

Mesangial Cell Immune Injury

Hemodynamic Role of Leukocyte- and Platelet-derived Eicosanoids

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Abstract

The role of leukocytes and platelets and of leukocyte- and platelet-derived eicosanoids in mediating acute changes in renal and glomerular hemodynamics was assessed in a model of antibody-induced mesangial cell injury in the rat. After a single intravenous injection (6 mg/kg) of the monoclonal antibody (ER₄) against the mesangial cell membrane antigen Thy 1, significant decrements in glomerular filtration rate (GFR) and renal blood flow (RBF) were observed at 1 h, and were associated with increments in glomerular LC (+) leukocyte counts and in the synthesis of thromboxane (Tx)₂, leukotriene (LT)₄, and 12-hydroxyeicosatetraenoic acid (HETE). In rats with immune leukopenia, the rise in glomerular LC (+) leukocytes and in eicosanoid synthesis were abolished and the fall in GFR and RBF after administration of ER₄ were completely ameliorated. Likewise, pretreatment of rats with both a thromboxane synthase and a 5-lipoxygenase inhibitor also blocked the fall in GFR and RBF and the rise in glomerular synthesis of Tx₂ and LTB₄ produced by ER₄ without changing glomerular LC (+) leukocyte counts. Selective inhibition of thromboxane or 5-lipoxygenase alone only partially ameliorated the decrements in GFR and RBF produced by ER₄. In animals with immune thrombocytopenia, the elevated glomerular synthesis of 12-HETE and fall in RBF but not GFR was ameliorated after administration of ER₄. The ER₄ antibody-induced fall in GFR was mainly caused by a marked decrement in the ultrafiltration coefficient, K_f , which was dependent on TxA₂ and 5-lipoxygenase products, since pretreatment of animals with a thromboxane receptor antagonist or with a 5-lipoxygenase inhibitor partially ameliorated this decrement. Structural changes such as infiltration of glomerular capillaries by leukocytes and endothelial cell damage may also have accounted for the fall in K_f . These observations indicate that in antibody-mediated mesangial cell injury, infiltrating leukocytes and platelets mediate the changes in renal hemodynamics via synthesis of thromboxane and arachidonate 5-lipoxygenation products. (*J. Clin. Invest.* 1992. 90:2304–2312.) Key words: glomerulonephritis • leukotriene • thromboxane • hydroxyeicosatetraenoic acid • hemodynamics

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Received for publication 11 February 1992 and in revised form 7 July 1992.

J. Clin. Invest.

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0021-9738/92/12/2304/09 \$2.00

Volume 90, December 1992, 2304–2312

Introduction

The glomerular mesangium is a specialized pericapillary tissue that consists of contractile smooth musclelike mesangial cells embedded in an extracellular matrix. Mesangial cells express the Thy 1 antigen and surface receptors for angiotensin II, arginine vasopressin, and eicosanoids. Their ability to contract in response to these and other agonists is believed to mediate changes in nephron filtration rate. In immune mesangial nephropathies, the mesangial cell becomes the target of immune injury and responds by proliferation, matrix synthesis, and may participate in the associated changes in renal hemodynamics. Of the various experimental models of antibody-mediated mesangial cell injury that have been developed, the model of anti-Thy 1 antibody-mediated injury has recently become the focus of several investigations (1, 2). We and others have described acute changes in glomerular filtration rate (GFR)¹ and renal blood flow (RBF) that occur after binding of this antibody to the phospholipid-anchored mesangial cell membrane antigen Thy 1 and have implicated vasoactive eicosanoids originating from blood borne leukocytes and platelets as mediators of these changes (3, 4). Yamamoto et al. have further demonstrated that in this model, lytic injury of the mesangial cell participates in the long term (1–4 d) decrements in the glomerular ultrafiltration coefficient, which accounts for the fall in glomerular filtration after binding of anti-Thy 1 antibody to mesangial cells (5). As eicosanoids can regulate mesangial cell contractility (6, 7), it can be proposed that in antibody-mediated mesangial injury, specific eicosanoids originating from infiltrating leukocytes and platelets may, in a paracrine manner, alter mesangial cell contractility, and mediate the initial (1–2 h) changes in glomerular filtration seen in this model. In the present study, we assessed whether leukocyte- and platelet-derived eicosanoids mediate the acute changes in glomerular hemodynamics that occur in anti-Thy 1 antibody-mediated mesangial cell injury in the rat.

Methods

Induction of mesangial cell immune injury

Mesangial cell injury was induced in male Munich-Wistar rats weighing 180–290 g (Harlan Sprague Dawley, Inc., Indianapolis, IN) by a single injection (6 mg/kg body wt i.v.) of the mouse monoclonal anti-

1. *Abbreviations used in this paper:* GBM, glomerular basement membrane; GFR, glomerular filtration rate; HETE, hydroxyeicosatetraenoic acid; K_f , ultrafiltration coefficient; LC, leukocyte common antigen; P_g , vascular pressure measured in capillaries of superficial glomeruli; P_{star} , vascular pressure measured in star vessels; P_{tub} , vascular pressure measured in free-flowing proximal tubules; P_{ur} , ultrafiltration pressure; R_a , afferent arteriolar resistance; RBF, renal blood flow; R_e , efferent arteriolar resistance.

body ER₄ raised against the rat thymocyte antigenic determinant Thy 1.1 as we reported previously (3). ER₄ antibody was prepared by Bagchus et al. (1) and was generously provided by Dr. W. Bakker (Department of Pathology, University of Groningen, The Netherlands). ER₄ antibody-induced mesangial nephritis was documented by proteinuria assessed on day 4 (urinary protein excretion 207±9 mg/24 h) and by histologic and ultrastructural examination of glomeruli using routine methods of light, immunofluorescence, and electron microscopy.

Renal hemodynamic studies

These studies assessed the effects of intravenous ER₄ antibody administration on changes in GFR and RBF. Moreover, they assessed the role of leukocytes and platelets and that of thromboxane and of arachidonate lipoxygenation products in mediating changes in GFR and RBF after ER₄ antibody-induced mesangial injury. Rats were anesthetized with an intraperitoneal injection of thiobutabarbital (Inactin; Byk Gulden, Konstanz, Germany) 12–15 mg/100 g body wt, and maintained at 37°C on a constant temperature table. A tracheostomy tube was placed and a catheter was inserted in the right jugular vein for infusion of 2% albumin (Sigma Chemical Co., St. Louis, MO) in 0.9% sodium chloride at a rate of 20 ml/kg per h throughout the experiment. [³H]inulin (0.5 μCi/ml) was added to the infusion solution and GFR was measured by the clearance of [³H]inulin. The right femoral artery was cannulated with a polyethylene catheter connected via a transducer to a digital pressure monitor for continuous measurement of systemic blood pressure and for collection of blood samples. Catheters were also inserted into the ureters for urine collection. RBF was measured by an electromagnetic flow meter (model 501; Carolina Medical Electronics, Inc., King, NC) connected to a flow probe placed around the left renal artery. After completion of surgery and a 60-min stabilization period, two baseline clearance periods (15 min each) were obtained. Animals subsequently received a single injection of ER₄ antibody (6 mg/kg body wt i.v.) or mouse IgG (6 mg/kg body wt i.v.) and urine and plasma samples were collected during two 30-min clearance periods.

In three additional groups of Munich-Wistar rats, micropuncture studies were performed to assess the effect of ER₄ antibody-mediated mesangial cell injury on changes in glomerular hemodynamic parameters and the effects of thromboxane receptor antagonism and of arachidonate 5-lipoxygenase inhibition on these changes. These rats were surgically prepared as described above and cannulas were placed in the carotid and femoral arteries for the collection of blood and in the jugular vein for infusions. The rats received replacement of fluid losses after surgery (1 ml of 6% BSA in saline) and an infusion of 0.9% sodium chloride solution at a rate of 1.2 ml/h. The left kidney was placed in a stainless steel cup and surrounded with a 5% solution of agar so that the renal cortical surface could be bathed with a 0.9% sodium chloride solution for measurement of vascular pressures using a servo-null micropressure device (model 900; World Precision Instruments, New Haven, CT). During control and experimental periods, pressures were measured in free-flowing proximal tubules (P_{tub}), star vessels (P_{star}), and in the capillaries of superficial glomeruli (P_g) using micropipettes (3 μm). GFR was determined from the clearance of [³H]inulin and RBF was continuously measured via an electromagnetic flow probe placed around the left renal artery as described above. Plasma protein concentration (C) was determined using a refractometer. Efferent arteriolar protein concentration (C_e) was calculated as C_e = C_a/FF, where FF is the filtration fraction derived from the GFR divided by renal plasma flow. Plasma oncotic pressure (P) was determined by the Landis-Pappenheimer equation as P = 0.0092 C³ + 0.16 C² + 2.1 C (8). Efferent arteriolar resistance (R_e) was calculated as R_e = (P_g - P_{star})/(RBF - GFR); afferent arteriolar resistance (R_a) was calculated as R_a = (RPP - P_g)/RBF, where RPP is the renal perfusion pressure derived from the mean systemic pressure. An iterative computer model (implemented by Dr. Harold Modell, Educational Consulting Materials Development, Seattle, WA) was used to calculate mean values of ultrafiltration pressure (P_{ur}) and Π_{gc} and to derive K_f values. This approach of

measuring changes in the determinants of glomerular filtration at the whole kidney rather than single nephron level has been previously described (9) and assumes that pressures measured in superficial nephrons are representative of those occurring throughout the kidney. Moreover, this approach most closely mimics the experimental design of our other clearance studies that correlate physiologic with biochemical changes (see below).

Cell depletion studies

To assess the extent that infiltrating leukocytes contributed to changes in GFR and RBF after induction of mesangial cell immune injury, leukocyte depletion was induced using a rabbit anti-rat leukocyte immune serum (Accurate Chem. & Sci. Corp. Westbury, NY). An injection of this immune serum (250–500 μl i.p./100 g body wt) induced a nonselective leukopenia and reduced the peripheral white blood cell count from 11.7±0.5 × 10³/mm³ to 3.2±0.5 × 10³/mm³ within 18 h. Total and differential white blood cell counts were determined using an automated hematology analyzer (Coulter Corp., Hialeah, FL). After peripheral leukopenia was documented, the rats received ER₄ (n = 6) or mouse IgG (n = 9), and GFR and RBF measurements were performed as described above.

To deplete circulating platelets, a rabbit anti-rat thrombocyte immune serum (Accurate Chem. & Sci. Corp.) was given as a single injection of 25–50 μl/100 g body wt i.p. This resulted in marked and selective systemic platelet depletion within 18 h (counts fell from 957±70 to 28±9 × 10³ platelets/mm³, n = 5). Rats were subsequently given ER₄ (n = 6) or mouse IgG (n = 6), and GFR and RBF were measured as described above.

Eicosanoid synthesis inhibition studies

To determine whether arachidonate 5-lipoxygenation metabolites mediate changes in GFR and RBF induced by administration of ER₄ antibody, the 5-lipoxygenase inhibitor, MK-886 [3-[1-(p-chlorobenzyl)-5-isopropyl-3-tert-butylthioindol-2-yl]-2,2-dimethylpropanoic acid obtained from Merck-Frosst Canada Inc. (Dorval, Quebec, Canada) was used (10). MK-886 (80 mg/kg body wt dissolved in 0.5% methyl cellulose) was given orally via a polyethylene tube inserted into the stomach. 2 h after administration of MK-886, baseline GFR and RBF measurements were obtained. ER₄ (n = 8) or mouse IgG (n = 6) was subsequently given, and measurement of GFR and RBF was repeated 1 h later. In an additional group of animals (n = 5), the effect of MK-886 pretreatment on changes in the renal and glomerular hemodynamic parameters GFR, RBF, P_{gc}, P_{tub}, P_{ur}, R_a, R_e, and K_f induced by ER₄ were assessed before and 1 h after administration of this antibody and compared to animals that received ER₄ alone (n = 8).

To assess the role of thromboxane in mediating changes in GFR and RBF induced by the ER₄ antibody, rats were pretreated with the thromboxane synthase inhibitor furegrelate (5-[3-pyridinylmethyl]-benzofurancarboxylic acid; Sigma Chemical Co.). This compound was given 20 mg/kg body wt i.v. 18 h and 1 h before administration of ER₄ antibody (n = 6) or mouse IgG (n = 6) and as a constant infusion (4 mg/kg per h) through the experiment.

The role of thromboxane in mediating changes in GFR and the hemodynamic mechanisms underlying these changes was also assessed using the thromboxane receptor antagonist, SQ-29,548 ([1s-[1α, 2β(5z), 3β, 4α]]-7-[3-[[2-[(phenylamino) carbonyl] hydrazino] methyl]-7-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic acid; Squibb Institute for Medical Research, Princeton, NJ) (11). SQ-29,548 was given as an intravenous bolus of 2 mg/kg followed by a constant infusion of 2 mg/kg per h. GFR, RBF, and the glomerular hemodynamic parameters P_{gc}, P_{tub}, P_{ur}, R_a, R_e, and K_f were measured before and 1 h after ER₄ antibody administration in rats pretreated with SQ-29,548 (n = 7) and in rats that received ER₄ alone (n = 8).

To assess the combined effect of 5-lipoxygenase and thromboxane synthase inhibition, 12 rats received both MK-886 and furegrelate pretreatment. In this group, furegrelate was given intravenously 18 h and 1 h before GFR and RBF measurement and as a constant infusion as

described above, while MK-886 was given via a gastric tube 2 h before GFR and RBF measurements. Doses of these inhibitors in the combined protocol were identical to the ones used in the single drug protocols described above.

Glomerular biochemical studies

Upon completion of renal hemodynamic measurements (1 h after ER₄ or mouse IgG antibody administration), animals were nephrectomized and the kidneys were placed in RPMI 1640 at 4°C. Glomeruli were isolated by differential sieving, suspended in 2 ml of RPMI 1640, and incubated under constant stirring at 37°C for 30 min. At the end of this incubation period, 200 μl of medium was sampled and assayed directly for TxB₂ using a specific RIA for this eicosanoid as previously described (12). The phospholipase A₂ activator A₂₃₁₈₇ was subsequently added to the glomerular suspension (final concentration 10 μM) and a second incubation was performed at 37°C for 45 min. This incubation was terminated by the addition of 2 vol of acidified ice-cold ethanol. After an 18-h extraction at 4°C, the supernatant was separated from the precipitated protein. The glomerular pellet was solubilized in NaOH and protein measured by a colorimetric assay. The supernatant was dried under nitrogen, suspended in 1 ml of HPLC solvent (methanol/water/acetic acid, 64:34:2, vol/vol/vol), and injected into a gradient HPLC system to separate and isolate 12-HETE and LTB₄ that were subsequently quantified using specific RIAs (13). Results were expressed in nanograms of eicosanoid per milligram glomerular protein. Sensitivities and cross-reactivities of the RIAs used for the above eicosanoids were previously assessed and reported (13). The antiserum for TxB₂ was a gift from Dr. W. Campbell (University of Texas Southwestern Medical School, Dallas, TX). The antiserum for LTB₄ was generously provided by Dr. A. Ford-Hutchinson (Merck-Frosst Canada Inc.). The antibody for 12-HETE was generously provided by Dr. L. Levine (Brandeis University, Waltham, MA).

Assessment of glomerular leukocyte infiltration

Cortical sections (7–9 μm thickness) were examined for the presence of leukocyte common (LC) antigen bearing cells by immunofluorescence microscopy using a fluorescein isothiocyanate conjugated monoclonal antibody against rat leukocyte common (LC) antigen (Accurate Chem. & Sci. Corp.). Sections were subsequently fixed and examined for the presence of LC antigen positive cells. 6–12 glomeruli from each rat in two to six rats from each group were studied, and results were expressed as mean ± SE of LC (+) cells per glomerulus.

Experimental groups

ER₄ and control group. 19 rats received ER₄ antibody (6 mg/kg body wt), and seven rats received nonimmune mouse IgG (6 mg/kg body wt).

Leukocyte depletion group. Leukocyte depleted rats received anti-leukocyte immune serum followed by ER₄ antibody (*n* = 6) or mouse IgG (*n* = 9).

Platelet depletion group. Platelet depleted animals received antithrombocyte immune serum followed by ER₄ antibody (*n* = 6) or mouse IgG (*n* = 6).

5-lipoxygenase inhibition group. Animals were pretreated with MK-886 and subsequently received either ER₄ antibody (*n* = 11) or mouse IgG (*n* = 8).

Thromboxane synthase inhibition group. Animals were pretreated with furegrelate and subsequently received ER₄ antibody (*n* = 6) or mouse IgG (*n* = 6).

Thromboxane receptor antagonism group. Animals were pretreated with SQ-29,548 and subsequently received ER₄ antibody (*n* = 7).

Combined 5-lipoxygenase and thromboxane synthase inhibition group. Animals were pretreated with MK-886 and furegrelate. Six rats subsequently received ER₄ antibody and six rats received mouse IgG.

Statistics

Results are expressed as mean ± SE. Statistical analysis was performed by the two-way analysis of variance for repeated measurements fol-

lowed by a Duncan multiple range test. The significance of differences in the synthesis of glomerular eicosanoids in the different groups was evaluated by a Student's *t* test for unpaired observations. Statistical significance was considered as *P* < 0.05.

Results

Fig. 1 demonstrates binding of ER₄ antibody to the mesangium (*a*) and glomerular LC (+) leukocyte infiltration 1 h after intravenous infusion of ER₄ (*b*).

In Fig. 2, the key ultrastructural changes observed by electron microscopy 1 h after administration of ER₄ are shown; namely, a mesangial cell with no apparent lytic injury (*a*), a polymorphonuclear leukocyte anchored to the glomerular basement membrane (GBM) (*b*), and a glomerular capillary demonstrating lifting of the endothelial lining (*c*).

Fig. 3 demonstrates the effect of leukocyte and platelet depletion on the ER₄ antibody-induced changes in GFR and RBF. Significant decrements in GFR occurred in both groups that received ER₄ antibody alone or were platelet depleted before administration of ER₄. GFR at 1 h was not statistically different between these two groups. Thus, platelet depletion afforded no protection in ER₄ antibody-mediated decrements in GFR, however it significantly reduced the fall in RBF (Fig. 3). In contrast, in the group with immune leukopenia, GFR and RBF 1 h after administration of ER₄ were not different from baseline values.

The effect of leukocyte and of platelet depletion on glomerular infiltration by LC (+) cells is shown in Fig. 4. An increased number of LC (+) cells per glomerulus were observed in rats 1 h after administration of ER₄, and this was effectively abolished in rats with immune leukopenia. Platelet depletion had no effect on glomerular LC (+) cell infiltration. The effect of leukocyte and of platelet depletion on glomerular synthesis of TxB₂, LTB₄, and 12-HETE is shown in Fig. 5. The synthesis of all three eicosanoids assessed in isolated glomeruli 1 h after administration of ER₄ was increased compared to control rats receiving mouse IgG. In the leukocyte-depleted group, the synthesis of these eicosanoids was no different than controls. In the platelet depleted group, the increment in 12-HETE synthesis was abolished. In contrast, TxB₂ synthesis was not different, and LTB₄ synthesis was found to be greater than the levels obtained in the group that received ER₄ antibody alone.

Fig. 6 demonstrates the effects of the thromboxane synthase and the 5-lipoxygenase inhibitors assessed individually and together on changes in GFR and RBF after administration of ER₄. Both the 5-lipoxygenase inhibitor MK-886 and the thromboxane synthase inhibitor furegrelate partially ameliorated the fall in GFR and RBF produced by ER₄, as both parameters remained significantly lower than baseline values in either group. In the group pretreated with a combination of furegrelate and MK-886, no significant changes in GFR or RBF were noted 1 h after administration of ER₄ antibody. Thus, combined thromboxane synthase and 5-lipoxygenase inhibition completely eliminated the decrements in GFR and RBF produced by ER₄.

Table I summarizes the effects of the TxA₂ receptor antagonist SQ-29,548 and of the arachidonate 5-lipoxygenase inhibitor MK-886 on ER₄-mediated changes in renal and glomerular hemodynamics. Of the determinants of glomerular filtration, P_{gc}, P_{tub}, and P_{ur} did not change, whereas there was a marked decrement in K_f and increments in R_a and R_e 1 h after adminis-

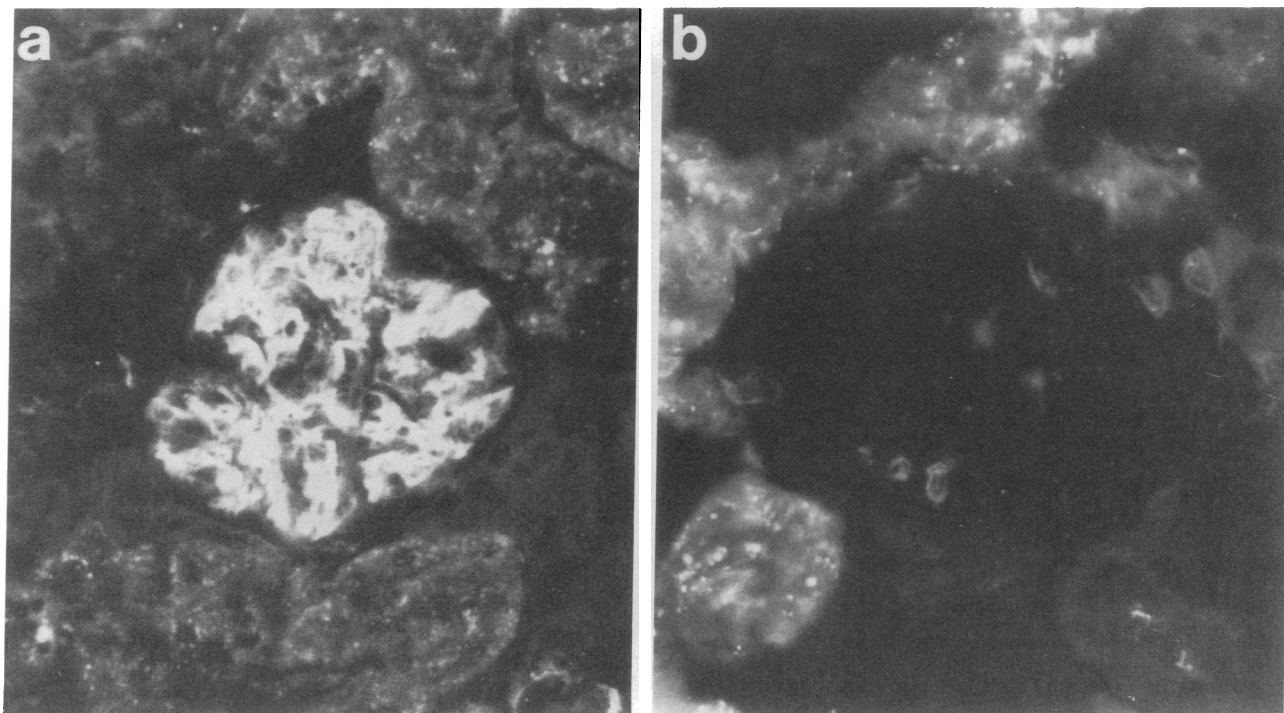


Figure 1. Immunofluorescence microscopy demonstrating deposition of the ER₄ antibody in the mesangium (a) and localization of LC (+) leukocytes (b) 1 h after antibody administration.

tration of ER₄ (Table I, *left column*). In animals pretreated with the thromboxane receptor antagonist SQ-29548, there was no change in R_a or K_f after ER₄, whereas R_e still increased significantly (Table I, *middle column*). Likewise, in animals pretreated with the 5-lipoxygenase inhibitor MK-886, there was no change in R_a or K_f but R_e increased significantly. In this group, mean P_{u,f} also increased after injection of ER₄ (Table I, *right column*).

The effect of thromboxane synthase and of 5-lipoxygenase inhibition on infiltration of leukocytes in glomeruli and on glomerular eicosanoid synthesis is shown in Figs. 4 and 7. The combination of furegrelate and MK-886 had no effect on glomerular leukocyte infiltration compared to the group receiving ER₄ alone (Fig. 4) but abolished the increments in glomerular LTB₄ and TxB₂ synthesis (Fig. 7). This indicates that the protective effect of MK-886 used in combination with furegrelate on GFR and RBF (Fig. 6) was caused by inhibition of synthesis of arachidonate 5-lipoxygenation products and of thromboxane synthesis rather than changes in leukocyte infiltration into the glomerulus. In the MK-886 pretreated group, the increments in glomerular LTB₄ synthesis were selectively abolished (Fig. 7) and glomerular LC (+) leukocyte counts were reduced compared to the group receiving ER₄ alone, but remained significantly elevated compared to the group that received mouse IgG (Fig. 4). Furegrelate selectively reduced glomerular TxB₂ synthesis (Fig. 7). In this group thromboxane inhibition was also associated with significant increments in LC (+) glomerular leukocyte counts and further enhancement of glomerular LTB₄ synthesis, compared to the group that received ER₄ alone (Figs. 4 and 7). Although furegrelate or MK-886 used individually had no effect on glomerular 12-HETE synthesis, the combined administration of these two inhibitors reduced the synthesis of 12-HETE.

Discussion

The model of anti-Thy 1 antibody-mediated mesangial immune injury is characterized by a lytic injury of the mesangial cell that is evident 24–48 h after formation of the Thy 1-anti-Thy 1 immune complex and a subsequent mesangioproliferative phase with enhanced mesangial matrix synthesis (1, 14). It resembles human mesangial nephropathies such as IgA and lupus nephritis in that the mesangial cell is the main target of immune injury and responds by proliferation, matrix synthesis, and renal hemodynamic impairment. After binding of the anti-Thy 1 antibody to the mesangium, acute decrements in GFR and RBF occur (3). We and others have demonstrated that these decrements in GFR and RBF are associated with elevated production of vasoactive eicosanoids such as TxA₂, and that these eicosanoids originate from infiltrating cells (platelets and leukocytes) rather than the injured mesangial cell (3, 4, 15). The present studies were undertaken to assess the hemodynamic role of the leukocyte- and platelet-derived eicosanoids TxA₂, LTB₄, and 12-HETE in mediating the acute changes in renal hemodynamics after mesangial cell injury induced by the monoclonal anti-Thy 1 antibody ER₄. Our observations indicate that the acute decrements in GFR and RBF that occur after binding of the anti-Thy 1 antibody to glomerular mesangium can be attributed to infiltrating leukocytes that mediate these changes via TxA₂ and arachidonate 5-lipoxygenation products. Thus, in leukocyte-depleted animals, the fall in GFR and RBF after ER₄ and the enhanced glomerular synthesis of TxB₂ and LTB₄ were abolished compared to the leukocyte-replete ER₄-treated rats (Figs. 3 and 5). Likewise, in animals that received combined thromboxane synthase and 5-lipoxygenase inhibition, the falls in GFR and RBF were also prevented (Fig. 6), and the elevations in TxB₂ and LTB₄ syn-

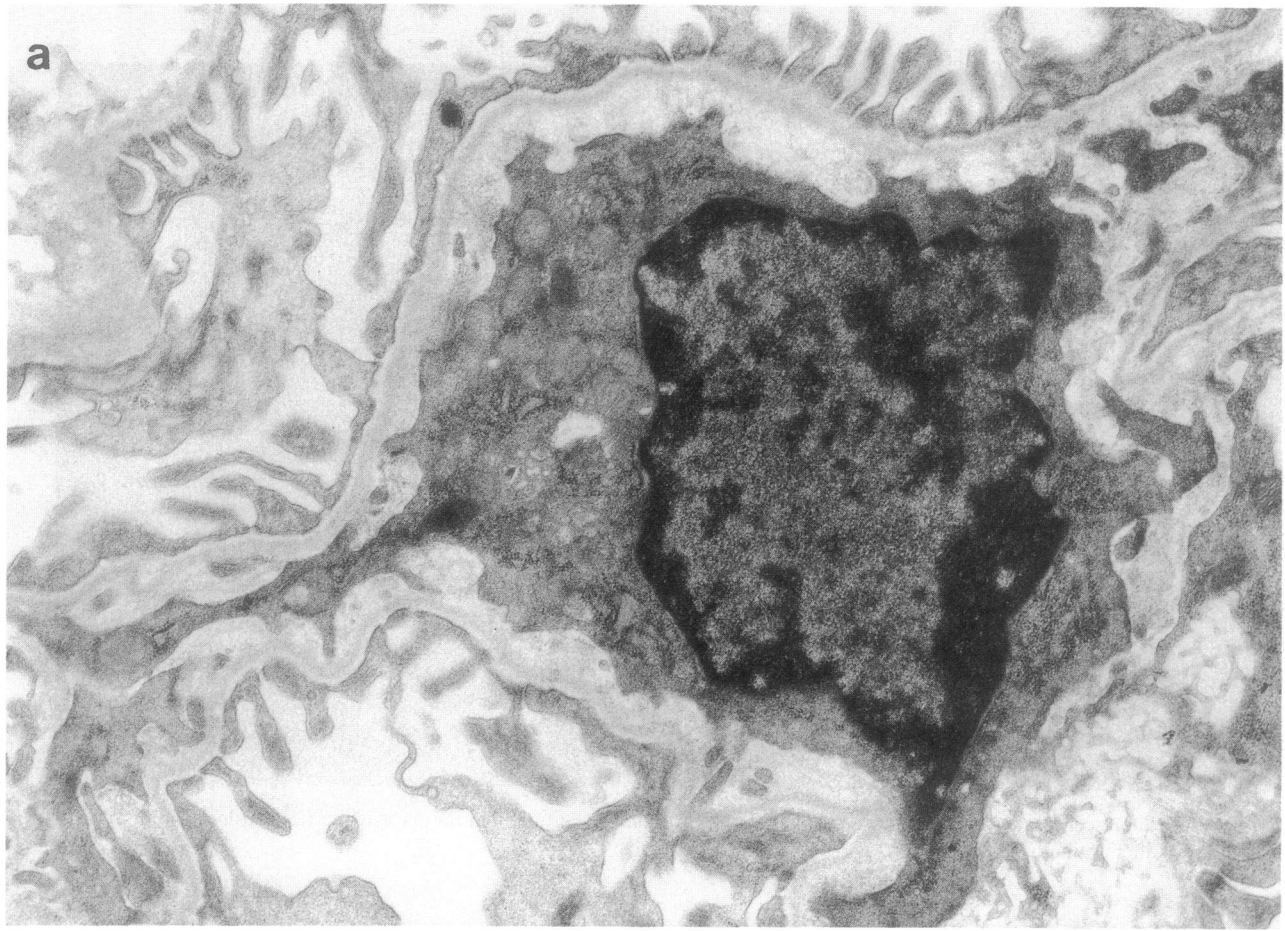


Figure 2. Electron micrographs demonstrating: a mesangial cell 1 h after administration of ER₄ without apparent lytic injury (a); a neutrophil anchored on the GBM (b); and detachment of the endothelial layer from the GBM (c).



Figure 2 (Continued)

thesis were abolished (Fig. 7), without a change in glomerular LC (+) cell counts (Fig. 4). Taken together, these observations indicate that the decrement in GFR after binding of ER₄ antibody to mesangial cells was caused by infiltrating leukocytes and was mediated by leukocyte-derived thromboxane and 5-lipoxygenation products. Thromboxane synthase and 5-lipoxygenase inhibition independently afforded partial protection in the changes in GFR and RBF produced by ER₄ (Fig. 6). On the other hand, inhibition of both enzymes together resulted in complete protection of these parameters (Fig. 6). This indicates that TxA₂ and arachidonate 5-lipoxygenation products both contribute to the fall in GFR and RBF, and their effects are additive.

To further explore the mechanism by which thromboxane and arachidonate 5-lipoxygenation products mediated decrements in GFR, we assessed the effect of TxA₂ receptor antagonism and of 5-lipoxygenase inhibition on the ER₄ antibody-mediated changes in glomerular hemodynamic parameters. As summarized in Table I, the most prominent change in these determinants was a fall in K_f that was abolished in animals pretreated with the Tx receptor antagonist SQ-29,548 or the 5-lipoxygenase inhibitor MK-886. These observations are in agreement with those made in the studies by Yamamoto et al., who assessed changes in glomerular hemodynamic parameters in antithymocyte antibody-mediated mesangial injury and demonstrated that the fall in single nephron filtration rates were mainly caused by a reduction in the glomerular ultrafil-

tration coefficient rather than by a fall in glomerular capillary pressure. Our results contrast with those of Yamamoto et al. in that both afferent and efferent arteriolar resistances increased

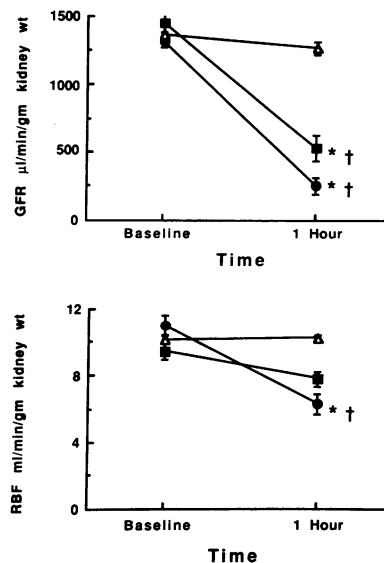


Figure 3. Effects of leukocyte depletion and platelet depletion on the ER₄ antibody induced decrements in GFR and RBF. 1 h after ER₄ administration, there was a marked decrement in GFR and RBF. Leukocyte depletion before ER₄ administration completely ameliorated the decrement in GFR and RBF. Platelet depletion did not alter the decrement in GFR, but did prevent the fall in RBF. **P* < 0.05 compared with baseline. †*P* < 0.05 compared with controls receiving mouse IgG (data not

shown). —●—, ER₄; —△—, leukocyte depletion + ER₄; and —■—, platelet depletion + ER₄.

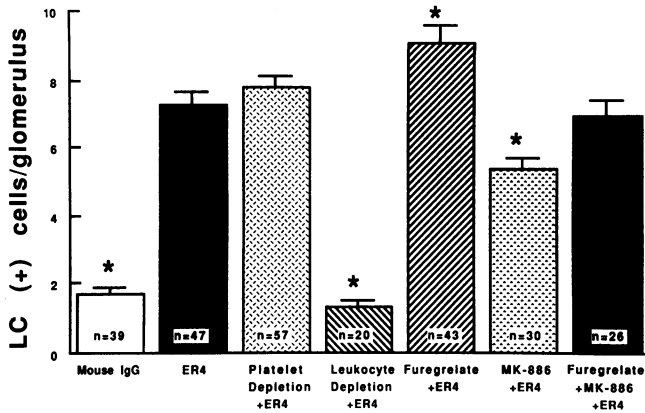


Figure 4. Effect of cell depletion and eicosanoid synthase inhibition on ER₄ antibody induced increments in LC (+) leukocytes per glomerulus. Administration of furegrelate and MK-886 before ER₄ antibody did not alter the influx of LC (+) cells into the glomerulus. Furegrelate alone, however, increased the number of LC (+) cells. *n* = number of glomeruli sampled, **P* < 0.05 compared with ER₄.

in our studies, whereas the afferent resistance fell without a change in the efferent resistance in the studies by Yamamoto et al. (5). The fall in afferent arteriolar resistance in the studies by Yamamoto et al. was proposed to be caused by impaired transmission of vasoconstrictor signals to the afferent arteriole by dysfunctional mesangial cells. Indeed, these previous observations were made 24 h and 6 d after anti-Thy 1 antibody administration at a time when evidence for mesangial cell injury and lysis was prominent (5). In contrast, our observations were made only 1 h after injection of ER₄, when evidence for mesangial cell injury is not apparent (Fig. 2 a). We attribute the changes in R_a to direct effects of vasoconstrictor eicosanoids on the afferent arteriole. Since SQ-29,548 and MK-886 ameliorated the increments in R_a (Table I), it can be proposed that PGH₂ or TxA₂ and an arachidonate 5-lipoxygenation product (i.e., a leukotriene) were the vasoconstrictors mediating this change. In contrast, neither SQ-29,548 nor MK-886 prevented

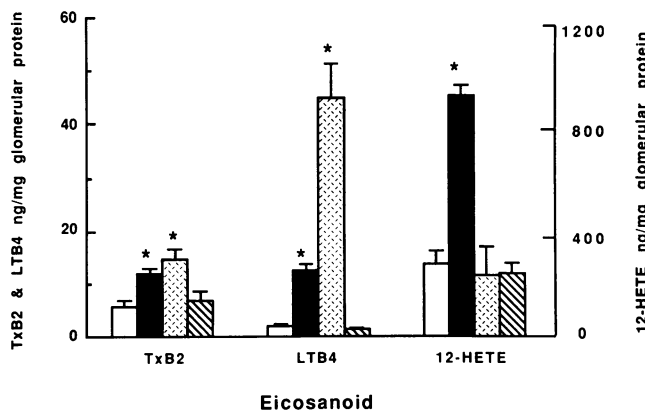


Figure 5. Effect of platelet depletion and leukocyte depletion on glomerular TxB₂, LTB₄, and 12-HETE synthesis. Platelet depletion before ER₄ reduced 12-HETE synthesis to control levels. Leukocyte depletion before ER₄ reduced the synthesis of TxB₂, LTB₄, and 12-HETE to control levels. **P* < 0.05 compared with mouse IgG. □, mouse IgG; ■, ER₄; ▨, platelet depletion + ER₄; and ▩, leukocyte depletion + ER₄.

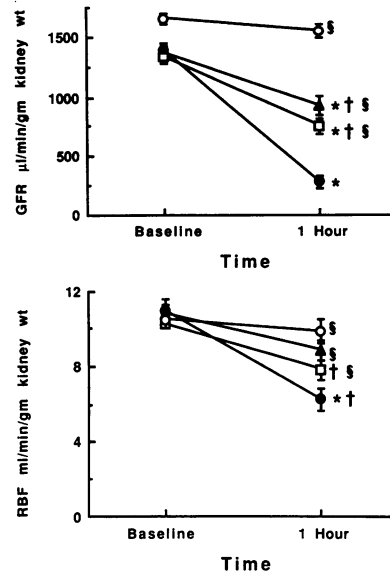


Figure 6. Effect of thromboxane synthase inhibition and 5-lipoxygenase inhibition on the ER₄ induced decrements in GFR and RBF. Furegrelate alone or MK-886 alone before ER₄ partially ameliorated the decrements in GFR and RBF. In combination, furegrelate and MK-886 pretreatment completely ameliorated the decrements in GFR and RBF. **P* < 0.05 compared with baseline. †*P* < 0.05 compared with controls receiving mouse IgG (data not shown). §*P* < 0.05 compared with ER₄. —●—, ER₄; —▲—, furegrelate + ER₄; —□—, MK-886 + ER₄; and —○—, furegrelate + MK-886 + ER₄.

the increments in R_e, indicating that a vasoconstrictor other than PGH₂ or TxA₂ or a 5-lipoxygenation product mediated the changes in R_e after ER₄. A likely noneicosanoid mediator for this effect may be angiotensin II, the production of which could be stimulated secondary to stimulated renin secretion from the macula densa as a result of the marked decrement in nephron filtration observed after ER₄.

As binding of ER₄ to the mesangium did not result in mesangial cell lysis in our studies (Fig. 2 a), we believe that the mesangial cell remained functional and responsive to factors such as TxA₂, LTC₄, and LTD₄, which have been shown to induce contraction of cultured mesangial cells in vitro (6, 7). If a similar response occurs in vivo, this could contribute to the decrements in the glomerular ultrafiltration coefficient. To this extent, both the TxA₂ receptor antagonist SQ-29,548 and the 5-lipoxygenase inhibitor MK-886 completely abolished the decrements in K_f indicating that PGH₂ or TxA₂ or an arachidonate 5-lipoxygenation product (i.e., a leukotriene) contributed to this event (Table I). Yet our studies cannot implicate thromboxane or the leukotrienes and mesangial cell contraction as the sole mediators of the changes in glomerular hemodynamic parameters induced by ER₄. Indeed, the structural alterations we observed in our electron microscopy evaluation of the glomerular capillary could also contribute to decrements in K_f. Thus, the glomerular infiltration and GBM anchoring of leukocytes (Fig. 2 b), and the detachment of the endothelial layer in glomerular capillaries (Fig. 2 c) observed in our study can reduce effective filtration surface area and thus contribute to the fall in K_f.

Our data do not implicate LTB₄ as the 5-lipoxygenation product responsible for the decrements in GFR. Indeed, LTB₄ has been shown to have minimal effects on the renal vasculature (16). Other products such as LTC₄ and LTD₄ may be involved, since both of these eicosanoids reduce GFR and K_f (17, 18) and induce mesangial cell contraction in vitro (7). Moreover, LTD₄ receptor antagonism has been shown to partially ameliorate decrements in GFR and K_f in glomerular immune injury induced by anti-glomerular basement membrane

Table 1. Effects of Thromboxane Receptor Antagonism and of 5-Lipoxygenase Inhibition on ER₄-Induced Changes in Renal and Glomerular Hemodynamics

	ER ₄ (n = 8)		ER ₄ + SQ29548 (n = 7)		ER ₄ + MK886 (n = 5)	
	Baseline	1 h	Baseline	1 h	Baseline	1 h
Plasma (Protein) mg/dl	4.9±0.2	4.6±0.2	5.2±0.2	4.9±0.2	5.2±0.4	5.2±0.2
GFR ml/min per g kidney wt	0.97±0.10	0.14±0.04*	1.15±0.08	1.04±0.14	0.92±0.05	0.65±0.07*
RBF ml/min per g kidney wt	11.3±1.1	7.9±0.9*	12.4±1.0	9.8±0.8*	9.9±0.8	8.8±0.6
Arterial pressure mmHg	129±4	125±4	128±4	124±6	123±7	120±6
P _{gc} mmHg	52.2±2.0	54.0±2.2	55.3±2.7	54.7±1.9	49.8±0.9	49.5±0.9
P _{tub} mmHg	11.7±0.7	9.6±0.8	15.0±2.0	11.4±0.5	19.0±1.9	14.4±1.4*
P _{cap} mmHg	12.8±0.8	12.1±1.4	17.8±1.7	15.0±0.9	21.4±1.4	13.8±1.8*
Π _{gc} mmHg	18.1±0.6	15.0±0.8*	18.6±0.4	18.1±0.8	19.7±2.2	18.1±0.9
P _{ur} mmHg	21.9±2.1	27.6±2.1	21.7±4.0	25.2±2.1	11.0±1.9	16.9±1.0*
R _a mmHg/ml per min per g kidney wt	7.3±0.9	9.6±1.2*	6.5±1.2	7.5±1.0	7.4±0.6	8.3±1.3
R _c mmHg/ml per min per g kidney wt	4.3±0.7	5.9±0.8*	3.3±0.2	4.7±0.3*	3.4±0.6	4.5±0.4*
R _v mmHg/ml per min per g kidney wt	0.8±0.1	1.1±0.3	1.3±0.3	1.1±0.9	1.8±0.1	1.1±0.2*
K _f ml/min per g kidney wt per mmHg	0.045±0.008	0.006±0.002*	0.078±0.019	0.042±0.005	0.098±0.028	0.039±0.004

Paired measurements obtained before and 1 h after administration of ER₄ antibody.

* *P* < 0.05 compared with baseline values.

antibody (19). Since the antileukocyte immune serum used induced a nonselective leukopenia, we cannot attribute the protective effect of immune leukopenia on GFR and RBF to a certain cell type. In previous studies, we and others have shown that the monocyte-macrophage infiltrates the glomerulus after binding of anti-Thy 1 antibody to the mesangium and accounts for the origin of TxA₂ and LTB₄ (3, 4, 20). However, neutrophils also infiltrate glomerular structures at early stages of anti-Thy 1 antibody-mediated mesangial injury (reference 3 and Fig. 2 *b*) and, therefore, a role of these cells in mediating decrements in GFR and RBF via arachidonate or 5-lipoxygenation products cannot be ruled out.

In the platelet-depleted group, glomerular 12-HETE synthesis was selectively abolished without a change in LC (+) cell counts (Figs. 4 and 5). Yet, GFR remained depressed and at levels no different than in the ER₄-treated group (Fig. 3), indicating that platelets or the platelet-derived 12-HETE did not contribute to decrements in glomerular filtration after ER₄

binding. In contrast, platelet depletion ameliorated the decrements in RBF. The protective effect of platelet depletion on RBF could be related to inhibition of 12-HETE synthesis, since 12-HETE has recently been shown to constrict preglomerular renal arteries (21). We do not have a satisfactory explanation for the failure of platelet depletion to blunt changes in GFR (Fig. 3). In reviewing the data in Figs. 4 and 5, platelet depletion did not alter glomerular LC (+) cell infiltration (Fig. 4) or the enhanced glomerular thromboxane synthesis (Fig. 5). Since the extent of leukocyte infiltration in glomerular capillaries plays a significant role in decreasing GFR (Fig. 3), it can be proposed that the failure of platelet depletion to blunt decrements in GFR is caused by an unchanged glomerular leukocyte infiltration and thromboxane synthesis in the thrombocytopenic animals.

In the group treated with furegrelate, both glomerular LC (+) cell counts and LTB₄ levels were increased compared with the group that received ER₄ alone (Figs. 4 and 7). The reason for these increments is not clear. Since infiltrating leukocytes apparently accounted for the increments in glomerular LTB₄ levels after mesangial cell immune injury (Fig. 5), the increments in glomerular LTB₄ levels found in the furegrelate-pretreated group might be caused by enhanced glomerular LC (+) leukocyte infiltration noted in this group (Fig. 4). The reason for the increased glomerular leukocyte infiltration is not clear and since thromboxane is a known leukocyte chemoattractant, one would expect either no change or a reduction in glomerular leukocyte infiltration in the furegrelate-pretreated group. To this extent, Nagamatsu et al. also found enhanced leukocyte infiltration in rats with glomerular immune injury (anti-GBM disease) pretreated with a cyclooxygenase inhibitor (22). The increments in glomerular LTB₄ levels in the furegrelate-treated group could be explained by a mechanism involving regulation of leukotriene synthesis by arachidonate cyclooxygenation products that have been shown to down regulate LTB₄ production (23), and by our previous observations, which indicate that TxA₂ may down regulate glomerular LTB₄ synthesis in mesangial cell immune injury mediated by the ER₄ antibody (24).

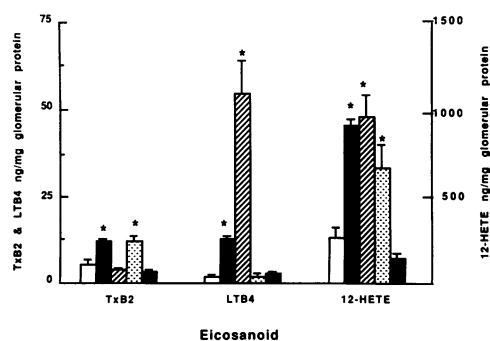


Figure 7. Effect of thromboxane synthase and 5-lipoxygenase inhibition on glomerular TxB₂, LTB₄, and 12-HETE synthesis. Pretreatment with furegrelate inhibited TxB₂ synthesis to control levels. Pretreatment with MK-886 decreased LTB₄ synthesis to control levels. * *P* < 0.05 compared with mouse IgG. □, Mouse IgG; ■, ER₄; ▨, furegrelate + ER₄; □, MK-886 + ER₄; ■, furegrelate + MK-886 + ER₄.

In summary, our studies indicate that in mesangial cell immune injury mediated by anti-Thy 1 antibody, the acute decrements in GFR and RBF are caused by infiltrating leukocytes and platelets that exert their effects via thromboxane and 5-lipoxygenation products by reducing the filtration surface area. Thromboxane and the 5-lipoxygenation products mediate the decrements in GFR mainly by reducing the ultrafiltration coefficient and to some extent by increasing afferent arteriolar resistance. Platelets do not participate in the fall in GFR, but they do contribute to the decrements in RBF, and this effect may be caused by the platelet-derived eicosanoid 12-HETE. Our observations are of significance in that they confirm that in an antibody-mediated mesangial immune injury specific vasoactive eicosanoids initiate the changes in renal hemodynamics via a paracrine effect.

Acknowledgments

MK-886 was generously provided by Dr. John Gillard of Merck-Frosst Canada Inc., Dorval, Quebec. Shelley Dufek and Beatrice Hucke provided expert technical assistance and Cindy Ziebell provided expert secretarial assistance. We thank Dr. Harold Modell (Educational Consulting Materials Development, Seattle, WA) for his help in implementing an iterative computer model for calculating K_f . We also thank Dr. Joseph Harb, Department of Pathology, Medical College of Wisconsin, for his valuable help in processing tissues for electron microscopy and comments in interpreting the findings.

This work was supported by U.S. Public Health Service grants DK-34793-05 to E. A. Lianos, DK-08243-02 to B. A. Bresnahan, and HL-36279 to R. J. Roman, and also an Arthritis and Prostaglandin Research Challenge Grant from G. D. Searle and Co. to E. A. Lianos. E. A. Lianos and R. J. Roman are Established Investigators of the American Heart Association.

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