treatment for six days. There was no difference in 24-hour proteinuria between UK 38,485-treated and nephrotic control animals in 11 rats per group observed on day three (data not shown). Five rats in each group were sacrificed after three days of treatment.

days and UK-38,485 40 mg/kg every 12 hours by oral gavage. UK-38,485 was dissolved in 0.1 N NaOH, then adjusted to pH 8.3 with 0.1 N HCl. Final dilutions to 4 mg/ml in the final solution were made into saline. Proteinuria was examined at day three and day six of intravenous antigen challenge.

Statistical analysis

All results were analyzed by Student's *t*-test (for 2 groups) or Fisher's protected *t*-test from analysis of variance (for 3 groups).

Results

Proteinuria

The rats that received NBGG ($N = 6$) over 21 days developed insignificant increments in protein excretion, 16.0 ± 3.0 mg/24 hr, compared to control ($N = 9$), 9.4 ± 0.5 mg/24 hours. In contrast, the rats that received CBGG ($N = 9$) developed marked proteinuria, 86.6 ± 18.8 mg/24 hours during the third week of antigen injection.

Pathology

Light microscopy was identical in both sham and antigen-injected rats and indistinguishable from normal age–matched controls. No mesangial proliferation or cellular infiltration were seen (Fig. 1, Table 1). The lack of cellular infiltration was a consistent finding up to two months after the last antigen injection. However, immunofluorescence microscopy showed conspicuous differences between rats receiving CBGG and NBGG (Fig. 2). Administration of NBGG resulted predominantly in mesangial deposits of BGG and rat IgG; however, no complement staining was seen. The injection of CBGG induced uniform, granular capillary–wall deposits of BGG, rat IgG, and complement C3. None of the glomeruli from control rats showed immunofluorescent staining for BGG, rat IgG, or C3. Electron microscopy of glomeruli from CBGG-injected animals showed broadening of the foot processes and numerous, regularly–spaced electron–dense subepithelial deposits (Fig. 3) as well as mesangial deposits. There was no thickening of the capillary basement membrane and no neutrophil or monocyte infiltration. In contrast, electron microscopy after injection of NBGG showed numerous, small mesangial deposits and rare, isolated subepithelial deposits, with no other abnormality evident.

Determination of serum antibody levels

By analysis of variance, there was no difference in the amount of antibody in the sera from rats immunized for 21 days with NBGG (mean ELISA optical density ± SE = 469 ± 75,

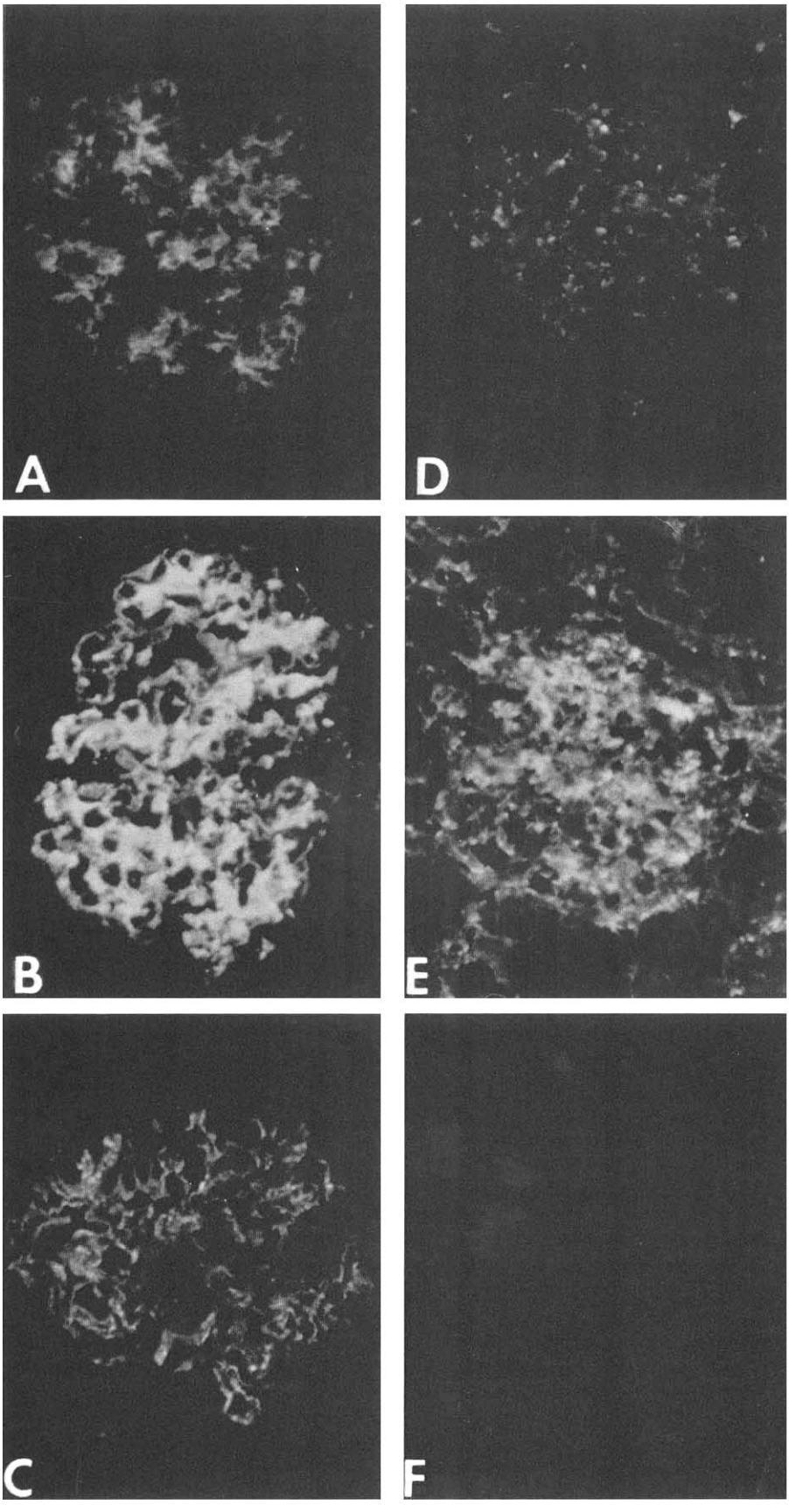

Fig. 2. *Immunofluorescence micrographs.* Immunofluorescence micrographs representative of rats immunized with CBGG (left column) or NBGG (right column) are shown. In rats immunized with CBGG, there were granular capillary wall and mesangial deposits of BGG (**A**), rat IgG (**B**), and rat complement C3 (**C**). Rats immunized with NBGG had purely mesangial deposits of BGG (**D**) and rat IgG (**E**); complement C3 was not observed (**F**). (final magnification 490×).

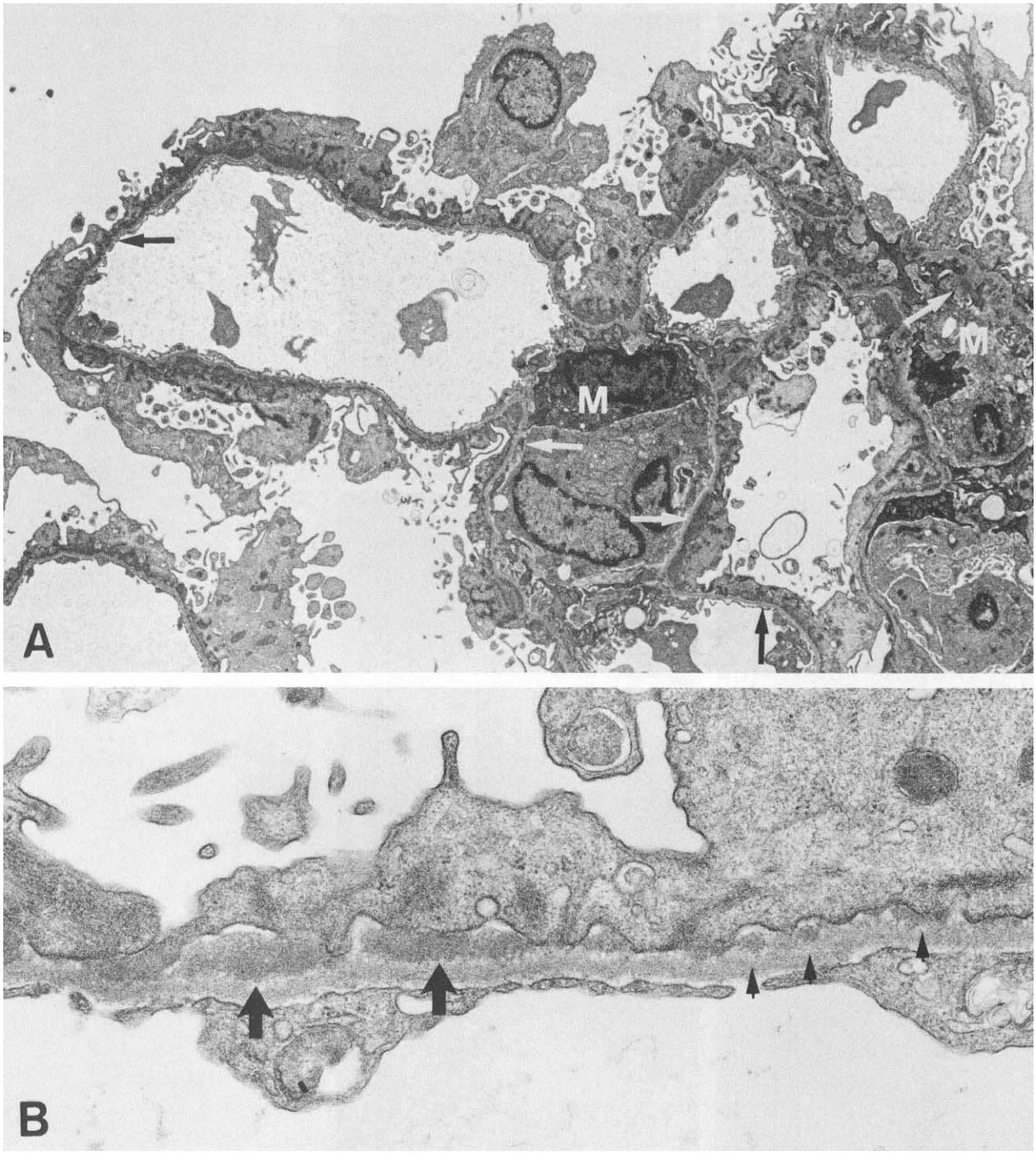

Fig. 3. *Electron micrographs.* By low power magnification (A), a glomerulus from a rat immunized with CBGG reveals numerous small to moderate distinct electron dense deposits (arrows) in mesangium (M) and in the capillary wall. There is extensive epithelial podocyte fusion; endothelium is intact and no leukocytic or thrombocytic infiltrate is present (final magnification 4,500×). Higher power examination (B) reveals regularly spaced electron–dense deposits (arrowheads) are widely distributed along the subepithelial side of the glomerular basement membrane. In most rats, focal areas of the capillary wall contain larger, coalescent epimembranous deposits (arrows). The epithelial podocytes are broadened to form elongate syncytia; parts of only two swollen podocytes are present in this field (final magnification 32,000×). No deposits were observed in the capillary walls of rats immunized with NBGG, or of sham immunized rats. NBGG rats did show numerous small mesangial deposits.

equivalent to 818 μg/ml Ab in standard serum) compared to rats immunized for 21 days with CBGG (560 ± 83, equivalent to 1821 μg/ml Ab in standard serum); both groups of immunized rats had approximately 30-fold increases in anti-BGG antibody compared to normal rat sera (90.0 ± 7.0, equivalent to <30 μg/ml Ab in standard serum) ($P < 0.01$). The optical densities of serial dilutions of sera from rats given CBGG and rats given NBGG were linear with the number of dilutions (correlation

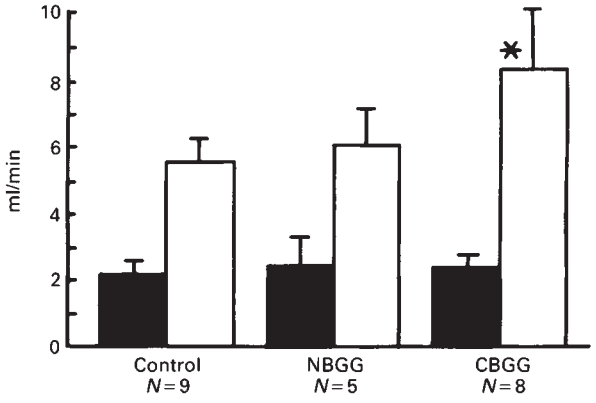

Fig. 4. *Renal hemodynamics.* Mean GFR (■) and RPF (□) (ml/min) are shown for the three groups of rats. Although there was no difference in GFR among the groups, RPF was increased in the CBGG group, 8.37 ± 0.90 vs. control, 5.54 ± 0.56, or NBGG, 6.05 ± 0.09 (*$P < 0.05$ vs. control or NBGG).

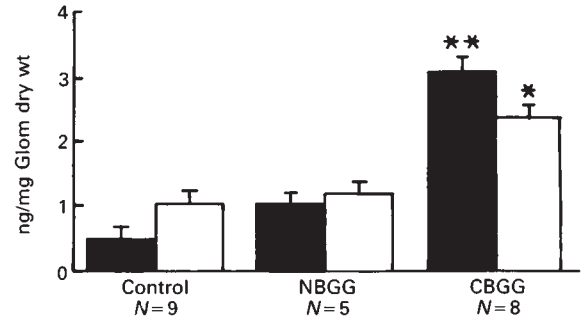

Fig. 5. *Eicosanoid synthesis.* Glomerular synthesis of PGE_2 (□) and TxB_2 (■) (ng/mg glomerular dry wt.) are indicated for control, NBGG and CBGG groups. Significant increases in PGE_2 (*$P < 0.01$) and TxB_2 (**$P < 0.001$) were observed in CBGG rats versus controls, whereas no statistically significant differences were observed between NBGG and control.

coefficients ranged from 0.89 to 0.97, all $P < 0.001$) and therefore the optical densities developed were linear with the logarithm of serum concentration. Titers were computed from the semilog regressions: the geometric mean titers were 1177 $\times\div$ 1.87 for NBGG sera and 1126 $\times\div$ 1.85 for CBGG sera; the geometric mean titer for three normal rat sera was less than 10, by definition.

Renal hemodynamics

Figure 4 illustrates the changes in renal hemodynamics. Clearances were performed five to seven days after the last antigen or sham injection. GFR was similar in all three groups (2.3 ± 0.22 ml/min, $N = 9$ for control, 2.46 ± 0.60, $N = 5$ for NBGG and 2.69 ± 0.23 ml/min, $N = 9$ for CBGG). RPF was significantly increased from 5.54 ± 0.56 ml/min for control to 8.37 ± 0.90 ml/min, $P < 0.05$ after 21 daily injections of CBGG. Therefore, the filtration fraction was reduced from 0.42 to 0.32. RPF after administration of NBGG was 6.05 ± 0.90 ml/min which was not different from control values.

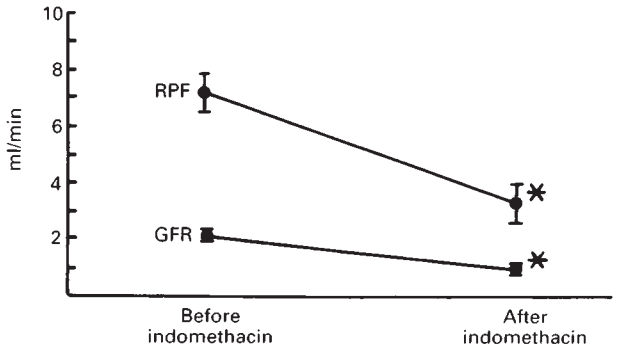

Fig. 6. *The effect of indomethacin on hemodynamics.* The cyclooxygenase inhibitor, indomethacin 3 mg/kg, reduced the GFR and RPF in rats given CBGG. Points represent mean ± SEM of five clearance experiments. Before administration of indomethacin, GFR was 1.91 ± 0.38 and RPF was 7.36 ± 1.0 ml/min. After indomethacin, both GFR and RPF were decreased to 1.22 ± 0.31 and 4.60 ± 0.97 ml/min, respectively (*$P < 0.05$).

Glomerular production of PGE_2 and TxB_2

The glomerular synthesis of PGE_2 and TxB_2 is shown in Figure 5. There was no statistical difference in either PGE_2 or TxB_2 production between control ($N = 9$) and NBGG ($N = 6$) rats. In rats that received CBGG ($N = 9$), however, the glomerular production of PGE_2 was increased (2.23 ± 0.37 vs. 1.03 ± 0.16 ng/mg glomerular dry wt for control, $P < 0.01$). TxB_2 increased also from 0.48 ± 0.07 to 3.1 ± 0.50 ng/mg glomerular dry wt ($P < 0.001$), and a significant correlation ($r = 0.82$, $P < 0.01$) was found between glomerular TxB_2 synthesis and the logarithm of 24 hours protein excretion.

Cyclo-oxygenase inhibition

To determine if the increment of RPF is secondary to increased PGE_2, we administered indomethacin, 3 mg/kg over 20 minutes, to five rats after they received CBGG injections for three weeks. The results are shown in Figure 6. Before administration of indomethacin, GFR was 1.91 ± 0.38 and RPF was 7.36 ± 1.0 ml/min. After indomethacin, both GFR and RPF were significantly decreased, to 1.22 ± 0.31 and 4.60 ± 0.97, respectively ($P < 0.05$). The decrements of GFR and RPF were of the same magnitude (37%); therefore, filtration fraction was unchanged. Glomerular PGE_2 synthesis was inhibited by 80% to 0.39 ± 0.19 ng/mg glomerular weight.

Thromboxane synthesis inhibition and receptor blockade

To examine the effect of increased glomerular TxA_2 on renal hemodynamics, we administered UK-38,485, a TxA_2 synthesis inhibitor, and EP-092, a TxA_2 receptor blocker, to four rats after they received CBGG for three weeks. There was no statistical difference in either GFR or RPF before and after both thromboxane synthetase inhibition and receptor blockade (pre-treatment GFR was 2.2 ± 0.42 and RPF was 8.1 ± 0.9; post-treatment GFR was 1.9 ± 0.32 and RPF was 7.8 ± 0.83).

Pre-immunized CBGG model

Proteinuria developed in 60 to 70% of the 11 untreated rats after four intravenous injections of CBGG and in 100% of rats after six injections (240 ± 45 mg/24 hr) in pre-immunized rats.

Light, immunofluorescence, and electron microscopy of the kidney was similar to rats which received three weeks of CBGG injections. Interestingly, although the antibody level in sera from rats pre-immunized and challenged with cationic BGG (484 ± 45.5, equivalent to 935 μg/ml Ab in standard serum) was not different from rats immunized for 21 days with the same antigen (560 ± 82.6, equivalent to 1821 μg/ml Ab in standard serum), the titers were substantially higher in sera from preimmunized rats (>9000) than that of rats immunized for 21 days ($1126 \overset{\times}{\div} 1.85$, $P < 0.001$). Furthermore, glomeruli isolated two to three days after six intravenous injections of CBGG showed increased production of TxB_2 (2.17 ± 0.16 vs. control 0.48 ± 0.07 ng/mg glomerular wt, $P < 0.01$). PGE_2 was also increased but the increment did not reach statistical significance (1.27 ± 0.29 vs. control 1.03 ± 0.16 ng/mg glomerular wt).

Hemodynamics and cyclooxygenase inhibition in the preimmunized model

In rats with nephrosis induced by preimmunization and subsequent i.v. challenge, the GFR was 1.96 ± 0.21 and the RPF 6.43 ± 0.55 ml/min. The filtration fraction was therefore 0.304 ± 0.027. Thus, GFR was no different from normal controls (2.3 ± 0.22 ml/min), but was significantly less than the mildly increased GFR (2.69 ± 0.23 ml/min) observed in the rats given CBGG for 21 days ($P < 0.05$). Likewise, the modest increase in RPF in pre-immunized rats relative to normal controls (5.54 ± 0.56 ml/min) was not significant, but was significantly less than the RPF in the rats given 21 i.v. doses of CBGG (8.37 ± 0.90 ml/min, $P < 0.05$). The filtration fraction in the pre-immunized rats was decreased to an extent similar to that observed in rats given 21 daily i.v. doses of CBGG.

After indomethacin infusion (3 mg/kg over 20 min), the GFR decreased to 1.65 ± 0.17, and the RPF decreased to 5.13 ± 0.28 ml/min. Both the GFR and RPF were significantly decreased (paired t's ≥ 2.6, P's < 0.05) from their pre-indomethacin levels, to a proportionate degree. The filtration fraction remained at 0.322 ± 0.05 after indomethacin treatment. The approximately 20% reduction in GFR and RPF by indomethacin observed in the pre-immunized model is significantly less than the nearly 40% reduction in these parameters observed in rats given CBGG for 21 days after identical indomethacin treatment, in keeping with the relatively lower RPF and PGE_2 synthesis observed in the pre-immunized model. Protein excretion before indomethacin (0.41 ± 0.05 mg/min) was unchanged after indomethacin (0.38 ± 0.03 mg/min).

Thromboxane synthetase inhibition and protein excretion in the preimmunized model

Pre-treatment with UK-38,485 in 11 preimmunized rats resulted in a slight reduction in proteinuria after three days of intravenous CBGG, but this decrement did not reach a statistical significance (77.3 ± 34 vs. control 169 ± 43 mg/24 hr). The difference in proteinuria disappeared after six days of CBGG injections, despite continuous administration of the thromboxane synthetase inhibitor, 80 mg/kg/day (263 ± 66 mg protein, 24 hr for UK-38,485 treated rats, vs. 249 ± 59 mg/24 hr for untreated rats). At the same time, the UK-38,485 treatment was accompanied by greater than 85% reduction in glomerular TxB_2 (0.27 ± 0.09 ng/mg glomerular wt after UK-38,485 treatment vs. 2.17 ± 0.18 ng/mg glomerular wt for the untreated).

Discussion

Previous studies implicate glomerular eicosanoids as important mediators of glomerular injury in toxic nephrosis induced by adriamycin [1], or in NSN glomerulonephritis [2, 3]. NSN is characterized by leukocytic infiltration of glomeruli, which could be the source of the eicosanoids. In the present work, we sought to evaluate glomerular eicosanoids and their hemodynamic role in a model of immune complex glomerulonephritis without infiltrating leukocytes. To this end, we adapted a previous murine model of membranous nephropathy induced by CBGG [16] to rats.

Cationic antigens or antibodies promote granular capillary–wall deposits of immune complexes. Like the active models in mice and rabbits [6, 16] and a passive in situ model in rats [7], the immune response to chronic cationic antigen administration elicited subepithelial deposits of BGG antigen and rat IgG and C3 associated with heavy proteinuria in our model. Border et al reported heavy mesangial deposits in rats given cationized albumin, and capillary wall deposits after injection of anionic or neutral albumin [19]. None of the rats immunized with any form of albumin developed proteinuria in excess of 50 mg/24 hr [19]. The reason for the divergence of our model from that of Border et al in the same strain of rat might be due to dissimilar immune responses related to the differences in antigens and immunization protocols.

Light and electron microscopic evaluation of glomeruli from rats given CBGG showed no leukocyte infiltration. Therefore, the enhanced synthesis of PGE_2 and TxA_2 we observed most probably represents production by endogenous glomerular cells since both glomerular epithelial and mesangial cells synthesize PGE_2 and TxA_2 [20, 21].

Increments of glomerular synthesis of PGE_2 and TxA_2 were observed only in the CBGG animals that developed proteinuria. Enhanced glomerular synthesis of PGE_2 and TxA_2 agrees with previous observations of arachidonic acid metabolism in experimental glomerular disease (NSN) at three to 24 hours and 14 days after immunologic injury [2, 3]. The mechanism underlying enhanced arachidonic acid metabolism by glomerular cells is unclear. PGE_2 and TxA_2 synthesis by cultured rat mesangial cells and rabbit peritoneal macrophages is stimulated by immune complexes and by activated complement [22–25]. Hence, it is possible that deposition of immune complexes in the glomerulus and perhaps subsequent complement activation can stimulate synthesis of PGE_2 and TxA_2 by glomerular mesangial and/or epithelial cells.

The hemodynamic effect of glomerular eicosanoids appeared to be vasodilation in both the chronically immunized and the pre-immunized models. While all animals maintained normal GFR, RPF was increased only in the rats given CBGG for 21 days. The modest increase in RPF in the pre-immunized rats was significantly less than the pronounced glomerular hyperemia observed in rats given CBGG for 21 days, although the decreased filtration fractions in the two groups were identical. The increment in RPF apparently maintained normal GFR in the rats given CBGG for 21 days. In contrast, we did not find any alteration in glomerular hemodynamics after administration of NBGG. Glomerular PGE_2 had important vasodilatory ac-

tions, as shown by the deleterious effect of indomethacin, a cyclooxygenase inhibitor, on renal function. Acute administration of indomethacin, which reduced glomerular PGE_2 synthesis by 80%, resulted in proportionate decreases in GFR and RPF, leaving filtration fractions unchanged, in both the 21 day model and the pre-immunized model. As predicted from the more modest increments in RPF and glomerular PGE_2 production in the pre-immunized model, the hemodynamic effects of indomethacin on the pre-immunized model were quantitatively less pronounced but qualitatively the same as in the 21 day model.

Glomerular TxA_2 was hemodynamically unimportant as demonstrated by the lack of changes in RPF and GFR after concurrent administration of both a thromboxane synthetase inhibitor, UK-38,485, and a thromboxane receptor antagonist, EP-092, which is a competitive inhibitor of the thromboxane receptor in smooth muscle and platelets [26, 27]. We used doses of these drugs previously demonstrated to be effective in glomerular disease in rats [2, 3] and we documented an 85% reduction in glomerular TxB_2 in our rats.

These results are in agreement with the findings of Stork and Dunn who found no increments of GFR and RPF in the autologous phase of rat NSN after treatment with thromboxane synthetase inhibitors (UK-38,485 and OKY-1581) and the receptor blocker EP-092, whereas indomethacin reduced both GFR and RPF by 50% [3]. Clinical reports have also documented the deleterious effects of cyclooxygenase inhibition on renal function in diverse types of glomerular disease. In patients with chronic glomerulonephritis [28, 29], lupus nephritis [30] and the nephrotic syndrome [31, 32] administration of nonsteroidal anti-inflammatory drugs can result in marked reduction of GFR.

The increment of glomerular TxA_2 after administration of CBGG was significantly correlated with protein excretion; this finding has been demonstrated in other models of immune glomerular disease [2, 33]. In the pre-immunized nephrotic rats, TxA_2 inhibition transiently reduced proteinuria at three days but, after six days of treatment with UK-38,485, protein excretion in both treated and untreated animals was identical, despite an 85% inhibition in TxA_2 synthesis. A primary, causal action of TxA_2 in proteinuria is therefore unlikely.

Our efforts to compare the effects of mesangial (NBGG) versus capillary wall (CBGG) deposits of immune complexes on glomerular eicosanoids and hemodynamics were complicated by the lack of complement deposition and objective signs of renal disease in the NBGG group. It is possible that the lack of renal injury in the NBGG group of rats is related to the degree of immune complex or complement deposition rather than the exclusive deposition of complexes in the mesangium. The lack of complement deposition in NBGG rats may also account for lack of increments in eicosanoids or hemodynamic changes. Mesangial cells synthesize more eicosanoids than glomerular epithelial cells [20, 21] and are stimulated to produce increased PGE_2 by complement [22, 23]. The explanation for the absence of complement deposition in the NBGG model may be related to fewer immune complexes deposited, diminished access of complement components to the mesangial area, greater catabolism of complement components in mesangial versus capillary sites, or some combination of these. Different subclass distribution of antibody elicited to NBGG versus CBGG may also be a factor.

In summary, we observed that immunization of rats with CBGG induced a proteinuric glomerular disease morphologically similar to membranous nephropathy, with concomitant increases of glomerular eicosanoid production, apparently from endogenous glomerular cells. Inhibition of PGE_2 resulted in reduction of both GFR and RPF, but inhibition of TxA_2 had no effect on glomerular hemodynamics or protein excretion. Therefore, the increment in RPF is most probably due to increased PGE_2, but the increment in TxA_2 does not exert a measurable vasoconstrictor effect and probably is not a component in the pathogenesis of the proteinuria.

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