



Review

Lipoxins and Aspirin-Triggered 15-epi-Lipoxins are Endogenous Components of Antiinflammation: Emergence of the Counterregulatory Side

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Abstract. Eicosanoids are known to play important roles in cell-cell communications and as intracellular signals that are critical components of multi-cellular responses such as acute inflammation and reperfusion injury. Recent findings have given rise to several new concepts that are reviewed here regarding the generation of eicosanoids and their impact in inflammation. Lipoxins (LX) are trihydroxytetraene-containing eicosanoids that can be generated within the vascular lumen during platelet-leukocyte interactions and at mucosal surfaces via leukocyte-epithelial cell interactions. During these cell-cell interactions, transcellular biosynthetic pathways are used as major LX biosynthetic routes, and thus, in humans, LX are formed *in vivo* during multi-cellular responses such as inflammation, atherosclerosis, and in asthma. This branch of the eicosanoid cascade generates specific tetraene-containing products that serve as stop signals, in that they regulate key steps in leukocyte trafficking and prevent leukocyte-mediated acute tissue injury. Of interest here are recent results indicating that aspirin's mechanism of action also involves the triggering of novel carbon 15 epimers of LX or 15-epi-LX that mimic the bioactions of native LX. Here, an overview of these recent developments is presented, with a focus on the cellular and molecular interactions of these novel antiinflammatory lipid mediators.

Key words: antiinflammation; lipids; leukocytes; signal transduction; resolution mediators.

Introduction

The inflammatory response involves the recognition of self and non-self by leukocytes. A diverse range of endogenous chemical mediators that orchestrate the host response²³ controls these events. These small chemical signals regulate leukocyte traffic and the

cardinal signs of inflammation. It is well established that the classic eicosanoids, such as prostaglandins and leukotrienes, play important roles and exert a wide range of actions in responses of interest in inflammation⁴⁵. In recent years, the scope and range of the chemical mediators identified have expanded considerably²³ to include (Fig. 1) novel lipid mediators, many

Abbreviations used: ALX – lipoxin A₄ receptor, ASA – aspirin, ATL – aspirin-triggered 15-epi-lipoxins, BLT – leukotriene B₄ receptor, COX – cyclooxygenase, LC/MS/MS – liquid chromatography-tandem mass spectrometry, LM – lipid mediators, LO – lipoxygenase, LT – leukotriene, LX – lipoxin, MPO – myeloperoxidase, PAF – platelet-activating factor, PMN – polymorphonuclear neutrophils.

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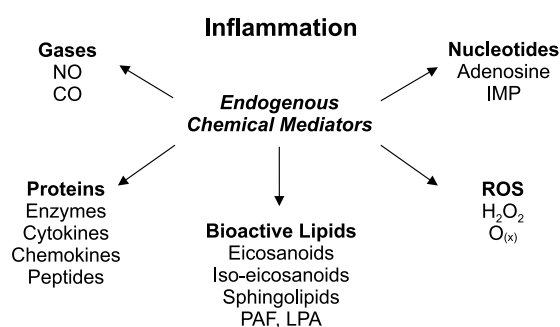


Fig. 1. Inflammation: endogenous chemical mediators. The classes of compounds now held to play important roles as mediators or signaling compounds in inflammation have increased in recent years to include gases and nucleotides as well as the expansion of the members of the well-established classes of lipid mediators, proteins (a wide range of chemokines and cytokines), and reactive oxygen species (ROS)

new cytokines and chemokines, gases (i.e. nitric oxide and carbon monoxide) and reactive oxygen species, as well as new roles for nucleotides as mediators, such as adenosine^{14, 15, 31} and the most recently uncovered of this class, namely inosine monophosphate (IMP), which also regulates neutrophil trafficking⁴³ (see <http://serhan.bwh.harvard.edu>). Many of these chemical signals are held to be proinflammatory when generated at elevated levels as in disease. Interactions between each of these classes *in vivo* remain for the most part largely unexplored and are likely to engage and produce many new levels of control as well as potential new signals. Along these lines, KRUMP et al.³¹ found that endogenous adenosine is a very potent inhibitor of leukotriene formation by polymorphonuclear neutrophils (PMN).

It is now clear from a body of results from the author's laboratory^{48, 50} and from other investigators that endogenous mediators are generated to dampen the host response and orchestrate resolution^{17, 18, 23}. In this regard, the lipoxins were the first to be identified and recognized as endogenous antiinflammatory lipid mediators of resolution in that they can function as "braking signals" or chalone in inflammation⁴⁸. Hence, it is of particular interest that aspirin (ASA), a widely used NSAID with many beneficial properties³⁸ in addition to its well-appreciated ability to inhibit prostaglandins⁶⁴, also triggers the endogenous generation of 15-epimeric LX via acetylation of cyclooxygenase (COX)-2 at sites of inflammation *in vivo*⁷ (*vide infra*) that carry antiinflammatory and antiproliferative actions^{8, 9}. This is a previously unappreciated and completely novel mechanism of drug action that has intriguing implications for targeted drug design. But more importantly, it helps to further illustrate the importance of endogenous generation of lipid mediators with antiinflammatory

properties. The traditional approach to developing anti-inflammatory drugs, as in other human conditions amenable to pharmacologic intervention, is the use of biosynthesis inhibitors and receptor antagonists of proinflammatory mediators, which indeed have enjoyed both considerable clinical and commercial successes^{23, 54}, but are not without significant unwanted side effects^{4, 37, 60}. Hence, the emergence of endogenous pathways and cellular mechanisms involved in counterregulation of responses that can lead to tissue injury and acute inflammation not only charts a relatively unappreciated side of human biology and acute inflammation^{49, 51}, but also provides an opportunity to explore new therapeutic approaches based on these new endogenous mechanisms, which may reduce the possibilities for unwanted toxic side effects.

Neutrophil-dependent vascular injury gives rise to increased vascular permeability, edema, and further release of chemoattractants. Leukotriene B₄ (LTB₄) is among the most potent PMN stimuli and, thus, participates in tissue injury via recruiting PMN in pathophysiologic scenarios⁶⁵. Lipoxins are trihydroxytetraene-containing eicosanoids that are, among other *in vivo* sites, rapidly produced within vascular lumen primarily via platelet-leukocyte^{20, 52} transcellular biosynthesis interactions that occur *in vivo* and which can now be visualized³³ by pathways (Fig. 2 and 3) that are activated during multicellular responses such as inflammation, atherosclerosis, and thrombosis (for a recent review, see SERHAN⁴⁹). In addition, ASA treatment triggers the transcellular biosynthesis of a new group of compounds termed 15-epi-LX or aspirin-triggered 15-epi-lipoxins (ATL), which are likely to contribute to some of the beneficial actions ascribed to ASA by serving as local endogenous antiinflammatory lipid mediators⁴⁹. Cell-cell interactions can evoke transcellular biosynthetic routes that can lead to amplification signals such as with leukotrienes² and prostaglandins³⁰, or to braking signals that can also involve novel compounds that have yet to be uncovered (Fig. 2). Thus, these LX branches, illustrated in Fig. 2 and 3, involve cell-cell interactions that appear to produce "endogenous stop signals" or "antiinflammatory compounds", while the pathways used to generate leukotrienes, for example, evoke cellular events that are considered to advance inflammation, namely proinflammatory mediators (Fig. 2).

Transcellular Generation of LX and 15-epi-LX

Platelet-leukocyte interactions and/or platelet-leukocyte microaggregates³³ promote the formation of LX by transcellular conversion of the leukocyte (Fig. 3,

Transcellular LM Biosynthesis

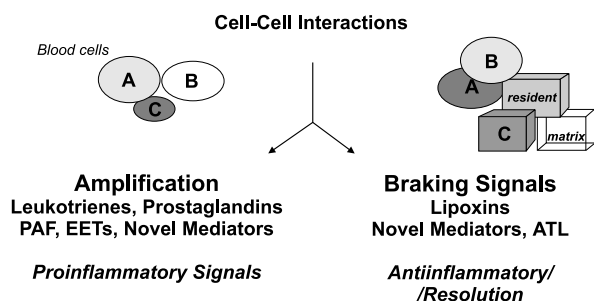


Fig. 2. Transcellular lipid mediators (LM) biosynthesis. During cell-cell interactions, LM can be amplified by transcellular biosynthesis to enhance the actions of signal molecules (left) or braking signals can be generated via the interactions of two or more cell types to limit further recruitment. Lipoxins and related compounds such as their endogenous ATL epimers appear to function *in vivo*, braking excess or overt congregation of cells

right side) 5-lipoxygenase (5-LO) epoxide product leukotriene A₄ (LTA₄). Once thought to be solely an intracellular intermediate in LT production, it is now clear that LTA₄ released by activated leukocytes is available

for enzymatic conversion by neighboring cell types^{22, 31}. When platelets are adherent, their 12-LO converts LTA₄ to LX A₄ and B₄ (Fig. 3). For a review and mechanistic details with recombinant 12-LO, see SERHAN⁴⁹. Hence, human platelets, which do not produce LX on their own (Fig. 2), become a major source of LX, given their abundance *in vivo* and their highly active 12-LO.

15-LO-initiated LX production is conveniently illustrated by airway epithelial cells, monocytes or eosinophils, which upregulate their 15-LO when exposed to cytokines such as IL-4 or IL-13^{34, 39}. When these cell types are activated, they generate and release 15S-HETE, which is rapidly taken up and converted by PMN to LX via the action of their 5-LO (Fig. 2, middle right pathway). This event not only leads to LX biosynthesis, but also “turns off” LT formation. 5-LO conversion of 15R-HETE also results in inhibition of LT biosynthesis⁸. 15R-HETE is a major product of arachidonic acid in several cell types when COX-2 is upregulated after acetylation by ASA. Thus, it is possible that ASA can regulate the *in vivo* production of LTs by 15R-HETE

Transcellular Lipoxin Biosynthesis

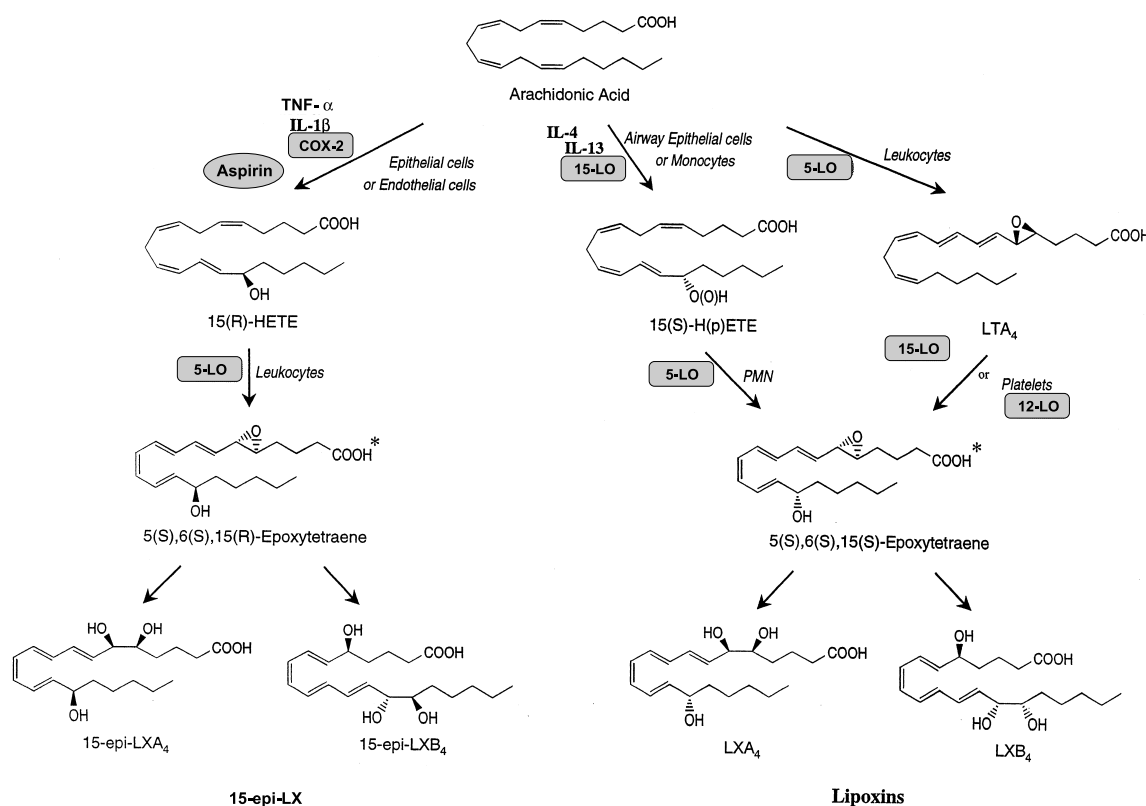


Fig. 3. Lipoxin and aspirin-triggered 15-epi-LX (ATL) biosynthesis. Illustration of the three main transcellular routes to generate lipoxins and ATL in mammalian tissues. Each of these is an independent route initiated by selective addition of molecular oxygen to arachidonic acid in the donor cell type of origin

conversion to 15-epi-LX, and 15-epi-LX can, in turn, also regulate the cellular actions of LTs.

We sought evidence for alternate explanations for ASA's therapeutic actions because many beneficial new actions have been documented in recent clinical studies. These new potential therapeutic indicators for ASA include decreasing incidence of lung, colon and breast cancer (reviewed by LEVY³⁵) and prevention of cardiovascular diseases⁴⁷. Inhibition of COX and biosynthesis of prostaglandins can account for many of ASA's therapeutic properties⁴⁴; however, its ability to regulate neutrophil-mediated inflammation or cell proliferation remains of interest. Along these lines, we uncovered a new action of ASA that involves COX-2-bearing cells, such as vascular endothelial cells or epithelial cells and their coactivation with PMN (Fig. 3). Hence, inflammatory stimuli (i.e. TNF- α , LPS, etc.) induce COX-2 to generate 15R-HETE when ASA is administered⁹. This intermediate carries a carbon-15 alcohol in the *R* configuration that is rapidly converted by activated neutrophils to 15-epimeric LX, or lipoxins that carry their 15 position alcohol in the *R* configuration⁴⁹, rather than 15S native LX, which results from LO : LO interaction (Fig. 3).

The native LXs regulate human PMN responses that are relevant to inflammation and reperfusion injury. These include: a) inhibition of FMLP and LTB₄-induced chemotaxis³², b) adhesion and transmigration with endothelial cells⁴¹, inhibition of cytokine formation such as IL-1 β ²⁹ and c) transmigration through epithelial cells¹³. These actions of LXA₄ and ATL, their endogenous epimeric counterpart (15-epi-LX), were first found in experiments with isolated cell types *in vitro*⁵¹ and also confirmed and demonstrated in several acute murine models of inflammation and second organ reperfusion injury (see TAKANO et al.^{61, 62}, HACHICHA et al.²⁹, CLISH et al.¹², BANDEIRA-MELO et al.¹, CHIANG et al.^{6, 7}). Some general points can now be made as follows: 1) PMN infiltration *in vivo* to lung, skin and sites of wound healing is dramatically and potently inhibited by both iv and topical application of stable analogues of both LXA₄ and ATL; 2) 15-epi-LXA₄ and LXA₄ analogs inhibit IL-1 β , TNF- α and IL-8 expression while stimulating IL-4 release *in vivo*^{25, 29}, interact in a stereoselective fashion with a common receptor on human and murine leukocytes; and 3) bioactive ATL and LXA₄ analogues compete with [³H]-LXA₄ binding to LXA₄ receptors (ALX)⁶². Thus, these inhibitory actions of LX analogues are likely to be mediated by these specific ALX present in rodent and human cells²⁸.

LXB₄ is a positional isomer of LXA₄, carrying alcohol groups at carbon 5S, 14R, and 15S positions, in-

stead of the C-5S, 6R, and 15S positions present in LXA₄. ASA-triggered LXB₄ carries a 15R alcohol, hence 15-epi-LXB₄ (see Fig. 3). Although LXA₄ and LXB₄ show similar activities in some biologic systems⁴¹, in many others they each show distinct actions (TAMAOKI et al.⁶³, reviewed in SERHAN⁴⁹). 15-epi-LXB₄, for example, is a more potent inhibitor of cell proliferation than LXA₄ or 15-epi-LXA₄⁴⁹. Next in this review we shall focus on findings indicating that 15-epi-LXA₄ is generated in inflammatory exudates in an ASA-dependent manner and that ASA-triggered LXA₄ and novel fluorinated LXA₄ as well as LXB₄ stable analogues are potent, topically active inhibitors of PMN-directed actions *in vivo*.

ASA-Dependent Generation of 15-epi-LXA₄ in Murine Exudates and Patients

To determine whether 15-epi-LXA₄ (Fig. 4) could be detected in animal experimental models or in patient-derived materials, experiments were first carried out with a mouse peritonitis model⁷. In this model, COX-2 protein levels were upregulated by intraperitoneal injection of LPS. Peritonitis was induced by intraperitoneal injection of casein. In these experiments, upregulation of COX-2 was also demonstrated by Western blot analysis, and the immunoreactive bands were observed at ~70 kDa in peritoneal lavage samples from LPS-treated mice. Four hours after leukocyte infiltration was initiated, approximately 25×10^6 cells were obtained from peritoneal lavage of each mouse. The exudate leukocyte populations were ~73% PMN and 10% monocytes (and/or macrophages) as determined by H/E staining and enumeration by light microscopy. To test whether ASA treatment of the mice results in the generation of 15-epi-LXA₄ during an inflammatory event, ASA was administered by intraperitoneal injection (see protocol timeline, Fig. 4, top). The collected peritoneal exudates from each mouse were incubated in the presence or absence of the agonist ionophore A23187 without addition of exogenous substrates, and samples from individual mice were analyzed separately using a newly developed specific ELISA method and LC MS MS system⁷.

Given one or two doses of ASA (Fig. 3), the mean values for 15-epi-LXA₄ production were ~1.5 and 1.8 ng per 5 ml peritoneal lavage per mouse. Without administration of ASA, approximately 0.5 ng of 15-epi-LXA₄ per 5 ml lavage was associated with peritoneal exudates from each mouse, suggesting that additional routes may be operative *in vivo* to produce 15-epi-LX

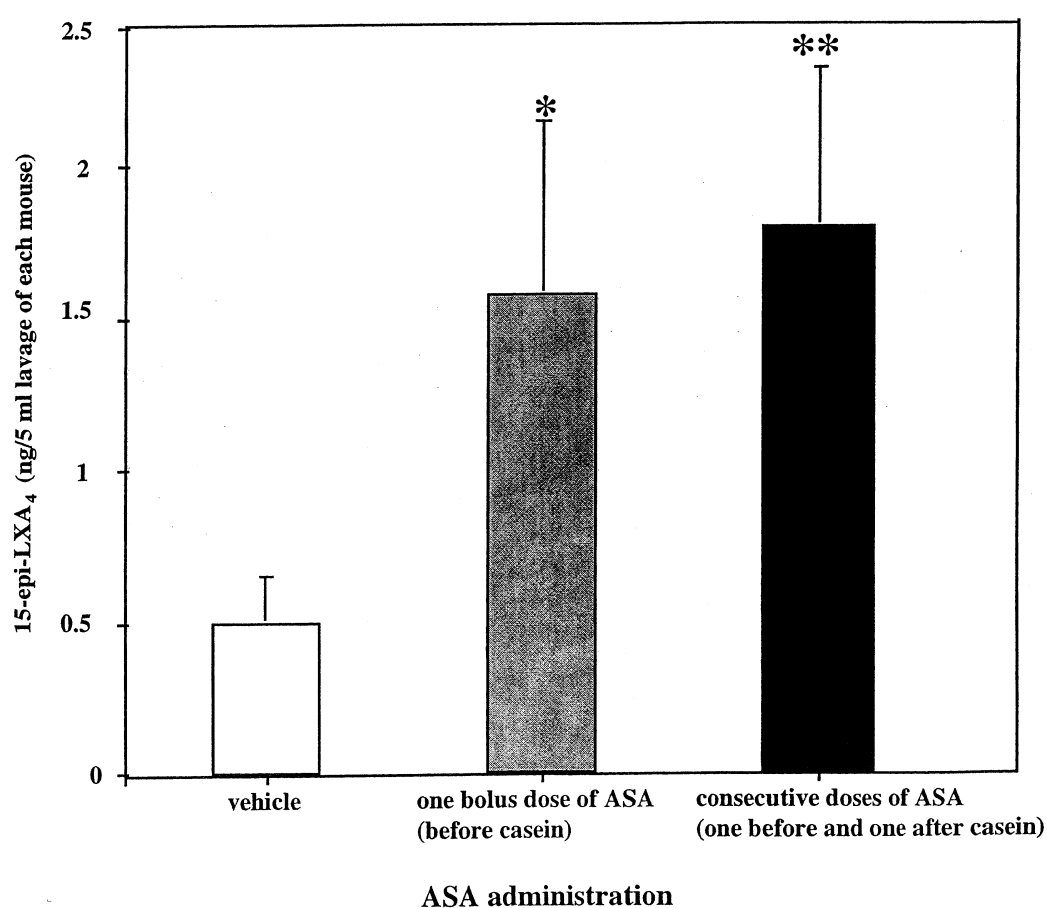
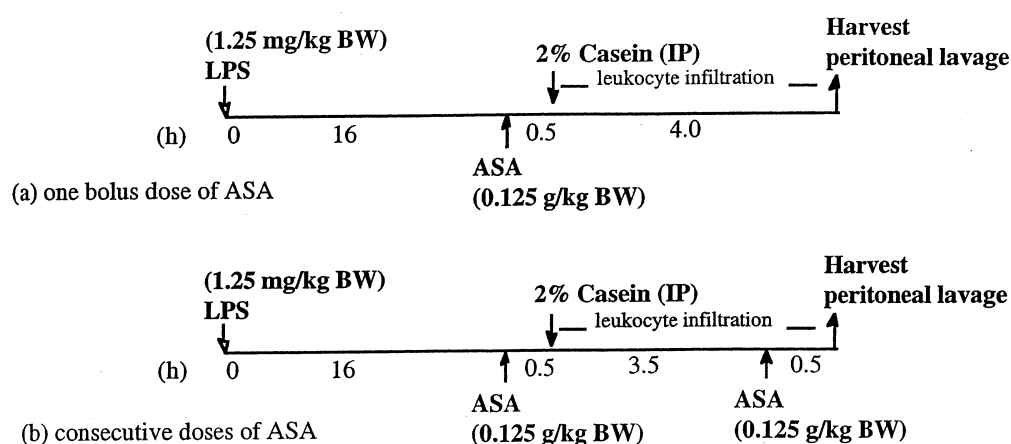
Experimental Timelines:

Fig. 4. Inflammatory exudates from ASA-treated mice generate 15-epi-LXA₄. Mice were injected with LPS (1.25 mg/kg BW) to induce COX-2 and 16 h later they were treated with vehicle or one bolus dose of ASA (0.125 g/kg BW) by IP 30 min prior to casein-initiated induction of neutrophil infiltration for 4 h (a). Consecutive doses of ASA were given in a separate group of animals that received ASA (0.125 g/kg BW) by IP 30 min prior to casein injection and before sacrificing the mice (b). The lavage exudates were collected and stimulated with 5 μ M of A23187 (30 min, 37°C). The values are expressed as the mean $\pm$ SEM from three independent experiments. The values from both treatment groups were statistically different (* p = 0.05 for group a, ** p = 0.03 for group b) from those obtained with animals that did not receive ASA (vehicle). The samples were extracted as reported in CHIANG et al.⁷ BW – body weight, IP – intraperitoneal injection

in an ASA-independent fashion. Naive animals (without any treatment) gave very low levels (< 0.2 ng/5 ml peritoneal lavage per mouse) of 15-epi-LXA₄. The physiological relevance of these values obtained in the absence of experimental challenge is currently not clear. Because LPS upregulates COX-2³⁵ and can induce neutrophil recruitment, the possibility was tested that animals treated with LPS could give rise to 15-epi-LXA₄ in the absence of casein. The results obtained showed a low level of 15-epi-LXA₄ generation in these LPS-treated animals in the presence or absence of ASA (0.25 ng and 0.30 ng per 5 ml peritoneal lavage per mouse, respectively), again suggesting that LPS alone is not sufficient to elicit neutrophil infiltration into peritoneal lavage. Casein-induced PMN infiltration and ASA are required in this scenario to generate statistically significant levels of 15-epi-LXA₄. These results demonstrated that ASA administration in murine peritonitis gives inflammatory exudates that generate 15-epi-LXA₄ in appreciable levels from endogenous substrate within these inflammatory cells, thus establishing a biosynthetic circuit for ATL/15-epi-LX generation *in vivo*.

Recently, these methods were used to evaluate ATL and LXA₄ formation in ASA-tolerant and ASA-intolerant asthmatics and their relation to leukotriene C₄ (LTC₄). Interestingly, the ASA-tolerant subjects generated both LX and ATL, but the ASA-intolerant patients proved to have a diminished capacity to generate ATL and LX upon ASA challenge⁴⁶. The lower levels of these potentially protective mediators could contribute to the pathobiology of this chronic disorder in that the disease state is not only characterized by the overproduction of proinflammatory mediators, but also by the loss or reduction in LX and ATL that may keep inflammation in check. Also, a reduction and alteration in LX generation was found in patients with chronic liver disease¹⁰ and chronic myelogenous leukemia^{56–59}. These diseases contrast with recent findings in localized juvenile periodontitis, namely that LXA production is upregulated⁴², following atherosclerotic plaque rupture³, and with nasal polyps¹⁹. Together, this result indicates that alterations in LX levels may be linked to the pathophysiology of several human diseases.

PMN Traffic in 5-Lipoxygenase Knock-Out and BLTR Transgenic Mice

To assess the role of 5-lipoxygenase (5-LO) products in the recruitment of PMNs to murine skin and in reperfusion injury, we evaluated the chemotactic capac-

ity for LTB₄ in 5-LO knock-out mice. Topical application at either 1 µg or 5 µg of LTB₄ per ear induced significant PMN infiltration into the mouse ear that reached a maximum at 24 h⁶¹. Both 5-LO (–/–) and (+/+) mice showed essentially equivalent levels of PMN infiltration into the ears, indicating that LTB₄ receptor signaling was intact in 5-LO (–/–) mice and suggesting that inhibitors of 5-LO might be of limited utility in this tissue, since after a 24 h exposure to topical LTB₄ approximately 5×10^5 PMN infiltrated per 6 mm punch biopsy, a value that was equivalent to the levels induced by 5 times more LTB₄ within 8 h. We selected topically-applied LTB₄ at 1 µg/ear for further experiments. We also prepared transgenic mice overexpressing the receptor for LTB₄ (denoted BLT) on leukocytes using a CD11B promoter construct to govern expression on leukocytes⁶. These mice not only gave heightened responses with LTB₄ *de novo* as well as with exogenous application, but showed that LXA₄ and ATL analogs given iv in low levels (µg/mouse) can limit PMN infiltration and protect from PMN-mediated lung injury (*vide infra*).

LXA₄ Stable Analogues Inhibit Both PMN Infiltration and PMN-Mediated Tissue Damage

Because LT biosynthetic enzyme inhibitors and LT receptor antagonists may be of limited use in certain clinical settings, we evaluated other approaches and prepared for *in vivo* studies LX stable analogues that were designed as potential mimetics of the inhibitory actions noted for LXA₄⁵¹ and, more recently, for LXB₄³⁶ *in vitro*. Several LXA₄ and ATL stable analogues were made using a recombinant dehydrogenase⁵¹ as a relatively inexpensive and rapid screen to design suitable analogues that might function *in vivo*. Several of these were scaled up via total organic synthesis to examine in more detail and were tested for their ability to inhibit PMN infiltration and changes in vascular permeability. 15(R/S)-methyl-LXA₄, having a methyl group at C-15 position (racemate 15R/S), is an analogue of both the ASA-triggered 15-epi-LXA₄ and native LXA₄; and 16-phenoxy-LXA₄, which has a phenoxy group at C-16 position, is an analogue of native LXA₄ that prevents enzymatic inactivation with recombinant 15-prostaglandin dehydrogenase *in vitro* (SERHAN et al. ⁵¹; some structures are given in Fig. 5). The analogues that proved active also act via competition at LXA₄ receptors⁶² and receptor chimeras⁵.

Applied topically to mouse ears, these LX stable analogues inhibit both PMN infiltration and vascular

-fluoro-phenoxy-LXA₄ ~5(S)-methyl-LXB₄ > 16-phenoxy-LXA₄ > 5(R)-methyl-LXA₄ for both PMN infiltration and vascular permeability changes. These also proved to be potent inhibitors of PMN infiltration in the dorsal air pouch with potency >100 times that of ASA¹² and redirecting cytokine formation and action²⁹.

Clearance of Topical Lipoxin Analogues

LX are rapidly inactivated by conversion to oxo- and dihydro-containing products (reviewed in SERHAN⁴⁹; CLISH et al.^{11, 12}). Each biologically active LX analogue tested also resisted rapid conversion by recombinant dehydrogenase *in vitro*^{36, 51}. To determine whether these analogues were cleared and/or present in an extractable form within ear skin tissues after topical application, biopsied ear tissues were taken for LC/MS/MS analysis at the time of application and after a time interval (i.e. 24 h), when high level inhibition was found for several LX analogues. The eicosanoids recovered after extraction of ear biopsies (i.e. immediately after application and at 24 h) were identified and quantitated using LC/MS/MS. LTB₄ was also monitored for purposes of direct comparison, and selected ion chromatograms of PGB₂ (internal standard) and LTB₄ were obtained after extraction from biopsies. Two of the more active LXA₄ analogues and an LXB₄ analogue were examined. Following initial applications to the ears, only ~10–20% of the applied compounds were present within the target area of the biopsies. At 24 h after application, this value was reduced to < 10%, indicating that > 95% of the added LX analogues were not recoverable in extractable forms and thus were likely cleared by the ear tissue.

Do LX Analogues Block other Inflammatory Stimuli?

A panel of known inflammatory mediators was examined to test the specificity and/or generality of the actions of LX analogues. PGE₂ dramatically augmented the LTB₄-induced PMN infiltration and vascular permeability change, although the effects of this prostaglandin by itself were minimal. PMA, a tumor promoter and topical irritant that bypasses cell surface receptors, caused concentration-dependent changes in both PMN infiltration and vascular permeability, and 100 ng PMA per ear was chosen for further evaluation. Several potent agents such as FMLP, C5a, IL-8, platelet-activating factor (PAF), or LTD₄ did not give sig-

nificant changes in these parameters at amounts applied as high as 1 to 25 µg compared with LTB₄, suggesting that they are not topically active, perhaps because they do not gain access in intact skin tissues to locations that would establish a chemotactic gradient⁶¹. *In vitro*, LX and ATL analogs block PAF, FMLP and IL-8 actions on human PMN^{24, 25} and in whole blood²¹.

We evaluated the impact of 15-epi-LXA₄ using a stable analogue, 15-epi-16-para-fluoro-phenoxy-LXA₄, synthesized to enhance iv stability by slowing metabolic inactivation¹². This LX analogue not only inhibited LTB₄, but also inhibited PGE₂-enhanced inflammation when used topically. Of interest is that it also inhibited PMA-induced PMN influx with little impact at 24 h on PMA-induced vascular leakage. Thus, LXA₄ stable analogues clearly inhibited native mediators as topically active agents and partially blocked PMA-evoked actions that were restricted to inhibition of PMN influx⁶¹. The observations with PMA indicate that a major component of the observed vascular leakage with PMA is not mediated by PMN-dependent mechanisms.

Discussion

To summarize, to date we developed a new ELISA and LC/MS/MS system for 15-epi-LXA₄ that proved highly sensitive as well as stereoselective, compared with its epimer LXA₄, at the level required to selectively interact with 15-epi-LXA₄⁷. Utilizing this ELISA, we established that 15-epi-LXA₄ generation proceeds via transcellular biosynthesis during heterotypic leukocyte-leukocyte interactions and the first evidence for ASA-dependent 15-epi-LXA₄ generation by inflammatory exudate cells from a murine model with LPS and casein-induced peritonitis (Fig. 3 and 4). The stable LX and ATL analogues reviewed here and found to be potent inhibitors of both PMN infiltration and PMN-mediated vascular permeability changes were designed (Fig. 5) to resist rapid inactivation and proved to resist conversion by cells and isolated recombinant enzymes *in vitro*^{36, 51}. It was of interest to determine whether the LX analogues remained within the ear tissues following topical application or if they were effectively cleared from ears and blood. Our results indicated that at 24 h after topical application more than ~95% of the LX analogues were not present in extractable or recoverable forms from the biopsies, suggesting that they were either cleared from the biopsied areas or were present within these tissues in forms that were not extractable using the current methodology⁶¹. We also did not find

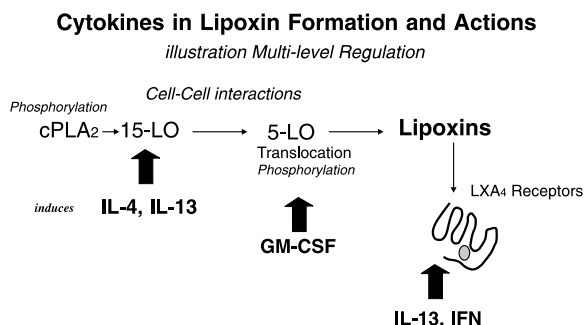


Fig. 6. Cytokines induce lipoxin formation and the ALX receptors. LX biosynthesis requires multi-level regulation. The cytosolic phospholipase A₂ (cPLA₂) required to release esterified arachidonic acid from phospholipid stores is phosphorylated upon cell activation. The 15-LO is induced in cells by IL-4 and IL-13 as is the LXA₄ receptor, which is also induced by interferon (IFN). Although the induction requires transcription and translation to bring the key biosynthetic enzymes into place in an uninvolved tissue, this can also be assembled via cell trafficking into regions where key enzymes are upregulated. The LX biosynthesis is rapid, within seconds to minutes, when the cells are exposed to a second challenge to release substrate into the LX circuit

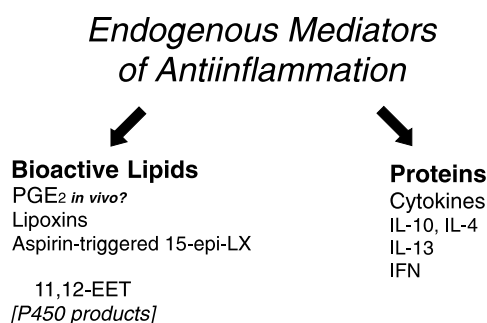


Fig. 7. Endogenous mediators of antiinflammation. Compounds that display downregulatory or inhibitor roles in events relevant to inflammation

evidence, using LC/MS/MS workstation-based analyses, for anticipated local metabolites of these LX analogues persisting within the biopsies. It is noteworthy that, in addition to local clearance, an alternate explanation may lie with the possibility that LX analogues could have been subject to local metabolism and/or covalent modification that results in their binding to tissue matrix components. Whether such matrix forms of LX analogues exist and whether they are in an inactive or bioactive configuration is of interest. Nevertheless, it is clear from the results to date that LX and ATL analogues are not retained in their native form within the local microenvironment (i.e. ear biopsies), and this may be another useful property of these LX analogues. They are stable in whole blood and are also iv active in murine systems^{1, 6, 12}, suggesting that they may provide a new approach to treatment and regula-

tion of leukocyte responses in a range of diseases including allergic disorders.

The activation of an LX biosynthetic circuit *in vivo* requires upregulation of key enzymes by cytokines such as IL-4 and IL-13 (Fig. 6) that also control the expression of the receptor ALX²⁷. Hence, both the temporal and spacial components in LX formation and actions are important determinants in their bioimpact. LX and ATL appear to be the first recognized members of a new class of mediator, namely, endogenous mediators of antiinflammation (Fig. 7). PGE₂ may display antiinflammation in certain settings¹⁶, but in most it enhances inflammation *in vivo*⁶¹. This is likely the result of numerous receptor isoforms and differential coupled mechanisms for PGE₂ and its diverse role in human physiology. Also, 11, 12-epoxy-eicosatetraenic acid (EET) was recently shown to display antiinflammatory actions, downregulating endothelial cell response via an NFκB mechanism that required a greater than micromolar range to evoke actions⁴⁰, which may prove to be of physiological and therapeutic interest in the near future.

The results with ATL and LX analogues reviewed here show highly potent stereoselective actions in the sub- to nano-molar range sustaining LX and ATL actions in several *in vivo* models, indicating that these pathways (Fig. 3 and 6) are likely to be important *in vivo* in human host defense. They join perhaps many mediators that govern this process *in vivo* such as select cytokines (IL-10, IL-4, IL-13), proteins of interest in resolution¹⁷. In this regard, LX and ATL receptor activation not only inhibits proinflammatory events such as IL-6 gene expression⁵⁵, but stimulates IL-4 generation *in vivo*²⁹ and stimulates the phagocytosis of apoptotic PMN by macrophages²⁶. The integrated response of the host is essential to health and disease; thus, it is important to achieve a more complete understanding of the molecular and cellular events governing the formation and actions of endogenous mediators of resolution that appear to control the duration of inflammation. In view of the present findings, it is not surprising that others have recently found a protective action for COX-2 in cardiovascular disease⁵³. Establishing useful experimental systems will also take a multidisciplinary approach and require a shift in our current thinking about inflammation and the role of lipid mediators.

In this context, the words of Francis Bacon (1561–1620) are of interest: “*Contemplation’s of nature and of bodies in their simple form break up and distract the understanding, while contemplation’s of nature and bodies in their composition and configuration overpower and dissolve the understanding... for that school*

is so busy with particles that it hardly attends to the structure, while the others are so lost in admiration of the structure that they do not penetrate to the simplicity of nature”.

Taken together, recent findings from the author's laboratory and those of others reviewed here indicate that ASA initiates the formation of 15-epi-LXA₄ in murine inflammatory exudates and *in vivo* in humans with asthma as well as native LX *in vivo*. Stable analogues of both the ATL class ASA-triggered 15-epi-LXA₄ and native LX (LXA₄ and LXB₄) designed to enhance their bio-half-life and availability proved to be very potent, *iv* and topically active agents that inhibit both PMN recruitment to murine skin and PMN-mediated changes in vascular permeability as well as second organ reperfusion injury. The findings to date provide new tools for investigating the actions of LX and also evidence indicating that the cell-cell interactions that generate LX and 15-epi-LX do so to limit further PMN recruitment *in vivo*, hence, uncovering new mechanisms of endogenous anti-inflammation and resolution relevant in human disease.

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