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Mechanisms in anti-inflammation and resolution: the role of lipoxins and aspirin-triggered lipoxins

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Abstract

Multicellular host responses to infection, injury or inflammatory stimuli lead to the formation of a broad range of chemical mediators by the host. 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. Lipoxins are trihydroxytetraene-containing lipid mediators that can be formed during cell-cell interactions and are predominantly counterregulators of some well-known mediators of inflammation. Since this circuit of lipoxin formation and action appears to be of physiological relevance for the resolution of inflammation, therapeutic modalities targeted at this system are likely to have fewer unwanted side effects than other candidates and current anti-inflammatory therapies. Here, we present an overview of the recent knowledge about the biosynthesis and bioactions of these anti-inflammatory lipid mediators.

Key words
• Inflammation
• Lipoxins
• Resolution

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Introduction

The vascular and cellular responses of both acute and chronic inflammation are mediated by endogenous chemical factors derived from plasma or cells and triggered by the inflammatory stimulus (1). These different factors play key roles, not only initiating but also regulating the host responses, like the recently discovered inosine monophosphate-AMP deaminase system (2). Such mediators, acting alone, in combination, or in tandem, then amplify the inflammatory response and influence its evolution and the outcome of the process (Figure 1).

Biosynthesis of mediators by transcellular and cell-cell interactions is recognized as an important means of amplifying and generating lipid-derived mediators, particularly those produced by lipoxygenases (LO). Arachidonic acid and its oxygenation products may transfer from one cell to another during cell-cell interaction undergoing further transformation to biologically active "pro-" and "anti-inflammatory" compounds. Results from numerous studies have shown that lipoxins are formed *in vitro* from endogenous sources of arachidonate in isolated cells and also *in vivo* and across many species, from fish to humans. This review addresses the major routes and biological actions of lipoxins and whether their formation and actions can be of therapeutic value in regulating inflammation and other pathophysiologic events of

interest in human disease.

Biosynthesis

Multicellular host responses to infection, injury or inflammation stimuli lead to the formation of lipoxins ("lipoxygenase interaction products"), trihydroxytetraene-containing bioactive lipid mediators that carry potent anti-inflammatory signals. First reported in 1984 in mixed suspensions of human leukocytes incubated with exogenous substrates (3), lipoxins are now known to be generated in humans during cell-cell interactions by one of at least three biosynthetic routes working independently or in concert, in particular biological settings or tissues.

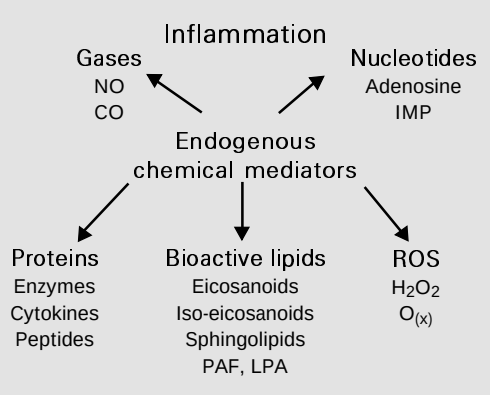

Figure 1. Inflammation: endogenous chemical mediators. Inflammatory responses are mediated by a range of endogenous mediators/signals as the well-established classes of lipid mediators, proteins and reactive oxygen species (ROS) and more recently also including gases and nucleotides. NO, nitric oxide; CO, carbon monoxide; IMP, inosine monophosphate; PAF, platelet-activating factor; LPA, lysophosphatidic acid.

During lipoxin formation, molecular oxygen is inserted at two sites in arachidonic acid (C20:4) by distinct LO that are often segregated into different cell types. The first report on lipoxin biosynthesis rationalized lipoxin generation by routes involving insertion of molecular oxygen into carbon 15 of C20:4, predominantly in the *S* configuration, which implied the involvement of a 15-LO (3). Eicosanoid products of 15-LO in airway epithelial cells or monocytes, 15*S*-hydroperoxyeicosatetraenoic acid (15*S*-H(p)ETE), or the reduced alcohol form 15*S*-hydroxyeicosatetraenoic acid (15*S*-HETE) can serve as substrates for neutrophil (PMN) 5-LO and lead to the formation of the trihydroxy-

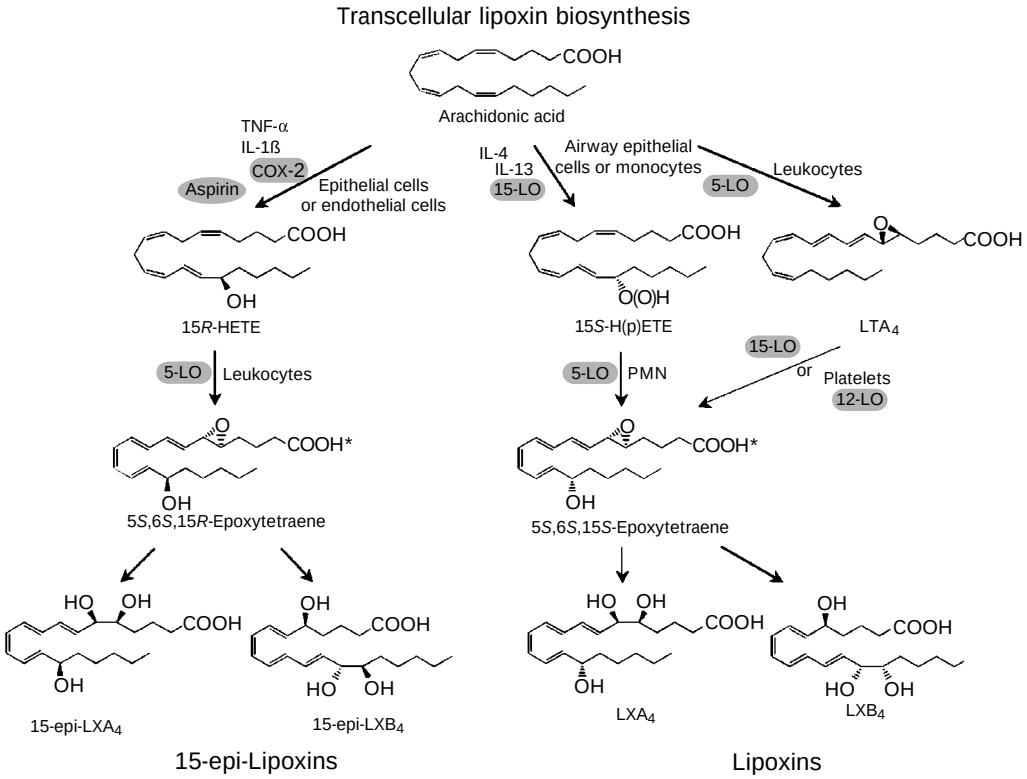

Figure 2. Lipoxin (LX) and aspirin-triggered 15-epi-lipoxin biosynthesis. Illustration of the three main transcellular routes to generate lipoxins and 15-epi-lipoxins in mammalian tissues. Each of these is an independent route initiated by site selective addition of molecular oxygen to arachidonic acid in the donor cell type of origin. LO, lipoxygenase; LT, leukotriene; PMN, neutrophils; 15*S*-H(p)ETE, 15*S*-hydroxyperoxyeicosatetraenoic acid; 15*R*-HETE, 15*R*-hydroxyeicosatetraenoic acid; COX-2, cyclooxygenase; IL-1ß, interleukin 1ß; TNF-α, tumor necrosis factor α.

tetraenes, lipoxin A_4 (LXA_4) and lipoxin B_4 (LXB_4) (Figure 2). These lipoxins retain their precursors' alcohol configuration to carry their carbon 15 alcohol in the *S* configuration.

The second pathway for lipoxin biosynthesis was determined for interactions that occur predominantly within the vasculature between 5-LO, present in myeloid cells, and 12-LO, present in platelets (3). The 5-LO product leukotriene A_4 (LTA_4) is rapidly taken up by the platelets and converted via a 12-LO-dependent mechanism to lipoxins (Figure 2). Since more than 50% is released from the cell of origin (3), LTA_4 serves as an intermediate for both intracellular and transcellular eicosanoid biosynthesis. During neutrophil-platelet interaction and co-activation, LTA_4 has multiple potential enzymatic and non-enzymatic fates, including a) conversion by 12-LO to LXA_4 and B_4, b) non-enzymatic hydrolysis (which occurs in seconds in aqueous environments), c) conversion by LTA_4 hydrolase to LTB_4 (a potent neutrophil and eosinophil chemoattractant) or d) converted by LTC_4 synthase to LTC_4 (slow reacting substance of anaphylaxis). Because LTB_4 and C_4 carry potent proinflammatory actions and lipoxins inhibit leukotriene-mediated responses *in vivo*, the balance of leukotriene to lipoxin formation is critical to cellular responses.

Aspirin-triggered 15-epi-lipoxin pathway

Recently, a third major pathway for lipoxin generation was discovered that involves aspirin and the actions of cyclooxygenase (COX) 2 and 5-LO (4). Endothelial and epithelial cells express COX-2 in response to diverse stimuli such as cytokines, hypoxia and bacterial infections. Aspirin acetylates COX-2 and switches its catalytic activity for conversion of C20:4 to 15*R*-HETE in lieu of prostanoid biosynthesis. 15*R*-HETE is released from endothelial and epithelial cells and transformed by leukocyte 5-LO, via transcellular routes, to 15-epimer lipoxins (or aspirin-triggered lipoxins, ATL) (Figure 3).

The route of lipoxin formation depends on the cells and enzymes present therein and can be subject to modulation by cytokines (reviewed in 5). For example, interleukin 4 (IL-4) and IL-13, which are thought to be negative regulators of the inflammatory response, both increase 15-LO expression and activity, thereby enhancing lipoxin formation. Proinflammatory cytokines up-regulate 5-LO (e.g., granulocyte/macrophage colony stimulating factor (GM-CSF) and IL-3) and COX-2 (e.g., IL-1ß and tumor necrosis factor (TNF)-α) activities (reviewed in 5) which

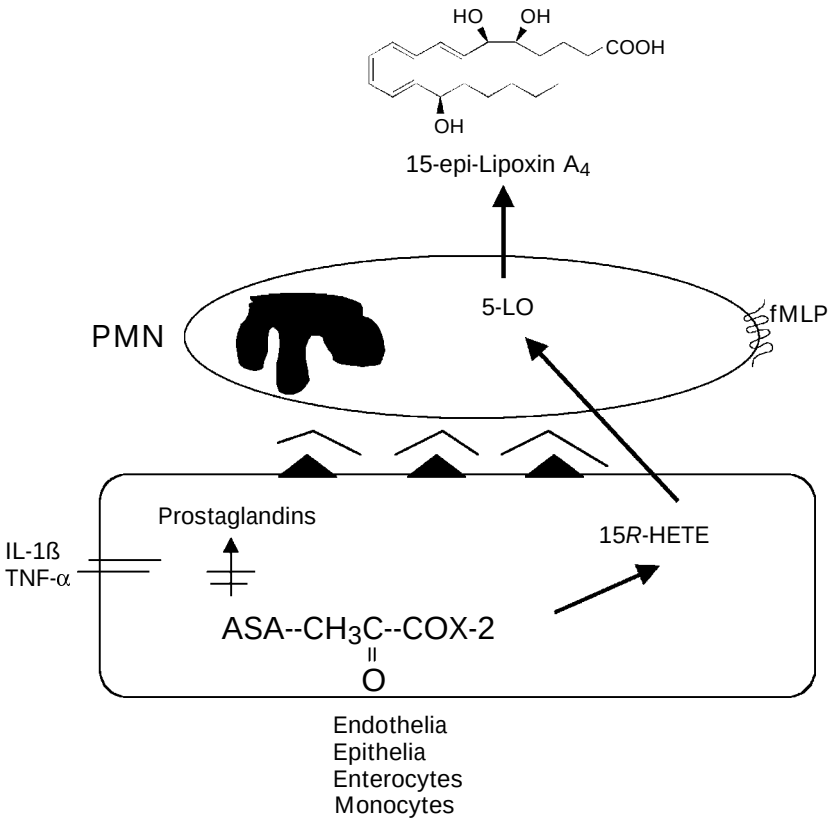

Figure 3. Aspirin (ASA)-triggered 15-epi-lipoxin pathway. Cytokine exposure induces COX-2 expression in vascular endothelial cells. This isozyme is acetylated by ASA, and cell activation via membrane receptors generates 15*R*-hydroxyeicosatetraenoic acid (15*R*-HETE) that is transformed by the 5-lipoxygenase (5-LO) of adhering neutrophils (PMN) to 15-epi-lipoxins. fMLP, formyl-methionyl-leucyl-phenylalanine; IL-1ß, interleukin 1ß; TNF-α, tumor necrosis factor α.

are crucial to the formation of both lipoxins and ATL. Furthermore, addition of exogenous C20:4 to GM-CSF-primed neutrophils co-incubated with platelets enhances receptor-triggered formation of LXA_4 with either formyl-methionyl-leucyl-phenylalanine (fMLP) or platelet-derived growth factor (6), establishing that lipoxins are indeed generated from endogenous sources of arachidonic acid following receptor-ligand interactions.

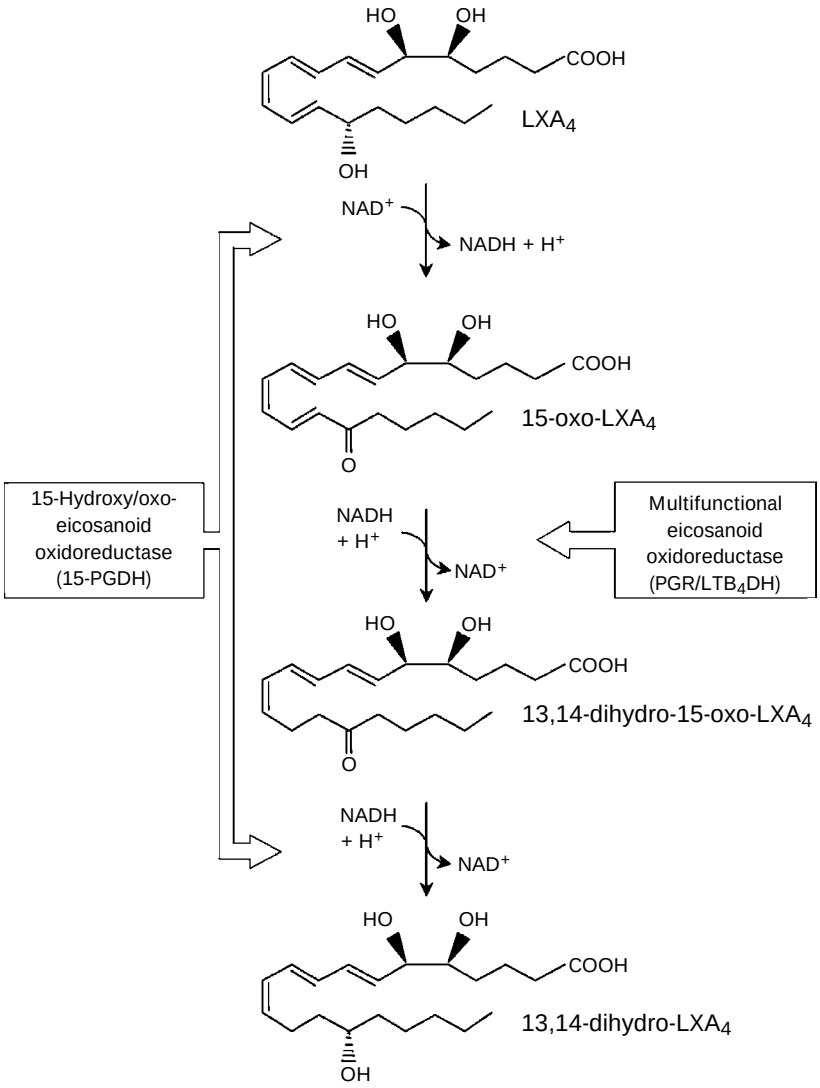

Figure 4. Lipoxin (LX) metabolic inactivation. The initial step in LXA_4 inactivation is dehydrogenation of the 15-hydroxyl group, catalyzed by 15-PGDH to yield 15-oxo-LXA_4. PGR/LTB_4DH catalyzes the reduction of the 13,14 double bond of 15-oxo-LXA_4 to give 13,14-dihydro-15-oxo-LXA_4. This product serves as a substrate for 15-PGDH, which catalyzes the reduction of the C15-oxo group to give 13,14-dihydro-LXA_4.

Although first described in human leukocytes (3), lipoxins are generated by bovine, porcine and rat cells, including basophils and macrophages (reviewed in 7). It appears that both the basic structure of lipoxins and the means of generating these compounds are conserved in the course of evolution. In this regard, it was shown that leukocytes or isolated phagocytic cells from several species of fish (trout, salmon, catfish) could generate substantial amounts of lipoxin from endogenous sources of substrate (8).

By taking advantage of LXA_4's unique overall three-dimensional conformation, an ELISA was developed for rapid detection of LXA_4 in biologically derived samples. This assay has proven to be sensitive and selective, showing no cross-reactivity for 5*S*-HETE, 12*S*-HETE, 15*S*-HETE, LTB_4, LTC_4, LTD_4 or arachidonic acid, and has an LXA_4 detection limit of 90 fmol/ml. Furthermore, a selective ELISA for the aspirin-triggered 15-epi-LXA_4 was also recently developed which shows little cross-reactivity with native LXA_4 or other eicosanoids (9). Thus, the availability of appropriate quantitative methods for lipoxins, including LC/MS/MS (9), as is the case for other eicosanoids, is a critical component in assessing the association between lipoxin formation and their potential involvement in both physiological and pathophysiological events.

Mechanisms of inactivation

Lipoxins, as other autacoids, are rapidly biosynthesized in response to stimuli, act locally and then are rapidly enzymatically inactivated. The major route of lipoxin inactivation is through dehydrogenation by monocytes that convert LXA_4 to 15-oxo-LXA_4, followed by specific reduction of the double bond adjacent to the ketone (10) (Figure 4). 15-Hydroxy/oxo-eicosanoid oxidoreductase (15-PGDH) catalyzes the oxidation of LXA_4 to 15-oxo-LXA_4. This compound is biologically inactive and is further converted to

13,14-dihydro-15-oxo-LXA_4 by the action of LXA_4/PGE 13,14-reductase/LTB_4 12-hydroxydehydrogenase (PGR/LTB_4DH). Moreover, reduction of the 15-oxo group by 15-PGDH yields 13,14-dihydro-LXA_4, revealing an additional catalytic activity for this enzyme (11). LXB_4 can also be dehydrogenated by 15-PGDH at carbon 5 to produce 5-oxo-LXB_4, therefore sharing a common route of inactivation (12). It has recently been shown that 15-oxo-LXA_4 is also produced from LXA_4 in mouse whole blood (13), suggesting that the mouse shares with humans a common pathway for LXA_4 inactivation.

Lipoxin and ATL stable analogs

In view of the rapid transformation and inactivation of lipoxin by monocytes and, potentially, other cells *in vivo*, it was highly desirable to design lipoxin analogs that would resist this metabolism and maintain their structural integrity and potential beneficial biological actions. Lipoxin analogs were constructed with specific modifications of the native structures of LXA_4 and LXB_4, such as the addition of methyl groups to carbon 15 and carbon 5 of the LXA_4 and LXB_4 structures, respectively, to block dehydrogenation by 15-PGDH. 15*R*/*S*-methyl-LXA_4 is a racemic stable analog of both LXA_4 and 15-epi-LXA_4. Additional analogs of LXA_4 were synthesized with a phenoxy group attached to carbon 16 and replacing the ω-end of the molecule. This design permits 16-phenoxy-LXA_4 to resist potential ω-oxidation and to be protected from dehydrogenation *in vivo*. Fluoride was added to the para-position of the phenoxy ring to yield 16-(para-fluoro)-phenoxy-LXA_4 in order to hinder degradation of the phenoxy ring. The aspirin-triggered counterpart of 16-(para-fluoro)-phenoxy-LXA_4, 15-epi-16-(para-fluoro)-phenoxy-LXA_4, was also synthesized. These modifications not only prolong the half-life of the compounds in blood but also enhance their bioavailability and bioactivity (13).

The ATL are less effectively converted *in vitro* to their 15-oxo metabolite than LXA_4 (10). This indicates that the dehydrogenation step is highly stereospecific and suggests that, when ATL are generated *in vivo*, their biological half-life is increased by about two-fold compared to that of native LXA_4, thereby enhancing their ability to evoke bioactions. Hence, biologically stable analogs of lipoxin and ATL can be engineered to enhance their bioactions, a fact suggesting that they are useful tools for the development of novel therapeutic modalities.

Bioactions

Vasoactive actions

Lipoxins display vasodilatory and counterregulatory roles in *in vivo* and *in vitro* models (7,14). LXA_4 and LXB_4 promote vasorelaxation and relax the aorta and pulmonary arteries (Table 1). LXA_4 reverses the precontraction of the pulmonary artery induced by prostaglandin $F_{2\alpha}$ and endothelin-1. The mechanisms of LXA_4- and LXB_4-induced vasodilatation involve endothelium-dependent vasorelaxation and involve prostaglandin-dependent and -independent pathways (reviewed in 14). In certain tissues, lipoxin can stimulate the formation of, for example, prostacyclin by endothelial cells (29), which can contribute to vasodilatation. These prostanoid-dependent actions of lipoxin are inhibited by COX inhibitors (14) and indicate that lipoxins can stimulate the biosynthesis of a second set of mediators. These also include lipoxin-stimulated generation of nitric oxide (33), which may mediate a component of lipoxin-regulated vascular tone.

Immunomodulatory actions

The actions of lipoxins contrast with those of most other lipid mediators that are prima-

rily proinflammatory, such as leukotrienes, platelet-activating factor (PAF) and prostanoids. It is now appreciated that lipoxins, LXA_4 in particular, are potent counterregulatory signals *in vitro* and *in vivo* for endogenous proinflammatory mediators, including lipids (leukotrienes, PAF) and cytokines (TNF-α, IL-6), resulting in inhibition of leukocyte-dependent inflammation (reviewed in 34). Lipoxins display selective actions on leukocytes (Table 1) that include a) inhibition of neutrophil chemotaxis (15), b) transmigration through epithelial cells (16), and c) adhesion and transmigration with endothelial cells (17). LXA_4 and ATL inhibit PMN-initiated second-organ injury in an ischemia-reperfusion model using LTB_4 receptor transgenic mice (35), suggesting an endogenous compensatory or protective role to limit PMN trafficking and PMN-mediated damage.

LXB_4 has not been studied as extensively as LXA_4; it is chemically and biologically less stable because it isomerizes rapidly *in vitro*. Therefore, it has been more difficult to handle previously, but now stable LXB_4 analogs have been prepared (12) that will help to expand the evaluation of their biological roles. There are specific and potent actions attributed to LXB_4, including stimulating proliferation and differentiation of granulocyte-monocyte colonies from human mononuclear cells and sleep induction. In addition to its specific actions, LXB_4 also shares actions with LXA_4, selectively stimulating human peripheral blood monocytes and inhibiting human neutrophil transmigration and adherence as well as PMN-mediated increases in vascular permeability in mice (3,34).

A considerable amount of data has well documented that lipoxin actions are closely linked with cytokine networks. In human enterocytes, LXA_4 and LXA_4 analogs inhibit IL-8 release at the gene transcriptional level (26). This report is consistent with recent findings

Table 1. Biological actions of lipoxins.

Cell type/tissue	Action	Reference
Neutrophils	- Inhibit chemotaxis, adherence and transmigration	10,15
	- Inhibit PMN-epithelial and endothelial cell interactions	16,17
	- Block superoxide anion generation	18
	- Inhibit CD11b/CD18 expression and IP_3 formation	19,20
	- Modulate L-selectin expression	21
Monocytes	- Stimulate chemotaxis and adhesion to laminin without increasing cytotoxicity	22
Eosinophils	- Inhibit migration/chemotaxis	23
Natural killer cells	- Block cytotoxicity	24
Myeloid progenitors	- Stimulate myeloid bone marrow-derived progenitors	25
Enterocytes	- Inhibit TNF-α-induced IL-8 expression and release	26
	- Inhibit *Salmonella typhimurium*-induced IL-8	27
Fibroblasts	- Inhibit IL-1ß-induced IL-6, IL-8 and MMP-3 production	28
Endothelia (HUVEC)	- Stimulate protein kinase C-dependent prostacyclin formation	29
	- Block P-selectin expression	30
Mesangial cells	- Inhibit LTD_4-induced proliferation	31
Pulmonary artery	- Induces relaxation and reverses precontraction by PGF_2 or endothelin-1	14
Bronchi	- Relaxation after precontraction by peptido-leukotrienes	32

showing that synthetic lipoxin analogs abolish the allergen-induced eotaxin formation (23) and suppress TNF-α-stimulated macrophage inflammatory peptide-2 and IL-1ß release but also concomitantly stimulate IL-4 (36) in *in vivo* models. It is thus likely that lipoxin bioactions *in vivo* are up-regulated by cytokines and that lipoxins directly modulate the cytokine composition in a local inflammatory milieu, a concept supported by recent findings showing that LXA_4 may be involved in a negative feedback loop opposing inflammatory cytokine-induced activation of human synovial fibroblasts (28).

Unlike PMN and eosinophils, lipoxins are potent stimuli for peripheral blood monocytes (3). While LXA_4 and LXB_4 stimulate monocyte chemotaxis and adherence, these cells do not degranulate or release reactive oxygen species in response to lipoxins, suggesting that the actions of these lipoxins are specific for locomotion and may be related to the recruitment of monocytes to sites of injury. These monocyte activities may be host-protective in view of the important role of these cells in wound healing and resolution of inflammatory sites. Along with these suggestions, LXA_4 and LXA_4 analogs were shown to accelerate the resolution of allergic pleural edema (37) and enhance phagocytosis of apoptotic PMN by monocyte-derived macrophages (38). It is increasingly appreciated that the resolution of inflammation is a dynamically regulated process and these different observations raise the possibility that lipoxins play pivotal roles in the resolution phase of PMN-mediated inflammation.

Lipoxins in disease models

Lipoxin formation is observed when cells are exposed to receptor-mediated soluble or phagocytic stimuli. Because cells routinely encounter these stimuli and lipoxins perform vasoactive and counterregulatory actions, LXA_4 and LXB_4 are likely to have

Table 2. Lipoxins (LX) and diseases.

Organ/system	Impact *in vivo*	Reference
Hematologic and oncologic	- Defect in LX production with cells from chronic myeloid leukemia patients in blast crisis	39
	- LX stimulate nuclear form of PKC in erythroleukemia cells	40
	- Formation of LX by granulocytes from eosinophilic donors	41
Vascular	- Angioplasty-induced plaque rupture triggers LX formation	42
Renal	- LX trigger renal hemodynamic changes generated in experimental glomerular nephritis	43
	- Increased LX excretion in rat kidney transfected with rh15-LO	44
Dermatologic	- LXA_4 regulates delayed hypersensitive reactions in skin	45
	- LX inhibit PMN infiltration and vascular permeability	46
Pulmonary	- LXA_4 detected in bronchoalveolar lavage fluids from patients with pulmonary disease and asthma	47
	- Production of LX by nasal polyps and bronchial tissue	48
	- LXA_4 inhalation shifts and reduces LTC_4-induced contraction in asthmatic patients	32
	- Aspirin-intolerant asthmatics display a lower biosynthetic capacity than aspirin-tolerant patients	49
Hepatic	- LX generation decreased in cirrhotic patients	50
Rheumatoid arthritis	- LX levels increase with recovery	51

physiologic roles during homeostatic responses even in the absence of illness. Lipoxins are generated *in vivo* in humans and in experimental animal models (reviewed in 34) and are also associated with diseases (Table 2). Currently, only limited data on the effects of lipoxins in clinical investigation are available. Nevertheless, in asthmatic patients, inhalation of LXA_4 inhibits LTC_4-induced airway obstruction (32). Lipoxins are generated from endogenous sources during provocative challenge in asthma (6), suggesting that they may play roles in modulating airway hyperresponsiveness. Asthmatic patients possess the capacity to generate both lipoxins and 15-epi-lipoxins, but aspirin-intolerant asthmatics display a lower biosynthetic capacity than aspirin-tolerant asthmatics for these potentially protective lipid mediators (49). In addition, lipoxins are formed in human airways *in vivo* during certain inflammatory lung diseases (e.g., sarcoidosis, alveolitis and resolving pneumonia) (52), in cirrhotic ascites (50) and intravascularly after percutaneous transluminal angioplasty of atherosclerotic coronary diseases (42). Together, these data support a physiological role for lipoxins *in vivo.*

Anti-inflammatory signaling and lipoxin-specific receptors

Lipoxin actions are cell type, species and organ specific. These actions can be assigned to one or a combination of three mechanisms: a) lipoxins act at their own specific cell surface receptors (i.e., LXA_4 specific and separate LXB_4 receptor) (53,54); b) LXA_4 interacts with a subclass of the peptido-leukotriene (LTC_4 and LTD_4) receptor that also binds LXA_4 (14), and c) lipoxins can act on intracellular targets after lipoxin transport and uptake or within their cells of origin (55).

LXA_4 and LXB_4 act at two distinct sites, and in some cell types, they evoke similar actions, but their actions are distinct in others. ^{3}H-LXA_4 specifically binds to intact PMN, the binding being modulated by stable guanosine analogs (53), suggesting that LXA_4-triggered responses in PMN are mediated by classical G-protein coupling of cell surface receptors. The bioactions of LXA_4, 15-epi-LXA_4 and stable analogs are transduced by this high affinity receptor (lipoxin A_4 receptor, ALXR) that has been sequenced and cloned for both mouse (46) and human leukocytes (19,22), as well as human enterocytes (26) and, more recently, for mesangial cells (31). In addition, LXA_4 actions on vascular endothelial cells are mediated via a distinct non-myeloid receptor that remains to be cloned.

Mouse ALXR isolated from a spleen cDNA library had a characteristic sequence of seven transmembrane spanning G protein-coupled receptors and its homology to the human ALXR (56) was 73% at the deduced amino acid level. Mouse ALXR showed high affinity binding to ^{3}H-LXA_4 (K_d 1.5 nM), with values similar to those obtained with the human receptor (1.7 nM) expressed in CHO cells (46). CHO cells stably transfected with mouse ALXR and exposed to LXA_4 selectively hydrolyzed guanine triphosphate (46,56), indicating that LXA_4 stimulates functional coupling of ALXR and G protein. Tissue distribution of mouse ALXR mRNA paralleled the appearance of human ALXR mRNA, and this mRNA was most abundant in mouse neutrophils, followed by spleen and lung.

In several tissues and cell types other than leukocytes, results of pharmacological experiments have indicated that LXA_4 can also interact with a subclass of peptido-leukotriene receptors ($cysLT_1$) as a partial agonist mediating their actions (31). Along these lines, both LTC_4 and LXA_4, albeit at high concentrations (>1 μM), induce contractions of guinea pig lung parenchyma and release of thromboxane A_2 which is sensitive to $cysLT_1$-receptor antagonists (57), an event which is not likely to be a physiologic action of LXA_4.

In addition to specific binding to membrane surface receptors, specific binding of labeled LXA_4 is associated with subcellular fractions including granules and nucleus (53). In this regard, it was recently reported that LXA_4 binds to and activates the aryl hydrocarbon receptor, a ligand-activated transcription factor, in a murine hepatoma cell line (58).

Our current understanding of the LXA_4 receptor's intracellular down-regulatory signals remains incomplete. In neutrophils, for example, lipoxins do not promote a sustained mobilization of intracellular Ca^{2+}, acidification of the intracellular milieu or generation of cAMP. But, LXA_4 binding to its receptor triggers the activation of GTPase, phospholipase A_2 and phospholipase D (reviewed in 7), responses that are inhibited by pretreatment of the cells with pertussis toxin. In addition, lipoxins are not receptor level antagonists for inflammatory stimuli such as fMLP or LTB_4. For example, lipoxins inhibit LTB_4 responses in neutrophils perhaps by uncoupling LTB_4 receptor-initiated proinflammatory signaling, as evidenced by down-regulation of CD11b/CD18, decreased IP_3 formation and changes in intracellular protein kinase C distribution (7). Recently, a novel polyisoprenyl phosphate signaling pathway was identified (59) with one of its components, presqualene diphosphate (PSDP), being a potent negative intracellular signal in PMN. Activation of ALXR inhibits PSDP remodeling, leading to an accumulation of PSDP and potent inhibition of both phospholipase D and superoxide anion generation (18) in PMN.

The receptor coupling in monocytes and PMN is similar to G-protein activation, being critical in both cell types. However, there could be different G-protein subtypes that diverge downstream in the signal transduction pathway to stimulate monocytes and inhibit PMN (Figure 5).

It was recently shown (54) that small peptides selectively compete for specific ^3H-LXA_4 binding with PMN and recombinant human ALXR, increasing extracellular acidification rates and inducing cell chemotaxis and migration *in vivo*. Chimeric receptors constructed from receptors with opposing functions, namely ALXR and LTB_4 (54), revealed that the seventh transmembrane segment and adjacent regions of ALXR are essential for LXA_4 recognition, and additional regions of this receptor are required for high affinity binding of the peptide ligands. It appears, however, that the G-protein interactions evoked by ligand-receptor binding and their intracellular amplification mechanisms are different for peptide versus lipid ligands of ALXR, and hence they can dictate different functional responses.

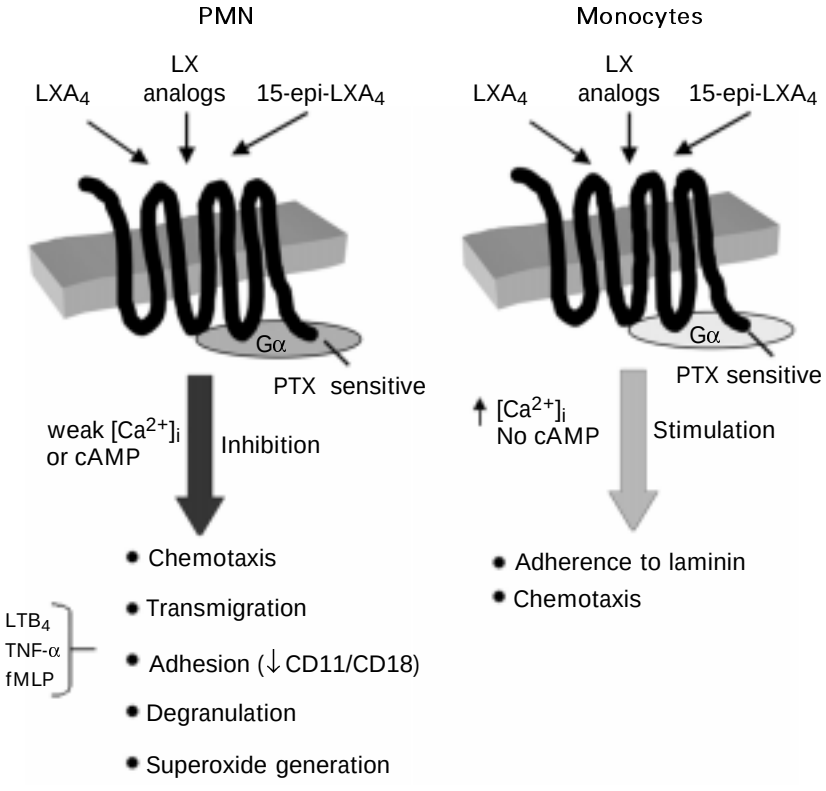

Figure 5. Activation of the lipoxin (LX) A_4 receptor (ALXR) evokes different responses in neutrophils (PMN) and monocytes. The activation of the ALXR inhibits PMN and stimulates monocyte function via pertussis toxin (PTX)-sensitive G proteins (Gα) upon activation by LXA_4, 15-epi-LXA_4 and lipoxin analogs. LTB_4, leukotriene B_4; TNF-α, tumor necrosis factor α; fMLP, formyl-methionyl-leucyl-phenylalanine.

Conclusions

Lipoxins are the trihydroxytetraene-containing class of eicosanoids primarily generated by cell-cell interactions via transcellular biosynthesis, serving as local endogenous anti-inflammatory mediators. These stop signals in inflammation and other related processes may be involved in switching the cellular response from additional PMN recruitment to monocytes that could lead to resolution of the inflammatory response or promotion of repair and wound healing. Aspirin impinges on this system and evokes the endogenous biosynthesis of the 15 epimers of lipoxins, namely ATL, that can modulate in part the beneficial actions of aspirin.

Lipoxins and ATL analogs represent useful tools to evaluate the potential of pharmacological manipulation of the inflammatory process as a means to develop new and selective anti-inflammatory therapies with reduced unwanted toxic side effects. In this context, it was recently described (60) that aspirin and other nonsteroidal anti-inflammatory drugs together with dietary omega-3 polyunsaturated fatty acid supplementation induce the generation of a novel array of bioactive compounds such as 5,12,18*R*-tri HEPE. Together with lipoxins and 15-epi-lipoxins, the identification of these novel endogenous anti-inflammatory lipid mediators opens new avenues in the therapeutics of inflammation, cardiovascular diseases and cancer.

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