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Immune-mediated mesangial cell injury—Biosynthesis and function of prostanoids

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Immune-mediated mesangial cell injury—Biosynthesis and function of prostanoids. We studied the formation of cyclo-oxygenase products in a rat model of mesangial cell injury, in order to determine a possible role of prostaglandin E_2 (PGE_2), prostaglandin I_2 (determined as 6-keto-$PGF_{1\alpha}$ and thromboxane A_2 (TxA_2) in immune-mediated glomerular disease. Selective immune-mediated mesangial cell injury was induced by i.v. administration of a rabbit anti-rat thymocyte antiserum (ATS). Intravenous ATS leads to immune deposits in the mesangium followed by mesangiolysis and the infiltration of polymorphonuclear granulocytes and monocytes. Glomerular TxB_2 formation two hours (292 ± 27 pg/mg/min) and 48 hours (396 ± 69 pg/mg/min) following antibody was significantly ($P < 0.05$) higher compared to animals receiving non-antibody rabbit IgG (TxB_2: 2 hr 143 ± 13; 48 hr 171 ± 32 pg/mg/min). Treatment with cobra venom factor (CVF) and the reduction of glomerular monocyte infiltration inhibited the increase of glomerular TxB_2 formation significantly. Depletion of granulocytes with a rabbit anti-rat granulocyte serum had no effect on glomerular prostanoid formation following ATS. Glomerular PGE_2 and 6-keto $PGF_{1\alpha}$ production was not altered following ATS. Inulin clearance in rats with immune-mediated mesangial cell injury was significantly ($P < 0.001$) lower at two hours (456 ± 24 µl/min/100 g body wt) and 48 hours (433 ± 54 µl/min/100 g body wt) compared to their corresponding control animals which were treated with non-antibody IgG (2 hr: 914 ± 51; 48 hr: 694 ± 79 µl/min/100 g body wt). Pretreatment of rats with indomethacin (Indo) or with the thromboxane synthetase inhibitor UK 38485 prevented the decrease in inulin clearance following ATS at two hours (Indo: 800 ± 67; UK 38485: 923 ± 115) and at 48 hours (Indo: 697 ± 60; UK 38485: 654 ± 99). The data demonstrate that selective, immune-mediated mesangial cell injury in rats is associated with increased glomerular TxB_2 formation. Complement and monocyte/macrophage depletion reduces TxB_2 production. The fall in inulin clearance following ATS is ameliorated when the rats receive indomethacin or the Tx synthetase inhibitor UK 38485. Thus, elevated TxB_2 formation might mediate the reduction in GFR in this model of glomerular immune injury.

Since Lianos, Andres and Dunn [1] observed that the induction of an immune-mediated glomerular injury stimulates glomerular prostanoid formation, several studies demonstrated the involvement of arachidonic acid metabolites in the pathophysiology of glomerular disease [2–8]. The effects of prostaglandins (PGs), thromboxane A_2 (TxA_2), and leukotrienes on glomerular hemodynamics and proteinuria in immune glomerular injury seem to be dependent on the type of animal models, the cellular sources of eicosanoid formation and on the relative pattern of the diverse cyclooxygenase products formed in the glomerulus.

The hemodynamic consequences in glomerular disease arising from altered eicosanoid formation are partially explained by the effects which arachidonic acid metabolites exert on glomerular mesangial cells. Prostaglandins [9], leukotrienes [10, 11] and thromboxane [12] modulate the contractile properties of mesangial cells and can mediate glomerular filtration rate due to changes of the ultrafiltration coefficient [13].

In most animal models where changes in glomerular hemodynamics are attributed to a function of eicosanoids, mesangial cells were not primary targets of the immune injury. It is unclear, however, which role eicosanoids might have on glomerular hemodynamics, when the mesangial cells are selectively injured by an immune mediated process.

Therefore, we studied prostaglandin E_2 (PGE_2) and thromboxane B_2 (TxB_2) formation in a rat model of selective immune-mediated mesangial cell injury. The lesion was induced with a polyclonal rabbit anti-rat thymocyte antiserum. We also examined the role of prostanoids on GFR in this model. Our findings demonstrate that increased TxA_2 formation following ATS depends on an intact complement system and that the stimulated TxA_2 biosynthesis mediates the decrease in GFR which appears in this model of glomerular mesangial cell injury.

Methods

Materials

Male Wistar rats and male Lewis rats, weighing 190 to 210 g were obtained from Ivanovas (Kisslegg, FRG). ^3H-PGE_2 and ^3H-TxB_2 were purchased from DuPont-New England Nuclear Research products (Dreieich, FRG). PGE_2 and TxB_2 standards were purchased from Upjohn (Kalamazoo, Michigan, USA).

Indomethacin was obtained from Sigma Chemicals (München, FRG). UK 38485 was a gift from Pfizer (Karlsruhe, FRG). Cobra venom factor was obtained from Cordis Laboratories (Miami, Florida, USA). Antisera to rat C3, rat IgG and rabbit IgG were from Camon (Wiesbaden, FRG).

Inactin was purchased from Byk-Gulden (Konstanz, FRG). Bolton-Hunter ^{125}I-reagent was purchased from DuPont, New

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England Nuclear Research Products. Sephadex G50 was from Pharmacia (Freiburg, FRG). Inulin was from Sigma Chemicals.

Experimental protocols

All studies followed a similar experimental protocol in which groups of rats had immune mediated mesangial cell injury. TxB_2 and PGE_2 were determined in isolated glomeruli at two hours and/or 48 hours following antibody or non-antibody-rabbit IgG injection. Renal tissue was obtained at two hours and/or 48 hours after ATS. In the same time intervals, inulin clearances were determined. The following groups of animals were studied.

Immune mediated mesangial cell injury (MCI). To assess the effect of the induction of mesangial cell injury (MCI) on glomerular PGE_2 and TxB_2 production, rats received either anti-thymocyte antiserum (see below) or equal amounts of non-antibody rabbit IgG. Prostanoid formation in glomeruli was assessed at two and 48 hours.

MCI and complement depletion. Two groups of rats ($N = 7$, each group) were complement depleted with cobra venom factor (CVF) prior to antibody injection to determine the effect of glomerular complement activation by immune complex formation on glomerular prostanoids. Two additional groups of rats ($N = 7$, each group) received the CVF carrier. MCI animals (one group with CVF, one group with CVF carrier) received rabbit anti-thymocyte antiserum (ATS). The remaining two groups of rats received non-antibody rabbit IgG. Glomerular prostanoid formation was assessed two hours after antibody.

MCI and polymorphonuclear granulocytes (PMN) depletion. Two groups of rats ($N = 7$, each group) were PMN depleted with a rabbit anti-rat PMN antiserum prior to ATS injection to assess a possible role of polymorphonuclear granulocytes on PGE_2 and TxB_2 formation in glomeruli of rat kidneys with MCI. Rats with MCI received ATS. Control rats were injected with non-antibody rabbit IgG. Glomeruli were isolated after two hours for prostanoid determination and histologic studies.

MCI and monocyte depletion. Two groups of rats ($N = 7$ each group) were monocyte depleted with a rabbit anti-rat monocyte antiserum prior to ATS injection in order to evaluate the possible role of monocytes/macrophages on glomerular prostanoid and thromboxane B_2 formation of rats with MCI. Since our combined in vivo in vitro studies revealed that monocytes were present in glomeruli at 48 hours after antibody, we studied glomerular cyclo-oxygenase production at this time.

Inulin clearance—The effects of indomethacin and UK 38485. Two groups of rats received ATS to assess the influence of MCI on inulin clearance. Control animals were treated with non-antibody rabbit IgG. Inulin clearance was evaluated at two hours (MCI: $N = 7$; IgG: $N = 7$) and 48 hours (MCI: $N = 14$; IgG: $N = 7$). Additional groups of animals received the cyclo-oxygenase inhibitor indomethacin or the thromboxane synthetase inhibitor UK 38485. Inulin clearance in Indo and UK treated rats was determined at two hours (Indo: $N = 7$; UK 38485: $N = 7$) and 48 hours (Indo: $N = 6$; UK 38485: $N = 7$).

Effect of complement and granulocyte depletion and indomethacin and UK 38485 on glomerular ^{125}I-labelled ATS binding. Five groups of rats ($N = 5$, each group) received either no treatment, CVF, anti-PMN antiserum, indomethacin or UK 38485 to assess whether complement or leucocyte depletion or the inhibition of cyclo-oxygenase or thromboxane synthetase might have an effect on ATS binding. Control animals remained untreated. Two hours after ATS antibody ^{125}I binding to isolated glomeruli was assessed.

Experimental procedures

Induction of mesangial cell injury. Selective immune-mediated mesangial cell injury (MCI) was induced in male Wistar rats (190 to 219 g) by i.v. injection of 5 mg/100 g body wt of an IgG preparation of rabbit anti-rat thymocyte antiserum (ATS). ATS was induced in New Zealand rabbits by repeated immunization with 2×10^8 thymocytes from Lewis rats, combined with Freund's complete or incomplete adjuvant. The IgG preparation of the rabbit serum was made by caprylic acid precipitation according to the method described by Steinbuch and Andran [14]. Reactivity of the antibody was tested on cultured mesangial cells grown as previously described [15] by indirect immunofluorescence.

Radiolabelling of ATS. IgG was labelled with ^{125}I using the Bolton-Hunter reagent (New England Nuclear, Dreieich, FRG) in order to study the effect of different interventions (complement and PMN depletion, cyclo-oxygenase and thromboxane synthetase inhibition) on ATS-IgG binding on glomeruli in vivo. Gel filtration (sephadex G 50) was used for separation of labelled protein from the ^{125}I-glycine conjugate. The final preparation of ^{125}I anti-ATS-IgG was 92% precipitable in 10% TCA. The specific activity was 4×10^5 cpm/μg protein. The ^{125}I labelled IgG was mixed with unlabelled ATS-IgG prior to i.v. injection. Glomeruli from the kidneys were isolated two hours after ^{125}I-labelled ATS IgG and the amount of antibody bound to glomeruli was assessed according to Salant, Darby and Couser [16]. Glomeruli were isolated as described below, counted visually in a Fuchs-Rosenthal hemocytometer and the amount of ^{125}I-labelled antibody was assessed by counting the glomeruli in gamma-counter (Packard). Results are expressed in counts/76,000 glomeruli. Seventy-six thousand glomeruli relate to the total number of two rat kidneys.

PMN depletion. Anti-PMN antiserum was raised in New Zealand White rabbits by immunization with 2×10^8 Wistar rat PMNs in complete and incomplete Freund's adjuvant [17]. The PMNs were collected from rat ascites three to five hours after instillation of 10 ml of 0.1% oyster-glycogen-heparin HBSS. Rabbit sera were heat inactivated at 56°C for 90 minutes. An IgG preparation was made according to Steinbuch and Andran [14]. Ten mg/100 g body wt of anti-PMN antiserum was given 14 hours before the administration of ATS. PMNs were 64 ± 11 cells/cm after PMN antiserum one hour prior to ATS. The mean total white cell count of rats without anti-PMN serum was 10800 $\pm$ 1470 cells/cm. To determine whether rabbit anti-rat PMN antiserum had an effect on complement prior to ATS, serum was collected from all the animals for the determination of complement CH50 activity.

Complement depletion. Rats were depleted of complement by injection of cobra venom factor (CVF) according to a protocol described by Cochrane, Müller-Eberhard, and Alken [18], to study the effect of mesangial cell immune complex-induced activation of the complement system on glomerular prostanoid formation. 300 U/kg/body wt of CVF was given i.p. the day prior to the antibody. Two hours before antibody injection the rats received 100 U/kg/body wt/CVF i.p. Prior to antibody injection and at the end of the experiment (2 hr), blood

was collected for determination of CH50 activity. CH50 measurements were performed according to the technique of Mayer [19].

For control of possible effects of CVF alone on glomerular PG formation, control rats ($N = 5$) received CVF, however, they did not receive ATS.

Depletion of glomerular monocytes. Anti-rat monocyte antiserum was raised in New Zealand white rabbits by the immunization with 10^7 Wistar rat monocytes in complete and incomplete Freund's adjuvant. The monocytes were collected from rat ascites three to four days after the instillation of 10 ml of 10% protease solution. The collected cells were cultured for five to six hours in RPMJ 1640 + 10% FCS in tissue culture flasks. The adherent cells were collected and frozen in HBSS at −20°C until utilized for immunization. Rabbit sera collected after the third or fourth immunization were heat inactivated at 56°C for 60 minutes. An IgG preparation was made according to Steinbuch and Andran [14] and the antiserum was tested. The antibody which was used to deplete monocytes, stained monocytes up to a titer of 1:1000 when evaluated by immunofluorescence but did not stain lymphocytes or granulocytes in a titer above 1:20. The antisera did not deplete significantly total white cell counts (11,666 ± 980 cells/mm^3) in Wistar rats after a 48 hour treatment protocol (3 × 8 mg antiserum/100 g body wt/day i.v.) compared to control values prior to the antiserum (11,750 ± 460 cells/mm^3). The used antisera, however, depleted total peripheral blood monocytes from 1.7 ± 0.07% to 0% after the treatment protocol. The antimonocyte serum did not stain glomerular epithelial and mesangial cells in culture and on cross sections from rat kidneys.

The antisera did not prevent glomerular binding of ATS when evaluated by immunofluorescence. In order to demonstrate the effectiveness of the antisera to reduce the number of infiltrating monocytes in the glomerulus of rats with MCI one group of rats ($N = 7$) was injected with ATS; a corresponding group of rats ($N = 7$) received 8 mg/100 g body wt of antimonocyte serum two hours prior to ATS and 3 × 8 mg/100 body wt every 12 hours after ATS. Forty-eight hours after ATS glomeruli were isolated, treated with collagenase and plated on culture glass chambers (Miles; 100 glomeruli/well) in RPMI 1640 plus 10% FCS and incubated at 37°C in 5% CO_2. After 12 hours in culture the non-adherent material was decanted and the adherent cells were stained with anti monocyte serum and evaluated by indirect immunofluorescence. Four wells from three animals of each group of rats were counted for positive adherent monocytes.

Isolation and superfusion of glomeruli. Glomeruli were isolated by a sieving technique as described earlier [20]. Glomeruli were transferred on a millipore filter and superfused for 30 minutes with Krebs-Ringer-HCO_3 buffer at 37°C in order to determine glomerular PGE_2 and TxB_2 production. The details of the superfusion technique were described earlier [21]. For quantification of glomerular protein content glomeruli were counted in a Fuchs Rosenthal chamber.

Four milliliters of buffer were used as perfusate in a closed superfusion system at a perfusion rate of 7 ml/min. The perfusates were collected and frozen at −20°C until analysis for PGE_2 and TxB_2. The glomeruli were solubilized in a 1 N NaOH and protein analysis was performed according to the method of Lowry et al [22].

Measurements of PGE_2 and TxB_2 and 6-keto-$PGF_{1\alpha}$. PGE_2, 6-keto-$PGF_{1\alpha}$ and TxB_2 were determined by direct radioimmunoassay in the perfusates without prior extraction or chromatographic separation. Urinary PGE_2, 6-keto-$PGF_{1\alpha}$ and TxB_2 excretion was determined after the extraction of urines with ethyl acetate and chromatographic separation on thin layer chromatography as described earlier [21]. The used assay procedures, sensitivity and specificity of the PGE_2 and TxB_2 antisera were described earlier [20, 21]. 6-keto-$PGF_{1\alpha}$ was measured with a commercially available radioimmunoassay kit purchased from NEN (Dreieich, FRG). Glomerular prostanoid production is expressed in pg/mg glomerular protein/min incubation time.

Inulin clearances—Effect of indomethacin and UK 38485. To determine the influence of antibody on GFR and inulin, clearances were performed in control rats receiving non-antibody IgG and animals receiving ATS. Inulin clearances were measured at two hours and 48 hours after antibody or IgG injection. The rats were anesthetized with inactin (100 mg/kg/body wt i.p.). A tracheostomy tube was inserted and both jugular veins were cannulated with PE-50 polyethylene catheters. The right ureter was cannulated with a PE-10 polyethylene catheter for urine collection. Each animal received 10 ml/kg/body wt of a 0.45% saline solution over 10 minutes to replace fluid losses during surgery. Then a bolus of a 1% inulin solution in 0.45% saline was given, followed by a 90 minute period where 0.9 ml/hr of a 1% inulin/0.45% saline infusion was given to reach constant urine flow. Control animals received non antibody IgG, experimental rats received ATS. I.v. infusion with 1% inulin in 0.45% saline was continued for two hours. A 30-minute clearance period followed. Blood for determination of inulin concentration was collected in the middle of the clearance period. In animals where GFR was assessed at 48 hours, a clearance period was determined directly following the 90 minute stabilization interval.

The day prior to antibody, groups of rats received either indomethacin (5 mg/kg body wt i.v.) or the thromboxane synthetase inhibitor UK 38485 (50 mg/kg body wt i.v.) to study a possible role of cyclo-oxygenase metabolites on GFR in rats with mesangial cell injury. The same dose was given 2 hours prior to antibody. Indomethacin (2 mg/kg body wt/hr) and UK 38485 (2.3 mg/kg body wt/hr) were continuously infused during the clearance studies. When inulin clearance was assessed at 48 hours, rats received 50 mg/kg body wt/day of UK 38485 or 5 mg/kg body wt/day of indomethacin in two divided doses orally, which was given every 12 hours. Inulin was determined according to the method of Führ, Kaczmarczyk and Krüttgen [23].

Morphologic studies

Kidney slices were obtained for light-, immuno- and electron-microscopy. For light- and electron microscopy tissue was fixed in 4% buffered formaldehyde. For light microscopy tissue was embedded in paraffin and stained with PAS. For electron microscopy tissue was embedded in methacrylate. Tissue for immunofluorescence was snap-frozen in liquid nitrogen, sectioned and stained with IgG fractions of antibodies to rabbit IgG and rat C3.

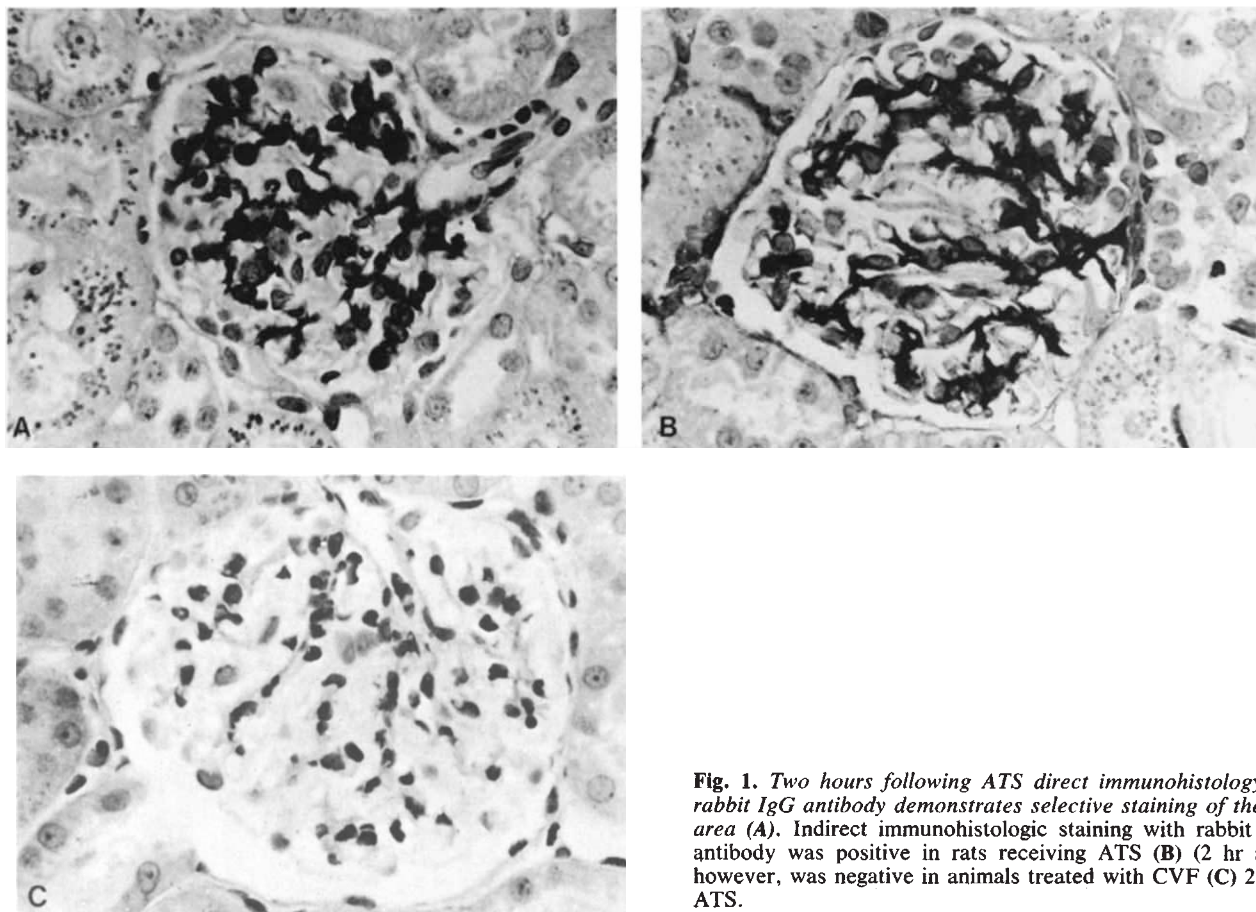

Fig. 1. *Two hours following ATS direct immunohistology with anti-rabbit IgG antibody demonstrates selective staining of the mesangial area (A).* Indirect immunohistologic staining with rabbit anti-rat C3 antibody was positive in rats receiving ATS (B) (2 hr after ATS), however, was negative in animals treated with CVF (C) 2 hours after ATS.

Statistical analysis

All data are given as means ± SEM. Statistical analysis was performed with the unpaired Student's t-test with correction for multiple comparisons when applicable [24]. A P value of <0.05 was considered significant.

Results

Morphologic characterization of the immune-mediated mesangial cell injury

At two hours after antibody injection, glomeruli stained positive for rabbit IgG and rat C3 (Fig. 1). The staining was selective and only positive in the mesangial area. The mesangial cells showed significant degeneration (Fig. 2A) and the mesangium was occasionally infiltrated with PMNs (Fig. 2B). By electron microscopy there was no evidence for subepithelial or subendothelial immune deposits.

Forty-eight hours after ATS, immunohistology for rabbit IgG and rat C3 was negative (not shown). By light microscopy, glomeruli at 48 hours appeared deprived of mesangial cells and there were aneurysma-like formations (Fig. 3A) and the appearance of mononuclear cells (Fig. 3B).

^{125}I-labelled ATS IgG binding to glomeruli

Table 1 summarizes the ^{125}I-labelled-IgG binding to glomeruli two hours after antibody injection. Neither complement depletion with CVF nor anti-PMN antiserum significantly influenced ^{125}I-labelled IgG binding to glomeruli. The inhibition of cyclooxygenase with indomethacin and the thromboxane synthetase inhibitor UK 38485 also did not alter antibody binding to the glomeruli.

Glomerular prostanoid formation in mesangial cell injury

Glomeruli, isolated from rats with mesangial cell injury two hours and 48 hours after antibody, produced significantly higher amounts of TxB_2 when compared to glomeruli from rats, which received non-antibody rabbit IgG (Table 2). Glomerular PGE_2 and 6-keto-$PGF_{1\alpha}$ biosynthesis was not different between rats with MCI and control animals (Table 2). The glomerular protein content/100 glomeruli averaged 9.08 ± 0.33 μg (2 hr, N = 11) and 8.11 ± 1.14 μg (48 hr, N = 9) for control rats and 8.08 ± 0.41 μg (2 hr, N = 14) and 7.66 ± 0.12 μg (48 hr, N = 9) for animals with MCI.

Effect of complement depletion on MCI

Treatment with CVF reduced complement CH50 activity to less than 1% compared to animals receiving non-antibody IgG (50% lysis was found with a dilution of 0.79 ± 0.17; N = 6). Immunohistologic studies at two hours revealed positive staining for rabbit IgG, however, glomeruli were negative for rat C3. The typical morphologic lesions and the cellular influx of PMNs did not appear. Complement depletion prevented increase in glomerular TxB_2 biosynthesis (150 ± 22, ATS + CVF; Fig. 4)

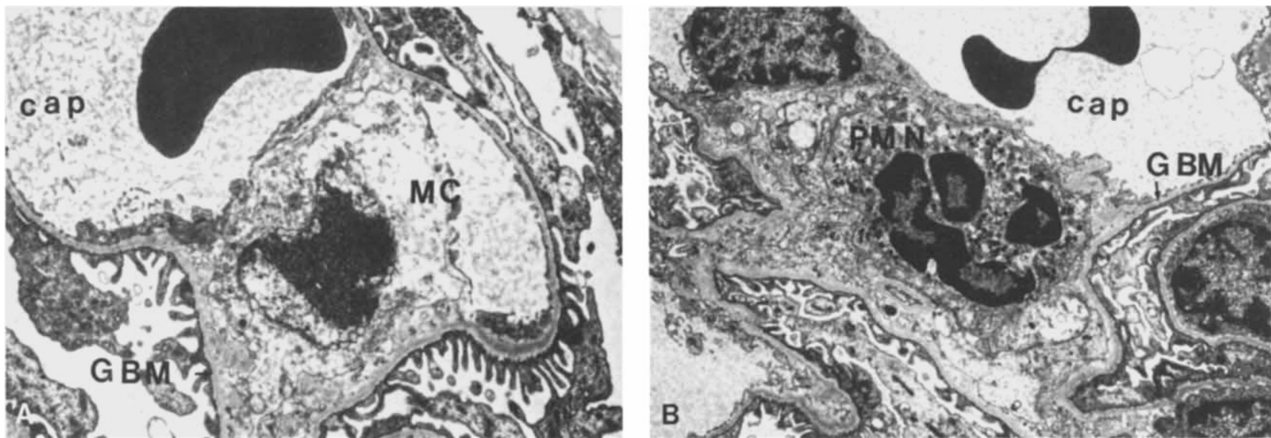

Fig. 2. A. *The mesangial cells (MC) show degenerative alterations in the cytoplasm 2 hours after ATS.* The glomerular basement membrane (GBM), the subendothelial and subepithelial spaces are free of immune deposits (cap = capillary lumen). **B.** The mesangium was occasionally infiltrated by polymorphonuclear granulocytes (PMN = polymorphonuclear granulocytes; GBM = glomerular basement membrane; cap = capillary lumen).

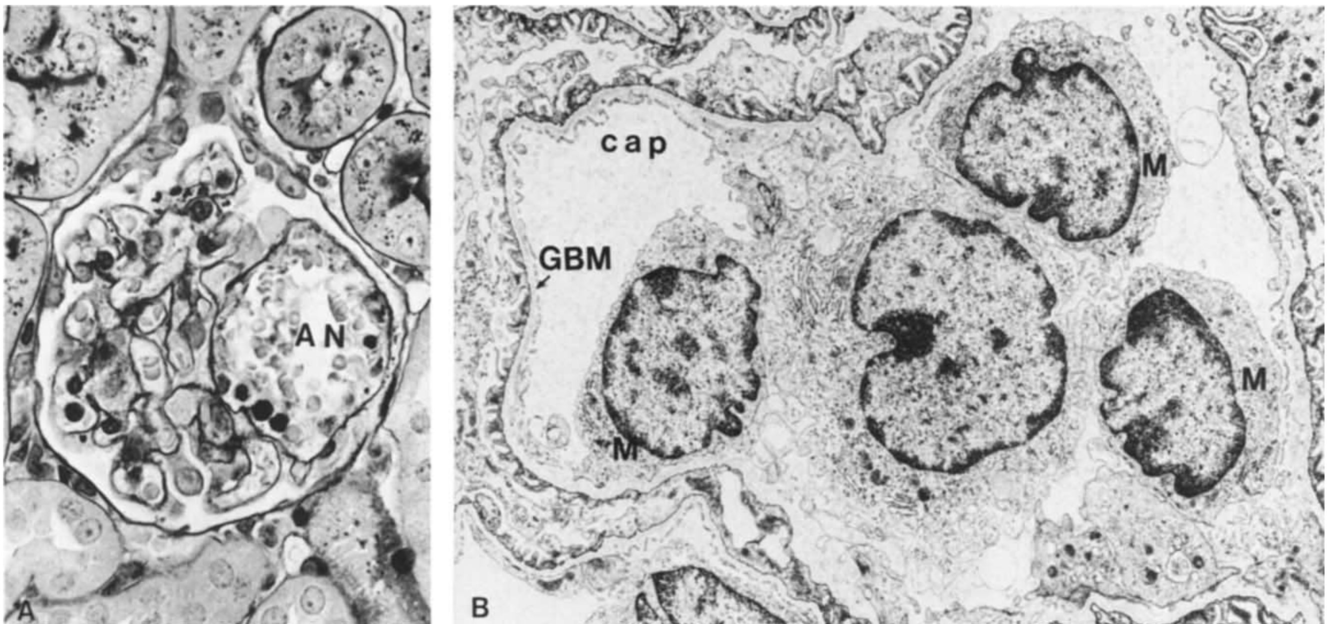

Fig. 3. A. *48 hours after ATS, glomeruli were cell depleted and had aneurysm (AN)-like formations.* **B.** 48 hours after antibody mononuclear cells were present in mesangial areas and capillary lumina (M = mononuclear cells; GBM = glomerular basement membrane; cap = capillary lumen).

which was found in animals receiving ATS alone (326 ± 24). TxB_2 production in rats which were complement depleted was not different from controls which received non-antibody-rabbit IgG (168 ± 24 pg/mg/min; Fig. 4). CVF treatment of normal control rats (IgG + CVF) did not influence glomerular TxB_2 formation (176 ± 36 pg/mg/min). Complement depletion had no effect on glomerular PGE_2 formation.

Effect of monocyte depletion on glomerular eicosanoid formation

Treatment of rats with anti-monocyte serum significantly ($P < 0.001$) reduced the number of adherent monocytes/macrophages of nephritic rats from 145 ± 30 cells/100 cultured glomeruli to 22 ± 17 cells/100 cultured glomeruli, when cells were evaluated according to the technique outlined above.

Glomerular PGE_2 and 6-keto-$PGF_{1\alpha}$ formation was unaffected by the treatment with the anti-monocyte serum (Table 3), however, glomerular TxB_2 formation was significantly reduced compared to untreated nephritic controls (Table 3). CH50 complement levels were not significantly reduced, 50% lysis was found with a dilution of 0.64 ± 0.18 ($N = 6$).

Effect of PMN depletion on MCI

Treatment with anti-PMN serum reduced PMNs in the peripheral blood from 2000 ± 214 cells/cmm to 64 ± 11 cells/cmm. Leucocyte depletion did not interfere with ^{125}I-anti-thymocyte

Table 1. ^{125}I-labelled rabbit anti-rat-anti-thymocyte IgG binding to rat glomeruli (cpm/76 000 glomeruli)

Non-antibody	ATS + IgG (N = 5)	ATS + CVF (N = 5)	ATS + a-PMN-IgG (N = 5)	ATS + indomethacin (N = 5)	ATS + UK 38485 (N = 5)
	16,825 ± 2,495 NS	13,832 ± 3,198 NS	13,790 ± 3,555 NS	15,127 ± 4,290 NS	16,480 ± 4,290 NS

Abbreviations are: CVF, cobra venom factor; a-PMN-IgG, rabbit anti-rat-polymorphonuclear granulocyte antibody; NS = non-significant from control ATS.

Table 2. Glomerular prostanoid formation in mesangial cell injury

	2 Hours			48 Hours		
	Control (N = 11)		MCI (N = 14)	Control (N = 9)		MCI (N = 9)
TxB_2 *pg/mg/min*	143 ± 13	$P < 0.001$	292 ± 27	171 ± 33	$P < 0.05$	396 ± 60
PGE_2 *pg/mg/min*	311 ± 33	NS	359 ± 66	345 ± 45	NS	330 ± 41
6-keto-$PGF_{1\alpha}$ *pg/mg/min*	not determined		not determined	159 ± 39	NS	219 ± 66

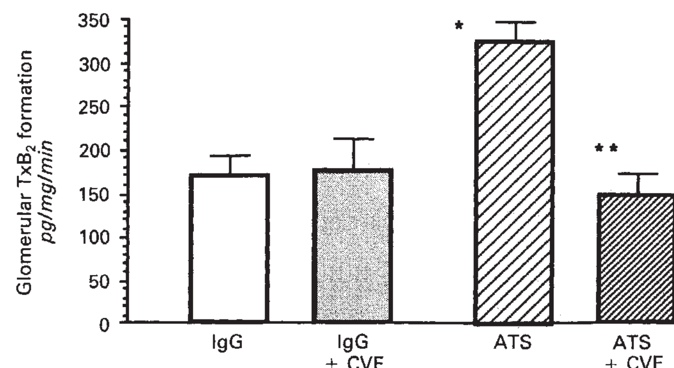

Fig. 4. *Glomerular TxB_2 formation 2 hours following ATS (N = 7) was significantly higher when compared with glomeruli of rats receiving IgG (N = 7; *P < 0.001).* Cobra venom factor (CVF) treatment of animals prior to ATS (N = 7) application prevented the increase in glomerular TxB_2 formation seen in ATS animals (**P < 0.001; ATS vs. ATS + CVF). CVF treatment did not alter TxB_2 formation in rats treated with non-antibody IgG (IgG + CVF, N = 7). Data are given as means ± SEM.

Table 3. Effect of glomerular monocyte depletion on prostanoid formation in rats with mesangial cell injury at 48 hours after antibody

	ATS (N = 7)		ATS + monocyte depletion (N = 7)
TxB_2 *pg/mg/min*	394 ± 32	$P < 0.05$	262 ± 24
PGE_2 *pg/mg/min*	344 ± 52	NS	424 ± 78
6-keto-$PGF_{1\alpha}$ *pg/mg/min*	177 ± 15	NS	213 ± 33

NS = not significant

IgG binding to glomeruli (Table 1) and did not effect complement CH50 activity (50% lysis with a serum dilution of 0.56 ± 0.13; N = 6). PMN depletion did not significantly reduce TxB_2 formation in rats with MC injury when studied two hours after ATS (ATS: 292 ± 27 pg/mg/min; ATS + aPMN: 253 ± 40 pg/mg/min). Glomerular TxB_2 production in normal rats receiving anti-PMN serum was also not effected (aPMN: 134 ± 14 pg/mg/min), when compared with untreated control rats (108 ± 24 pg/mg/min).

Inulin clearance in rats with mesangial cell injury—Effects of indomethacin and UK 38485

Two hours following non-antibody rabbit IgG, GFR was 914 ± 51 μl/100 g body wt/min and was 784 ± 69 μl/100 g body wt/min when rats were studied at 48 hours. Induction of mesangial cell injury significantly reduced GFR at two hours (456 ± 24 μl/100 body wt/min) and at 48 hours (368 ± 40 μl/100 g body wt/min; Fig. 5). When rats were pretreated either with the cyclo-oxygenase inhibitor indomethacin or the thromboxane synthetase inhibitor UK 38485, the fall in inulin clearance did not appear (2 hr: Indo: 800 ± 67, UK 38485: 923 ± 15; 48 hr: Indo: 697 ± 60, UK 38485: 654 ± 99 μl/100 g body wt/min). The thromboxane synthetase inhibitor reduced glomerular TxB_2 formation in nephritic glomeruli after 48 hours to 59 ± 7 pg/mg/min (N = 5) versus 396 ± 69 pg/mg/min, $P < 0.001$, but had no significant effect on glomerular PGE_2 (350 ± 47 pg/mg/min) and 6-keto $PGF_{1\alpha}$ (171 ± 29 pg/mg production, when evaluated at 48 hr and compared with nephritic untreated animals; Table 2).

Indomethacin significantly ($P < 0.05$) reduced glomerular TxB_2 (120 ± 30 pg/mg/min), PGE_2 (183 ± 37 pg/mg/min) and 6-keto-$PGF_{1\alpha}$ 96 ± 20 pg/mg/min) formation in rats with MCI after 48 hours, when compared with untreated nephritic rats (Table 2). UK 38485 reduced urinary TxB_2 excretion in nephritic rats from 927 ± 84 pg/30 min to 127 ± 11 pg/30 min, but had no effect on urinary PGE_2 (134 ± 17 pg/30 min) excretion, when compared to untreated nephritic animals (116 ± 11 pg/30 min). The cyclo-oxygenase inhibitor indomethacin inhibited both ($P < 0.001$) urinary TxB_2 (227 ± 42 pg/30 min) and PGE_2 formation in nephritic rats (47 ± 19 pg/30 min) after the 48 hour treatment, compared to untreated nephritic animals.

Discussion

The immune glomerular injury which appears in rats after rabbit anti-rat thymocyte antiserum is morphologically charac-

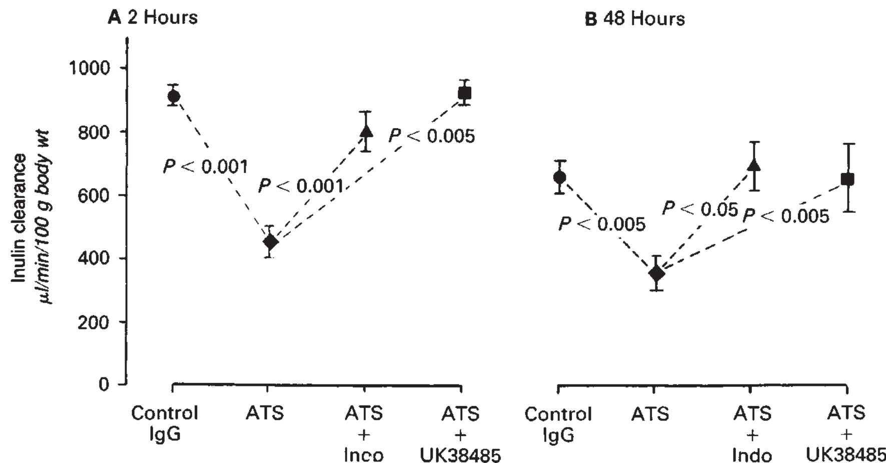

Fig. 5. *Mesangial cell injury following ATS reduced the inulin clearance at 2 hours (ATS: N = 7, IgG: N = 7) and 48 hours (ATS: N = 14, IgG: N = 7) significantly compared with rats receiving IgG (control IgG).* Pretreatment of rats either with the cyclo-oxygenase inhibitor indomethacin (ATS + Indo: $N = 7$) or the thromboxane synthetase inhibitor UK 38485 (ATS + UK 38485: $N = 7$) prevented the fall in the inulin clearance. GFR was not significantly different from rats receiving IgG. Data are given as means ± SEM.

terized by a selective positive immunohistologic staining of the mesangium for rabbit IgG and rat C3 (Fig. 1). This positive immunohistology at two hours is associated with the degeneration of mesangial cells (Fig. 2A). Electron microscopy revealed that the subendothelial, the subepithelial and the glomerular basement membrane were free of immune deposits (Fig. 2A). At two hours the mesangial area was occasionally infiltrated by PMNs (Fig. 2B). At 48 hours mononuclear cells appeared in the glomerular tuft (Fig. 3B). At 48 hours the severity of the lesion had aggravated and the glomeruli were still cell depleted with the appearance of microaneurysm (Fig. 3A).

The morphology of this immune glomerular injury is similar to the model described by Bagchus et al [25] in the Wistar rat and the lesion reported by Yamamoto and Wilson [26, 27] in the Lewis rat. Bagchus et al [25] induced the glomerular injury with a monoclonal antibody which was directed against the Thy-1 antigen, a cell surface antigen on thymocytes which is also present in a number of different other tissues [28, 29], including glomerular mesangial cells [30]. The antigenic structures to which ATS binds on the rat mesangial cells in our animal model are not yet defined.

The induction of immune mediated mesangial cell injury in the rat is associated with an increase in TxB_2 formation by isolated glomeruli. TxB_2 production was elevated at two hours and 48 hours after antibody. No difference in glomerular PGE_2 and 6-keto-$PGF_{1\alpha}$ formation was found between rats with mesangial cell injury and control rats.

The increase in glomerular production of TxB_2 was about threefold (at 2 hr and 48 hr). The ratio of glomerular PGE_2/TxB_2 formation in control animals was 3:1 and changed to 1:1 following antibody. The enhanced glomerular TxB_2 formation in the model of mesangial cell injury raises the question regarding the responsible mechanisms of increased thromboxane formation and the cellular sources which account for the increased thromboxane production.

There is evidence from recent studies by Chen et al [31] that an antibody directed against epitopes on mesangial cells stimulates in vitro PGE_2 and TxB_2 formation in these cells. The stimulatory effect of the antibody on mesangial cell prostanoid biosynthesis is amplified when complement is present in the in vitro system. Earlier studies by Lovett et al [32] demonstrate that the terminal complement components might be significant in the stimulation of prostanoids in mesangial cells. Moreover, we found that glomerular activation of the complement system stimulated prostanoid formation in a model of membranous nephropathy [6]. Since Yamamoto et al [26] reported that the activation of the complement system following anti-thymocyte antiserum is an important mediator of the degenerative changes of mesangial cells, we evaluated the relative role of the glomerular activation of complement on the formation of prostanoids following ATS. When rats were treated with CVF, which reduced CH50 levels of complement to almost undetectable levels, the increase in glomerular TxB_2 formation did not appear, which suggests that the activation of the complement system in the glomerulus is a mediator of elevated glomerular TxB_2 following ATS.

Furthermore, there is additional evidence that monocytes/macrophages which infiltrate in the glomerulus during the process of the immune injury might participate in the formation of thromboxane [3]. Therefore, we treated rats with anti-monocyte serum which reduced monocytes in nephritic rats. The released amount of TxB_2, however, was still higher than TxB_2 formation in non-nephritic controls. These data suggest that infiltrating monocytes might participate in the elevated TxB_2 formation of this animal model. The infiltration of PMNs in the mesangium is a prominent histologic feature following ATS. PMNs can synthesize prostanoids [33] and might in addition to resident glomerular cells be a source of elevated thromboxane formation. PMN depletion, however, did not influence glomerular biosynthesis of TxB_2. This makes PMNs an unlikely source of elevated TxB_2 production.

Induction of the mesangial cell injury is associated with a fall of glomerular filtration rate, when the nephritic animals were compared with rats which received non-antibody rabbit IgG. The GFR of nephritic rats was about 40% lower than in control animals. The difference in GFR was present at two hours and at 48 hours. This difference in GFR of control animals might be due to the experimental protocols. The animals studied at two hours had a longer pre-equilibration period than rats studied at 48 hours which might have influenced baseline GFR.

The decrease in GFR following ATS was prevented when the rats were pretreated either with the cyclo-oxygenase inhibitor indomethacin or the thromboxane synthetase inhibitor UK

38485. Both drugs were used in concentrations which inhibited intraglomerular prostanoid and thromboxane formation.

The amelioration of the decrease in GFR following indomethacin in rats with mesangial cell injury is a rather unexpected observation. In several other animal models of glomerular injury we and others found, that indomethacin or other cyclo-oxygenase inhibitors reduce GFR [2, 7, 34, 35], which suggests that vasodilatory cyclo-oxygenase products might maintain GFR in some situations of glomerular disease. In these animal models, in addition to TxB_2, vasodilating prostaglandins were also enhanced.

Induction of immune-mediated mesangial cell injury, however, exclusively increases TxB_2 formation in isolated glomeruli. TxB_2 is a vasoconstrictor which might reduce GFR due to a contraction of mesangial cells [12] and could decrease glomerular plasma flow [36]. The inhibition of this vasoconstrictor by indomethacin may therefore improve GFR. The observation that the thromboxane synthetase inhibitor also improves GFR supports the suggested role for TxB_2. Furthermore, the increase in GFR seen at 48 hours following UK 38485 treatment was higher compared to the effect after indomethacin. This suggests that vasodilating cyclo-oxygenase products might still play a role in the regulation of GFR in this state of disease. Our studies cannot define, however, the hemodynamic factors which are influenced by TxB_2. But recent data by Yamamoto et al [37] demonstrate a decrease in the K_f in a similar model of mesangial cell injury.

In summary we conclude that induction of immune-mediated mesangial cell injury stimulates glomerular TxB_2 formation. This stimulatory effect is dependent on complement activation and infiltration of monocytes/macrophages in the glomerulus. The reduction of the glomerular filtration rate following antithymocyte antibody is mediated by TxB_2, which makes this cyclo-oxygenase product a mediator responsible for the decrease in GFR in this animal model.

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