

Mesangial Cell Immune Injury

Synthesis, Origin, and Role of Eicosanoids

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Abstract

The synthesis, cell origin, and physiologic role of eicosanoids were investigated in a model of mesangial cell immune injury induced by a monoclonal antibody against the rat thymocyte antigen Thy 1.1 also expressed in rat mesangial cells. A single intravenous injection of the antibody resulted in enhanced glomerular synthesis of thromboxane (Tx)B₂, leukotriene (LT)B₄, and 12-hydroxyeicosatetraenoic acid (HETE), whereas that of PGE₂ and PGF₂α was either unaltered or impaired. The enhanced eicosanoid synthesis was associated with decrements in glomerular filtration rate (GFR) and renal blood flow (RBF). Complement activation mediated both the increments in TxB₂, LTB₄, and 12-HETE and the decrements in GFR and RBF. The decrements in GFR were abolished by the TxA₂ receptor antagonist SQ-29,548. Although both neutrophils and Ia (+) leukocytes infiltrated glomeruli, glomerular LTB₄ originated mainly from the latter. Platelets entirely accounted for the enhanced 12-HETE synthesis in isolated glomeruli and to a lesser extent for that of LTB₄ and TxB₂. Glomerular PGE₂ and PGF₂α originated from mesangial cells as their impaired synthesis coincided with extensive mesangial cell lysis. The observations indicate that in mesangial cell immune injury vasoactive and proinflammatory eicosanoids originate from recruited or activated Ia (+) leukocytes and platelets and may exert paracrine effects on mesangial cells. (*J. Clin. Invest.* 1991; 88:623–631.) Key words: mesangial cell • nephritis • eicosanoids • leukocytes • platelets

Introduction

The Thy-1 gene is a member of the immunoglobulin “super family” of genes and is expressed in a number of different cell types (1). The expression product is a 17-kD cell surface antigenic glycoprotein (the Thy-1 antigen) that is expressed in thymocytes, young lymphocytes, and their precursor hematopoietic stem cells, in fibroblasts and in neurons of the central nervous system (1). Rat glomerular mesangial cells have also been shown to express the Thy-1.1 antigen (2). In Thy-1-bearing cells this antigen is anchored to the acyl chains of membrane phosphatidylinositol and can be cleaved by a phosphatidyli-

sitol-specific phospholipase C (3). A monoclonal antibody (ER₄, IgG2A), raised against the rat thymocyte antigenic determinant Thy 1.1, binds to rat glomerular mesangial cells and induces a complement-dependent injury resulting in mesangial cell lysis, followed by a mesangioproliferative phase (4). This model of glomerular immune injury can, therefore, be regarded as the experimental equivalent of nephropathies involving primarily the glomerular mesangium, and provides the opportunity to investigate the biology of mesangial cell injury as it relates to synthesis of proinflammatory mediators and renal hemodynamic perturbations. We, therefore, investigated the biosynthesis, cell origin, and role of vasoactive and proinflammatory arachidonate cyclooxygenation and lipoxygenation products.

Methods

Induction of mesangial cell immune injury. Mesangial cell injury was induced by a single intravenous administration of the mouse monoclonal antibody (ER₄) raised against the rat thymocyte antigenic determinant Thy 1.1 and generously provided by Dr. W. Bagchus, Department of Pathology, University of Groningen, The Netherlands. This antibody (molecular weight 25 kD) binds to thymocytes, bone marrow cells, peripheral blood lymphocytes, and to mesangial cells, and also demonstrates complement binding capacity (4). Male Munich-Wistar rats (165–290 g body wt) were employed and, in most experiments, two antibody doses were employed: a 2.5 and a 6 mg/kg body wt. Both doses induced proteinuria (urine protein excretion: 207±9 mg/24 h) and glomerulonephritis. The 6-mg/kg dose also induced decrements in glomerular filtration rate and renal blood flow (see below). Glomerular and mesangial cell lesions were assessed in cortical sections by routine methods of light and immunofluorescence microscopy. Light microscopy assessed changes in glomerular and mesangial cell morphology in 4–5-μ cortical sections stained with the Hematoxylin-Eosin and Periodic acid Schiff stains as well as the presence of blood-borne leukocytes in sections stained with the Giemsa stain. Immunofluorescence microscopy (direct) was performed in 7–8-μ cortical sections and assessed deposition and distribution of the ER₄ antibody using FITC-labeled goat anti-mouse IgG and of rat complement component C₃ using FITC-labeled goat anti-rat C₃. The presence of Ia (+) cells was also assessed by direct immunofluorescence using an FITC-labeled mouse monoclonal antibody against the rat monocyte/macrophage Ia determinant (Sera-Lab, Sussex, England). The presence of Ia (+) and blood-borne leukocytes (neutrophils) was expressed as number of cells per glomerulus (mean±SEM, n = 20 glomeruli).

Biochemistry studies. These studies were performed at two early points (1 and 2 h) and at two late points (day 4 and day 14) after ER₄ antibody administration. At these time points, 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, an aliquot of the medium (100–200 μl) was sampled and assayed directly for PGE₂, PGF₂α, and TxB₂ using specific RIA for these eicosanoids as previously described (5). In the remaining glomerular suspension, the phospholipase A₂ activator A₂₃₁₈₇ was added (final concentration 2 μM) in order to assess synthesis of the

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Received for publication 28 September 1990 and in revised form 21 March 1991.

J. Clin. Invest.

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0021-9738/91/08/0623/09 \$2.00

Volume 88, August 1991, 623–631

arachidonate lipoxygenation products 12-hydroxyeicosatetraenoic acid (HETE)¹ and leukotriene (LT)₄. A second incubation in the presence of A₂₃₁₈₇ was performed at 37°C for 45 min and was terminated by the addition of 2 vol of acidified absolute ethanol. After prolonged agitation at 4°C, the ethanolic mixture of the glomerular suspensions was centrifuged in order to separate glomeruli and precipitated proteins. The supernatant was dried under vacuum, suspended in 1 ml of methanol:water:acetic acid (64:34:2 vol/vol/vol), and injected in a gradient HPLC system in order to separate and isolate HETE and leukotrienes, as previously described (6). HPLC-isolated 12-HETE and LT₄ were subsequently measured using specific RIAs as previously described (6). The glomerular pellet was solubilized in 0.1 N sodium hydroxide and used for protein determination by a colorimetric method. Results were expressed in nanograms of eicosanoid/mg glomerular protein. Sensitivities and cross-reactivities of the RIAs employed for the above eicosanoids were previously assessed and reported (6). The antiserum for PGE₂ was purchased from the Institute Pasteur (Paris, France). The antiserum for thromboxane (Tx)₂ was a gift from Dr. W. Campbell (University of Texas Southwestern Medical School, Dallas, TX). The antiserum for PGF₂α was a gift from Dr. A. Hassid (New York Medical College, Valhalla, NY). The antiserum for LT₄ was generously provided by Dr. A. Ford-Hutchinson (Merck-Frosst/Canada, Dorval, Quebec, Canada). The antibody for 12-HETE was generously provided by Dr. L. Levine (Brandeis University, Waltham, MA).

Physiology studies. These studies assessed the effect of intravenous ER₄ antibody administration on changes in glomerular filtration rate (GFR) and renal blood flow (RBF). Animals were anesthetized with intraperitoneal thiobutabarbital (Inactin; Byk, Gulden Konstanz, FRG), 12–15 mg/100 g body wt, and maintained at 37°C by warming on a constant temperature table. After tracheostomy, a catheter was placed in the right jugular vein for infusion of 2% albumin in 0.9% sodium chloride at a rate of 20 ml/kg per h throughout the experiment. Tritiated inulin, 0.5 μCi/ml, was added to the infusion solution for measurement of GFR. The right femoral artery was cannulated for measurement of systemic blood pressure via a transducer connected to a digital pressure monitor and for collection of blood samples. Catheters were inserted into the right and left ureters for urine collection. A flow probe (1.5–2.0 mm) was placed around the left renal artery and RBF was measured using an electromagnetic flow meter (model 501; Carolina Instruments, King, NC). After completion of surgery and a 60-min stabilization period, two baseline clearance periods (15 min each) were obtained. Animals subsequently received a single intravenous injection of ER₄ antibody (6 mg/kg) or mouse IgG (controls, 6 mg/kg) and urine and plasma samples were collected for determination of GFR at 1 h after administration of ER₄.

In a second group of animals, GFR and RBF were assessed after decomplexation in order to determine the role of complement in mediating the ER₄ antibody-induced changes in these parameters. Decomplexation was achieved by the intraperitoneal injection of cobra venom factor (CVF; Cordis, Miami, FL) purified by ion exchange and gel filtration chromatography (7). 100 U per 100 g body wt was administered in four divided doses over the 24-h period preceding measurements of GFR and RBF. Animals were subsequently instrumented and GFR and RBF were measured as described above. Upon completion of these measurements rats were nephrectomized and glomeruli were isolated for assessment of eicosanoid synthesis as described above. Systemic decomplexation was assessed by a modified hemolytic assay, performed in serum samples obtained from the tail vein at baseline and before initiation of the physiologic studies. To perform the assay, sensitized sheep red blood cells, 1 × 10⁹ cells/ml, were incubated with varying dilutions of serum samples at 37°C for 1 h and subse-

quently centrifuged for 10 min. Absorbance (spectrophotometry) was measured at λ = 540 nm. Decomplexation was considered complete when absorbance readings from CVF-treated animals were less than 5% baseline samples. Decomplexation was also confirmed by the absence of immunofluorescence staining for glomerular C₃ deposits.

In a third group of animals, the ER₄ antibody was given after pretreatment with the Tx_{A2} 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). This compound was given as an intravenous bolus of 2 mg/kg, followed by a constant infusion of 2 mg/kg per h for 20 min before the administration of ER₄ antibody (6 mg/kg). GFR and RBF were determined before the infusion of SQ-29,548, before ER₄ antibody, and at 1 h after infusion of ER₄. Upon completion of these measurements, glomeruli were isolated for analysis and quantification of eicosanoids as described above.

Leukocyte and platelet depletion studies. Glomerular Ia (+) leukocytes were depleted by the use of a whole animal x irradiation protocol as previously described (8). Specifically, animals were subjected to 250 Kvp orthovoltage x rays with a half value of 1 mm Cu at a dose rate of 133 rad/min for a total dose of 900–1,100 rad, using parallel opposed fields. Kidneys were shielded with 6-mm thick lead blocks that covered the kidneys within 5 mm margins. Positioning of the blocks was verified with diagnostic x rays done simultaneously with treatment (port fields). Dosimetry was done in a plexiglass phantom using a Farmer-type ionization chamber. The effect of x irradiation was assessed on peripheral leukocyte counts determined by an automated hematology analyzer (Coulter Electronics, Hialeah, FL) before administration of ER₄ and on glomerular Ia (+) cells, neutrophil counts, and LT₄ synthesis at 2 h after ER₄. These studies employed three groups of rats that were studied at 24, 72, and 96 h after the x irradiation dose.

To deplete animals of circulating platelets, a rabbit anti-rat thrombocyte immune serum was employed (Accurate Co., Westbury, NY). A single intraperitoneal injection of 1.5 ml of this serum resulted in marked and selective systemic platelet depletion within 18–24 h and was associated with prolongation in the bleeding time. After platelet depletion was confirmed in each animal by an automated hematology analyzer (Coulter Electronics), ER₄ antibody (2.5 mg/kg) was injected and glomeruli were isolated 2 h later for determination of eicosanoid synthesis as described above.

In vitro eicosanoid synthesis. To assess whether binding of anti-Thy 1 antibody in the glomerular mesangium in vitro induces eicosanoid synthesis, glomeruli isolated from normal animals were subjected to mild enzymatic permeabilization in Hank's balanced salt solution containing 1% Hepes, collagenase Type II, 50 U/ml (Sigma Chemical Co., St. Louis, MO), and DNase 0.01% (Sigma Chemical Co.). After washing off the permeabilization solution, glomeruli were incubated with ER₄ antibody (2 mg/ml; n = 4) or mouse IgG (2 mg/ml; n = 4) in the presence of 10% rat serum or plasma as a source of complement. Incubations were performed at 37°C for 45 min in a manner similar to that employed in glomeruli isolated from animals that had received ER₄ injections. Binding of ER₄ antibody and of rat complement C₃ to the glomerular mesangium was documented by immunofluorescence microscopy in an aliquot of glomeruli at the end of the incubations. Glomerular incubations were terminated by the addition of 2 vol of absolute ethanol, and extracts were processed for isolation and quantification of LT₄ and 12-HETE using HPLC and RIA as described above.

EXPERIMENTAL GROUPS

1. Time course studies. Animals receiving 2.5 mg/kg of antibody ER₄ and killed at 1 h (n = 6), 2 h (n = 10), 4 d (n = 4), and 14 d (n = 4) after antibody administration. Parallel controls receiving mouse IgG and studied at 1 h (n = 5), 2 h (n = 10), 4 d (n = 8), and 14 d (n = 4).

2. Physiology studies. (a) Animals receiving ER₄ antibody 6 mg/kg (n = 8) or nonimmune mouse IgG (controls, n = 6) and studied at 1 h. (b) Decomplexed animals receiving ER₄ antibody 6 mg/kg (n = 8) and decomplexed controls receiving nonimmune mouse IgG (n

1. *Abbreviations used in this paper:* CVF, cobra venom factor; GFR, glomerular filtration rate; GBM, glomerular basement membrane; HETE, hydroxyeicosatetraenoic acid; LT, leukotriene; PG, prostaglandin; RBF, renal blood flow; Tx, thromboxane.

= 6) and studied at 1 h. (c) Animals pretreated with the TxA_2 receptor antagonist SQ-29,548, subsequently given ER_4 antibody 6 mg/kg ($n = 6$) and studied at 1 h.

3. *Studies in x irradiated animals.* These were performed at 24 h ($n = 6$), at 72 h ($n = 6$), or at 96 h ($n = 6$) after x irradiation. At these time points animals received ER_4 antibody (2.5 mg/kg) and glomerular eicosanoid synthesis was assessed 2 h later.

4. *Platelet depletion studies.* Animals received a single injection of anti-thrombocyte serum 18–24 h before ER_4 antibody (2.5 mg/kg; $n = 6$). Glomerular eicosanoid synthesis was assessed at 2 h after injection of ER_4 .

Statistical analyses employed the two-way analysis of variance for repeated measurements followed by a Duncan multiple range test and the Student's *t* test for unpaired observations, as dictated by the experimental designs. Results were expressed as mean \pm SEM.

Results

Effects of ER_4 on glomerular histopathology. Fig. 1 demonstrates glomerular deposition of the monoclonal antibody ER_4 at 1 h (Fig. 1 A) and 14 d (Fig. 1 B) after a single intravenous injection. The antibody initially localized in the glomerular mesangium (Fig. 1 A). It subsequently redistributed towards peripheral glomerular capillary loops (Fig. 1 B). Fig. 2 demonstrates the presence of glomerular Ia (+) cells in a control glomerulus (Fig. 1 A), at 2 h (Fig. 2 B), and on day 14 (Fig. 2 C) after a single intravenous injection of the ER_4 antibody. There was an increase in Ia (+) cells throughout the glomerulus at 2 h and a sustained increment in these cells on day 14, at which point their location was peripheral. Fig. 3 demonstrates the changes in glomerular cellularity at various time points after ER_4 antibody administration. Increased cellularity was noted at 1 to 2 h (Fig. 3 B) compared with control (Fig. 3 A). On day 4 (Fig. 3 C), glomerular hypocellularity with marked absence of mesangial cells was apparent. On day 14 (Fig. 3 D), glomerular cellularity recovered and there was also an increase in mesangial matrix.

Effects of ER_4 on glomerular eicosanoid synthesis: correlation with leukocyte infiltration. In Fig. 4 the changes in glomerular synthesis of TxB_2 , PGE_2 , and $\text{PGF}_2\alpha$ compared with pooled synchronous controls are shown at the various time points after a single intravenous administration of ER_4 antibody (2.5 mg/kg). There was a progressive and sustained increment in TxB_2 that spanned all time points of study. In contrast, the synthesis of PGE_2 and $\text{PGF}_2\alpha$ progressively declined and reached a nadir on day 4. The synthesis of these two eicosanoids subsequently recovered (day 14) to control levels.

In Fig. 5 the glomerular synthetic profiles of LTB_4 and 12-HETE, at the various time points after a single intravenous injection of ER_4 (2.5 mg/kg), are shown in parallel with LTB_4 and 12-HETE values obtained in synchronous controls. The synthesis of 12-HETE in animals receiving ER_4 was significantly enhanced at 2 h after antibody administration and remained increased at statistically significant levels at all subsequent time points compared with synchronous controls. In contrast to 12-HETE, significant increments in glomerular LTB_4 synthesis occurred earlier (1 h) but spanned a shorter period of time and returned toward control levels on day 4. LTB_4 synthesis remained significantly higher than synchronous controls on day 4 (0.51 ± 0.10 vs. 0.24 ± 0.04 ; $P < 0.05$) and was no different than controls on day 14. Fig. 6 demonstrates changes in glomerular Ia (+) and neutrophil cell counts.

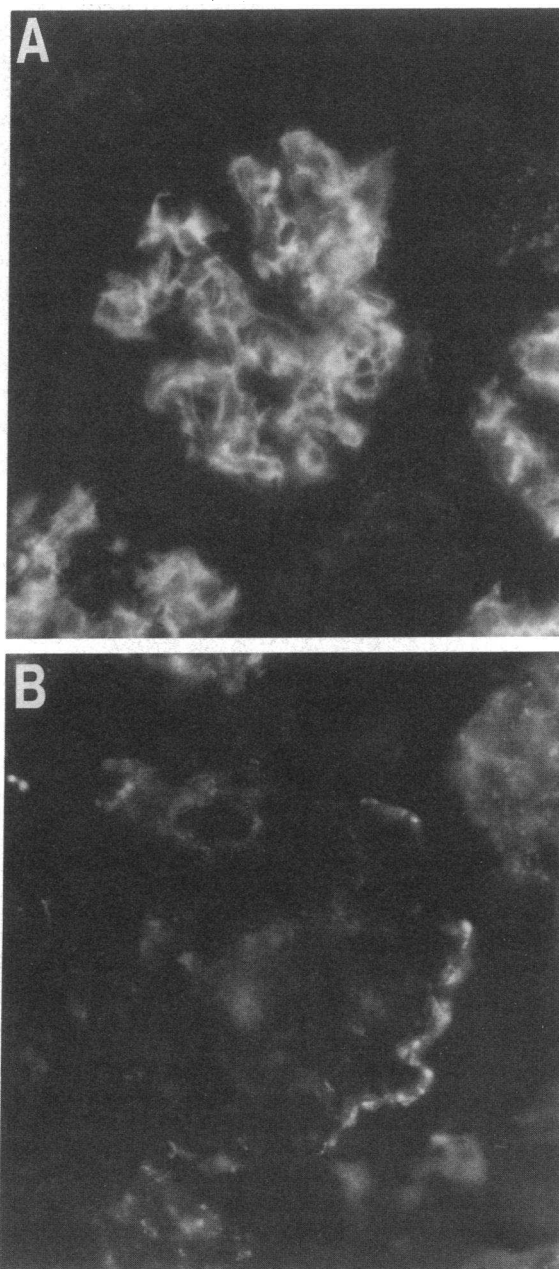


Figure 1. Immunofluorescence microscopy employing FITC-labeled goat anti-mouse IgG demonstrating glomerular ER_4 antibody deposition after a single intravenous dose. The antibody deposits in the mesangium at 1 h (A), with subsequent localization toward peripheral glomerular capillary loops on day 14 (B).

Counts of both cell types were significantly higher at all time points compared with synchronous controls. Whereas the increments in glomerular 12-HETE and LTB_4 were temporarily associated with the increments in glomerular Ia (+) and neutrophil cell counts at 1 and 2 h after administration of ER_4 (Figs. 5 and 6), at later time points (days 4 and 14), LTB_4 values returned toward control levels (Fig. 5) despite a sustained increase in glomerular Ia (+) cell and neutrophil counts (Fig. 6). 12-HETE synthesis remained significantly increased at all time points.

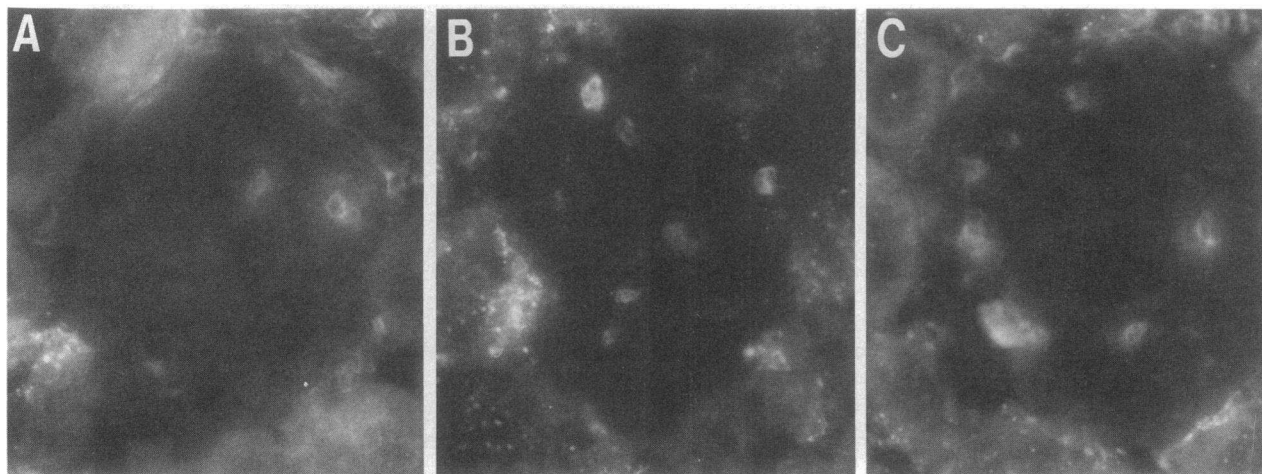


Figure 2. Immunofluorescence localization of glomerular Ia (+) cells in a control glomerulus (A), at 2 h (B), and on day 14 (C) after ER₄ administration.

Effects of x irradiation and of platelet depletion on glomerular eicosanoid synthesis. Table I demonstrates the correlation between glomerular LTB₄ synthesis and glomerular neutrophil and Ia (+) cell counts determined 2 h post-ER₄ and peripheral leukocyte counts assessed before ER₄ administration in the three groups of animals studied at 24, 72, and 96 h after x irradiation dose. Peripheral leukopenia and a relative reduction in glomerular neutrophils after x irradiation were present in all groups. Post-ER₄ glomerular LTB₄ synthesis was signifi-

cantly reduced only in glomerular preparations isolated from animals studied 96 h after x irradiation. In this group glomerular Ia (+) cell counts were also abolished. In the groups studied 24 and 72 h post-x irradiation, although marked peripheral leukopenia and glomerular reduction of neutrophils were present, glomerular LTB₄ synthesis measured 2 h post-ER₄ was not different compared with levels obtained in glomeruli isolated from nonirradiated rats studied 2 h after ER₄ antibody administration (Table I).

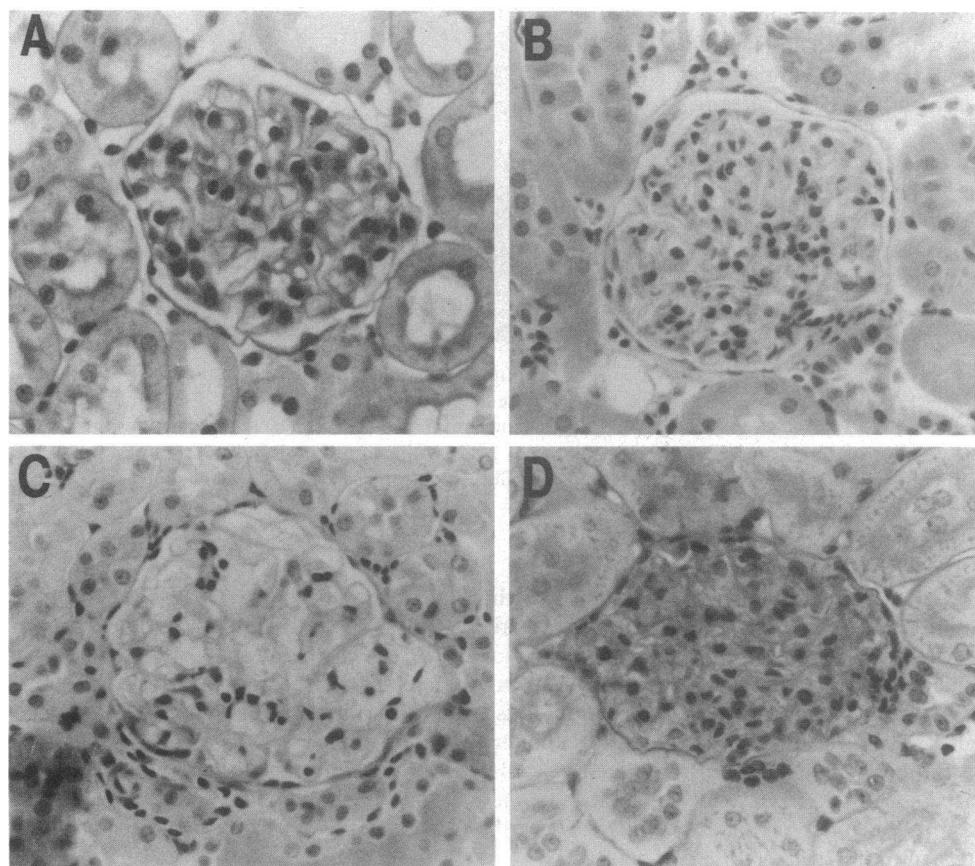


Figure 3. Light microscopy demonstrating changes in glomerular cellularity after a single administration of ER₄ antibody. (A) Control glomerulus; (B) 2 h post-ER₄; (C) day 4, post-ER₄; (D) day 14, post-ER₄.

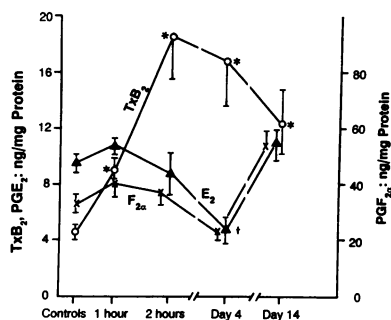


Figure 4. Effects of ER₄ administration (2.5 mg/kg) on glomerular TxB₂, PGE₂, and PGF_{2α} synthesis compared with pooled synchronous controls. There was no difference in the synthesis of these eicosanoids among controls at the various time points.

There is a dichotomy in synthetic profiles of

these three eicosanoids most notable on day 4. * $P < 0.05$, TxB₂ compared with pooled controls. † $P < 0.05$, PGE₂ and PGF_{2α} compared with pooled controls.

The effect of platelet depletion and of x irradiation on glomerular TxB₂, 12-HETE, and LTB₄ synthesis assessed at 2 h after administration of ER₄ (2.5 mg/kg) is shown in Fig. 7. The ER₄-induced increments in glomerular 12-HETE synthesis were abolished in the platelet-depleted group compared with the group that received ER₄ antibody alone (Fig. 7). In this group, glomerular TxB₂ and LTB₄ synthesis were also significantly reduced; however, they remained significantly higher compared with control values. In the x irradiated group (96 h postirradiation), glomerular TxB₂ and 12-HETE synthesis were not significantly different compared with the group that received ER₄ antibody alone. In contrast, LTB₄ synthesis was significantly reduced.

The antiplatelet antibody employed markedly reduced circulating platelet counts (from 929 ± 37 to $8.3 \pm 2.1 \times 10^3$ per μl ; $n = 6$) and had an insignificant effect on circulating leukocyte counts (from $10.9 \pm 0.8 \times 10^3$ to $8.6 \pm 1.3 \times 10^3$ per μl ; $n = 6$) assessed at 18 h after a single intraperitoneal injection of the antibody. X irradiation had no effect on circulating platelet counts assessed at 96 h ($940 \pm 15 \times 10^3/\mu\text{l}$) or on serum complement assessed by hemolytic assay at the same time point (hemolysis of sensitized sheep RBCs measured spectrophotometrically as hemoglobin absorbance units: base line = 0.700 ± 0.005 vs. post-x irradiation = 0.727 ± 0.024).

In vitro effect of ER₄ antibody on glomerular eicosanoid synthesis. Fig. 8 demonstrates binding of ER₄ antibody (Fig. 8 A) and of rat C₃ (Fig. 8 B) in isolated enzymatically permeabilized glomeruli incubated with the monoclonal antibody ER₄ (2 mg/ml) in the presence of 10% rat serum (source of complement). Fig. 8 C is a control glomerulus incubated with mouse

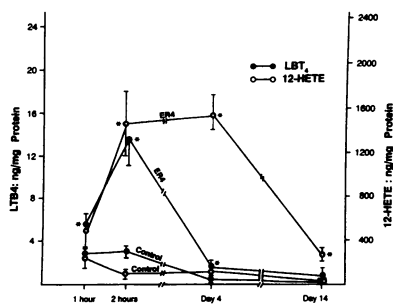


Figure 5. Effects of ER₄ administration (2.5 mg/kg) on glomerular LTB₄ and 12-HETE synthesis compared with synchronous controls. Significant increments in 12-HETE synthesis occurred at 2 h after ER₄ administration and were sustained on days 4 and 14. Significant increments in LTB₄ synthesis occurred at 1, 2 h, and 4 d and they were no different than controls on day 14.

ments in LTB₄ synthesis occurred at 1, 2 h, and 4 d and they were no different than controls on day 14.

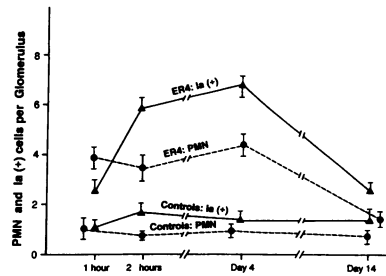


Figure 6. Changes in glomerular Ia (+) cell and neutrophil (PMN) counts at 1 h, 2 h, 4 d, and 14 d. The Ia (+) and neutrophil cell counts were significantly higher at all time points compared with synchronous controls.

IgG and stained for rat C₃. There was no change in glomerular LTB₄ synthesis in permeabilized glomeruli incubated with ER₄ antibody compared with control glomeruli incubated with mouse IgG (LTB₄: 0.9 ± 0.2 ng/mg glomerular protein in glomeruli incubated with ER₄ and 1.7 ± 0.2 ng/mg glomerular protein in glomeruli incubated with mouse IgG). Because the rat serum used as a source of complement was found to contain high levels of 12-HETE (866 ng/ml of serum containing medium), two additional experiments using rat plasma as a complement source were performed in order to assess changes in glomerular 12-HETE synthesis in response to ER₄ binding. The levels of 12-HETE in rat plasma containing media were 47 ng/ml. In permeabilized glomeruli incubated with ER₄ (2 ng/ml) in the presence of 10% rat plasma 12-HETE synthesis was 206 ng/mg glomerular protein. In control glomeruli incubated with mouse IgG 12-HETE synthesis was 260 ng/mg protein.

Effects of ER₄ on GFR and RBF: role of complement and of Tx receptor antagonism. Fig. 9 demonstrates the effect of complement depletion and of thromboxane receptor antagonism with SQ-29,548 on ER₄ antibody-induced decrements in GFR and RBF. The glomerular eicosanoid synthetic profiles corresponding to the four groups of animals studied and determined upon completion of the GFR and RBF measurements are shown in Table II. In both the complement-depleted group and the SQ-29,548-treated group the decrements in GFR and RBF were ameliorated to levels not different from each other and significantly higher than the group receiving ER₄ alone. In the SQ-29,548-pretreated rats, GFR and RBF values were no different compared with the decompartmented group of rats receiving mouse IgG (controls). In the decompartmented group receiving ER₄, the glomerular synthesis of TxB₂, LTB₄, and 12-HETE were significantly lower compared with the complement replete group receiving ER₄ (Table II). In the SQ-29,548-pretreated group the synthesis of these eicosanoids was no different than the group receiving ER₄ alone.

Discussion

Enhanced eicosanoid synthesis in isolated glomeruli has been demonstrated in various forms of experimental and clinical glomerulopathies. Studies have focused on identifying the type of eicosanoid(s) synthesized after initiation of immune injury and their role in mediating vasoactive and proinflammatory events occurring in the progression of injury. Increased synthesis of both arachidonate cyclooxygenation and lipoxygenation metabolites has been demonstrated (9). Of the former, increased glomerular synthesis of thromboxane has been consistently shown in most forms of antibody-mediated renal injury and has been implicated in effecting adverse hemodynamic effects such as decrements in GFR and RBF (9). Of the arachi-

Table 1. Effect of X-Irradiation on Peripheral Leukocyte Counts (WBC) before ER₄ Administration and on Glomerular Ia (+) Cell and Neutrophil Counts and Glomerular LTB₄ Levels, 2 h after ER₄ Administration

	WBC × 10 ³ /μl	Ia(+) cells/glomerular	PMN/glomerular	LTB ₄ :ng/mg protein
	Pre-ER ₄	2 h Post-ER ₄	2 h Post-ER ₄	2 h Post-ER ₄
No irradiation (n = 10)	10.45±0.4	7.3±2.1	5.0±0.7	13.7±2.7
24 h post-irradiation (n = 8)	2.20±0.7*	4.8±2.2	1.6±0.5*	12.9±0.1
72 h post-irradiation (n = 6)	1.90±0.4*	2.5±1.4*	1.4±0.4*	12.3±1.7
96 h post-irradiation (n = 6)	1.97±0.3*	0.8±0.6*	2.1±0.3*	4.6±0.3*

PMN, polymorphonuclear leukocyte. * $P < 0.05$ compared with no irradiation.

donate lipoxygenation metabolites, HETE and leukotrienes have been best characterized (6, 10). Both vasoactive and proinflammatory roles have been attributed to these metabolites. Thus, antagonism of the LTD₄ receptor prevents anti-glomerular basement membrane (GBM) antibody-mediated acute decrements in GFR (11), and essential fatty acid-deficient diets inhibit glomerular LTB₄ synthesis and ameliorate the hemodynamic and histopathological severity of anti-GBM glomerular injury (12).

The cell of origin of "glomerular" eicosanoids has been an issue of debate. In antibody-mediated models of glomerular immune injury, it seemed reasonable to assume that the injured glomerular cell was a likely source of eicosanoids. This was a particularly reasonable assumption in models employing antibodies directed against antigens of specific glomerular cells (i.e., antibodies against the Fx1A antigen of glomerular epithelial cells) that, when cultured, were capable of eicosanoid synthesis. This assumption was strengthened further when the role of hematogenous cells capable of eicosanoid synthesis was assessed in infiltrative models of glomerular immune injury. Thus, in anti-GBM disease, an infiltrative form of experimental glomerulonephritis, it was demonstrated that platelets did not contribute to the enhanced glomerular 12-HETE synthesis (13) and, in the same disease model, neutrophil depletion only partially contributed to the enhanced glomerular LTB₄ synthesis (6). Likewise, in a different model of infiltrative glomerulonephritis, induced by administration of cationized gamma globulin, enhanced glomerular LTB₄ synthesis persisted in neutropenic rats (10). Several lines of evidence, however, indicate that cells other than those on which immunologic reactants (i.e., antibody and complement components) bind may account for the enhanced eicosanoid synthesis observed in glomeruli isolated after initiation of injury. (a) Isolated normal glomeruli

synthesize measurable amounts of LTB₄ and 12-HETE when incubated with the phospholipase A₂ activator, A₂₃₁₈₇ (6), yet, none of the glomerular cell populations in culture (epithelial, mesangial, endothelial) express the arachidonate 5-lipoxygenase gene (14, 15). (b) In glomerular epithelial cell injury mediated by anti-Fx1A antibody, there is enhanced glomerular LTB₄ synthesis despite the fact that glomerular epithelial cells in culture do not synthesize LTB₄ and glomeruli do not show infiltration by leukotriene-producing cells (i.e., leukocytes) after anti-Fx1A administration (16). (c) Diets deficient in essential fatty acids, or bone marrow depletion using whole animal x irradiation, abolish glomerular LTB₄ synthesis, and this event was convincingly correlated with depletion of glomerular Ia-bearing monocytes (17), an observation that points to this cell type as a likely source of arachidonate lipoxygenation products in glomeruli.

On the basis of these controversial observations, we reasoned that because the eicosanoid synthetic profile of mesangial cells is well characterized (18) and the role of mesangial cells in regulating glomerular filtration emphasized (19), the model of anti-Thy 1 antibody-induced mesangial cell injury is most suitable to study origin and physiologic role of eicosanoids. The demonstration that the Thy-1 antigen is specifically linked to membrane phosphatidylinositol in Thy-1-bearing cells (3), lends further suitability to this model of immune injury for study of the origin and role of eicosanoids.

Our observations indicate that in mesangial cell immune injury induced by the monoclonal anti-Thy 1 antibody ER₄, cells other than the injured mesangial account for the enhanced glomerular eicosanoid synthesis. Moreover, different cells account for the synthesis of different eicosanoids. This is best illustrated in Fig. 4, where the dichotomy in the synthesis of the three arachidonate cyclooxygenation products, PGE₂, PGF₂α, and TxB₂ is shown. This dichotomy is most apparent on day 4, when TxB₂ synthesis was at a maximum, while that of PGE₂ and PGF₂α was at a minimum and at levels lower than controls. As the nadir in PGE₂ and PGE₂α synthesis (Fig. 4) correlated with glomerular hypocellularity and mesangial cell depletion (Fig. 3 C), we propose that the origin of these two eicosanoids is the mesangial cell. This contention is supported by the well established profile of eicosanoids in cultured mesangial cells that includes primarily PGE₂ and PGF₂α (18). The recovery in PGE₂ and PGF₂α synthesis on day 14 after administration of ER₄ (Fig. 4) could be due to a mesangioproliferative phase (Fig. 3 D), which follows the mesangiolytic phase as previously described in this model (4, 20).

That the Ia (+) leukocyte is a likely source of glomerular LTB₄ is supported by the following two observations: (a) In x

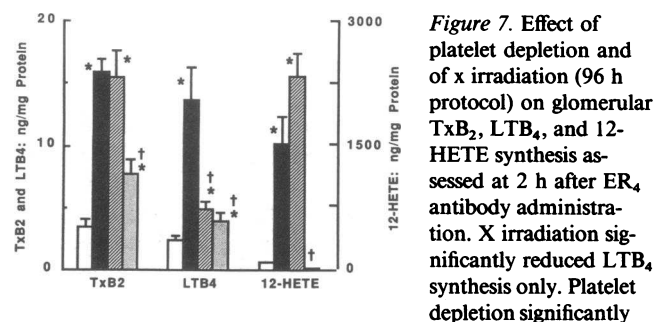


Figure 7. Effect of platelet depletion and of x irradiation (96 h protocol) on glomerular TxB₂, LTB₄, and 12-HETE synthesis assessed at 2 h after ER₄ antibody administration. X irradiation significantly reduced LTB₄ synthesis only. Platelet depletion significantly

reduced TxB₂ and LTB₄ synthesis and abolished 12-HETE synthesis. Control (□); ER₄ (■); X irradiated (▨); platelet depleted (▩). * $P < 0.05$ compared with controls. † $P < 0.05$ compared with ER₄.

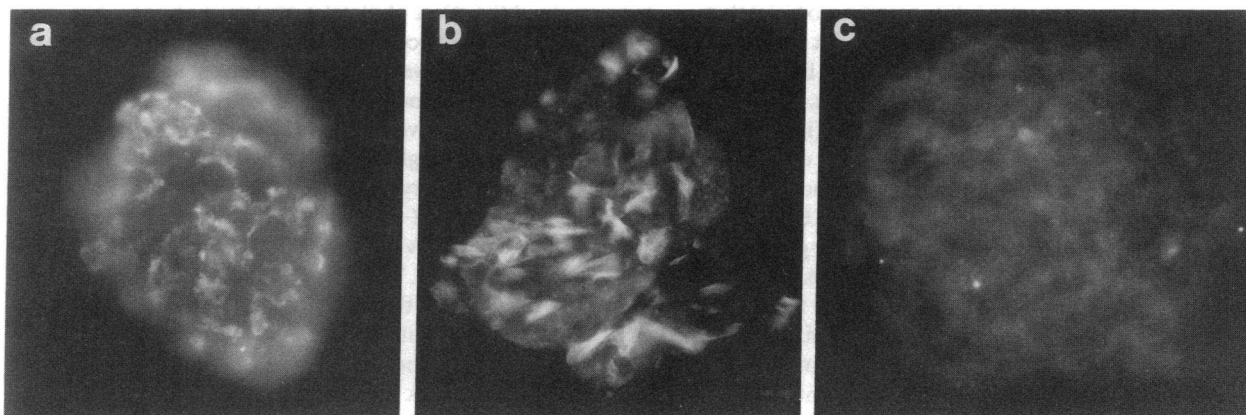


Figure 8. Binding of the ER₄ antibody (A) and rat C₃ (B) in isolated enzymatically permeabilized normal glomeruli. C is a control glomerulus stained for C₃.

irradiated animals, enhanced glomerular LTB₄ synthesis determined 2 h post-ER₄ continued in a manner independent of peripheral leukocyte counts and of glomerular neutrophil counts (Table I). It was abolished only in animals studied 96 h after x irradiation, when glomerular Ia (+) cell counts were also abolished (Table I); (b) binding of ER₄ in permeabilized glomeruli (Fig. 8) originating from normal animals and in the presence of rat serum did not result in enhanced LTB₄ or 12-HETE synthesis. Cell types possessing the Ia antigen that could account for the enhanced glomerular LTB₄ synthesis include: elicited monocyte-macrophages, activated lymphocytes, activated glomerular endothelial cells, and the mesangial cell itself (21). As there is no evidence that lymphocytes synthesize 5-HETE or LTB₄ (22), or that glomerular epithelial, endothelial, or mesangial cells express the 5-lipoxygenase gene (14), we propose that the Ia (+) monocyte-macrophage is the likely source of LTB₄. Our observations do not entirely rule out the neutrophil as a potential source of LTB₄. As shown in Figs. 5 and 6, the increments in glomerular LTB₄ at 1 and 2 h coincided with

increments in both glomerular Ia (+) and neutrophil counts. However, on days 4 and 14 after ER₄, LTB₄ synthesis returned toward control levels despite significant glomerular infiltration by neutrophils (Fig. 6). Moreover, in x irradiated leukopenic animals, glomerular depletion of neutrophils did not result in decreased glomerular LTB₄ synthesis after ER₄ (Table I). These observations indicate that the infiltrating neutrophil does not entirely account for the enhanced glomerular LTB₄ synthesis after administration of ER₄. The reason for the discrepancy between glomerular Ia (+) and neutrophil cell counts and LTB₄ levels on days 4 and 14 (Figs. 5 and 6) is not clear. Specifically, it is not clear why LTB₄ synthesis returned toward control levels despite the sustained increase in glomerular Ia (+) and neutrophil counts. A similar phenomenon was previously described in anti-GBM antibody-induced injury, in which the enhancement in glomerular LTB₄ synthesis was again short lived and did not correlate with glomerular neutrophil infiltration (6).

The enhanced glomerular LTB₄ synthesis was complement dependent (Table II) and this might be accounted for by the effect of complement activation and anaphylatoxin release on glomerular leukocyte infiltration. In addition, it is possible that binding of anaphylatoxin (C5a), generated after intraglomerular complement activation, on Ia-bearing macrophages results in enhanced LTB₄ synthesis either directly or via a mechanism involving IL-1. Specific receptors for C5a have been demonstrated on murine Ia-bearing macrophages and binding of C5a on these receptors induces IL-1 secretion (23). IL-1, in turn, is a known eicosanoid synthesis agonist.

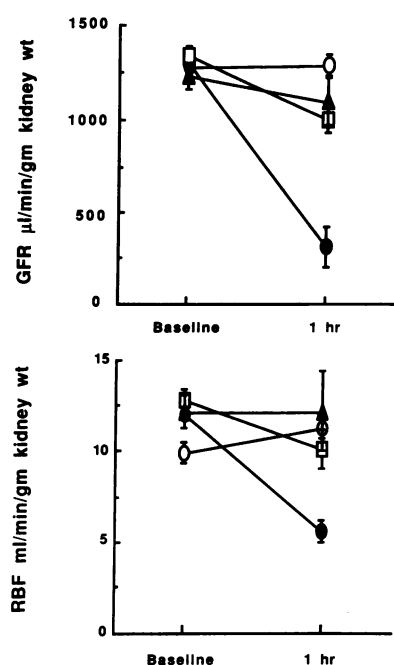


Figure 9. Effects of complement depletion and of pretreatment with the TxR₂ receptor antagonist SQ-29,548 on the decrements in GFR and RBF induced by administration of ER₄. In the group pretreated with SQ-29,548, GFR and RBF were ameliorated to levels no different than those of the decompartmented group. There were no differences in GFR and RBF between complement replete (n = 6) and decompartmented rats (n = 6) that received mouse IgG. Decompartmented controls (○), controls, ER₄ (●), decompartmented + ER₄ (-□-), and SQ-29,548 + ER₄ (-▲-).

Table II. Glomerular Eicosanoid Synthesis (ng/mg Glomerular Protein) 1 h after ER₄ or Mouse IgG Administration in Normal, Decompartmented (CVF + ER₄), and Tx Receptor Antagonist (SQ-29,548 + ER₄)-pretreated Rats

	Controls n = 6	ER ₄ n = 8	CVF + ER ₄ n = 8	SQ-29,548 + ER ₄ n = 6
TxB ₂	4.9±0.6*	11.4±1.0	7.4±1.1*	14.4±2.0
LTB ₄	0.7±0.1*	11.4±2.6	4.9±0.8*	11.1±3.0
12-HETE	46±12*	988±75	263±57*	1532±308

* P < 0.05 compared with ER₄.

The dependence of glomerular LTB₄ synthesis on platelets is intriguing (Fig. 7), mainly because platelets do not synthesize LTB₄. Thus, this observation raises the possibility for a platelet/leukocyte interaction resulting in LTB₄ synthesis. The platelet could generate an eicosanoid synthesis agonist (i.e., PDGF or TGF- β). It may provide arachidonate, which can subsequently undergo 5-lipoxygenation to LTB₄ by 5-lipoxygenase-containing leukocytes (24), or it may augment LTB₄ production by leukocytes through a mechanism involving generation of 12-HPETE (25). In the latter case, however, one would expect the enhanced glomerular 12-HETE synthesis to precede that of LTB₄, an event that was not noted in our time course studies (Fig. 5), which demonstrate that the enhanced LTB₄ synthesis preceded that of 12-HETE. The complement system may also play a role in mediating platelet recruitment and/or activation, thereby promoting platelet-leukocyte interactions. In mesangial cell injury induced by a polyclonal rabbit anti-rat thymocyte antibody, it was demonstrated that complement depletion using CVF reduced glomerular platelet infiltration (26). As can be concluded from Fig. 7, platelets accounted for the glomerular 12-HETE and TxB₂ synthesis. Of these two eicosanoids, the effect on 12-HETE was the most striking. In contrast, TxB₂ levels remained elevated compared with controls. In contrast to platelet depletion, x irradiation, followed by a 96-h waiting period, had no effect on glomerular TxB₂ or 12-HETE synthesis (Fig. 7). As this x irradiation protocol depletes glomeruli of Ia-bearing monocytes (reference 8 and Table I), our observations indicate that this cell population does not entirely account for the sustained increments in glomerular TxB₂ or 12-HETE synthesis after administration of ER₄. This contrasts with the observations of Stahl and co-workers, who reported a monocyte dependence of glomerular TxB₂ synthesis in a model of mesangial cell injury using rabbit polyclonal antibody against the rat thymocyte (27). This discrepancy could be due to the different experimental approaches employed in order to deplete monocytes, namely, immune monocytopenia (27) versus x irradiation, which causes bone marrow depletion and progressive glomerular Ia (+) cell depletion.

Our data indicate that the protective effect of complement depletion on GFR and RBF was associated with decreased glomerular synthesis of TxB₂, LTB₄, and 12-HETE (Table II). The protective effect of deplementation can be accounted for by the decrements in thromboxane synthesis, as pretreatment with the TxA₂ receptor antagonist SQ-29,548 ameliorated the ER₄-induced decrements in GFR to an extent similar to that noted in deplemented animals (Fig. 9) and had no effect on TxB₂, LTB₄, or 12-HETE synthesis (Table II). That thromboxane mediates the acute decrements in GFR is also supported by the recent observations of Stahl and co-workers, who demonstrated that thromboxane synthase inhibition ameliorated the anti-thymocyte antibody-induced decrements in GFR (27). Whether the enhanced synthesis of LTB₄ or 12-HETE have a vasoactive effect and contribute to the decrements in GFR is speculative. A synergistic effect to thromboxane is likely because 12-HETE has recently been shown to vasoconstrict the rat vasculature (28) and essential fatty acid-deficient diets inhibit glomerular LTB₄ and ameliorate decrements in GFR and RBF in glomerular immune injury induced by anti-GBM antibody (12). The availability of specific 12-lipoxygenase or 5-lipoxygenase inhibitors should allow definitive conclusions to be made on the role of 12-HETE and LTB₄ in mediating the decrements in GFR and RBF. More-

over, study of the effect of platelet depletion on renal hemodynamic perturbations occurring in mesangial cell immune injury would allow unravelling of the role of platelet-derived vasoactive eicosanoids, such as Tx and 12-HETE.

In summary, we have demonstrated that the enhanced synthesis of eicosanoids in glomeruli isolated from rats with mesangial cell immune injury originates from cells other than the mesangial cell. The Ia (+) leukocyte is a likely source of LTB₄ by a mechanism involving interactions with the complement system and the platelet. The stimulus for LTB₄ synthesis, however, is short lived and the participation of the neutrophil cannot be entirely ruled out. The platelet entirely accounts for the enhanced 12-HETE and partially accounts for the enhanced thromboxane synthesis. Complement activation mediates the acute decrements in GFR and RBF after mesangial cell injury via a mechanism involving enhanced eicosanoid synthesis and specifically thromboxane.

Our observations are of significance in that they implicate the platelet and the leukocyte as sources of proinflammatory and vasoactive eicosanoids in mesangial cell immune injury. Both cell types are capable of synthesizing and releasing potent noneicosanoid proinflammatory factors that can mediate proliferation (i.e., PDGF), matrix synthesis (i.e., TGF- β), cytotoxicity (i.e., TNF), and immunoregulation (interleukins). The timed or sustained synthesis of specific eicosanoids by these cells may, therefore, play an important role in regulating the synthesis or release of these proinflammatory factors in an autocrine or paracrine manner.

Acknowledgments

Cindy Ziebell provided expert secretarial assistance.

This work was supported by a United States Public Health Service Grant, RO1-DK-34793, and an American Heart Established Investigatorship to Dr. Lianos, and a United States Public Health Service National Research Service Award F32-DK-08243 to Dr. Bresnahan.

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