Kidney International, Vol. 41 (1992), pp. 1245–1253

Essential fatty acid deficiency normalizes function and histology in rat nephrotoxic nephritis

KIHITO TAKAHASHI, TETSUO KATO, GEORGE F. SCHREINER, JOAN EBERT, and KAMAL F. BADR

Department of Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee; and Departments of Medicine and Pathology, Washington University, St. Louis, Missouri, USA

Essential fatty acid deficiency normalizes function and histology in rat nephrotoxic nephritis. The central lipid abnormality in essential fatty acid deficiency (EFAD) is the lack of availability of arachidonic acid. To examine the role of total eicosanoid's biosyntheses in the pathology and pathophysiology of glomerulonephritis, EFAD was induced in weanling rats, which were then subjected to antiglomerular basement membrane antibody (NTS)-induced injury in adulthood. Glomerular dynamics (as assessed by micropuncture), quantitative histology, and eicosanoid generation rates were measured at two hours and two weeks post-NTS, and compared to those of standard diet-fed (STD) controls. Two hours post-NTS, and despite the occurrence of proteinuria in both EFAD and STD animals, glomerular dynamics were essentially normal in EFAD rats, whereas STD animals had reduced values for glomerular filtration rate (GFR) and renal plasma flow rate (RPF). At two weeks, severe histologic changes were observed in STD animals including mesangial and stalk hypercellularity, moderate sclerosis, and interstitial nephritis, coupled with heavy proteinuria and reduced GFR and RPF. In dramatic contrast, EFAD rats displayed totally normal glomerular structures and functions. In parallel, glomerular generation rates of prostaglandin E_2 and thromboxane A_2 were suppressed markedly in EFAD rats. Thus, EFAD confers complete protection against the histopathologic and functional sequelae of immune-initiated injury in the glomerulus. The data suggest that the initial wave of complement-induced neutrophil infiltration (with resultant proteinuria) is not sufficient to perpetuate injury into the more destructive chronic phases. The results provide strong impetus for the design of more specific interventional therapies targeting the various enzymes and products of arachidonic acid metabolism in the attempts to control glomerular inflammation.

Clinical and experimental studies over the past three decades have established a central role for biologically active eicosanoids in the pathophysiology of glomerular immune injury. The local synthesis and release of oxygenated metabolites of arachidonic acid exerts profound effects on renal microvascular tone, glomerular cell functions, glomerular filtration rate, and, most significantly, histopathologic expression, during the evolution of glomerulonephritis [1]. In addition to the known renal actions of cyclooxygenase and lipoxygenase-derived products of arachidonate metabolism (prostaglandins, thromboxanes, leukotrienes, lipoxins, and hydroxyeicosatetraenoic acids), recent studies have documented highly potent renal vasoconstrictor actions for a non-enzymatically generated class of prostaglandin F_2-like compounds, synthesized by a free radical-catalyzed mechanism [2], as well as cytochrome P450-derived arachidonate epoxidation products [3, 4]. In its totality, therefore, enzymatic and non-enzymatic oxygenation of arachidonic acid during glomerular inflammation appears to play a central role in the mediation of glomerular dysfunction and ultimate destruction. A formal evaluation and definition of this proposed role for total arachidonate metabolism in experimental glomerulonephritis, however, remained lacking.

In the present study, we employed essential fatty acid deficient (EFAD) animals to dissect the impact of total arachidonate depletion on the course of anti-glomerular basement membrane (anti-GBM) antibody [nephrotoxic serum (NTS)]-induced glomerulonephritis in the rat. It is well established that, when animals are rendered deficient in essential fatty acids, the cellular levels of arachidonic acid decrease markedly [5, 6]. EFAD offers the added advantage, in addition to depleting a precursor for eicosanoids biosyntheses, of inhibiting macrophage elicitation without the use of systemic immunosuppressive manipulations [7]. The latter phenomenon is itself likely due to the abrogation of the local generation of an arachidonate-related macrophage chemoattractant lipid, the structural identification of which is currently underway [8]. NTS glomerulonephritis was therefore induced in standard diet-fed (STD) and EFAD rats, and glomerular functional alterations were assessed by glomerular micropuncture, in parallel with histopathologic examinations and measurements of glomerular eicosanoids productions during both the acute (heterologous), as well as the chronic (autologous) phases of injury.

Methods

Preparation of NTS

NTS was produced by repeated immunization with basement membrane-rich sediment of rat cortex, as previously described [9]. The dosage of NTS was determined in a preliminary study by examining the dose required to induce heavy proteinuria (200 to 300 mg protein/day) in the first 48 hours after intravenous injection. All rats received a standardized dose of 0.05 ml.

Received for publication September 23, 1991
and in revised form November 18, 1991
Accepted for publication November 21, 1991

Induction of EFAD

Weanling male Munich-Wistar rats were obtained and fed either a standard lab diet or a fat-free diet (Purina Test Diets, Richmond, Indiana, USA) for three months. The fatty acid analysis of these diets has been published previously [10]. A previous study [5] indicated that this protocol results in liver and glomeruli being deficient in fatty acids as assessed by 20:3(n-9) to arachidonate ratio of >0.4, which is the biochemical criterion for EFAD [11]. Individual experiment was performed with age-matched controls that had been on a standard diet for an equivalent period to the EFAD animals.

Induction of NTS nephritis and experimental protocol

Acute study (heterologous phase)

All experiments were performed on anesthetized male Munich-Wistar rats weighing 150 to 180 g which were prepared for micropuncture according to protocols described below. In all experiments, micropuncture measurements were started 120 to 150 minutes after the administration of either non-immunized serum or NTS. In this study, rats were divided into four experimental groups as follows.

*Group IA (N = 6).*This group of animals were STD rats to which 0.05 ml of non-immunized serum, the vehicle for NTS, was administered intravenously.

*Group IIA (N = 5).*This group of animals were STD rats, but received 0.05 ml of NTS.

*Group IIIA (N = 5).*These animals were EFAD rats which received 0.05 ml of non-immunized serum as in Group IA.

*Group IVA (N = 5).*These animals were EFAD rats which received 0.05 ml of NTS as in Group IIIA.

Chronic study (autologous phase)

Rats weighing 150 to 180 g were maintained in metabolic cages on either a standard or a fat-free rat diet and allowed free access to water. After 24-hour urine collection for the measurement of baseline protein excretion, NTS or non-immunized serum was administered intravenously and 24-hour urine protein excretion was monitored daily until 14 days after NTS injection, when micropuncture and morphologic studies were performed.

In this study, rats were divided into four experimental groups as follows.

*Group IC (N = 7).*This group of animals were STD rats to which 0.05 ml of non-immunized serum was administered intravenously.

*Group IIC (N = 6).*This group of animals were STD rats, but received 0.05 ml of NTS.

*Group IIIC (N = 6).*These animals were EFAD rats which received 0.05 ml of non-immunized serum as in Group IC.

*Group IVC (N = 6).*These animals were EFAD rats which received 0.05 ml of NTS as in Group IIIC.

Micropuncture study

Rats were prepared for micropuncture according to protocols described previously [9]. In brief, following Inactin anesthesia (Andrew Lockwood & Associates, Sturtevant, Wisconsin), the left femoral artery catheter was used to monitor mean system arterial pressure (AP) by means of a pressure transducer (P23Db, Statham Instruments, Oxnard, California, USA) connected to a direct writing recorder (Gould Instruments Inc., Cleveland, Ohio, USA) and for sampling of blood. Jugular veins were catheterized for infusion of plasma and a solution of ^{3}H-inulin (300 μCi/experimental period in 0.9% NaCl) and para-aminohippurate (PAH) (0.32 mg/min) at 1.2 ml/hr. The left kidney was exposed on a Lucite holder. The kidney surface was illuminated with a fiberoptic light source and bathed with isotonic NaCl. Homologous rat plasma was administered intravenously to replace surgically-induced plasma losses, thus maintaining euvolemia [12], at a rate of 10 ml/kg/hr for 45 minutes followed by a reduction in infusion rate to 1.5 ml/kg/hr for the remainder of the experiment. The plasma from EFAD rats was used for the experiments in EFAD rats. In all experiments micropuncture measurements were started 45 minutes after the onset of plasma infusion and carried out as follows: two-minute samples of fluid were collected from surface proximal convolutions for determination of flow rate and inulin concentration. Concomitantly, femoral arterial blood was obtained in each period for determination of systemic arterial hematocrit (Hct) and plasma protein and inulin concentrations. In addition, two or three samples of urine from the experimental kidney were collected for the determination of flow rate, inulin and PAH concentrations, and for the calculation of whole kidney glomerular filtration rate (GFR) and plasma flow rate (RPF). Three samples of blood were obtained from surface efferent arterioles (star vessels) for determination of efferent arteriolar protein concentration. Time-averaged hydraulic pressures were measured in surface glomerular capillaries (P_{GC}), proximal tubules (P_T), and surface efferent arterioles (P_E) using a continuous recording, servo-null micropipette transducer system (Model 5, Instrumentation for Physiology and Medicine, San Diego, California, USA) and micropipettes with outer tip diameters of 2 to 3 μm and containing 2.0 M NaCl. Colloid osmotic pressures of plasma entering and leaving glomerular capillaries, single nephron glomerular filtration rate (SNGFR), single nephron filtration fraction (SNFF), glomerular capillary ultrafiltration coefficient (K_f), resistance of single afferent (R_A) and efferent (R_E) arterioles, and initial glomerular capillary plasma flow (SNPF), were determined using equations described in detail elsewhere [13]. The concentrations of inulin in tubule fluid, plasma and urine were determined by measuring the radioactivity of ^{3}H-inulin in a scintillation counter (Beckman Instruments, Fullerton, California, USA). The concentration of PAH in urine and plasma was determined according to the method of Smith, Finkelstein and Aliminosa [14]. Protein concentration in efferent arteriolar and femoral arterial blood plasmas were determined using a fluorometric method developed by Viets et al [15]. In Group IC-IVC, glomeruli were isolated right after the experiment, for in vitro experiments and morphologic studies described below.

Glomerular isolation, eicosanoid generation measurements

In the chronic study, following micropuncture studies, removal of kidneys, a small section was dissected for morphologic study. Following separation of cortices from medullae, glomeruli were isolated by a modification of the differential sieving technique [9], aimed at optimizing glomerular viability. In brief, the removed kidneys were immediately placed in Krebs buffer consisting of 105 mM NaCl, 24 mM $NaHCO_3$, 5 mM

Table 1. Summary of values for systemic and single nephron parameters

Group	Hct *vol %*	MAP *mm Hg*	GFR *ml/min*	RPF *ml/min*	FF	SNGFR *nl/min*	SNPF *nl/min*	SNFF	ΔP *mm Hg*	R_A *10^{10} dyn · sec · cm^{-5}*	R_E *10^{10} dyn · sec · cm^{-5}*	K_f *nl/(sec · mm Hg)*
IA	43	112	0.96	3.86	0.26	38.8	142	0.28	35	2.19	1.48	0.055
	± 1	± 2	± 0.04	± 0.18	± 0.01	± 3.4	± 6	± 0.01	± 1	± 0.19	± 0.09	± 0.006
IIA	47[b]	121[a]	0.30[b]	1.04[b]	0.30[a]	19.5[b]	62[b]	0.35[a]	40[a]	4.18[b]	3.32[b]	0.019[b]
	± 2	± 3	± 0.08	± 0.42	± 0.01	± 0.8	± 3	± 0.03	± 1	± 0.35	± 0.39	± 0.002
IIIA	45	109	1.03	4.10	0.25	39.4	155	0.26	38	1.80	1.31	0.049
	± 1	± 4	± 0.09	± 0.10	± 0.01	± 1.9	± 6	± 0.01	± 1	± 0.22	± 0.13	± 0.007
IVA	44	110	0.87	3.96	0.24	36.8	160	0.24	36	1.83	1.13	0.037
	± 1	± 2	± 0.07	± 0.14	± 0.01	± 1.8	± 10	± 0.01	± 1	± 0.12	± 0.06	± 0.004

[a] $P < 0.05$, [b] $P < 0.01$ vs. Group IA

KCl, 2 mM Na_2HPO_4, 1 mM $MgSo_4$, 1.5 mM $CaCl_2$, 5 mM glucose, 10 mM HEPES, and 0.2% BSA at pH 7.4, which was pre-bubbled with 95% O_2/5% CO_2 for 30 minutes. The cortical tissue was then minced at 4°C and transferred to another solution containing 0.3 mg/ml collagenase Sigma, Type I, (Sigma Chemical Co., St. Louis, Missouri, USA) in Krebs buffer, and bubbled in this solution with 95% O_2/5% CO_2 for 45 minutes at 37°C. Differential sieving was then carried out by passing cortical tissue sequentially through 150 and 75 micron sieves, with warming and gentle centrifugation. The glomerular preparation that was obtained was >95% pure. This modification of the conventional sieving technique does indeed assure greater viability of glomerular cells [5]. The isolated glomeruli were then incubated in Krebs buffer maintained at 37°C and 5% CO_2 for 30 minutes for measurement of prostaglandin E_2 (PGE_2) and thromboxane B_2 (TxB_2), the stable metabolite of TxA_2. The preparation was then centrifuged and divided into the supernatant and the glomerular pellet. The supernatant was frozen at −70°C for eicosanoids measurements at a later time, and the glomeruli were used for protein assay. The protein content of the pelleted glomeruli was assayed by a colorimetric method (Bio-Rad protein assay). The amounts of PGE_2 and TxB_2 generated by these glomeruli over the time period of incubation were determined by radioimmunoassay (Amersham) [9].

Histological analysis

Kidney sections from each animal in Groups IC-IVC were fixed in 10% buffered formalin. Six-micrometer paraffin sections at least 100 μm apart were then evaluated by several criteria. The number of glomeruli displaying focal segmental sclerosis was expressed as a percentage of total glomeruli. This lesion was distinctive for its hypercellularity and asymmetrical involvement of the affected glomerulus (Fig. 2B). The sclerotic area consisted of mesangial expansion, obliteration of the capillary bed, and increased numbers of cells with the nuclear morphology of macrophages. Many of the glomeruli in the nephritic groups manifested hypercellularity in the juxtaglomerular (JG) region, between the mesangial stalk and macula densa. Those glomeruli in which this region could be clearly visualized and which manifested at least a doubling of the nuclear counts normally observed in this region were scored as (+) for JG hypercellularity and expressed as a % ($N = 25$). Intraglomerular cellularity was assessed by enumeration of mononuclear cells within glomeruli whose size was arbitrarily chosen to be 125 to 150 μm in both the horizontal and vertical axes. This was done to provide a standardized glomerular size for reference and to minimize the statistical variability arising from enumeration of glomeruli heterogenously sized due to the level at which they were sectioned. We observed that most of the hypercellularity observed in nephritic glomeruli was attributable to increases in mesangial cellularity that was readily apparent even in glomeruli not exhibiting sclerosis. The increased mesangial cellularity was typically asymmetrically distributed. In order to quantitate this, we counted the nuclei in the most cellular mesangial region of the peripheral loops of glomeruli not exhibiting segmental sclerosis. The mesangial nuclei were counted as the number of nuclei per 62.5 nm^2 of mesangium. The enumeration was restricted to peripheral capillary loops in order to avoid the mesangial stalk region, and is defined as maximum mesangial cell accumulation. Finally, PMN counts were conducted assaying glomeruli with the same dimensions noted above. Results were expressed as mean PMN counts per glomeruli. Interstitial nephritis was evaluated by counting the number of extratubular interstitial cells per 25 μm^2 high power field in 10 randomly selected fields lacking a glomerulus. Results are expressed as the arithmetic mean ± SEM.

Statistical

Intergroup comparisons were performed by one-way analysis of variance, followed by Neumann-Keul multiple comparison. Differences were considered significant at a P value ≤0.05. All values are reported as mean ± SEM.

Results

Acute study

Morphologic and biochemical studies were not performed in the acute study, since these have been published previously [7].

Micropuncture studies (Table 1). The pattern of systemic, whole kidney, and glomerular microcirculatory hemodynamic parameters observed in STD rats two hours following administration of non-immunized serum (Group IA) was similar to that previously established for baseline control values in the euvolemic Munich-Wistar rat [9] and is presented in the top panels of Table 1. Additionally, no significant difference was noted in any parameters between EFAD non-nephritic animals (Group IIIA) and STD non-nephritic rats (Group 1A). Administration of NTS to STD rats (Group IIA) was associated with an elevated value of AP and Hct (AP: 121 ± 3 vs. 112 ± 2 mm Hg in Group IA; $P < 0.005$ Hct: 47.0 ± 2.0 vs. 43.0 ± 1.0 vol% in Group 1A; P

< 0.005), a phenomenon not observed in EFAD nephritic animals in Group IVA (AP: 110 ± 2 vs. 109 ± 4 mm Hg in Group IIIA, NS. Hct: 43.6 ± 0.4 vs. 45.0 ± 1.0 in Group IIIA, NS). Further, this induction of NTS nephritis in STD rats (Group IIA) was associated with reductions in GFR and RPF, and an elevation in filtration fraction (FF) as compared to STD non-nephritic rats in Group IA. The values were: GFR, 0.30 ± 0.08 vs. 0.96 ± 0.04 ml/min, $P < 0.005$; RPF, 1.04 ± 0.42 vs. 3.86 ± 0.18 ml/min, $P < 0.005$; FF, 0.30 ± 0.01 vs. 0.26 ± 0.02; $P < 0.05$, respectively. In contrast, administration of NTS to EFAD rats in Group IVA was associated with complete preservation of GFR, RPF and FF as compared to EFAD non-nephritic rats in Group IIIA (GFR: 0.87 ± 0.07 vs. 1.03 ± 0.09 ml/min, NS; RPF: 3.96 ± 0.14 vs. 4.10 ± 0.10 ml/min, NS; FF: 0.24 ± 0.01 vs. 0.25 ± 0.01, NS, respectively).

In parallel with these changes in whole kidney GFR, RPF and FF, single nephron measurements revealed a fall in SNGFR, SNPF and an elevation in SNFF in STD nephritic rats in Group IIA as compared to STD non-nephritic rats in Group IA (SNGFR: 19.5 ± 0.8 vs. 38.8 ± 3.4 nl/min, $P < 0.005$; SNPF: 62 ± 3 vs. 142 ± 6 nl/min, $P < 0.005$; SNFF: 0.35 ± 0.03 vs. 0.28 ± 0.01, $P < 0.005$, respectively). The administration of NTS in EFAD rats in Group IVA was associated with preservation of SNGFR, SNPF and SNFF (SNGFR: 36.8 ± 1.8 vs. 39.4 ± 1.9 nl/min, NS; SNPF: 160 ± 10 vs. 155 ± 6 nl/min, NS; SNFF: 0.24 ± 0.01 vs. 0.26 ± 0.01, NS, respectively). Measurements in P_{GC}, P_T, and P_E revealed that induction of acute NTS-induced glomerular injury in STD animals (Group IIA) was associated with an elevation in mean transcapillary hydraulic pressure difference, ΔP (40 ± 1 vs. 35.1 mm Hg in Group IA, $P < 0.005$), a phenomenon not observed in Group IVA EFAD nephritic rats (36 ± 1 vs. 37 ± 1 mm Hg in Group IIIA, NS). Mean values of superficial cortical pressure measurements in these groups of animals are summarized in Table 1. Calculation of R_A and R_E revealed that STD nephritic rats in Group IIA were characterized by significant increases in both of these parameters as compared to STD non-nephritic rats in Group IA (R_A: 4.18 ± 0.35 vs. 2.19 ± 0.19 10^{10} dyn · sec · cm^{-5}, $P < 0.005$; R_E: 3.32 ± 0.39 vs. 1.48 ± 0.09 10^{10} dyn · sec · cm^{-5}, $P < 0.005$). Hence, a proportionally greater increase was attained in R_E as compared to R_A. These NTS-induced changes in R_A and R_E were not observed in EFAD rats in Group IVA (R_A: 1.83 ± 0.12 vs. 1.80 ± 0.22 10^{10} dyn · sec · cm^{-5} in Group IIIA, NS; R_E: 1.13 ± 0.06 vs. 1.31 ± 0.13 10^{10} dyn · sec · cm^{-5} in Group IIIA, NS). The presence of filtration pressure disequilibrium allowed for the calculation of unique values for K_f in all the experimental animals. In Group IIA, a dramatic reduction in the mean value for this parameter was observed [0.019 ± 0.002 vs. 0.055 ± 0.006 nl/(sec · mm Hg) in Group IA, $P < 0.005$]. This marked fall in K_f was nearly absent in EFAD nephritic rats in Group IVA when compared to EFAD non-nephritic animals (Group IIIA) [0.037 ± 0.004 vs. 0.049 ± 0.007 nl/(sec · mm Hg), respectively, NS].

Urinary protein excretion

Total urinary protein excretion (U_{pr}) two hours following administration of NTS in STD rats in Group IIA averaged 574 ± 93 μg/min, a value significantly higher than 10 ± 2 μg/min in STD non-nephritic animals in Group IA ($P < 0.005$; Fig. 1). In contrast to hemodynamic parameters, this increase in U_{pr} was only partially attenuated in EFAD nephritic rats in Group IVA, though the value was significantly lower than that of Group IIA [IVA: 336 ± 36 μg/min, $P < 0.05$ vs. Group IIA, and $P < 0.005$ vs. EFAD non-nephritic rats in Group IIIA (7.2 ± 2 μg/min)].

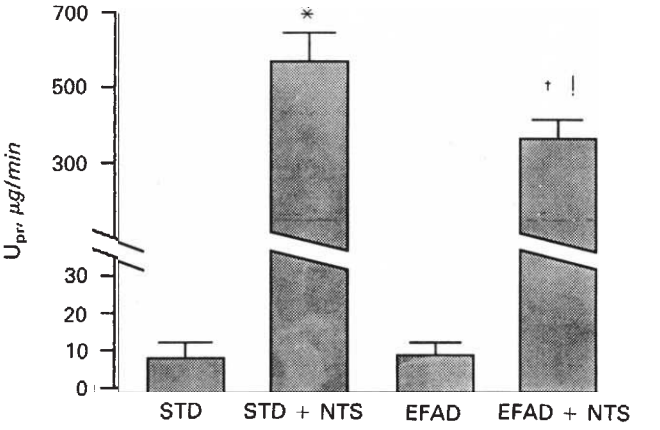

Fig. 1. *Urinary protein excretion (U_{pr}) in Groups IA to IVA.* Abbreviations are: STD, standard-diet fed rats (Group 1A); STD + NTS, standard-diet fed rats receiving NTS (Group IIA); EFAD, essential fatty acid deficient rats (Group IIIA); EFAD + NTS, essential fatty acid deficient rats receiving NTS (Group IVA). * $P < 0.005$ vs. Group IA, † $P < 0.05$ vs. Group IIA, ! $P < 0.001$ vs. Group IIIA.

Chronic study

*Morphologic examination.*Compared to the normal glomeruli of STD rats receiving non-immunized serum (Fig. 2A), STD rats receiving NTS manifested a mesangioproliferative form of glomerulonephritis in the autologous phase of the disease (Fig. 2B). Diffuse mesangial hypercellularity and focal segmental sclerosis were present. The sclerotic regions were associated with mesangial expansion, obliteration of the capillary bed locally, and increased cellularity. Many of the cells demonstrated a nuclear morphology characteristic of macrophages. The induction of nephritis was associated with an increase in the percent of glomeruli with focal sclerosis from 1.5 ± 1.5% for STD non-nephritic animals in Group IC to 26.0 ± 2.3% for STD nephritic rats in Group IIC ($P < 0.01$). In marked contrast, EFAD nephritic animals in Group IVC (Fig. 2C) failed to develop the lesion (0.8 ± 0.5 vs. 0.7 ± 0.7% for EFAD non-nephritic animals in Group IIIC, NS).

EFAD rats receiving NTS (Group IVC) were also protected against the glomerular hypercellularity exhibited by STD nephritic rats in Group IIC (131.4 ± 3.6 vs. 83.9 ± 3.5 for EFAD nephritic animals in Group IVC, $P < 0.01$). Indeed, their glomerular cell counts were not statistically different from non-nephritic rats on either a standard or EFAD diet. In parallel to these findings, EFAD nephritic glomeruli experienced no significant mesangial cell increase when compared to STD nephritic rats (3.3 ± 0.4 peak mesangial cell accumulation compared to 10.9 ± 0.4 for STD nephritic rats, $P < 0.01$), and did not statistically differ from non-nephritic animals from either dietary group.

Interestingly, STD nephritic animals in Group IIC demonstrated a marked increase in the cellularity of the JG region (36.0 ± 10.6 vs. 8.0 ± 4.0% for STD non-nephritic rats in Group IC, $P < 0.05$). EFAD animals exhibited a decrease in JG region

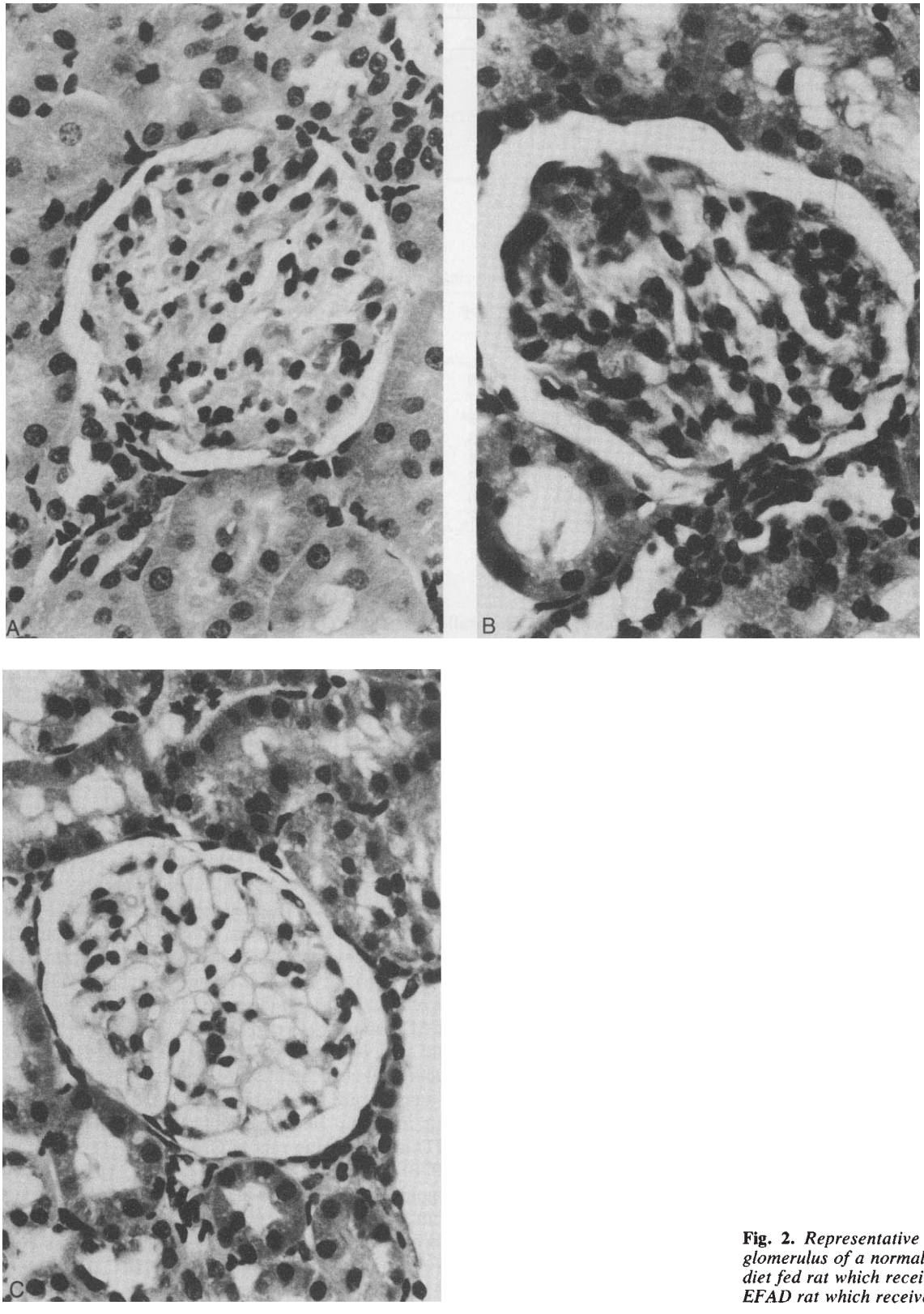

Fig. 2. *Representative light micrograph of a glomerulus of a normal rat* (**A**)*, a standard-diet fed rat which received NTS* (**B**) *and a EFAD rat which received NTS* (**C**).

cell accumulation both as non-nephritic (2.7 ± 1.3%, Group IIIC) and after receiving NTS (3.2 ± 1.5%, Group IVC).

As expected for the autologous phase of glomerulonephritis, STD nephritic rats displayed only a mild PMN infiltrate (1.5 ± 0.1 vs. 0.2 ± 0.1 per glomerulus for STD non-nephritic rats in Group IC, $P < 0.01$). EFAD nephritic animals showed no

Table 2. Summary of morphologic quantification

Group	IC	IIC	IIIC	IVC
% Glomeruli displaying focal segmental sclerosis	1.5 ± 1.5	26.0 ± 2.3[b]	0.7 ± 0.7	0.8 ± 0.5
% Glomeruli displaying JG hypercellularity	8.0 ± 4.0	36.0 ± 10.6[a]	2.7 ± 1.3	3.2 ± 1.5
Glomerular nuclear counts	83.6 ± 2.1	131.4 ± 3.6[b]	83.4 ± 0.7	83.9 ± 3.5
Peak concentration of mesangial cell accumulation	3.4 ± 0.1	10.9 ± 0.4[b]	2.3 ± 0.2	3.3 ± 0.4
Number of PMN per glomerulus	0.2 ± 0.1	1.5 ± 0.1[b]	0.3 ± 0.1	0.3 ± 0.1
Interstitial infiltrate (Interstitial cells 25 μ^2 field)	8.5 ± 0.2	20.5 ± 2.0[a]	9.8 ± 0.1	10.7 ± 1.1

Explanations for each parameter are in the text.
[a] $P < 0.05$, [b] $P < 0.01$ vs. Group IC

Table 3. Summary of values for systemic and single nephron parameters

Group	Hct	MAP	GFR	RPF	FF	SNGFR	SNPF	SNFF	ΔP	R_A	R_E	K_f
		mm Hg	*ml/min*			*nl/min*			*mm Hg*	10^{10} *dyn · sec · cm*$^{-5}$		*nl (sec · mm Hg)*
IC	47	107	1.17	4.24	0.28	50.6	179	0.28	34.5	1.44	1.10	0.064
	± 1	± 5	± 0.08	± 0.27	± 0.01	± 2.6	± 7	± 0.01	± 1.3	± 0.10	± 0.04	± 0.008
IIC	44	102	0.88[b]	5.07[a]	0.19[b]	33.1[b]	198[a]	0.17[b]	41.8[b]	1.20[a]	1.25[a]	0.026[b]
	± 1	± 2	± 0.04	± 0.20	± 0.01	± 1.4	± 8	± 0.01	± 0.7	± 0.11	± 0.07	± 0.002
IIIC	46	110	1.10	3.92	0.27	46.0	166	0.28	38.0	1.43	1.15	0.050
	± 1	± 4	± 0.17	± 0.33	± 0.03	± 1.9	± 12	± 0.01	± 0.7	± 0.13	± 0.12	± 0.007
IVC	45	104	1.03	3.98	0.24	44.3	170	0.26	38.0	1.40	1.16	0.047
	± 1	± 4	± 0.09	± 0.02	± 0.01	± 5.0	± 17	± 0.01	± 1.4	± 0.11	± 0.17	± 0.008

[a] $P < 0.05$, [b] $P < 0.01$ vs. Group IC

increase in PMN infiltrate compared to EFAD non-nephritic animals.

Finally, we noted that this form of glomerulonephritis was associated with a non-necrotizing interstitial infiltrate (20.5 ± 2.0 interstitial cells per 25 μm^2 field compared to 8.5 ± 0.2 for STD non-nephritic rats in Group IA, $P < 0.05$). Again, EFAD nephritic rats were completely protected.

These results are summarized in Table 2.

Micropuncture studies

As in acute study, the pattern of systemic, whole kidney, and glomerular microcirculatory hemodynamic parameters observed 14 days following the administration of non-immunized serum in STD (Group IC) and EFAD animals (Group IIIC) was similar to that previously established for baseline control values in the euvolemic Munich-Wistar rat [9] and is presented in Table 3. The induction of the autologous phase of NTS-nephritis was without effect on AP. No significant differences in AP and Hct were observed among the four Groups. GFR was mildly but significantly depressed in Group IIC animals as compared to Group IC controls, the values were 0.88 ± 0.04 versus 1.17 ± 0.08 ml/min, respectively, $P < 0.05$. However, the induction of nephritis in EFAD rats in Group IVC was not associated with a significant reduction in GFR as compared to Group IIIC rats (1.03 ± 0.09 vs. 1.10 ± 0.17 ml/min, respectively, NS). RPF was significantly greater in Group IIC animals than that of Group IC controls (5.07 ± 0.20 vs. 4.24 ± 0.27 ml/min, respectively, $P < 0.05$). However, RPF in EFAD nephritic rats in Group IVC was not significantly different from those in EFAD non-nephritic animals in Group IIIC. Thus FF in Group IIC animals was lowered to 0.19 ± 0.01, values significantly lower than those of Group IC (0.28 ± 0.01, $P < 0.05$ vs. Group IIC), whereas no significant difference in FF was observed between Group IIIC and IVC.

In parallel with these changes in whole kidney GFR, RPF and FF, single nephron measurements revealed a fall in SNGFR and SNFF, an increase in SNPF in STD nephritic rats in Group IIC as compared to STD non-nephritic rats in Group IC (SNGFR: 33.1 ± 1.4 vs. 52.6 ± 2.6 nl/min, respectively, $P < 0.01$; SNFF: 0.19 ± 0.01 vs. 0.27 ± 0.01, respectively, $P < 0.05$; SNPF: 196 ± 8 vs. 179 ± 7 nl/min, respectively, $P < 0.05$). Similarly, the induction of autologous nephritis in EFAD rats in Group IVC was associated with a preservation of SNGFR, SNPF and SNFF as compared to EFAD non-nephritic rats in Group IIIC (SNGFR: 44.3 ± 5.0 vs. 46.0 ± 1.9 nl/min, respectively, NS; SNPF: 170 ± 17 vs. 166 ± 12 nl/min, respectively, NS; SNFF: 0.25 ± 0.01 vs. 0.26 ± 0.01, respectively, NS). The mean value of ΔP in autologous NTS-nephritis in STD rats in Group IIC was significantly higher than that of STD non-nephritic rats Group IC (41.8 ± 0.7 vs. 34.5 ± 1.3 mm Hg, respectively, $P < 0.05$). However, again, no significant difference in ΔP was noted between EFAD nephritic (Group IVC, 38.0 ± 1.4 mm Hg) and non-nephritic rats (Group IIIC, 38.0 ± 0.7 mm Hg). Mean values of superficial cortical pressure measurements in the four Groups of animals are summarized in Table 3. R_A in STD nephritic rats in Group IIC was significantly reduced as compared to STD non-nephritic rats in Group IC (1.17 ± 0.11 vs. 1.44 ± 0.10 10^{10} dyn · sec · cm^{-5}, $P < 0.05$), while R_E was elevated significantly (1.25 ± 0.07 vs. 1.10 ± 0.04 10^{10} dyn · sec · cm^{-5}, $P < 0.01$). However, both resistances in EFAD nephritic rats in Group IVC were unchanged as compared to EFAD non-nephritic rats in Group IIIC (R_A: 1.40 ± 0.11 vs. 1.43 ± 0.13 10^{10} dyn · sec · cm^{-5}, respectively, NS; R_E: 1.16 ± 0.17 vs. 1.15 ± 0.12 10^{10} dyn · sec · cm^{-5}, respectively, NS). K_f of Group IIC showed a dramatic reduction in the mean value for this parameter (0.026 ± 0.002 vs. 0.064 ± 0.008 nl/(sec · mm Hg) in Group IC, $P < 0.01$). However, this marked fall in K_f was completely prevented in EFAD nephritic rats in

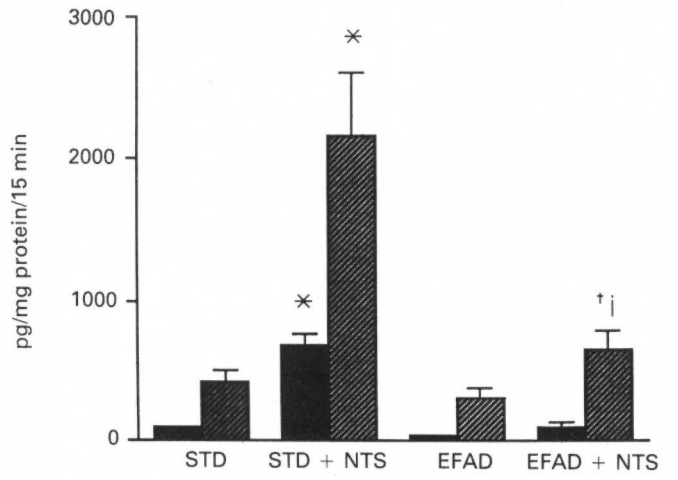

Fig. 3. *Mean values for glomerular generation rates of TxB_2 (solid bars) and PG_2 (hatched bars) in Groups IC to IVC.* Abbreviations are: STD, standard-diet fed rats (Group IC); STD + NTS, standard-diet fed rats receiving NTS (Group IIC); EFAD, essential fatty acid deficient rats (Group IIIC); EFAD + NTS, essential fatty acid deficient rats receiving NTS (Group IVC). * $P < 0.005$ vs. Group IC, † $P < 0.05$ vs. Group IIIC, ¡ $P < 0.001$ vs. Group IIC.

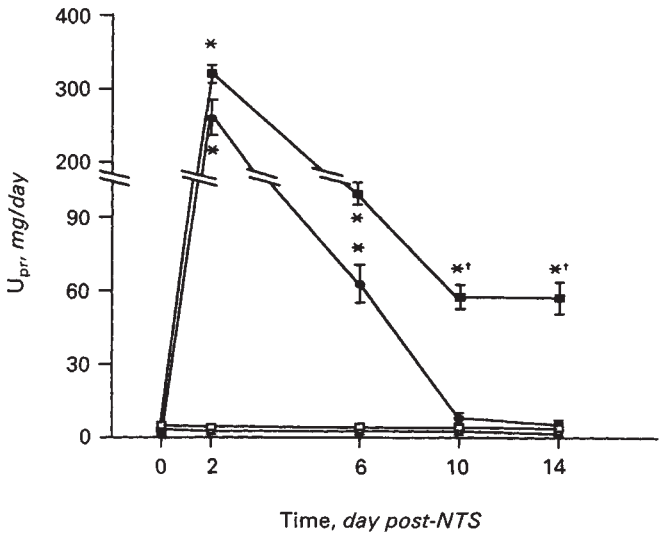

Fig. 4. *Time course of urinary protein excretion (U_{pr}) in Groups IC to IVC.* Symbols are: open square, standard-diet fed rats (Group IC); closed square, standard-diet fed rats receiving NTS (Group IIC); open circle, essential fatty acid deficient rats (Group IIIC); closed circle, essential fatty acid deficient rats receiving NTS (Group IVC) * $P < 0.001$ vs. basal values (Day 0), † $P < 0.01$ vs. correspondent values in Group IC.

Group IVC as compared to EFAD non-nephritic animals in Group IIIC (0.047 ± 0.008 vs. 0.050 ± 0.007 nl/(sec · mm Hg), respectively, NS).

Glomerular eicosanoids production

The glomeruli freshly isolated from STD nephritic rats in Group IIC showed increased productions of PGE_2 and TxB_2 expressed as pg/mg glomerular protein by as much as six- and eight-fold, respectively (Fig. 3). PGE_2 and TxB_2 production of glomeruli from EFAD non-nephritic rats in Group IIIC was suppressed by 72 and 56%, respectively, as compared to those from STD non-nephritic rats in Group IC. NTS-induced increased production of these eicosanoids was also suppressed markedly in the EFAD nephritic rats in Group IVC (Fig. 3).

Urinary protein excretion (U_{pr})

U_{pr} of STD nephritic rats in Group IIC expressed as mg/day increased from baseline at 5.8 ± 1.4 to 327.8 ± 17.6 ($P < 0.001$) in two to three days following NTS injection and reduced rapidly until seven to nine days. The U_{pr} remained constant and was 44.2 ± 11.5 at 14 days, a value which was significantly higher than that observed at 14 days in Group IC rats (9.7 ± 0.6, $P < 0.01$). In contrast, U_{pr} in EFAD nephritic animals in Group IVC fell to normal level by 10 days and was 10.1 ± 1.5 at 14 days (NS, vs. 8.0 ± 1.0 in EFAD non-nephritic animals in Group IIIC animals; Fig. 4).

Discussion

Dietary deprivation of essential fatty acids exerted profound effects on every aspect of glomerular immune injury in this model of glomerulonephritis in the rat. These included complete normalization of glomerular filtration function and histology, as well as marked improvement in glomerular permselectivity. The data on inhibition of prostaglandin and thromboxane syntheses presented in Figure 3, coupled with the previously demonstrated marked suppression of the early increase in LTB_4 synthesis in EFAD nephritic glomeruli under basal as well as angiotensin II-stimulated conditions [5] attest to the efficacy of this dietary manipulation in inhibiting eicosanoids generation under normal and inflamed conditions.

Normalization of glomerular hemodynamics

The pattern of glomerular dynamics observed in our STD animals during both the acute and the chronic phases of NTS injury is identical to that published previously by us and others [1]. Studies performed during both the heterologous and autologous phases demonstrate that the principal factor accounting for the fall in filtration rates in this model of injury is a marked reduction in K_f [1, 9, 16]. While K_f is low throughout both phases of the disease process, renal vascular resistance (RVR) is initially elevated during the heterologous phase [16], but eventually falls as the lesion transforms into the autologous phase and, at 10 to 14 days post-initiation of injury, RVR may be equal to or even less than that observed in control animals [9]. Of interest, micropuncture measurements reveal that in the heterologous phase, both R_A and R_E are elevated, but R_E is proportionately greater so that P_{GC} and ΔP are higher than normal [9, 16]. In the late phase, however, RVR is low due to a dramatic reduction in R_A with relative maintenance of R_E, resulting in persistently elevated P_{GC} and ΔP [9, 16]. Evidence from a number of laboratories suggests strongly that the evolving changes in arteriolar tone during the course of this inflammatory lesion are mediated principally by locally released cyclooxygenase and lipoxygenase arachidonate metabolites of arachidonic acid [9, 16–19].

The studies of Lianos, Andres and Dunn [17] and of Stork and Dunn [18] have established an important role for TxA_2 release in mediating the increased RVR observed during the early phase of this disease. Their studies also suggest that by

day 1, the increasing rate of PGE_2 generation may account for the progressive dilatation of renal arterioles leading to progressive increases in renal blood flow (RBF) at this and later stages [17, 18]. In these studies, TxA_2 antagonism appeared to ameliorate the falls in RBF and GFR two hours post-NTS, but not at one day [17, 18]. Antagonism of the LTD_4 receptor, on the other hand, normalizes K_f without affecting vascular resistances during acute NTS nephritis, suggesting that lipoxygenase products play a significant role with regard to the falls in K_f and GFR during this early phase [16]. In the present study, deprivation of arachidonic acid through EFAD prevents the biosyntheses of both cyclooxygenase and lipoxygenase products of arachidonic acid, thereby totally normalizing vascular resistances as well as K_f.

As noted above, the autologous phase of this form of experimental glomerulonephritis is characterized by renal vasodilation, and not vasoconstriction, though the GFR remains depressed. Of interest, human glomerulonephritis is also often associated with moderate to severe reductions in GFR despite relative preservation, or even augmentation, of RPF [20, 21]. Current evidence favors a predominant role for cyclooxygenase products in mediating both the renal vasodilation as well as the reduction in K_f [9], and hence GFR, which characterize this phase. Again, the total absence of significant alterations in glomerular dynamics in EFAD nephritic animals provides conclusive evidence for the central role of lipid-derived mediators in the pathogenesis of these abnormalities. Of interest, selective inhibition of cyclooxygenase in this experimental model results in marked increases in R_A to values far exceeding those of normal animals [9]. This had suggested the presence of potent non-cyclooxygenase dependent vasoconstrictors, acting primarily at preglomerular resistance sites, at this stage of injury, whose actions are masked by the marked stimulation of vasodilator cyclooxygenase derivatives (likely PGE_2) [9], but which become evident during cyclooxygenase inhibition. The predominant afferent action of these putative mediators argued against the involvement of angiotensin II or peptidoleukotrienes, which exert their constrictor actions predominantly on postglomerular arterioles [22, 23]. Since EFAD abolishes all changes in glomerular vascular resistance in these animals, such vasoconstrictive influences could also be derived from arachidonic acid, and might well represent shunting of this precursor into nonenzymatic formation of the recently described PGF_2-like compounds, which are potent renal vasoconstrictors [2].

Normalization of glomerular histology

NTS nephritis is characterized by initial influx of neutrophils in the first two to three hours which is followed by a complement-independent infiltration of macrophages over the subsequent 72 hours [7]. The latter is maintained for at least 14 days after the injection of NTS [9]. The influence of EFAD on the histologic changes of the heterologous phase of injury have been published previously [7], and were not addressed in the present study. In that study, EFAD appears to have little effect on the initial infiltration by neutrophils, a process which is largely complement-dependent [7]. Here, we focused our histologic studies on the more relevant chronic phase of injury. At two weeks post-NTS, the glomerular lesions of STD nephritic rats included focal segmental hypercellular sclerosis, mesangial hypercellularity and intense interstitial infiltrate, which represent a more intense form of injury than that reported in our previous study [9]. This may be attributed to the more potent NTS batch used in the current study, as evidenced by the greater degree of initial proteinuria and more profound reductions of SNGFR and K_f. These changes were also associated with greater increases in glomerular production rates of TxB_2 and PGE_2 which play important roles in mediating glomerular functional alterations in autologous NTS nephritis [9]. As the summary of quantitative histology presented in Table 2 indicates, EFAD was associated with a dramatic absence of histologic abnormalities two weeks after the induction of injury. This occurred despite clear presence of stigmata of initial injury (that is, antibody binding, early neutrophil infiltration, and early proteinuria) at two hours post-NTS when this disease model is induced in EFAD rats [5] (Fig. 1). The marked absence of histopathology two weeks after the induction of immunologic injury in these animals brings into focus the potent anti-inflammatory action of this dietary intervention. The mechanisms underlying this effect of EFAD on renal and nonrenal forms of immunologic injury have been addressed in previous studies [5–8]. As has been documented [8], the number of resident glomerular macrophages is diminished in EFAD animals and the influx of macrophages into a focus of glomerular inflammation is markedly attenuated, as is glomerular eicosanoid synthesis. These effects on macrophage function are clearly central to the physiological protection conferred by EFAD. The mechanism by which EFAD interferes with macrophage elicitation remains to be clarified. Evidence from previous studies excludes depletion of circulating monocytes or impairment of macrophage chemotactic responsiveness [5–8]. Recently, a novel arachidonate-linked lipid monocyte chemotactic factor has been identified from glomeruli of NTS nephritic rats [8]. Its structure is as yet uncharacterized.

Effects on proteinuria

U_{pr} increased dramatically in STD animals three hours after receiving NTS. In EFAD nephritic animals, U_{pr} was only slightly, but significantly, diminished as compared to STD nephritic rats. However, in contrast to glomerular hemodynamic changes, U_{pr} remained markedly increased as compared to STD non-nephritic animals. Neutrophil activation leads to enzymatic-induced damage to the glomerular capillary wall [24], which may play a role in mediating the proteinuria accompanying the early phase of nephrotoxic nephritis. Morganroth et al [25] reported that neutrophils obtained from EFAD rats exhibited slightly decreased superoxide production, and similar rates for release of degradative enzymes as compared to those observed in STD rats. As we have previously reported, despite depleting glomerular leukocytes under basal conditions, the EFAD status does not diminish the degree of neutrophil infiltration characteristic of this phase of the lesion [5], suggesting that neutrophils damage the basement membrane via mechanisms independent of arachidonate. Perpetuation of neutrophil-initiated capillary wall damage and persistence of proteinuria into the chronic phase, however, appears to require the activation of lipid-mediator dependent responses. This is evidenced by the marked attenuation of proteinuria in this disease at two weeks by cyclooxygenase inhibition [9], and its complete absence at that time point in EFAD animals in the present study (Fig. 3).

In summary, the present study provides evidence that EFAD confers complete protection against the histopathologic as well as the functional sequelae of immune-initiated injury in the glomerulus. The data suggest that the mere deposition of antibody, and the initial wave of complement-induced neutrophil infiltration, with its attendant proteinuria, are not sufficient to perpetuate injury into the more destructive chronic phases. The central lipid abnormality in EFAD is the lack of availability of arachidonic acid. Since the enzymatic and non-enzymatic products of arachidonate metabolism have been implicated in the pathogenesis of the histologic and the functional sequelae of glomerulonephritis, the present studies provide strong impetus for the design of more specific interventional therapies, targetting the various enzymes and products of these pathways, in the attempts to control glomerular inflammation.

Acknowledgments

This work was supported by NIH Grants DK43883, AM-36277, DK-39261, HL-19153, and HL-27274. This work was presented in abstract form at the 23rd Annual Meeting of the American Society of Nephrology, December 11–14, Washington DC.

Reprint requests to Kihito Takahashi, M.D., S-3223 Medical Center North, Vanderbilt University Medical Center, Nashville, Tennessee 37232-2372, USA

References

1. Badr KF: Arachidonic acid metabolites in glomerular injury, in *Seminars in Nephrology: Mechanisms of Glomerular Injury*, edited by Shah SV, Kurtzman N, Vol 11(3):332–339, 1991
2. Takahashi K, Nammour TM, Fukunaga M, Ebert J, Morrow JD, Roberts LJ II, Badr KF: Glomerular actions of a free radical-generated novel prostaglandin, 8-epi-Prostaglandin $F_{2\alpha}$, in the rat: Evidence for interaction with thromboxane A_2 receptors. *J Clin Invest* (in press)
3. Takahashi K, Capdevila J, Karara A, Falck JR, Jacobson HR, Badr KF: Cytochrome P-450 arachidonate in the rat kidney: Characterization and hemodynamics responses. *Am J Physiol* 258:F781–F789, 1990
4. Katoh T, Takahashi K, Capdevila J, Karara A, Falk JR, Jacobson JR, Badr KF: Glomerular stereospecific synthesis and hemodynamic actions of 8,9-epoxyeicosatrienoic acid in rat kidney. *Am J Physiol* 261:F578–F586, 1991
5. Lefkowith JB, Schreiner G: Essential fatty acid deficiency depletes rat glomeruli of resident macrophages and inhibits angiotensin II-induced eicosanoid synthesis. *J Clin Invest* 80:947–956, 1987
6. Lefkowith JB, Morrison AR, Schreiner GF: Murine glomerular leukotriene B_4 synthesis: Manipulation by (n-6) fatty acid deprivation and cellular origin. *J Clin Invest* 82:1655–1660, 1988
7. Schreiner GF, Roven B, Lefkowith JB: The antiinflammatory effects of essential fatty acid deficiency in experimental glomerulonephritis: The modulation of macrophage migration and eicosanoid metabolism. *J Immunol* 143:3192–3199, 1989
8. Rovin BH, Lefkowith JB, Schreiner G: Mechanisms underlying the anti-inflammatory effects of essential fatty acid deficiency in experimental glomerulonephritis: Inhibited release of monocyte chemoattractant by glomeruli. *J Immunol* 145:1238–1245, 1990
9. Takahashi K, Schreiner GF, Yamashita K, Christman BW, Blair I, Badr KF: Predominant functional roles for thromboxane A_2 and prostaglandin E_2 during late nephrotoxic serum glomerulonephritis in the rat. *J Clin Invest* 85:1974–1982, 1990
10. Hurd ER, Johnston JM, Okita JR, Macdonald PC, Ziff M, Gilliam JN: Prevention of glomerulonephritis and prolonged survival in New Zealand Black/New Zealand White F1 hybrid mice fed an essential fatty acid-deficient diet. *J Clin Invest* 67:476–485, 1981
11. Holman R: The ratio of trienoic:tetraenoic acids in tissue lipids as a measure of essential fatty acid requirement. *J Nutrition* 70:405–410, 1960
12. Maddox DA, Prince DC, Rector FC Jr: Effect of surgery on plasma volume and salt and water excretion in rat. *Am J Physiol* 233:F600–F606, 1977
13. Deen WM, Troy JL, Robertson CR, Brenner BM: Dynamics of glomerular ultrafiltration in the rat. IV. Determination of the ultrafiltration coefficient. *J Clin Invest* 52:1500–1508, 1973
14. Smith HW, Finkelstein N, Aliminosa L: The renal clearance of substituted hippuric acid derivatives and other aromatic acids in dog and man. *J Clin Invest* 24:388–391, 1945
15. Viets JW, Deen WM, Troy JL, Brenner BM: Determination of serum protein concentration in nanoliter blood samples using fluorescamine or o-phthaldehyde. *Anal Biochem* 88:513–521, 1978
16. Badr KF, Schreiner GF, Wasserman M, Ichikawa I: Preservation of the glomerular capillary ultrafiltration coefficient during rat nephrotoxic serum nephritis by a specific leukotriene D_4 receptor antagonist. *J Clin Invest* 81:1702–1709, 1988
17. Lianos EA, Andres GA, Dunn MJ: Glomerular prostaglandin and thromboxane synthesis in rat nephrotoxic serum nephritis. Effects on renal hemodynamics. *J Clin Invest* 72:1439–1448, 1983
18. Stork JE, Dunn MJ: Hemodynamic roles of thromboxane A_2 and Prostaglandin E_2 in glomerulonephritis. *J Pharmacol Exp Ther* 233:672–678, 1985
19. Kaizu K, Marsh D, Zipser R, Glassock RJ: Role of prostaglandins and angiotensin II in experimental glomerulonephritis. *Kidney Int* 28:629–635, 1985
20. Myers BD, Okarma OB, Friedman C, Bridges C, Ross J, Asseff S, Deen WM: Mechanism of proteinuria in human glomerulonephritis. *J Clin Invest* 70:732–746, 1982
21. Friedman S, Strober S, Field EH, Silverman E, Myers BD: Glomerular capillary wall function in human lupus nephritis. *Am J Physiol* 15:F580–F591, 1984
22. Blantz RC, Konnen KS, Tucker BJ: Angiotensin II effects upon the glomerular microcirculation and ultrafiltration coefficient of the rat. *J Clin Invest* 57:419–434, 1976
23. Badr KF, Brenner BM, Ichikawa I: Effects of leukotriene D_4 on glomerular dynamics in the rat. *Am J Physiol* 253:F239–F243, 1987
24. Kreisberg JI, Wayne DB, Karnovsky MJ: Rapid and focal loss of negative charge associated with mononuclear cell infiltration early in nephrotoxic serum nephritis. *Kidney Int* 16:290–300, 1979
25. Morganroth ML, Schoeneich SO, Till GO, Ward PA: Lung injury caused by cobra venom factor is reduced in rats raised on an essential fatty acid-deficient diet. *Am J Physiol* 257:H1192–H1199, 1989