Growth Factors, Cytokines, Cell Cycle Molecules

Elevated Expressions of 15-Lipoxygenase and Lipoxin A₄ in Children with Acute Poststreptococcal Glomerulonephritis

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Anti-inflammatory effects of the 15-lipoxygenase (15-LO) derivatives lipoxin A₄ (LXA₄) and 15-S-hydroxy-eicosatetraenoic acid (15-S-HETE) have been documented in many experimental models of acute inflammation. However, the expression levels of 15-LO and its products in human renal diseases remain unknown. This study investigated the expression levels of LXA₄, leukotriene B₄ (LTB₄), and 15-LO in leukocytes and glomeruli obtained from 22 children with acute poststreptococcal glomerulonephritis (APSGN), and determined the modulatory effects of both 15-S-HETE and LXA₄ on LTB₄ synthesis in leukocytes and LTB₄-evoked chemotaxis of polymorphonuclear leukocytes (PMNs) obtained from children during the first 3 days after onset of APSGN. Expression levels of both LXA₄ and 15-LO in leukocytes and glomeruli were up-regulated during the acute phase of disease, further peaking between days 10 and 14, and remained increased after 6 to 8 weeks of APSGN onset. In contrast, blood and urinary levels of LTB₄ as well as the number of glomerular PMNs peaked during the acute phase of disease and then decreased during the resolution phase. Administration of both 15-S-HETE and LXA₄ *in vitro* inhibited LTB₄-induced chemotaxis of PMNs and production of LTB₄ from leukocytes obtained from patients with APSGN. The current study provides further support for an anti-inflammatory role for 15-LO products in human nephritis through both antagonism and inhibition of leukotriene synthesis and its biological activity. (*Am J Pathol* 2009, 174:115–122; DOI: 10.2353/ajpath.2009.080671)

Acute poststreptococcal glomerulonephritis (APSGN) is a disease that primarily affects children, and children with APSGN have better prognosis than adults.¹ Most children with APSGN undergo a spontaneous diuresis within 7 to 10 days after onset of their illness.² The clinical manifestations of APSGN usually resolve in 1 to 2 weeks as the edema and hypertension disappear after diuresis.^{1,2} In most cases, the serum complement levels return to normal by 4 weeks.¹ Dome-shaped subepithelial electron-dense deposits usually resolve within 4 to 6 weeks,¹ and disappear by 8 weeks.² The reason for the self-limited course of APSGN in children, however, remains unclear. It is likely that several processes must act in a coordinated manner to suppress inflammation and promote renal recovery. In this regard, O'Meara and Brady³ pointed out seven possibilities that may lead to resolution of acute inflammation of APSGN. One of these possibilities is that endogenous braking signals inhibit further recruitment of leukocytes.³ Accumulating evidence demonstrates that lipoxins, which serve as endogenous braking signals that inhibit neutrophil recruitment and promote resolution of inflammation, are potential anti-inflammatory and proresolution mediators in several experimental renal injuries.^{3,4} Maintaining the balance between lipoxin and leukotriene formation is likely to be a critical determinant of the resolution of inflammation.⁴ Apart from lipoxins, another 15-lipoxygenase (15-LO) derivative, 15-S-hydroxy-eicosatetraenoic acid (15-S-HETE), has been also shown to antagonize the chemotactic action and glomerular synthesis of leukotriene B₄ (LTB₄) in rats, and therefore play an anti-inflammatory role in experimental glomerulonephritis.⁵ However, the expressions of 15-LO and its products in human renal diseases remain unknown. The present studies were therefore undertaken to find tempo-

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ral changes and a possible role of lipoxin A₄ (LXA₄) and 15-S-HETE in the course of APSGN in children.

Materials and Methods

Patients

Twenty-two patients with APSGN were enrolled in these studies from the Department of Pediatrics and Nephrology, First Affiliated Hospital of Nanjing Medical University, Nanjing First Hospital Affiliated to Nanjing Medical University, and Central Hospital of Tengzhou in Shandong Province. The age range was 5.5 to 12 years, with a mean of 8.6 years. The diagnosis of APSGN was based on prodromal pharyngeal infection with group A streptococci, edema, hematuria, oliguria, hypertension, increased serum anti-streptolysin-O titer, and decreased serum C₃,^{1,2} without severe manifestation such as acute renal failure, hypertensive encephalopathy, and severe hyperemia. The patients were treated with penicillin for 2 weeks. Venous blood specimens were obtained from the patients on the first 3 days before the antibiotic treatment, at 10 to 14 days, and at 6 to 8 weeks after onset of the disease. Renal biopsies were taken in the first 3 days (22 patients), 10 to 14 days (6 patients), and 6 to 8 weeks (4 patients) after onset of the disease. Glomerular filtration rate was evaluated by creatinine clearance adjusted for body surface area in ml/minute/1.73 m². Twenty-two normal healthy children (NHC) served as the first group of controls. The age range was 6 to 12 years, with a mean of 9 years. The second group of controls included 12 children with acute pharyngeal infection by streptococci, but no glomerulonephritis. The age range was 4.5 to 14.8 years, with a mean of 8.2 years. The third group of controls included six children with minimal change disease (MCD) who were treated with prednisone. The age range was 3.2 to 8.8 years, with a mean of 5.2 years. The fourth group of controls included four patients with IgA nephropathy (IgAN) and microscopic hematuria, and without acute infection or gross hematuria during the period of the studies. The age range was 8.5 to 15.8 years, with a mean of 11.2 years. Biopsied tissues of the kidney were taken during the first week of MCD and IgAN, which were processed and investigated by light microscopy. Venous blood specimens were obtained from the above controls on the first 3 days, at 10 to 14 days, and at 6 to 8 weeks after the beginning of the study. The parents or guardians of all enrolled children had signed their names on the informed consent to a protocol approved by the committee of ethics of our three hospitals.

Assessment of Whole Blood LXA₄ and LTB₄

Whole blood samples were drawn into 5-ml tubes containing heparin and exposed to the calcium ionophore A23187 (50 μmol/L; Sigma, St. Louis, MO) 37°C for 30 minutes. After stimulation, reactions were stopped with 5 volumes of ice-cold methanol.⁵ The samples were centrifuged and the supernatants were diluted and acidified to pH 3.5 with HCl. Subsequently, eicosanoids were ex-

tracted with C₁₈ Sep-Pak light column (Waters, Milford, MA) preactivated with methanol and deionized water. The column was washed with deionized water followed by petroleum ether. Materials in the methyl formate eluate were dried under a stream of N₂ and kept at -80°C until LXA₄ and LTB₄ were measured by enzyme-linked immunosorbent assay (Neogen, Lexington, KY) following the manufacturer's instructions.

Measurement of Urinary LXA₄ and LTB₄

The extraction methods were performed as described earlier.⁶ Briefly, urinary samples were centrifuged, acidified, and applied to a C₁₈ Sep-Pak light column (Waters). Materials were eluted with hexane/ethyl acetate and then dried. Residues were resuspended and applied to silica cartridges. Columns were washed with benzene/ethyl acetate and materials eluted with benzene/ethyl acetate/methanol. Eluates were dried and kept at -80°C until LXA₄ and LTB₄ were measured by enzyme-linked immunosorbent assay (Neogen) following the manufacturer's instructions. Urinary creatinine was measured with enzymatic reaction. The results were expressed as LXA₄/mg creatinine and LTB₄/mg creatinine.

Expression of 15-LO in Leukocytes

Whole blood samples were drawn into 5-ml tubes containing heparin. Leukocyte-rich plasma was extracted after sitting for 60 minutes in the presence of physiological saline containing 3% gelatin. The erythrocytes in the separation were lysed by osmotic shock. Subsequently, leukocytes were resuspended and adjusted to a density of 2×10^6 cells/ml in phosphate-buffered saline (PBS). After centrifugation, the cells were collected and the total RNA extraction was performed using Trizol reagent (Life Technologies, Inc., Grand Island, NY) followed by chloroform-isopropanol extraction and ethanol precipitation. Subsequently, RNA was reverse-transcribed using the reverse transcription system (Promega, Madison, WI). Reverse transcription-polymerase chain reaction (RT-PCR) analysis was performed with the following sets of primers: for 15-LO-1, 5'-CCGACCTCGCTATCAAAGAC-3' (sense) and 5'-GGATGACCATGGGCAAGAG-3' (antisense), amplifying a 402-bp fragment. β-Actin was used as an internal control, 5'-GGTCAGTCCATGCCATTAG-3' (sense) and 5'-CCGGAATCACCCCTGTTGCC-3' (antisense) generating a 247-bp fragment. Amplification protocols for 15-LO-1 and β-actin consisted of 35 and 30 cycles, respectively. Amplified cDNA was separated by 3% agarose gel electrophoresis and visualized with ethidium bromide. Semiquantitative analysis was performed by using UVP-gel densitometry (UVP Co., San Gabriel, CA). Arbitrary units = $(A_{15-LO}/A_{\beta-actin}) \times 100\%$.

Determination of LTB₄ Released from Leukocytes

The leukocytes (2×10^6 cells/ml) obtained from eight patients with APSGN were exposed to the calcium ionophore

phore A23187 (2 μ mol/L), A23187 plus 15-S-HETE (30 nmol/L; BIOMOL Intl., Inc., Plymouth Meeting, PA), A23187 plus LXA₄ (30 nmol/L, Sigma), or A23187 plus 12-S-HETE (30 nmol/L, BIOMOL Intl., Inc.) at 37°C for 45 minutes. After stimulation, the supernatants were acidified and extracted with C₁₈ Sep-Pak light column (Waters) preactivated with methanol and deionized water. The column was washed with deionized water and petroleum ether. Materials in the methyl formate eluate were dried and kept at -80°C until LTB₄ were measured by enzyme-linked immunosorbent assay (Neogen) following the manufacturer's instructions.

Chemotactic Assays

Chemotactic assays were performed as described previously.^{7,8} PBS containing LTB₄ (10 nmol/L, Sigma) were placed in the lower compartment of Transwell cluster plates (Costa Corning, Cambridge, MA) with two compartment chambers separated by a polycarbonate membrane filter. The leukocytes (2 $\times$ 10⁶ cells/ml) obtained from eight patients with APSGN in the acute phase were pretreated with the vehicle (1% ethanol in PBS), 15-S-HETE (30 nmol/L), or LXA₄ (30 nmol/L) or 12-S-HETE (30 nmol/L) for 20 minutes in a humidified incubator at 37°C, respectively, and then added to the upper chamber and incubated at 37°C for 30 minutes. Subsequently, the membrane filters were fixed and stained with hematoxylin and eosin (H&E). The polymorphonuclear leukocytes (PMNs) that migrated from the upper chamber were counted in duplicate per $\times$ 400 high-power field with a light microscope. Pretreatment with 15-S-HETE, LXA₄, and 1% ethanol did not alter PMN viability, which was measured as 95% by trypan blue vital dye exclusion.

Glomerular PMN Counts and 15-LO Expression

Cortical sections of formalin-fixed renal tissues were stained with H&E. Infiltrating PMNs in three to six glomeruli obtained from each patient were counted in duplicate per $\times$ 1000 high-power field with a light microscope. Methanol-Carnoy-fixed tissue were deparaffinized and treated with normal goat serum. The goat anti-human 15-LO polyclonal IgG (V-17; Santa Cruz Biotechnology, Santa Cruz, CA) was overlaid on the sections. Subsequently, biotinylated rabbit anti-goat IgG (Vector Laboratories, Burlingame, CA) was applied and endogenous peroxidase activity was blocked by hydrogen peroxide. The sections were exposed to avidin-biotinylated horseradish peroxidase and diaminobenzidine tetrahydrochloride (Vector Laboratories). Methyl green was used for counterstaining. Control sections were treated identically, but without the primary antibody, and did not demonstrate any positive staining. The mean ratio of 15-LO-positive cellular area (deep brown, not green) to single glomerulus in three to six glomeruli of each patient was assessed by JD-801 computer-aided image analyzer (JEDA Co., Nanjing, China) under high-power magnification ($\times$ 1000).

Statistical Analysis

Results are expressed as mean $\pm$ SD. Experimental data were analyzed using one-way analysis of variance followed by *F* and *q* test by SPSS 10.0 statistical software (SPSS Inc., Chicago, IL). Spearman rank correlation analysis was undertaken. Differences were considered to be statistically significant when *P* < 0.05.

Results

Changes of Blood and Urinary LXA₄

As shown in Figure 1A, blood LXA₄ levels in 22 patients were higher than those of NHC on the first 3 days (acute phase) after onset of APSGN, further increased at 10 to 14 days (early resolution phase), and still higher at 6 to 8 weeks (late resolution phase) as compared to those of NHC. Blood LXA₄ in 12 patients with acute pharyngeal infections by streptococci were not higher than those of NHC on the first 3 days, but peaked at 10 to 14 days, and returned to normal at 6 to 8 weeks after infection. Blood LXA₄ in four patients with IgAN were higher than those of NHC but lower than those of APSGN at three time points. Blood LXA₄ in six children with MCD were higher than those of NHC but lower than those of APSGN on the first 3 days and at 10 to 14 days after the onset of MCD, and returned to normal at 6 to 8 weeks. As shown in Figure 1B, the dynamic changes of urinary LXA₄ in all patients were similar to those of the blood LXA₄, except the urinary and blood LXA₄ in patients with streptococcal infections.

Changes of Blood and Urinary LTB₄

As indicated in Figure 1C, blood LTB₄ in patients with APSGN were markedly higher than those of NHC in the first 3 days after onset of APSGN, and gradually decreased from then on. However, the values at 6 to 8 weeks were still higher than those of NHC. Blood LTB₄ in patients with streptococcal infections were higher than those of NHC on the first 3 days and at 10 to 14 days after infection but lower than those of APSGN, and returned to baseline at 6 to 8 weeks. Blood LTB₄ in patients with IgAN were higher than those of NHC but lower than those of APSGN at three time points. Blood LTB₄ in patients with MCD were higher than those of NHC but lower than those of APSGN on the first 3 days and at 10 to 14 days after the onset of MCD, but returned to normal at 6 to 8 weeks. As shown in Figure 1D, the dynamic changes of the urinary LTB₄ in all patients were similar to those of the blood LTB₄ except the urinary and blood LTB₄ in patients with streptococcal infections.

Expression of 15-LO in Leukocytes

As shown in Figure 2, the dynamic changes of arbitrary units of 15-LO in patients with APSGN, streptococcal infections, IgAN, MCD, and NHC were similar to those of the blood LXA₄ in all patients as indicated in Figure 1A. Spear-

Figure 1. Temporal changes of whole blood and urinary LXA₄ and LTB₄. The samples were obtained from 22 children with APSGN, 12 children with acute pharyngeal infections by streptococci (infection), 4 patients with IgA nephropathy (IgAN), and 6 children with MCD in the first 3 days, 10 to 14 days, and 6 to 8 weeks after the onset of the diseases, or from 22 NHC who served as controls. The extraction methods were performed as described in the Materials and Methods; whole blood and urinary LXA₄ and LTB₄ were assessed by enzyme-linked immunosorbent assay following the manufacturer's instructions. **P* < 0.05 as compared to the same kind of eicosanoid levels of NHC at the same time point. ***P* < 0.05 as compared to the same kind of eicosanoid levels of APSGN at the same time point.

man rank correlation analysis between the temporal changes of blood LXA₄ and 15-LO expression in leukocytes of the patients with APSGN brought out a positive correlation and the correlation coefficient (*r_s*) was 0.682, *P* < 0.05.

Figure 2. Expression of 15-LO mRNA was assessed by RT-PCR in leukocytes. mRNA was derived from leukocytes obtained from eight children with APSGN, four children with acute pharyngeal infection by streptococci (infection), four patients with IgA nephropathy (IgAN), and four children with MCD in the first 3 days, 10 to 14 days, and 6 to 8 weeks after the onset of the diseases, or from eight NHC who served as controls. **A:** RT-PCR for 15-LO mRNA expression; β-actin served as an internal control. **B:** Data of semi-quantitative analysis using UVP-gel densitometry are shown. Arbitrary units = (*A*_{15-LO}/*A*_{β-actin}) × 100%. **P* < 0.05 as compared to eicosanoid levels of NHC at the same time point. ***P* < 0.05 as compared to eicosanoid levels of APSGN at the same time point.

Release of LTB₄ from Leukocytes

To determine whether 15-S-HETE or LXA₄ inhibit production of LTB₄ in leukocytes isolated from children with APSGN, we assessed the levels of LTB₄ released from leukocytes after 15-S-HETE or LXA₄ stimulation. As indicated in Figure 3, treatment of the cells with A23187 plus 15-S-HETE or plus LXA₄ inhibited the release of LTB₄, respectively. However, 12-S-HETE did not inhibit the levels of LTB₄ released from leukocytes exposed to A23187, as compared with that of the cells exposed to A23187 plus vehicle (1% ethanol in PBS) that served as controls.

Chemotactic Assays

To investigate if 15-S-HETE or LXA₄ inhibit the LTB₄-evoked chemotaxis of PMNs obtained from children with APSGN in the acute phase, we performed chemotactic assays. As indicated in Figure 4, pretreatment of the cells with 15-S-HETE and LXA₄ both inhibited the LTB₄-induced chemotaxis of PMNs. In addition, pretreatment of

Figure 3. Production of LTB₄ in leukocytes. The leukocytes were obtained from eight children in the first 3 days (acute phase) after the onset of APSGN, and exposed to the calcium ionophore A23187 (2 μ mol/L) plus vehicle, served as controls, or A23187 plus 15-S-HETE (30 nmol/L), or A23187 plus LXA₄ (30 nmol/L), or A23187 plus 12-S-HETE (30 nmol/L) 37°C for 45 minutes, respectively. Release of LTB₄ in supernatants was assessed by enzyme-linked immunosorbent assay following the manufacturer's instructions. * $P < 0.05$ as compared to levels of controls.

the cells with 12-S-HETE and vehicle had no effect on LTB₄-induced PMN migration.

Glomerular PMN Counts

As shown in Figure 5, A and C, peaked infiltration of PMNs was found in the acute phase of APSGN. From that time on, the infiltration of PMNs gradually decreased. Spearman rank correlation analysis between the temporal changes of urinary LTB₄ and glomerular PMN counts of the patients brought out a positive correlation and the correlation coefficient (r_s) was 0.664, $P < 0.05$.

Glomerular 15-LO Expressions

As indicated in Figure 5, B and C, the expressions of 15-LO gradually increased from the acute phase and peaked in the early resolution phase of APSGN. From that time on, the

Figure 4. Chemotactic assays of PMNs in response to LTB₄. PMNs were obtained from eight children with APSGN. LTB₄ (10 nmol/L) was placed in the lower chamber of Transwell cluster plates. The leukocytes were pre-treated with the vehicle (1% ethanol as a control), 15-S-HETE (30 nmol/L), LXA₄ (30 nmol/L), or 12-S-HETE (30 nmol/L) for 20 minutes and then added to the upper chamber and incubated at 37°C for 30 minutes. The membrane filters were fixed and stained with H&E. PMNs that migrated from the upper chamber were counted in duplicate per $\times 400$ high-power field with a light microscope. * $P < 0.05$ as compared to levels of controls.

Figure 5. Glomerular PMN counts and 15-LO expression. The glomeruli were obtained from children with APSGN in the first 3 days (22 patients, acute phase), 10 to 14 days (6 patients, early resolution phase), and 6 to 8 weeks (4 patients, late resolution phase) after the onset of the disease. **A** and **C:** Infiltrating PMNs from three to six glomeruli per patient were counted in duplicate H&E-stained sections per $\times 1000$ high-power field with a light microscope. **B:** Glomerular 15-LO expressions were determined by immunohistochemical staining. Methyl green was used for counterstaining. **C:** The mean ratio of the 15-LO-positive cellular area (deep brown, not green) to the single glomerulus was assessed by a JD-801 computer-aided image analyzer under high-power magnification ($\times 1000$). * $P < 0.05$ as compared to eicosanoid levels in the acute phase of APSGN.

expressions of 15-LO gradually decreased. Spearman rank correlation analysis between the temporal changes of urinary LXA₄ and glomerular 15-LO expressions of the patients brought out a positive correlation and the correlation coefficient (r_s) was 0.725, $P < 0.05$.

Clinical Data Analysis

Glomerular filtration rate in the 22 patients with APSGN was 75.2 ± 12.4 ml/minute/1.73 m², 96.3 ± 16.1 ml/minute/1.73 m², and 102.7 ± 18.6 ml/minute/1.73 m² on the first 3 days, at 10 to 14 days, and at 6 to 8 weeks after onset of the disease, respectively. Macroscopic hematuria was found in 8 patients and gross hematuria in 14 patients in acute phase of APSGN. Spearman rank correlation analysis between the temporal changes of urinary LXA₄ and glomerular filtration rate of the patients brought out a positive correlation and correlation coefficient (r_s) was 0.528, $P < 0.05$. However, no significant correlations were found between the temporal changes of blood LXA₄ and the glomerular filtration rate, and the degree of hypertension, or between the temporal changes of urinary LXA₄ and degree of proteinuria, and hematuria of the patients with APSGN.

Discussion

APSGN is a self-limited disease especially occurring in children. Within the first 2 weeks of APSGN, acute histological change is characterized by an influx of PMNs that results in diffuse global hypercellularity.^{1,2} After 4 to 6 weeks, PMNs are no longer present.¹ As shown in Figure 1, C and D, there was an early peak of blood and urinary LTB₄ on the first 3 days after onset of APSGN and a subsequent decrement along with the time. The temporal concordance and positive correlation ($P < 0.05$) between the disappearance of PMNs in glomeruli (Figure 5, A and C) and suppression of urinary LTB₄ generation (Figure 1D) brings into focus the potential role of locally generated anti-chemotactic mediators in arresting LTB₄-evoked PMN infiltration and possibly suppressing LTB₄ synthesis. The 15-LO products 15-S-HETE and LXA₄ may be capable of exerting counterregulatory effects to inhibit the proinflammatory actions of LTB₄ in children with APSGN. This observation is relevant in view of the previous investigations, which have demonstrated that 15-S-HETE inhibited production of LTB₄ by isolated glomeruli obtained from rats with nephrotoxic nephritis⁷ and by human PMNs,⁹ a LXA₄ analog inhibited the production of cysteinyl leukotrienes in murine model of asthma,¹⁰ 15-S-HETE inhibited LTB₄-evoked chemotaxis of PMNs obtained from rat with nephrotoxic nephritis,⁷ and LXA₄ inhibited LTB₄-induced chemotaxis of PMNs obtained from humans.¹¹

The blood and urinary LXA₄ were ~10-fold greater than the blood and urinary LTB₄ (Figure 1). As indicated in Figures 1, 2, and 5, in children with APSGN, the dynamic changes of blood and urinary LXA₄ were temporally concordant with the dynamic changes of 15-LO expression in leukocytes and glomeruli, respectively, both positive correlations ($P < 0.05$) indicating blood and urinary LXA₄ came from leukocyte 15-LO and glomerular 15-LO, respectively. Urinary LXA₄ may be the overflow from blood LXA₄. Furthermore, blood and urinary LXA₄, leukocytic, and glomerular 15-LO expressions were up-regulated in the acute phase, peaked in the early resolution phase and still increased in the late resolution phase of APSGN. To the contrary, blood and urinary LTB₄ and glomerular infiltration of PMNs peaked in the acute phase but gradually decreased in the early and late resolution phase of APSGN. Contrary temporal changes were found between the levels of LXA₄, 15-LO expression, and the levels of LTB₄ and glomerular infiltration of PMNs in the early resolution phase of APSGN, suggesting that the suppressed LTB₄ production and LTB₄-evoked PMN infiltration in glomeruli in the early resolution phase may be attributed to enhanced 15-LO expression at the same time point because 15-LO derivatives, LXA₄, and 15-S-HETE could inhibit LTB₄ synthesis and LTB₄-evoked PMN infiltration.^{7,12,13} Consistently, similar time courses of production of LTB₄, 15-LO expression, and its products to our data were also reported in rats with experimental glomerulonephritis.^{7,12} In rats with nephrotoxic nephritis, the production of LTB₄ by isolated glomeruli peaked early in the first 3 hours after initial injury and subsequently returned to baseline levels, whereas the enhanced production of 15-S-HETE was greatest be-

yond 72 hours and fivefold greater than LTB₄ production, and increased steadily throughout the 2-week period while the levels of LTB₄ fall dramatically.⁷ Rat glomerular 15-LO mRNA increased significantly over control values 24 and 48 hours after induction of nephrotoxic nephritis and then decreased at 72 hours.¹²

As shown in Figures 3 and 4, 15-S-HETE and LXA₄ inhibited the production of LTB₄ from leukocytes isolated from children with APSGN in the acute phase, and pretreatment of the leukocytes obtained from the patients with 15-S-HETE and LXA₄ but not 12-S-HETE, inhibited the LTB₄-induced chemotaxis of PMNs. These results suggest an *in vivo* interaction between 15-LO products and LTB₄ synthesis and its actions. Our data coupled with previous investigations,^{7,9,11} indirectly demonstrated that 15-LO derivatives could inhibit leukocyte LTB₄ synthesis and LTB₄-induced infiltration of PMNs in glomeruli of children with APSGN. Our results were consistent with the observation reported previously by us and other investigators.^{7,10,13–15} Preincubation of nephritic glomeruli with 15-S-HETE resulted in abrogation of LTB₄ production,⁷ and 15-S-HETE but not 12-S-HETE inhibited LTB₄-evoked chemotaxis of PMNs obtained from rat with nephrotoxic nephritis.⁷ Transfection of rat kidney with human 15-LO increased the renal production of LXA₄ and decreased glomerular LTB₄ synthesis in rats with passive nephrotoxic nephritis.¹³ Treatment of rats with a LXA₄ analogue reduced the leukocyte accumulation in glomeruli obtained from rats with nephrotoxic nephritis¹ and mesangioproliferative nephritis.¹⁵

The cellular sources of the enhanced 15-LO expression in glomeruli (Figure 5, B and C) were unclear in the present studies. In the rats with nephrotoxic nephritis, macrophages/monocytes are a potent source of 15-LO products, and the suppression of LTB₄ synthesis accompanied the shift in the glomerular cellular infiltration from neutrophils to macrophages/monocytes.^{7,16} Similarly, within the first 2 weeks of APSGN, neutrophils, eosinophils, and lymphocytes are present in glomeruli, and endothelial and mesangial cell proliferation is prominent.¹ After 4 to 6 weeks, PMNs are no longer present, and hypercellularity with mononuclear cells (mesangial cells and monocytes) predominates.¹ Accordingly, the present studies showed that 15-LO expression in glomeruli increased on 14 days and 8 weeks after onset of APSGN (Figure 5, B and C), suggesting that cellular sources of the enhanced 15-LO expression were mainly monocytes and mesangial cells, both of which are a rich source of 15-LO products.^{17,18} The reason for up-regulation of 15-LO expression in glomeruli and leukocytes in children with APSGN remains unclear. Interleukin-4 (IL-4) and IL-13 both induce 15-LO expression in blood monocytes and epithelial cells.^{18,19} In rats with nephrotoxic nephritis, glomerular IL-4 mRNA expression increased 16 hours after injection of nephrotoxic serum.¹² In children with APSGN, production of IL-13 in mononuclear cells was increased in acute phase.²⁰ Collectively, these investigations support the speculation that IL-4 and IL-13 could up-regulate the expression of 15-LO in glomeruli obtained from children with APSGN (Figure 5, B and C).

Apart from LTB₄, other leukoattractant mediators and proinflammatory cytokines also played a role in pathogenesis of APSGN. Urinary and serum IL-8 were significantly elevated in the acute phase of APSGN.²¹ IL-6 and tumor necrosis factor- α (TNF- α) are increased in serum and renal biopsies, and serum platelet-activating factor (PAF) is up-regulated in the patients with APSGN.²² LXA₄ may inhibit the production and/or proinflammatory actions of IL-6, IL-8, TNF- α , and PAF in patients with APSGN. Previous investigations have demonstrated LXA₄ and its analogue inhibited renal IL-6 expression in mice with renal ischemic reperfusion injury,²³ reduced IL-8 expression in human leukocytes,²⁴ attenuated TNF- α -initiated neutrophil responses and trafficking,²⁵ and blocked PAF-induced eosinophil trafficking.²⁶ We have demonstrated that LXA₄ inhibits TNF- α -induced production of IL-6 in rat mesangial cells.²⁷ In addition, LXA₄ and its analogue activate monocytes and promote phagocytosis of apoptotic PMNs by macrophages without proinflammatory cytokine release.²⁸ The engulfment of apoptotic PMNs leads to the expression of 15-LO in macrophages that released LXA₄ to limit inflammation further by stopping PMN influx and accelerating the macrophage removal of apoptotic PMNs,²⁹ suggesting that LXA₄ may be involved in the dynamic regulation of the resolution phase of APSGN. Consistently, in the current study, glomerular 15-LO expression was up-regulated in the resolution phase of disease (Figure 5, B and C).

In conclusion, our studies provided the first observation of the dynamic changes of levels of blood and urinary LXA₄ and LTB₄, and of 15-LO expressions in leukocytes and glomeruli in children with APSGN. The contrary temporal changes were found in the early resolution phase of APSGN between gradually increased blood and urinary LXA₄, leukocytic and glomerular 15-LO expressions and gradually reduced blood and urinary LTB₄, glomerular PMN counts. Furthermore, 15-S-HETE and LXA₄ both inhibited the LTB₄-induced chemotaxis of PMNs and production of LTB₄ from leukocytes obtained from the patients in the acute phase of APSGN. Coupled with previous demonstrations of counterregulatory interactions between 15-LO products and leukotrienes,^{7,10,11} the current studies provide further support for a anti-inflammatory and proresolution role for 15-LO products in human nephritis through antagonism and/or inhibition of leukotriene synthesis and biological activities. Our results further expand on the anti-inflammatory and proresolution repertoire of lipoxins,⁴ which represent useful tools for the development of a new therapy in treatment of glomerulonephritis.

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