Leukotrienes and lipoxins: lipoxygenase-derived modulators of leukocyte recruitment and vascular tone in glomerulonephritis

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Introduction

Glomerulonephritis is a leading cause of end-stage renal failure worldwide. The therapeutic options for patients with immune-mediated glomerular diseases have changed little in recent times, and consist mainly of broad spectrum immunosuppressive regimens involving corticosteroids, azathioprine, cyclophosphamide, and cyclosporine. The pressing need for more effective and less toxic therapies has focused attention on the diverse mediators that regulate the initiation, amplification, maintenance, and resolution phases of glomerular inflammation. Amongst these mediators, the leukotrienes and lipoxins are particularly intriguing as they appear to play counter-regulatory roles within inflamed glomeruli that influence leukocyte recruitment, vascular tone and other pivotal pathophysiological events [1–6].

Leukotrienes (LT) and lipoxins are eicosanoids, i.e. 20-carbon derivatives of membrane-derived arachidonic acid, that are generated via biochemical pathways catalyzed by lipoxygenase enzymes [1–6]. They are components of the pleiotropic ‘arachidonic acid cascade’, which include prostaglandins, prostacyclins, and thromboxanes, among other lipid mediators [1–6]. LT are potent pro-inflammatory compounds and have been detected in serum, urine and renal tissue during glomerular inflammation [7–12]. In this setting, they have been implicated as triggers for neutrophil recruitment and activation, intrarenal vasoconstriction, mesangial cell contraction, and proliferation of resident glomerular cells [13–20]. Lipoxins antagonize many responses evoked by LT (and indeed other mediators), and are putative endogenous inhibitors of inflammation [18–22]. Here we review briefly the major biosynthetic pathways for leukotriene and lipoxin synthesis in vitro and in vivo, we summarize their bioactions with specific reference to glomerulonephritis, and we discuss the therapeutic opportunities spawned by recent advances in this field.

Biosynthesis and metabolism of leukotrienes and lipoxins

LT biosynthesis and metabolism. LT are generated by several different cell types from endogenous arachidonate or from arachidonate intermediates derived from neighbouring cells during cell-cell interactions [1–6; Figure 1]. LT biosynthesis is initiated, in part, by increments in cytosolic calcium concentration triggered by receptor activation or physical stimuli (e.g. trauma, burns) [1–6,23]. Increased cytosolic calcium levels stimulate translocation of the cytosolic enzyme phospholipase A2 to cell membranes where it cleaves arachidonic acid from membrane-bound phospholipid [23]. The major bioactive LTB4, and the sulfidopeptide leukotrienes, LTC4, LTD4, and LTE4, are derived from a common precursor, LTA4 [1–6]. The latter is generated by the insertion of molecular oxygen and abstraction of hydrogen at carbon-5 of arachidonic acid, a reaction catalyzed by 5-lipoxygenase when associated with 5-lipoxygenase activating protein (FLAP) [24,25]. 5-Lipoxygenase expression appears to be limited to leukocytes, with the exception of colon cancer cells and some cell lines [1–6]. LTB4 is generated from LTA4 by net addition of water, a reaction catalyzed by LTA4 hydrolase. Omega-oxidation of LTB4 to 20-OH-LTB4 and 20-COOH-LTB4 through a P450 enzymatic system represents the major pathway for LTB4 degradation in mammalian cells and is generally associated with loss of biological activity [1,6]. Interestingly, LTB4 is an activating ligand for the transcription factor peroxisome proliferator-activated receptor-α (PPARα). Activated PPARα upregulates expression of enzymes that oxidatively degrade fatty acids and their derivatives, including LTB4, suggesting the presence of a feedback loop that may limit the duration of LTB4-mediated inflammatory responses.
Fig. 1. Summary of major pathways for leukotriene and lipoxin generation by human cells. Abbreviations: LT, leukotriene; LX, lipoxin; LO, lipoxygenase; HETE, hydroxyeicosatetraenoic acid; GSH, glutathione; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; PDGF, platelet-derived growth factor; [Ca]i, intracellular calcium concentration. Reprinted with permission from Brady and Serhan [22].

Sulfdopeptide leukotrienes are formed by the addition of glutathione to carbon-6 of LTA4 to form LTC4, a reaction catalyzed by LTC4 synthase/glutathione-S-transferase [1,5,6]. The sequential removal of glutamic acid and glycine residues by specific dipeptidases yields LTD4 and LTE4, respectively. In general, LTC4 and LTD4 are bioactive, and metabolism of LTD4 to LTE4 is associated with loss of biological activity.

Whereas relatively homogenous populations of leukocytes generate leukotrienes in abundance when activated in vitro (e.g. eosinophils, basophils, mast cells), transcellular biosynthetic pathways are being increasingly recognized as important routes for amplification of the quantities and profile of lipoxygenase-derived eicosanoids within a multicellular inflammatory milieu [26]. Upon cell activation, leukocytes release some LTA4 into extracellular fluid where it is transiently available for transformation by neighbouring cells into bioactive products. LTA4 hydrolase is expressed by cells other than leukocytes, including platelets, glomerular endothelial cells and glomerular mesangial cells [27–34]. Glutathione-S-transferase and dipeptidase enzymatic activities are also widely distributed in renal and non-renal cells, including platelets and glomerular endothelial cells [27–34]. These non-leukocyte cells can convert leukocyte-derived LTA4 to LTB4 and/or peptidoleukotrienes in mixed cell suspensions in vitro, and transcellular leukotriene formation is postulated to be a major source of leukotrienes during glomerular inflammation in vivo [26].

Leukocyte adhesion to vascular endothelium and other glomerular cells is a key event in leukocyte recruitment during glomerular inflammation and promotes other pro-inflammatory activities such as free radical generation and degranulation, cytolysis and antigen presentation [35,36]. Interestingly, these adhesive events also promote transcellular leukotriene biosynthesis during interaction between neutrophils and glomerular endothelial cells in vitro [33; Figure 2]. Potential mechanisms by which eicosanoid production is augmented in this setting include approximation of cell membranes, thereby facilitating transfer of lipophilic arachidonate intermediates between lipid bilayers, and priming of neutrophil lipoxigenase pathways through cell signalling events activated by engagement of adhesion molecules (so-called ‘outside-in’ cell signalling through adhesion molecules) [22]. Given that leukotrienes are themselves potent stimuli for leukocyte-endothelial cell adhesion (vide infra), these observations suggest the presence of complex leukotriene-adhesion networks that, if allowed to proceed unchecked, would ultimately amplify leukocyte recruitment and tissue injury.

In support of the in vitro observations discussed in the preceding paragraphs, LTB4 and the sulfdopeptide leukotrienes LTC4 and D4 have been detected in several experimental models of glomerulonephritis. LTB4 is generated in passive Heymann nephritis (PHN), anti-Thy-1 nephritis, nephrotoxic serum nephritis and glomerular injury induced by cationic bovine gamma globulin [7,8,10,37–41]. The detection and measurement of the peptidoleukotrienes in glomerular isolates has been hampered by methodological constraints; nevertheless, peptidoleukotrienes have been detected in experimental immune complex glomerulonephritis in rats, in mice with spontaneous lupus nephritis, and in human glomerulonephritis [9,42]. The relative contributions of leukocyte-restricted and transcellular pathways to leukotriene biosynthesis during glomerular inflammation is still being defined. Prior depletion of neutrophils in animals markedly reduces LTB4 levels and inflammatory injury in experimental glomerulonephritis confirming that leukocytes are the major sources of LTA4 in this setting [41]. In vitro assessment of transcellular leukotriene formation during interactions of cytokine-primed neutrophils and glomerular endothelial cells suggests that transcellular routes play a major role in peptidoleukotriene generation and a lesser role in LTB4 biosynthesis [32].

Lipoxin biosynthesis and metabolism. Lipoxins, initially isolated in 1984 by Serhan and colleagues, are trihydroxytetraene-containing eicosanoids generated by biochemical pathways that initially involve the sequential actions of two lipoxygenases with arachidonic acid [1–4,43; Figure 1]. Lipoxins are potential ‘braking signals’ for neutrophil recruitment induced by leukotrienes and several other mediators of inflammation [3,4,22]. Multiple pathways of lipoxin biosynthesis...
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Fig. 2. Some pathways for transcellular generation of leukotrienes, lipoxins and aspirin-triggered epi-lipoxins during neutrophil-platelet and neutrophil-endothelial cell adhesion. Abbreviations as in Figure 1.

have been described in model systems in vitro. Lipoxins (LX) are generated by activated granulocytes through sequential lipoxygenation of arachidonic acid by 15- and 5-lipoxygenase to yield an unstable epoxide intermediate, 5,6-epoxytetraene [1,4]. The latter is converted to the major bioactive lipoxins LXA$_4$ and LXB$_4$ via reactions catalyzed by epoxide hydrolases [1,4]. Lipoxin biosynthesis is greatly augmented, however, through transcellular pathways if granulocytes are activated during co-incubation with platelets [44–50]. Platelets cannot generate lipoxins from arachidonic acid. During cell-cell interactions, however, platelets convert neutrophil-derived LTA$_4$ to 5,6-epoxytetraene through the action of platelet 12-lipoxygenase [44–50].

Within this framework, the term ‘12-lipoxygenase’ is a misnomer as it refers to the enzyme’s enzymatic activity with arachidonic acid. The same enzyme functions as a 15-lipoxygenase (LX synthase) when its substrate is LTA$_4$ [44–50]. Thus, within a multicellular inflammatory environment, LTA$_4$ can serve as a key intermediate for both leukotriene and lipoxin formation. As with peptidoleukotriene generation during interaction of neutrophils and glomerular endothelial cells, leukocyte adhesion promotes transcellular lipoxin generation during platelet-neutrophil interaction [39,50,51; Figure 2]. The cytokines interleukin-4 and interleukin-13 induce 15-lipoxygenase expression in some cells, raising the possibility that resident tissue cells also cooperate with granulocytes to generate lipoxins [52,82].

The ‘anti-inflammatory’ potential of the lipoxins has been underscored by exciting reports that aspirin triggers production of lipoxin epimers during co-incubations of neutrophils and endothelial cells in vitro [53,54; Figure 2]. In the presence of aspirin, prostaglandin production by cyclooxygenase-2 (COX-2) is inhibited; however, aspirin-acetylated COX-2 retains the ability to convert arachidonic acid to 15(R)-HETE. The latter compound has anti-inflammatory potential by virtue of its ability to inhibit neutrophil-endothelial cell adhesion [55,56]. During cell-cell interactions, some endothelial-derived 15(R)-HETE is converted by neutrophil 5-lipoxygenase to either 15-epi-LXA$_4$ or 15-epi-LXB$_4$ which have anti-inflammatory properties similar to native lipoxins [53,54]. Given that cytokines induce COX-2 expression at sites of inflammation, it is intriguing that aspirin, the prototype non-steroidal anti-inflammatory drug, diverts arachidonate metabolism into production of a novel class of lipoxygenase-derived eicosanoids that are potent inhibitors of leukocyte-endothelial cell adhesion. These observations suggest a novel mechanism by which aspirin may exert its anti-inflammatory effects in vivo.

Lipoxin generation has been demonstrated in a variety of human diseases including asthma, sarcoidosis, psoriasis, pneumonia and post-coronary angio-
plasty [3,57,58]. With regard to renal disease, lipoxin biosynthesis has been demonstrated in experimental immune complex glomerulonephritis in rats and mice [39,50]. The dominant pathway for lipoxin generation during the initial stages of acute glomerulonephritis appears to involve neutrophil-platelet biosynthetic interactions [39,50]. In the concanavalin A-ferritin model of acute immune complex-mediated glomerulonephritis, robust LXA₄ generation is observed coincident with electron microscopic evidence of neutrophil-platelet adhesion within glomerular capillaries [50; Figures 2 and 3]. Depletion of animals of either neutrophils or platelets prior to induction of glomerulonephritis attenuates lipoxin generation, suggesting involvement of both cell types in lipoxin generation [50]. Monoclonal antibodies directed against platelet P-selectin, that inhibit platelet-neutrophil adhesion and transcellular lipoxin production during platelet-neutrophil interactions *in vitro*, also attenuate the generation of lipoxin A₄ *in vivo* [50]. Studies of acute passive anti-GBM nephritis model in P-selectin ‘knockout’ mice support a role for adhesion-dependent platelet-neutrophil interactions as a major source of LXA₄ within the vascular lumen [39,59]. The latter site would be strategically advantageous for inhibition of further neutrophil recruitment during inflammation (*vide infra*) [39]. The relative importance of non-haematogenous cells in transcellular lipoxin generation *in vivo* is unclear, but a role is possible given that conversion of neutrophil-derived intermediates by renal cells expressing 12- or 15-lipoxygenase activity has been described [82].

The pathways for lipoxin degradation are still being appreciated. The major route in leukocytes involves dehydrogenation of alcohols and reduction of double bonds through enzymatic pathways analogous to those involved in prostanoid metabolism. Within this framework, LXA₄ is metabolized by phorbol myristate acetate-differentiated HL-60 cells and monocytes to 15-oxo-LXA₄ and 13,14-dihydro-15-oxo-LXA₄ [60]. Human neutrophils also metabolize LXA₄ to 20-hydroxy-LXA₄ and 20-hydroxy-LXB₄, respectively [61]; however, LTB₄ is the preferred substrate for this P₄₅₀ pathway and it is unlikely to be a major contributor to lipoxin degradation. The metabolic fate of lipoxins *in vivo* has yet to be determined. Future research in this area should enhance our understanding of the mechanisms by which tissue levels are regulated [50]. Monoclonal antibodies directed against platelet P-selectin, that inhibit platelet-neutrophil adhesion and transcellular lipoxin production during platelet-neutrophil interactions *in vitro*, also attenuate the generation of lipoxin A₄ *in vivo* [50]. Studies of acute passive anti-GBM nephritis model in P-selectin ‘knockout’ mice support a role for adhesion-dependent platelet-neutrophil interactions as a major source of LXA₄ within the vascular lumen [39,59]. The latter site would be strategically advantageous for inhibition of further neutrophil recruitment during inflammation (*vide infra*) [39]. The relative importance of non-haematogenous cells in transcellular lipoxin generation *in vivo* is unclear, but a role is possible given that conversion of neutrophil-derived intermediates by renal cells expressing 12- or 15-lipoxygenase activity has been described [82].

**Biological activities: regulators of leukocyte recruitment and vascular tone in glomerulonephritis**

**Leukotriene bioactivities.** LTB₄ is a potent stimulus for neutrophil activation and induces chemotaxis, adhesion to endothelial cells, some superoxide anion generation and degranulation, and release of IL-8 [1–6]. LTB₄ also increases endothelial permeability and adhesiveness for neutrophils [62,63]. Specific to the kidney, intrarenal administration of LTB₄ into healthy rats leads to mild vasorelaxation and natriuresis [11].

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**Fig. 3.** Neutrophil-endothelial cell and neutrophil-platelet adhesion during acute immune complex-mediated glomerulonephritis (concanavalin A-ferritin-induced glomerulonephritis). Representative electron micrograph in (A) shows a glomerular capillary loop from a nephritic animal with infiltrating polymorphonuclear neutrophils (PMN) and platelets (P) in close contact and occluding the lumen. These adhesive interactions were observed in association with biochemical evidence of transcellular lipoxin biosynthesis during P-selectin-dependent adhesion of neutrophils and platelets. Prior exposure of neutrophils to LXA₄ attenuates their recruitment to inflamed glomeruli in this model, suggesting the presence of a negative feedback loop that inhibits further neutrophil infiltration and promotes resolution of glomerulonephritis. (B) micrograph of a control animal (CL, capillary lumen; US, urinary space; Ep, visceral epithelial cell; RBC, red blood cell; End, endothelial cell). Reprinted with permission from A. Papayianni *et al.* [50].
In vitro studies reveal that LTβ4 triggers adhesion of neutrophils and monocytes to glomerular endothelial and mesangial cells through adhesive events involving neutrophil CD11/CD18 β integrins [20,32,64]. Intrarenal infusion of LTβ4 in rats with nephrotoxic serum nephritis markedly increases neutrophil infiltration and amplifies the corresponding fall in renal blood flow and glomerular filtration rate (GFR) [11].

The profile of biological activities triggered by sulfidopeptide leukotrienes differs from LTβ2. Originally classified as the ‘slow-reacting substance of anaphylaxis’, LTC4 and LTD4 are potent stimuli for contraction of bronchial and vascular smooth muscle. Infusion of LTC4 into the renal artery leads to a marked reduction in renal blood flow, glomerular ultrafiltration coefficient and GFR [15,16,18]. LTC4 and LTD4 contract mesangial cells in vitro, a response that presumably underpins the fall in the glomerular filtration coefficient (Kf) in vivo [13,14]. LTC4 and LTD4 alter vascular permeability and enhance endothelial adhesiveness for neutrophils by provoking synthesis of platelet-activating factor and mobilization of P-selectin from Weibel-Palade bodies to the cell surface [2,5,19,65,66]. Adhesion of phagocytes to glomerular endothelial and mesangial cells is also enhanced by sulfidopeptides [20]. Leukotriene C4 induces proliferation of renal epithelial cells in vitro, and both LTC4 and LTD4 have been implicated as stimuli for mesangial cell proliferation in animal models of proliferative glomerulonephritis [8,9,67].

Leukotrienes modulate diverse cellular activities through interaction with high affinity cell surface receptors. Receptor engagement triggers cell signalling events that include G-protein coupled activation of phospholipase C, generation of inositol triphosphate and diacylglycerol, elevation of cytosolic calcium concentrations, activation of kinase activities and phosphorylation of intracellular proteins [1–6]. Cloning and characterization of the human leukocyte LTβ4 receptor has been reported recently [68]. Interestingly, the human LTβ4 receptor shows little homology to prostanoid receptors and, like the previously described myeloid LXA4 receptor (vide infra), is a member of the chemokine receptor superfamily [68–70]. In addition to localization on the cell surface, some LTβ4 receptors are expressed at the nuclear envelope, raising the possibility that LTβ4 plays intracellular roles such as regulation of gene transcription [68]. Smooth muscle cells, endothelial cells and a variety of other cell types express high affinity binding sites for LTC4 and LTD4; however, receptors for these lipoxigenase products have yet to be characterized at the molecular level [6]. It is likely that multiple signalling pathways are evoked by sulfidopeptide leukotrienes given a recent report demonstrating two distinct pathways in human monocytic leukaemia THP-1 cells, namely a pertussis toxin-sensitive chemotactic response and a pertussis toxin-insensitive mitogen-activated protein kinase activation through protein kinase Cx [71].

Lipoxin bioactivities. Lipoxins attenuate many of the pro-inflammatory effects of leukotrienes and other mediators in vitro, and are putative endogenous ‘stop signals’ that inhibit neutrophil recruitment and promote resolution of inflammation [2–4]. LXA4 and LXβ4 attenuate LTβ4-induced neutrophil chemotaxis, neutrophil-endothelial cell adhesion, and migration of neutrophils across endothelial monolayers [2–4,19,72,73]. LXA4 also blunts basolateral-to-luminal migration of neutrophils across epithelial cell monolayers induced by the synthetic peptide chemotactic attractant N-formyl-L-methionine-L-leucine-L-phenylalanine (fMLP) [74], chemotaxis of eosinophils in response to fMLP and platelet-activating factor [75], and fMLP-triggered upregulation of neutrophil CD11/CD18 integrins [70]. With regard to peptide-leukotrienes, LXA4 and LXβ4 attenuate LTC4- and LTD4-triggered endothelial hyperadhesiveness for neutrophils, in part by inhibiting mobilization of P-selectin [19]. The latter lipoxin bioactivity may, in turn, be mediated through stimulation of endothelial cell prostacyclin production [19]. A recent report indicates that LXA4 and stable analogues of LXA4 attenuate interleukin-8 release by colonic cell lines activated by TNFα, raising the possibility that lipoxins also modulate cytokine responses in some cell types [76].

In keeping with in vitro assessments of neutrophil trafficking, LXA4 inhibits LTβ4-induced neutrophil adhesion, diapedesis, and vascular permeability changes in the hamster cheek pouch model of the microcirculation in vivo [73]. With regard to glomerular disease, treatment of rat neutrophils with LXA4 ex vivo blunts their subsequent trafficking to inflamed glomeruli in the concanavalin A-ferritin model of immune complex glomerulonephritis [50]. Depressed LXA4 biosynthesis is associated with exaggerated neutrophil infiltration in nephrotoxic serum nephritis in P-selectin knockout mice, and measures that restore lipoxin generation bring glomerular neutrophil counts in ‘knockout’ animals to levels comparable with wild-type animals [39]. With regard to vascular tone, LXA4 is a vasodilator, opposes the vasoconstrictor properties of LTD4, and attenuates LTD4-triggered decrements in renal blood flow, glomerular Kf and GFR [18]. These vasoactive properties of lipoxins appear to be mediated through production of vasodilatory prostaglandins and nitric oxide, and/or partial agonist activity at peptidoleukotriene receptors [18,19,77,78]. Finally, LXA4 blunts bronchial smooth muscle contraction in vitro and in vivo [79,80]. Together, these observations suggest potential immunomodulatory roles for lipoxins during host defense, inflammation and hypersensitivity disorders.

The molecular basis for these lipoxin-evoked responses is still being appreciated. Neutrophils, monocytes and gastrointestinal epithelial cells express high affinity binding sites for lipoxins [69,70,76]. Given the biological activities of lipoxins (see below), it is likely that endothelial cells and mesangial cells also express lipoxin receptors. Cloning of the cDNA for the myeloid and enterocyte LXA4 receptors indicated that they are the same seven transmembrane spanning G-protein-coupled receptor, and homologous to...
members of the chemokine superfamily [69,76,90]. Engagement of the myeloid LXA₄ receptors triggers a profile of cell signalling events distinct from that evoked by other eicosanoids [4,69–70,81]. This profile includes GTP hydrolysis, activation of phospholipases A₂, C and D, arachidonic acid release without oxygenation, and generation of phosphatidic acid [4,69–70,81].

The more downstream signal transduction events remain to be defined. Intriguingly, LXA₄ receptor expression is induced in enterocytes by interleukin-13, another mediator proposed to down-regulate inflammatory responses [76]. LXB₄ also exhibits high affinity binding to several cell types. Stereospecific, spatial and functional considerations suggest that the LXB₄ receptor is structurally different from the LXA₄ receptor; however, the LXB₄ receptor has yet to be cloned and characterized [4]. Neither LXA₄ nor LXB₄ compete with LTB₄ for binding at the myeloid LTB₄ receptor, suggesting that lipoxins modulate LTBB₄-triggered neutrophil trafficking at a post-receptor level [4,69,70]. LXA₄ may exert partial agonist activity at peptideluokotriene receptors on mesangial and smooth muscle cells [4,18]. It remains to be determined if the ability of lipoxins to block leukotriene-stimulated contraction of these cell types hinges on competition at a common receptor or post-receptor signalling events triggered by distinct lipoxin receptors.

**Leukotriene/lipoxin homeostasis: potential molecular switches that shift the balance towards an ‘anti-inflammatory’ phenotype**

If lipoxins serve as endogenous inhibitors of leukotrienes and promote resolution of inflammation, it is likely that 'molecular switches' are thrown that enhance lipoxin levels or bioactivity, relative to leukotrienes, as inflammatory responses evolve [2–4,21,22]. These switches would shift the balance from a leukotriene-dominated 'pro-inflammatory’ phenotype to a lipoxin-dominated 'anti-inflammatory’ phenotype. Several factors have been identified that may serve this purpose within an inflammatory microenvironment. As highlighted above, both leukotrienes and lipoxins are generated in abundance by transcellular routes during neutrophil-platelet interactions. Exposure of neutrophil-platelet co-incubations to granulocyte-macrophage colony-stimulating factor and platelet-derived growth factor shifts arachidonate metabolism towards a lipoxin phenotype [47,51]. Changes in cellular redox potential that lower glutathione levels (necessary for LTC₄ synthesis) have a similar effect suggesting a modulatory role for local hypoxia and oxidant injury [47–49]. Disruption of platelet cell membranes, as occurs with time following platelet adhesion, also boosts lipoxin biosynthesis [48]. As discussed previously, the Th2-derived cytokines interleukin-4 and -13 induce 15-lipoxygenase activity in a variety of cell types, including glomerular endothelial and epithelial cells [82], providing the enzymatic potential for enhanced lipoxin production through interaction of neutrophils and resident glomerular cells. Finally, cyto-kine-triggered expression of LXA₄ receptor expression could enhance lipoxin bioactivity even without a major shift in lipoxin generation [76].

**Potential therapeutic implications for renal disease**

Given that impressive quantities of leukotrienes and lipoxins are generated during inflammation and hypersensitivity reactions, the potential for therapeutic gain by pharmacological manipulation of the leukotriene/lipoxin network is manifest [1–6,22]. Whereas most investigators have focused their efforts on blockade of leukotriene biosynthesis and receptor engagement, recent advances in lipoxin biology raise the possibility that this putative endogenous anti-inflammatory eicosanoid system could be amplified or mimicked for therapeutic gain.

With regard to abrogation of leukotriene synthesis and bioactivity, prior exposure of rats to pharmacologic inhibitors of 5-lipoxygenase activity ameliorates the fall in GFR and reduces proteinuria during experimental immune complex glomerulonephritis in rats [83,84]. In addition, treatment of rats with the FLAP inhibitor MK886 during the heterologous phase of nephrotic serum nephritis in rats virtually abolishes proteinuria during the subsequent autologous phase [85], presumably by attenuating neutrophil-mediated injury to the glomerular filtration barrier triggered by LTB₄. Dietary deficiency of essential fatty acids (EFA) has salutary effects on the heterologous phase of nephrotic serum nephritis [86,87]. EFA deficient animals suffer less oliguria, azotemia, sodium retention, neutrophil infiltration of glomeruli, and proteinuria than control animals [86,87]. EFA deficiency is also associated with impaired intraglomerular LTB₄ biosynthesis, thereby providing a potential explanation for its renoprotective effects. LTD₄ receptor antagonists, in contrast to 5-lipoxygenase inhibitors, do not prevent leukocyte infiltration of the glomerular tuft, but markedly attenuate the fall in Kᵣ and GFR that typifies PHN and nephrotic serum nephritis [88].

More recently, synthetic analogs of LXA₄ and LXB₄ have been designed and synthesized that are resistant to enzymatic degradation in vitro [80]. These compounds have proved useful tools for probing lipoxin biology further and for exploring the therapeutic potential of lipoxin pathways [89–91]. Initial studies indicate that some stable analogues of LXA₄ (e.g. 16-phenoxy-LXA₄) and LXB₄ (e.g. 5(S)-methyl-LXB₄) retain the ability to inhibit neutrophil-endothelial cell and neutrophil–epithelial cell adhesion in vitro, and attenuate neutrophil recruitment and vascular permeability changes induced by topical application of LTBB₄ to mouse ear [89–91]. It will be intriguing to determine if these lipoxin mimetics also blunt neutrophil recruitment and cytotoxicity in more complex multi-mediator settings, such as glomerulonephritis. The future description of the pathways of lipoxin inactivation in vivo may also offer alternative strategies for boosting lipoxin levels and testing their anti-inflammatory potential.
Summary and conclusions

With the gradual elucidation of the cellular and molecular events that underpin the inflammatory process, the pathogenetic complexities of glomerulonephritis are slowly being unravelled. Lipooxygenase-derived eicosanoids play important counter-regulatory roles within inflamed glomeruli. Leukotrienes, derived from the 5-lipoxygenase pathway, are potent stimuli for leukocyte infiltration, intrarenal vasoconstriction, and mesangial cell contraction in many forms of experimental glomerulonephritis and probably in human disease. The recruitment of 12- and 15-lipoxygenase pathways, particularly during cell-cell interactions, promotes the formation of lipoxins. The latter compounds antagonize many leukotriene effects, attenuate neutrophil recruitment, and are potential ‘braking signals’ within the inflammatory cascade that promote resolution of inflammation. The generation and metabolism of leukotrienes and lipoxins is regulated independently, and each family of eicosanoids mediates its biological activities through distinct cell surface receptors and signal transduction pathways. Leukotriene biosynthesis inhibitors and leukotriene receptor antagonists are protective in several experiments of glomerulonephritis. Initial studies with lipoxins and synthetic lipoxin stable analogues suggest that it may be possible to harness this and other putative anti-inflammatory system for therapeutic gain [3, 22, 92].

References

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