Murine Glomerular Leukotriene B₄ Synthesis
Manipulation by (n-6) Fatty Acid Deprivation and Cellular Origin

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Abstract

Leukotriene (LT) B₄ is an important pro-inflammatory autacoid. 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 LTB₄ when provided with exogenous substrate in a dose-dependent fashion in the presence of ionophore A23187. Ionophore, although by itself a weak agonist for LTB₄ formation, was required for LT B₄ production from exogenous arachidonate. The identity of LT B₄ was confirmed by specific radioimmunoassay, high pressure liquid chromatography, and gas chromatography/mass spectrometry. The synthesis of LTB₄ 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 LT B₄. 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 LT B₄ synthesis. To test this hypothesis, glomeruli were depleted of macrophages using x-irradiation. Glomeruli from these animals exhibited a marked decrease in LT B₄ synthesis. Glomerular synthesis of cyclooxygenase products was unaffected by irradiation. In sum, glomeruli have the capability to synthesize LT B₄, and this capacity is correlated with the presence of glomerular macrophages. EFA deficiency attenuates the ability of glomeruli to synthesize LT B₄ by depleting them of macrophages.

Introduction

Leukotriene (LT) B₄ is an important pro-inflammatory mediator. This autacoid 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 E₂ (4). The potential role of LT B₄ 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) deficiency and (n-3) fatty acid enrichment have been shown to inhibit LT B₄ 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 LTA₅ (9). This reactive intermediate binds to LTA₅ hydrolase and prevents the synthesis of LTB₄ 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 LTA₅, which is a relatively poor substrate and an inhibitor of LTA₅ 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 hydrenephrosis (15). Additionally, this metabolic alteration appears to have pathophysiologic significance in these conditions (12–15). In order to assess the potential role of LT B₄ in renal inflammation, the capability of glomeruli to synthesize this eicosanoid was assessed. This study establishes that glomeruli are indeed able to synthesize LT B₄, 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) E₂ and thromboxane (Tx) B₂, 12S-1-PGE₂, 12S-TxB₂, and specific antisera were a gift from Dr. Philip Needleman (Dept. of Pharmacology, Washington University); LT B₄, 20-hydroxy LT B₄, and 20-carboxy LT B₄ were obtained from Merck-Frosst Canada, Inc. (Pointe-Claire-Dorval, Quebec, Canada; gifts of Dr. J. Rokach); [³H]LT B₄ (32 Ci/mmol) was obtained from Du Pont-New England Nuclear (Boston, MA). Octadecylsil columns were obtained from J. T. Baker Chemical Co. (Phillipsburg, NJ); BW755C was obtained from Burroughs-Wellcome (Research Triangle Park, NC). Polyclonal rabbit anti-rat macrophage antiseraum 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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Received for publication 7 December 1987 and in revised form 23 June 1988.

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

© The American Society for Clinical Investigation, Inc.
0021-9738/88/11/1655/06 $2.00
Volume 82, November 1988, 1655–1660
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/DNase 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 DNase 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 10^6 glomeruli was equal in control and EFA-deficient animals (23±3 and 21±2 µg/10^6 glomeruli, respectively). Glomerular eicosanoid production (PGE_2, Tx_B, 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 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 glomeruli 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-leucocyte 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 C18 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)trifluoroacetic acid 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-18]^+ 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
consistent with the double-lipoxygenation product, 5S, 12S-
dihydroxyeicosatetraenoic acid. This isomer incompletely sep-
arrates from LTB4 by high pressure liquid chromatography (9)
but has negligible cross-reactivity in the radioimmunoassay
(0.25%). Additional verification of the presence of LTB4 was
obtained via the use of inhibitors of arachidonate metabolism.
BW755C (a mixed lipoxygenase/cyclooxygenase inhibitor),
but not indomethacin, inhibited the generation of LTB4 by
glomeruli (Table I). Both drugs inhibited the simultaneous
production of TxB2 (Table I).

The synthesis of LTB4 from arachidonate was dose depen-
dent but required higher concentrations of substrate to obtain
maximal production relative to cyclooxygenase metabolites
(Table II). The synthesis of LTB4 also required the presence
of ionophore A23187 (Figure 2). Exogenous arachidonate alone
was not efficiently converted to LTB4. Ionophore alone was a
weak agonist for LTB4 production. Only with the combination
of ionophore and exogenous arachidonate was substantial
LTB4 generated. In contrast, arachidonate was converted to
PGE2 efficiently in the presence or absence of ionophore.

Production of LTB4 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-
cell to glomerular LTB4 synthesis, however, was more directly
tested by comparing the LTB4 production by perfused glomer-
uli to that of unperfused glomeruli. Unperfused glomeruli syn-
thesized identical quantities of LTB4 to perfused glomeruli: 33
and 25 fmol/103 glomeruli at 10 µM arachidonate, respect-
ively, and 170 and 168 fmol/103 glomeruli at 30 µM arach-
donate, respectively. Thus, blood-cell borne products contributed little
to the observed LTB4 production.

The issue of whether perfused glomeruli contained any
contaminating leukocytes (e.g., polymorphonuclear neutro-
phils or lymphocytes) was also addressed by labeling disso-
ciated 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

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

<table>
<thead>
<tr>
<th>Product formation</th>
<th>Control</th>
<th>+Indomethacin</th>
<th>+BW755C</th>
</tr>
</thead>
<tbody>
<tr>
<td>fmol/103 glomeruli</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LTB4</td>
<td>32</td>
<td>26,31</td>
<td>3,3</td>
</tr>
<tr>
<td>TxB2</td>
<td>49</td>
<td>2,1</td>
<td>1,1</td>
</tr>
</tbody>
</table>

Glomeruli were isolated from four animals using the protocol de-
tailed in Methods and pooled. Glomeruli were then aliquoted into
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 pro-
duction of TxB2 and LTB4 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 LTB4 and
PGE2 from Exogenous Arachidonate by Glomeruli

<table>
<thead>
<tr>
<th>Arachidonate (µM)</th>
<th>LTB4</th>
<th>PGE2</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>&lt;1</td>
<td>49</td>
</tr>
<tr>
<td>1</td>
<td>&lt;1</td>
<td>526</td>
</tr>
<tr>
<td>5</td>
<td>19</td>
<td>803</td>
</tr>
<tr>
<td>10</td>
<td>48</td>
<td>696</td>
</tr>
<tr>
<td>20</td>
<td>71</td>
<td>744</td>
</tr>
</tbody>
</table>

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 sub-
sequently assessed for the production of PGE2 and LTB4 by specific
radioimmunoassays and normalized for the number of glomeruli
present in the incubations.

Effects of (n-6) fatty acid deprivation on glomerular LTB4
synthesis. Since EFA deficiency exerts an anti-inflammatory
effect in renal inflammation (5), the effects of the deficiency
state on glomerular LTB4 synthesis were examined. EFA deficiency
inhibited the production of LTB4 by 50–80% (Table III). This effect contrasted with the effects of EFA deficiency
on cyclooxygenase metabolites. EFA-deficient glomeruli syn-
thesized greater amounts of TxB2 and PGE2 (Table III) than
control glomeruli when provided with exogenous arachidon-

![Figure 2](image-url)
Table III. Effect of EFA Deficiency on Glomerular Eicosanoid Production

<table>
<thead>
<tr>
<th>Product formation</th>
<th>EFAD</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>fmol/10^4 glomeruli</td>
<td></td>
</tr>
</tbody>
</table>

Experiment 1 (arachidonate 10 μM)
- LTB₄: 15±3 38±2
- TxB₂: 163±20 77±16
- PGE₂: Not done Not done

Experiment 2 (arachidonate 10 μM)
- LTB₄: 13±2 60±3
- TxB₂: 40±3 23±3
- PGE₂: 563±24 199±7

Experiment 3 (arachidonate 20 μM)
- LTB₄: 39, 43 84, 87
- TxB₂: 67, 78 24, 32
- PGE₂: 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 TxB₂, PGE₂, and LTB₄ 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 LTB₄ 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.

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 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.

Discussion

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 LTB₄, TxB₂, and PGE₂ 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 LTB₄ 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.

Table IV. Effect of Irradiation on Glomerular Eicosanoid Production

<table>
<thead>
<tr>
<th>Product formation</th>
<th>Irradiated</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>fmol/10^4 glomeruli</td>
<td></td>
</tr>
</tbody>
</table>

Experiment 1 (arachidonate 10 μM)
- LTB₄: 23±2 65±8
- TxB₂: 28±3 32±3
- PGE₂: 468±19 448±41

Experiment 2 (arachidonate 10 μM)
- LTB₄: 7±1 35±2
- TxB₂: 55±9 51±2
- PGE₂: 261±40 397±47

Experiment 3 (arachidonate 20 μM)
- LTB₄: 20, 18 84, 87
- TxB₂: 30, 50 24, 32
- PGE₂: 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 LTB₄, TxB₂, and PGE₂ 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 LTB₄ 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 IL-1 determinants, to be phagocytic, and to react with lymphocytes in a genetically restricted fashion (20). Their number and IL-1 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 macrophages are capable of LTB4 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 LTB4 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 LTB4 to perfused glomeruli showing that endovascular cells contributed little to the observed LTB4 production. Secondly, the leukocytes within perfused glomeruli were categorized by morphology, and found to consist solely of resident macrophages. Thus, glomerular LTB4 production could not be attributed to contaminating leukocytes.

The requirement for ionophore in glomerular LTB4 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 LTB4. 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 LTB4 synthesis appears to differ from the mechanism by which EFA deficiency diminishes the synthesis of LTB4 from isolated leukocytes. Isolated EFA-deficient leukocytes, when stimulated with an agonist that releases endogenous fatty acids (e.g., zymosan), synthesize markedly less LTB4 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 LT A1, it has been shown that this particular metabolite of 20:3(n-9) binds to and inactivates LTA hydrolyase, thus preventing the metabolism of arachidonate to LTB4 (10). Therefore, the decrease in LTB4 formation by EFA-deficient leukocytes requires the prior release of endogenous 20:3(n-9) and presumably its metabolism to LTA1.

In the current study, however, EFA-deficient glomeruli were impaired in their ability to produce LTB4 even when provided with exogenous substrate. In contrast, EFA-deficient macrophages are able to synthesize LTB4 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 LTB4 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 LTB4 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 LTB4 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 LTB4 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, linoleate 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 LTB4 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 LTB4, and to mitigate the influx of leukocytes during the inflammatory response (29). A causal connection between the effects of EFA deficiency on LTB4 generation and leukocyte influx was supported by experiments using the cyclooxygenase/lipoxygenase inhibitor, BW755C. This inhibitor prevented LTB4 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 LTB4 and that the mesangial macrophage appears to be the predominant cell source of this eicosanoid. EFA deficiency impairs the generation of LTB4 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 LTB4 synthesis may be the basis of the salutary effect of the deficiency state on renal inflammatory states.

Acknowledgments

The authors appreciate the technical assistance of Margery Halstead and Kathleen Grapperhaus.

This work was supported by grants HL-01313 (Dr. Lefkowith), DK-37879 (Dr. Lefkowith), AM-36277 (Dr. Schreiner), AM-09976 (Dr. Morrison), and DK-38111 (Dr. Morrison) from the National Institutes of Health and a grant from the Communities Foundation of Texas (Dr. Schreiner).

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