Murine Glomerular Leukotriene B_4 Synthesis

Manipulation by (n-6) Fatty Acid Deprivation and Cellular Origin

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Abstract

Leukotriene (LT) B_4 is an important pro-inflammatory autocoid. In order to investigate the potential role of this eicosanoid in renal inflammation, in this study we determined the capability of glomeruli to synthesize this mediator. Glomeruli were able to synthesize LTB_4 when provided with exogenous substrate in a dose-dependent fashion in the presence of ionophore A23187. Ionophore, although by itself a weak agonist for LTB_4 formation, was required for LTB_4 production from exogenous arachidonate. The identity of LTB_4 was confirmed by specific radioimmunoassay, high pressure liquid chromatography, and gas chromatography/mass spectrometry. The synthesis of LTB_4 was inhibited by BW755C (a lipoxygenase/cyclooxygenase inhibitor) but not indomethacin. Essential fatty acid (EFA) deficiency, obtained by the deprivation of (n-6) fatty acids, is known to exert a protective effect in renal inflammatory states. This dietary manipulation markedly attenuated the ability of glomeruli to synthesize LTB_4. In contrast, the synthesis of cyclooxygenase products from exogenous arachidonate was increased by EFA deficiency. Because EFA deficiency has been shown to deplete glomeruli of resident mesangial macrophages, it was hypothesized that this effect accounted for the diminished LTB_4 synthesis. To test this hypothesis, glomeruli were depleted of macrophages using x-irradiation. Glomeruli from these animals exhibited a marked decrease in LTB_4 synthesis. Glomerular synthesis of cyclooxygenase products was unaffected by irradiation. In sum, glomeruli have the capability to synthesize LTB_4, and this capacity is correlated with the presence of glomerular macrophages. EFA deficiency attenuates the ability of glomeruli to synthesize LTB_4 by depleting them of macrophages.

Introduction

Leukotriene (LT) B_4 is an important pro-inflammatory mediator. This autocoid has been shown to induce leukocyte chemotaxis (1), to cause leukocyte adherence to endothelial cells (2), to activate and aggregate leukocytes (3), and to cause vascular changes in synergy with prostaglandin E_2 (4). The potential role of LTB_4 in inflammation is underscored by studies which show that dietary polyunsaturated fatty acid modulation exerts an anti-inflammatory effect (5–7). Both essential fatty acid (EFA)[1] deficiency and (n-3) fatty acid enrichment have been shown to inhibit LTB generation by leukocytes. In the former case, 20:3(n-9), the fatty acid that uniquely accumulates in the deficiency state (8), is metabolized to LTA_3 (9). This reactive intermediate binds to LTA hydrolase and prevents the synthesis of LTB of either the 3 or 4 series, that is, from either 20:3(n-9) or arachidonate (10). A similar mechanism is operative in the latter case. 20:5(n-3), the fatty acid that prominently accumulates with dietary (n-3) fatty acid enrichment, is metabolized to LTA_5, which is a relatively poor substrate and an inhibitor of LTA hydrolase (11).

Renal eicosanoid metabolism is enhanced in a number of renal pathologic states such as glomerulonephritis (12), adriamycin-induced nephrosis (13), acute renal failure (14), and hydronephrosis (15). Additionally, this metabolic alteration appears to have pathophysiologic significance in these conditions (12–15). In order to assess the potential role of LTB_4 in renal inflammation, the capability of glomeruli to synthesize this eicosanoid was assessed. This study establishes that glomeruli are indeed able to synthesize LTB_4, and that EFA deficiency inhibits the generation of this eicosanoid. The mechanism by which EFA deficiency exerts its inhibitory effect, however, appears to be different than the aforementioned mechanism operative in isolated leukocytes in vitro, and suggests a unique cellular source for this mediator.

Methods

Reagents and materials. Weanling Lewis rats were obtained from Charles River Breeding Laboratories, Inc. (Wilmington, MA) and fed either a standard lab diet or a fat-free diet purchased from Purina Test Diets (Richmond, IN) for at least 8 wk. The fatty acid analysis of these diets has been previously published (16). Arachidonic acid, ionophore A23187, and DNAase type I were obtained from Sigma Chemical Co. (St. Louis, MO). Prostaglandin (PG) E_2 and thromboxane (Tx) B_2, ^{125}I-PGE_2, ^{125}I-TxB_2, and specific antisera were a gift from Dr. Philip Needleman (Dept. of Pharmacology, Washington University). LTB_4, LTB_3, 20-hydroxy LTB_4, and 20-carboxy LTB_4 were obtained from Merck-Frosst Canada, Inc. (Pointe Claire-Dorval, Quebec, Canada; gifts of Dr. J. Rokach). [^{3}H]LTB_4 (32 Ci/mmol) was obtained from Du Pont-New England Nuclear (Boston, MA). Octadecylsilyl columns were obtained from J. T. Baker Chemical Co. (Phillipsburg, NY). BW755C was obtained from Burroughs-Welcome (Research Triangle Park, NC). Polyclonal rabbit anti-rat macrophage antiserum was obtained from Accurate Chemical and Scientific Corp. (Westbury, NY). FITC-labeled goat anti-rabbit antisera were purchased from Cappel Laboratories (Cochranville, PA). Collagenase type II was purchased from Cooper Biochemical, Inc. (Malvern, PA). Tissue culture plates were purchased from Costar (Cambridge, MA). Tissue culture media were prepared in the Washington University Cancer Research Center. Organic chemicals were high performance liquid chromatography grade.

EFA deficiency and x-irradiation. Animals were made EFA deficient by feeding them a fat-free diet for at least 8 wk. Animals exhibited the characteristics of the deficiency state both clinically and biochemically. A detailed analysis of liver and glomerular phospholipid fatty acid composition from these animals has been published (17). Hepatic lipid fatty acid analysis was performed to periodically monitor for the

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1. *Abbreviations used in this paper:* EFA, essential fatty acid.

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degree of deficiency. 20:3(n-9)/arachidonate ratios ranged from 2 to 4, exceeding by far the minimum criterion of EFA deficiency (i.e., ratio > 0.4 [8]).

Animals were x-irradiated to deplete glomeruli of macrophages as described (18). Briefly, anesthetized animals were exposed to 1,315 rad of x-irradiation spread over 10 min. After 4 d the animals were leukopenic (< 90% depletion of peripheral leukocyte count) and were subsequently used in the experiments below.

Glomerular isolation, incubations, and labeling. Glomeruli were isolated from saline-perfused kidneys using a sieving protocol detailed previously (17). Preparations were typically > 90% pure. No difference in purity was observed between preparations from normal or EFA-deficient animals. Because of previous findings that collagenase/DNAase treatment of glomeruli was necessary to preserve glomerular viability and response to agonists (17), glomeruli were treated with 20 U/ml of collagenase type II and 0.01 mg/ml DNAase for 20 min at room temperature before use. They were then washed several times with cold oxygenated Kreb's-Henseleit buffer.

Glomerular incubations were then carried out as previously described (17) except that the agent used to stimulate glomeruli was solely arachidonate (with or without ionophore A23187). Incubations were terminated by pelleting the glomeruli and solubilizing them in 0.62 N NaOH for protein determinations. The protein content per 10^3 glomeruli was equal in control and EFA-deficient animals (23±3 and 21±2 $\mu g/10^3$ glomeruli, respectively). Glomerular eicosanoid production (PGE_2, TxB_2, and LTB_4) was determined by previously described radioimmunoassays (19) and normalized for the number of glomeruli in the incubation.

In several experiments, glomerular incubations were pooled, adjusted to pH 6.2, and applied to an octadecylsilyl column prepared as described (19). The column was eluted with methanol and the eluate later analyzed by high performance liquid chromatography as described below.

Glomeruli were labeled for the presence of macrophages as detailed before (17). The labeled cell content of isolated glomeruli was evaluated by microscopic examination with a Universal microscope (Carl Zeiss, Inc., Thornwood, NY). Cells were quantified by focusing through the glomerulus and counting cells as they appeared in the plane of focus. In each experiment, 50–100 glomeruli were counted, and the results are expressed as mean number of macrophages per glomerulus±SEM.

Glomeruli were also dissociated into a single-cell suspension in order to perform differential leukocyte counts. Glomeruli were dissociated into single cells by means of a previously described enzymatic protocol (17). Leukocytes were then stained with an immunoperoxidase method which used an anti-leukocyte common antigen primary antibody (OX14LK, Accurate Chemical and Scientific Corp.) and a Zymed Histostain-SP kit (Zymed Laboratories, San Francisco, CA). Positively-labeled cells were visualized by light microscopy and categorized by nuclear morphology.

High performance liquid chromatography and gas chromatography/mass spectrometry. Glomerular incubations, extracted as above, were analyzed for the presence of leukotrienes of the B series using a high performance liquid chromatography system (Beckman Instruments, Inc., Palo Alto, CA) with an Ultrasphere C_{18} column. The mobile phase was methanol/water/acetic acid 50:50:0.05, pH 6.2, with a step gradient to 65% methanol at 10 min. 1-min fractions were collected and subsequently analyzed for LTB_4 content by radioimmunoassay. Fractions containing LTB_4 by radioimmunoassay were then pooled for gas chromatography/mass spectrometry.

Gas chromatography was carried out on a 25-m Ultra 1 (cross-linked OV-1) column in a gas chromatograph (model 5830A, Hewlett Packard Co., Palo Alto, CA), which was temperature programmed to operate from 85 to 250°C at 30°C/min. Mass spectrometry was carried out on a Hewlett-Packard 5985B equipped for negative ion chemical ionization. Reagent gas was methane and ionization pressure was kept at 0.6 torr. Compounds of interest were converted to the pentafluorobenzyl ester by addition of 10 μl of 35% pentafluorobenzyl bromide in acetonitrile (Pierce Chemical Co., Rockford, IL) and 10 μl of diisopropylethylamine followed by heating in a reactivial to 45°C for 30 min. Excess reagent was removed in a stream of N_2 and the trimethylsilyl ether prepared by addition of 10 μl of *bis*-(trimethyl-silyl)trifluoroacetamide with 1% trimethylchlorosilane (Pierce Chemical Co.) and 10 μl of pyridine, heated to 45°C for 15 min. Excess reagent was removed under a stream of N_2 and the derivative dissolved in 10 μl of heptane. The mass spectrometer was operated in the selected ion mode monitoring m/z 479 $[M-181]^-$ due to the loss of the pentafluorobenzyl radical from LTB_4 pentafluorobenzyl ester trimethylsilyl ether.

Results

Glomerular LTB_4 synthesis. Glomeruli, when provided with exogenous arachidonate and ionophore A23187, were able to synthesize detectable LTB_4 by radioimmunoassay. The identity of this eicosanoid was initially verified by pooling several incubations, extracting the pool using an octadecylsilyl column, and then analyzing the methanol eluate by high performance liquid chromatography (Fig. 1). LTB_4 immunoreactivity from the incubations comigrated with authentic standard. The fractions containing immunoreactivity were subsequently pooled, derivatized, and analyzed by gas chromatography/negative ion chemical ionization mass spectrometry. The material from these fractions generated a peak with a retention time and base peak at m/z 479 consistent with LTB_4 (Fig. 1, *inset*). An additional peak with a slightly later retention time was also detected by gas chromatography/mass spectrometry which was

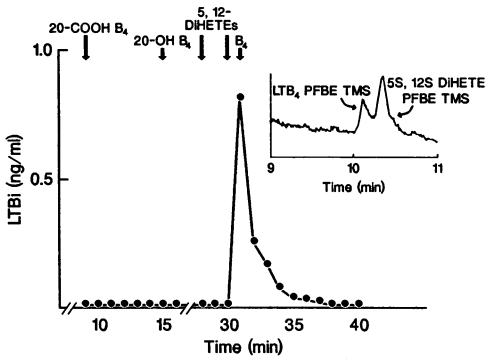

Figure 1. Validation of glomerular LTB_4 formation by high pressure liquid chromatography and gas chromatography/mass spectrometry. Glomeruli were isolated from three animals as detailed in Methods, aliquoted into three tubes, and incubated with arachidonate 10 μM in the presence of ionophore. The supernatants from the incubations were then pooled, extracted using an octadecylsilyl column, and subjected to high pressure liquid chromatography as detailed in Methods. Aliquots from 1-min fractions of mobile phase were subsequently assayed for LTB_4 immunoreactivity using a specific radioimmunoassay. The migration of immunoreactivity was compared to the migration of authentic standards as shown. The immunoreactivity from the peak comigrating with LTB_4 was pooled, derivatized, and analyzed by gas chromatography/mass spectrometry using the negative ion chemical ionization mode as described in Methods. A peak at m/z 479 with a retention time consistent with authentic LTB_4 was obtained (*inset*). A later eluting peak consistent with the pentafluorobenzylester trimethylsilyl ether derivative of the double-lipoxygenation product 5S,12S-dihydroxyeicosatetraenoic acid was also observed. Abbreviations: 20-COOH B_4, 20-carboxy LTB_4; 20-OH B_4, 20-hydroxy LTB_4; 5,12-DiHETEs, nonenzymatic 5,12-dihydroxyeicosatetraenoic acids; B_4, LTB_4; PFBE TMS, pentafluorobenzyl trimethylsilyl derivative.

consistent with the double-lipoxygenation product, 5S, 12S-dihydroxyeicosatetraenoic acid. This isomer incompletely separates from LTB_4 by high pressure liquid chromatography (9) but has negligible cross-reactivity in the radioimmunoassay (0.25%). Additional verification of the presence of LTB_4 was obtained via the use of inhibitors of arachidonate metabolism. BW755C (a mixed lipoxygenase/cyclooxygenase inhibitor), but not indomethacin, inhibited the generation of LTB_4 by glomeruli (Table I). Both drugs inhibited the simultaneous production of TxB_2 (Table I).

The synthesis of LTB_4 from arachidonate was dose dependent but required higher concentrations of substrate to obtain maximal production relative to cyclooxygenase metabolites (Table II). The synthesis of LTB_4 also required the presence of ionophore A23187 (Figure 2). Exogenous arachidonate alone was not efficiently converted to LTB_4. Ionophore alone was a weak agonist for LTB_4 production. Only with the combination of ionophore and exogenous arachidonate was substantial LTB_4 generated. In contrast, arachidonate was converted to PGE_2 efficiently in the presence or absence of ionophore.

Production of LTB_4 was apparently from an endogenous cell in the glomerulus rather than a blood element in that glomeruli were perfused free of blood before use. As has been shown before, perfused glomeruli are virtually devoid of blood cell contamination: < 1% of dissociated glomerular cells are red blood cells (20). The potential contribution of blood-borne cells to glomerular LTB_4 synthesis, however, was more directly tested by comparing the LTB_4 production by perfused glomeruli to that of unperfused glomeruli. Unperfused glomeruli synthesized identical quantities of LTB_4 to perfused glomeruli: 33 and 25 fmol/10^3 glomeruli at 10 μM arachidonate, respectively, and 170 and 168 fmol/10^3 glomeruli at 30 μM arachidonate, respectively. Thus, blood-borne cells contributed little to the observed LTB_4 production.

The issue of whether perfused glomeruli contained any contaminating leukocytes (e.g., polymorphonuclear neutrophils or lymphocytes) was also addressed by labeling dissociated glomerular cells for the leukocyte common antigen. In cell preparations from normal glomeruli 4.4% of the cells stained positively for the leukocyte common antigen. Of the positive cells, 100% were macrophages by morphology. These macrophages are the resident mesangial macrophages described previously (17, 20). No polymorphonuclear neutrophils, lymphocytes, or other leukocytes were seen.

Table I. Effects of Indomethacin and BW755C on Glomerular Eicosanoid Synthesis

	Product formation		
	Control	+Indomethacin	+BW755C
	fmol/10^3 glomeruli		
LTB_4	32	26,31	3,3
TxB_2	49	2,1	1,1

Glomeruli were isolated from four animals using the protocol detailed in Methods and pooled. Glomeruli were then aliquoted into five tubes and incubated in the presence of arachidonate 10 μM and ionophore A23187 10 μM also as detailed in Methods. In two tubes indomethacin 10 μM was added for 10 min before the addition of arachidonate and during the incubation with arachidonate. In two tubes BW755C 30 μM was used in place of indomethacin. The production of TxB_2 and LTB_4 in the glomerular supernatants was then determined by specific radioimmunoassays and normalized for the number of glomeruli in the incubation.

Table II. Dose-Response Curve for the Production of LTB_4 and PGE_2 from Exogenous Arachidonate by Glomeruli

	Product formation	
	LTB_4	PGE_2
	fmol/10^3 glomeruli	
Arachidonate (μM)		
0	<1	49
1	<1	526
5	19	803
10	48	696
20	71	744

Glomeruli from four rats were isolated as detailed in Methods, pooled, and then aliquoted into four tubes. Glomeruli were then incubated with various concentrations of arachidonate in the presence of ionophore A23187 10 μM. Glomerular supernatants were subsequently assessed for the production of PGE_2 and LTB_4 by specific radioimmunoassays and normalized for the number of glomeruli present in the incubations.

Effects of (n-6) fatty acid deprivation on glomerular LTB_4 synthesis. Since EFA deficiency exerts an anti-inflammatory effect in renal inflammation (5), the effects of the deficiency state on glomerular LTB_4 synthesis were examined. EFA deficiency inhibited the production of LTB_4 by 50–80% (Table III). This effect contrasted with the effects of EFA deficiency on cyclooxygenase metabolites. EFA-deficient glomeruli synthesized greater amounts of TxB_2 and PGE_2 (Table III) than control glomeruli when provided with exogenous arachidon-

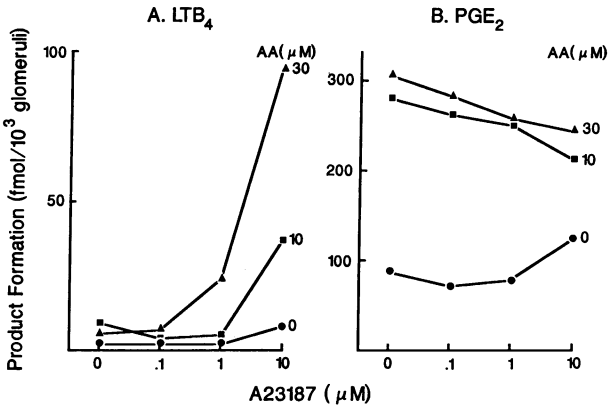

Figure 2. Effect of ionophore A23187 on glomerular LTB_4 formation. Glomeruli from four animals were isolated as detailed in Methods, pooled, and aliquoted into four tubes. Glomeruli were then sequentially incubated with ionophore alone (0.1–10 μM), arachidonate 10 μM with ionophore (0.1–10 μM), and arachidonate 30 μM with ionophore (0.1–10 μM). Supernatants were then analyzed for LTB_4 and PGE_2 content by specific radioimmunoassay. Results were normalized for the number of glomeruli in the incubation. The entire experiment was repeated with similar results.

Table III. Effect of EFA Deficiency on Glomerular Eicosanoid Production

	Product formation	
	EFAD	Control
	fmol/10³ glomeruli	
Experiment 1 (arachidonate 10 μM)		
LTB_4	15±3	38±2
TxB_2	163±20	77±16
PGE_2	Not done	Not done
Experiment 2 (arachidonate 10 μM)		
LTB_4	13±2	60±3
TxB_2	40±3	23±3
PGE_2	563±24	199±7
Experiment 3 (arachidonate 20 μM)		
LTB_4	39, 43	84, 87
TxB_2	67, 78	24, 32
PGE_2	2,609, 2,010	700, 827

Glomeruli from both EFA-deficient (EFAD) and control rats were isolated as detailed in Methods and pooled. Glomeruli were then aliquoted into tubes and incubated with arachidonate in the presence of ionophore A23187 10 μM. Supernatants were then assayed for the production of TxB_2, PGE_2, and LTB_4 by specific radioimmunoassays. Results were normalized for the number of glomeruli in the incubation. Several experiments were performed at different concentrations of arachidonate and the decrease in LTB_4 seen with EFA deficiency varied from 50 to 80%. Representative experiments at arachidonate 10 and 20 μM are shown. The former two experiments used three rats in each group and means±SEM are shown. The latter experiment used two rats and both replicates are shown.

ate as has been observed previously (17). Incubations from EFA-deficient glomeruli were also pooled and analyzed by high pressure liquid chromatography. LTB_4 immunoreactivity from these incubations comigrated solely with authentic LTB_4 (data not shown). No immunoreactivity comigrated with authentic LTB_3. As for control glomeruli, LTB_4 synthesis was unaffected by the addition of indomethacin at a dose that inhibited cyclooxygenase product production by > 90% (data not shown).

Cellular source of glomerular LTB_4. Since ionophore alone was a weak stimulus for arachidonate metabolism, and since exogenous arachidonate was used to obtain LTB_4 production, it was unlikely that the inhibitory effects of EFA deficiency were due to the release and metabolism of 20:3(n-9) to LTA_3. It was subsequently hypothesized that EFA deficiency might have impaired the generation of glomerular LTB_4 due to a depletion of its cellular source rather than by the inhibition of LTA hydrolase. Prior studies have demonstrated that EFA deficiency markedly diminishes the population of resident mesangial macrophages in the glomerulus (17), and macrophages are known to have the capability to synthesize this eicosanoid (19). In order to test this assumption, glomeruli were depleted of macrophages using whole-body irradiation. This procedure leads to a profound depletion of macrophages from the glomerulus (Fig. 3). Glomeruli from irradiated animals exhibited a 50-80% decrease in LTB_4 synthesis from exogenous arachidonate (Table IV). In contrast, the synthesis of PGE_2 and TxB_2 from arachidonate was relatively unaffected

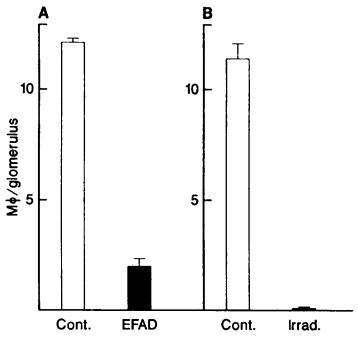

Figure 3. Depletion of glomerular macrophages by irradiation and EFA deficiency. Glomeruli from control, EFA-deficient, and irradiated rats were isolated and stained for the presence of macrophages as detailed in Methods. (*A*) Control vs. EFA-deficient rats. (*B*) Control vs. irradiated rats. Each bar represents the mean±SEM from a pool of glomeruli from three rats.

by irradiation. These data served to support the hypothesis that the mesangial macrophage is the major source of glomerular LTB_4, and that EFA deficiency inhibited glomerular LTB_4 synthesis by depleting the glomerulus of this cell.

Discussion

Glomeruli have long been known to synthesize a host of eicosanoids, including the prostaglandins and hydroxyeicosatetraenoic acids (21, 22). The various component cells of the glomerulus (e.g., mesangial cells and epithelial cells) also have

Table IV. Effect of Irradiation on Glomerular Eicosanoid Production

	Product formation	
	Irradiated	Control
	fmol/10³ glomeruli	
Experiment 1 (arachidonate 10 μM)		
LTB_4	23±2	65±8
TxB_2	28±3	32±3
PGE_2	468±19	448±41
Experiment 2 (arachidonate 10 μM)		
LTB_4	7±1	35±2
TxB_2	55±9	51±2
PGE_2	261±40	397±47
Experiment 3 (arachidonate 20 μM)		
LTB_4	20, 18	84, 87
TxB_2	30, 50	24, 32
PGE_2	1,447, 1,268	700, 827

Glomeruli from irradiated and control animals were isolated as in Methods, pooled, and aliquoted for incubation. Glomeruli were then incubated with arachidonate in the presence of ionophore A23187 10 μM. The supernatants from the incubations were analyzed for the presence of LTB_4, TxB_2, and PGE_2 by specific radioimmunoassays. Results were normalized for the number of glomeruli in the incubation. Several experiments at different concentrations of arachidonate were performed. The decrease in LTB_4 production seen with irradiation varied from 50 to 80%. Representative experiments at arachidonate 10 and 20 μM are shown. The former two experiments were performed with three animals and means±SEM are shown. The latter experiment was performed with two animals and both replicates are shown.

been cultured in vitro and were found to synthesize these metabolites to greater or lesser degrees (23). Only recently, however, has it been appreciated that the glomerulus contains a resident mesangial macrophage (20). These cells have been shown to express Ia determinants, to be phagocytic, and to react with lymphocytes in a genetically restricted fashion (20). Their number and Ia expression are also altered in a variety of renal inflammatory states (24). The present study underscores the importance of this cell type to glomerular pathophysiology. This study establishes not only that glomeruli are capable of LTB_4 synthesis, but that the mesangial macrophage appears to be the major glomerular source of this eicosanoid. Glomerular cyclooxygenase activity in comparison appears to reside for the most part in the other glomerular cells.

The issue of whether the LTB_4 synthesized by glomeruli in this study could be from blood-borne cells, such as polymorphonuclear neutrophils or lymphocytes, was addressed in two ways. First, unperfused glomeruli were shown to synthesize an identical amount of LTB_4 to perfused glomeruli showing that endovascular cells contributed little to the observed LTB_4 production. Secondly, the leukocytes within perfused glomeruli were categorized by morphology, and found to consist solely of resident macrophages. Thus, glomerular LTB_4 production could not be attributed to contaminating leukocytes.

The requirement for ionophore in glomerular LTB_4 synthesis from exogenous substrate is similar to a previously described phenomenon observed in macrophages. Macrophages synthesize only cyclooxygenase products in response to soluble stimuli, such as phorbol myristate acetate, and inefficiently convert exogenous arachidonate to leukotrienes (25). However, in the presence of ionophore, soluble stimuli lead to the substantial production of leukotrienes (25). Additionally, the conversion of exogenous arachidonate to leukotrienes is markedly facilitated (25). These phenomena parallel the findings in the current study and support the contention that the mesangial macrophage is the major source of glomerular LTB_4. The basis of these observations may be the calcium requirement of 5-lipoxygenase (26), the initial enzyme in the leukotriene synthetic pathway.

The mechanism by which EFA deficiency diminishes glomerular LTB_4 synthesis appears to differ from the mechanism by which EFA deficiency diminishes the synthesis of LTB_4 from isolated leukocytes. Isolated EFA-deficient leukocytes, when stimulated with an agonist that releases endogenous fatty acids (e.g., zymosan), synthesize markedly less LTB_4 than control cells (19, 27). By using exogenous fatty acids, it can be demonstrated that this inhibition requires the prior metabolism of 20:3(n-9) (19, 27). Using chemically synthesized LTA_3, it has been shown that this particular metabolite of 20:3(n-9) binds to and inactivates LTA hydrolase, thus preventing the metabolism of arachidonate to LTB_4 (10). Therefore, the decrease in LTB_4 formation by EFA-deficient leukocytes requires the prior release of endogenous 20:3(n-9) and presumably its metabolism to LTA_3.

In the current study, however, EFA-deficient glomeruli were impaired in their ability to produce LTB_4 even when provided with exogenous substrate. In contrast, EFA-deficient macrophages are able to synthesize LTB_4 from exogenous arachidonate comparably to control macrophages (Lefkowith, J., unpublished observations). These findings led to the alternative hypothesis that EFA deficiency inhibited the generation of glomerular LTB_4 by depleting the glomerulus of macrophages. This hypothesis was supported by the studies using irradiation to deplete glomeruli of macrophages. Glomeruli from irradiated animals were deficient in their ability to produce LTB_4 from exogenous substrate but were able to produce amounts of cyclooxygenase products comparably to control.

The residual capacity of glomeruli from irradiated animals to synthesize LTB_4 suggests that another cell type within the glomerulus (other than the macrophage) may be a source of this eicosanoid, since irradiation eliminates virtually all of the mesangial macrophages. A potential candidate is the smooth muscle–like mesangial cell. The mesangial cell has been shown to have the capacity to synthesize lipoxygenase products (28), although LTB_4 synthesis by these cells has not been established.

The mechanism by which EFA deficiency leads to a depletion of glomerular macrophages is unknown. However, the presence of these cells in the mesangium is clearly a specific function of (n-6) fatty acids (17). Supplementing EFA-deficient animals with linoleate restores glomerular arachidonate, decreases glomerular 20:3(n-9), and leads to a restocking of the glomerulus with macrophages. In comparison, linolenate supplementation, which increases levels of glomerular (n-3) fatty acids but also suppresses glomerular 20:3(n-9), does not lead to a normalization in the numbers of glomerular macrophages.

The depletion of glomerular resident macrophages and consequent decrease in the ability of glomeruli to synthesize LTB_4 may be central to the anti-inflammatory effect of the deficiency state. In a recent study on acute inflammation, EFA deficiency was shown to decrease numbers of resident macrophages, to diminish the generation of LTB_4, and to mitigate the influx of leukocytes during the inflammatory response (29). A causal connection between the effects of EFA deficiency on LTB_4 generation and leukocyte influx was supported by experiments using the cyclooxygenase/lipoxygenase inhibitor, BW755C. This inhibitor prevented LTB_4 synthesis and also decreased the influx of leukocytes during acute inflammation (29).

In summary, the current study establishes that glomeruli have the potential to synthesize LTB_4 and that the mesangial macrophage appears to be the predominant cell source of this eicosanoid. EFA deficiency impairs the generation of LTB_4 by glomeruli, apparently not by inhibiting the cellular synthetic pathway, but by depleting glomeruli of the major cellular source of this eicosanoid. The effects of EFA deficiency on glomerular LTB_4 synthesis may be the basis of the salutary effect of the deficiency state on renal inflammatory states.

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