Review Article
TheScientificWorldJOURNAL (2002) 2, 169–204
ISSN 1537-744X; DOI 10.1100/tsw.2002.81

Lipid-Derived Mediators in Endogenous Anti-Inflammation and Resolution: Lipoxins and Aspirin-Triggered 15-epi-Lipoxins[†]

Charles N. Serhan* and Nan Chiang

Center for Experimental Therapeutics and Reperfusion Injury, Department of Anesthesiology, Perioperative and Pain Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts 02115

Received September 7, 2001; Revised October 31, 2001; Accepted November 2, 2001; Published January 22, 2002

It is well appreciated that lipid-derived mediators play key roles in inflammation and many other physiologic responses where multicellular processes are involved. Among them, lipoxins (LX) and aspirin-triggered LX (ATL) evoke actions of interest in a range of physiologic and pathophysiologic processes, and these two series have emerged as founding members of the first class of lipid/chemical mediators "switched on" in the resolution phase of an inflammatory reaction. These unique compounds possess a trihydroxytetraene structure and are both structurally and functionally distinct among the many groups of lipid-derived bioactive mediators. LXA_4 and 15-epi-LXA_4 (a member of the ATL series) display leukocyte-selective actions that enable them to serve as endogenous "stop signals" in multicellular events in that they modulate adherence, transmigration, and chemotaxis. Both LXA_4 and 15-epi-LXA_4 elicit these responses via a G protein-coupled receptor (GPCR), termed ALXR, identified in human and murine tissues. Among eicosanoids, ALXR is stereoselective for LXA_4 (5*S*,6*R*,15*S*-trihydroxy-7,9,13-*trans*-11-*cis*-eicosatetraenoic acid). Its aspirin-triggered 15*R* epimer (15-epi-LXA_4) and their bioactive stable analogs act in the subnanomolar to nanomolar range in human cellular systems and murine models of acute inflammation and reperfusion. ALXR also has the ability to interact with a wide panel of small peptides that give different signaling responses *in vitro* than LXA_4 or its analogs, suggesting that ALXR is capable of serving as a multirecognition receptor in immune responses. Characterization of ALXR and development of metabolically stable LX and ATL analogs that are mimetics rapidly advanced our appreciation of the mechanism of LX actions and the potential utility of these counter-regulatory biocircuits in the quest to control local inflammatory events. In this on-line update, LX and ATL biosynthesis and the LXA_4 specific receptor, termed ALXR, are reviewed with a focus on their roles in inflammation and resolution with respect to pharmacology, molecular biology, and signal transduction in several cell types and animal models investigated thus far.

[†] For additional information and updates, see: http://letheon.bwh.harvard.edu/research/overview/cet+ri.phtml and http://serhan.bwh.harvard.edu/

*Corresponding author. Email: cnserhan@zeus.bwh.harvard.edu; Tel.: 617-732-8822; Fax: 617-278-6957.
Email for Dr. Chiang: nchiang@zeus.bwh.harvard.edu

KEY WORDS: lipid mediators, leukocytes, lipoxins

DOMAINS: inflammation

INTRODUCTION*

Inflammation initially involves the recognition of self and nonself by leukocytes. It is now clear that a diverse range of endogenous chemical mediators control these events and orchestrate the host response[1]. These small chemical signals regulate leukocyte traffic as well as the cardinal signs of inflammation. It is well established that the classic eicosanoids such as prostaglandins (PG) and leukotrienes (LT) play important roles and exert a wide range of actions in responses of interest in inflammation[2]. In recent years, the scope and range of chemical mediators identified has expanded considerably[1] to include novel lipid mediators, many 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[3,4,5] and the most recently uncovered of this class, namely inosine monophosphate (IMP), that also regulates neutrophil (PMN) trafficking[6] (see Illustration 1 and ***http://serhan.bwh.harvard.edu***). Many of these chemical signals are held to be proinflammatory when generated in 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 novel signals (Illustration 2). Along these lines, Krump et al.[5] found that endogenous adenosine is a very potent inhibitor of LT formation by PMN. Also, IMP inhibited cytokine-initiated PMN infiltration and attenuated PMN rolling in microvessels[6].

A body of results from the author's laboratory[7,8] and other investigators demonstrated that endogenous mediators are generated to dampen the host response and orchestrate resolution[1,9,10]. In this regard, the lipoxins (LX) were the first to be identified and recognized as endogenous anti-inflammatory lipid mediators relevant in resolution in that they can function as “braking signals” or chalones in inflammation[7]. Most recent evidence with clinical and experimental exudates revealed early coordinate appearance of LT and PG with PMN recruitment. This was followed by LX biosynthesis, which was concurrent with spontaneous resolution. Human peripheral blood PMN exposed to PGE_2 (as in exudates) switched eicosanoid biosynthesis from predominantly LTB_4 and 5-lipoxygenase(LO)-initiated pathways to LXA_4, a 15-LO product that “stopped” PMN infiltration (Illustrations 3 and 5). These results indicate that functionally distinct lipid mediator profiles switch during acute exudate formation to “reprogram” the exudate PMN to promote resolution.

It is of particular interest that aspirin (ASA), a widely used nonsteroidal anti-inflammatory drug (NSAID) with many beneficial properties[11] in addition to its well-appreciated ability to inhibit PG[12], also triggers the endogenous generation of 15-epimeric LX, termed ASA-triggered LX (ATL)(Illustration 5, 6, and 7). This occurs via acetylation of cyclooxygenase-2 (COX-2) at sites of inflammation *in vivo*[13] (*vide infra*) that carry anti-inflammatory and antiproliferative actions[14,15]. This is a previously unappreciated and novel mechanism of drug action that has intriguing implications for targeted drug design. But more importantly, they help to further illustrate the importance of endogenous generation of lipid mediators with anti-inflammatory properties. The traditional approach to developing anti-inflammatory drugs, as in other human conditions amenable to pharmacologic interventions, is the use of biosynthesis inhibitors and receptor antagonists of proinflammatory mediators, which indeed have enjoyed

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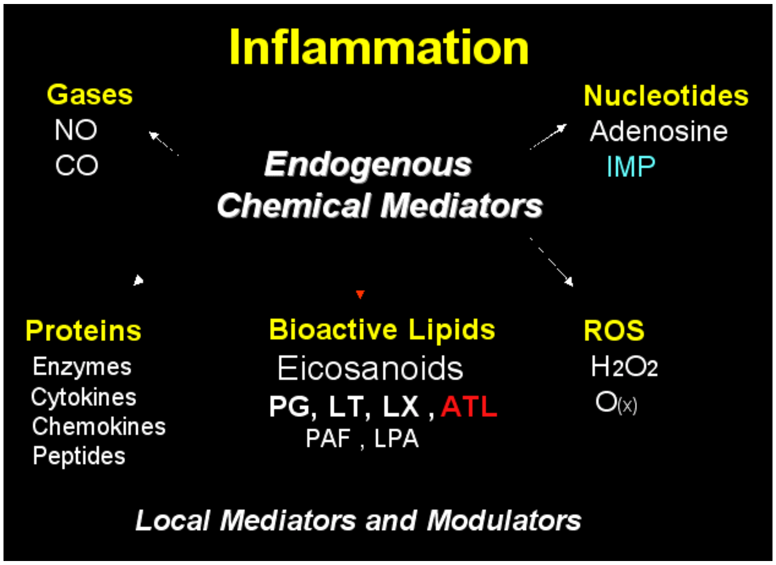
*****ABBREVIATIONS:*** ALXR, lipoxin A_4 receptor; ASA, aspirin; ATL, aspirin-triggered 15-epi-lipoxins, BLT, leukotriene B_4 receptor; COX, cyclooxygenase; GPCR, G protein-coupled receptor; LC/MS/MS, liquid chromatography-tandem mass spectrometry; LM, lipid mediators; LO, lipoxygenase; LT, leukotriene; LX, lipoxin; MPO, myeloperoxidase; PAF, platelet-activating factor; PMN, neutrophils



Inflammation

Gases
NO
CO

Nucleotides
Adenosine
IMP

Endogenous Chemical Mediators

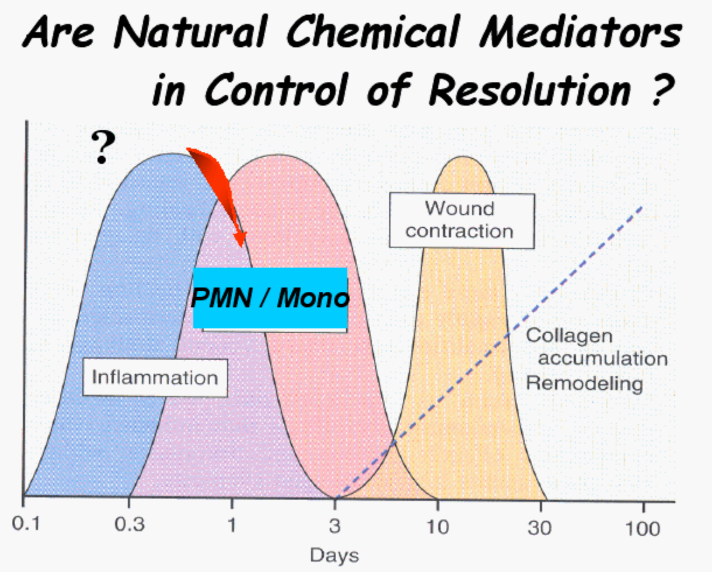
Proteins
Enzymes
Cytokines
Chemokines
Peptides

Bioactive Lipids
Eicosanoids
PG, LT, LX , ATL
PAF , LPA

ROS
H_2O_2
$O_{(x)}$

Local Mediators and Modulators

Illustration 1.*[*] 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 members of the well established classes of lipid mediators, proteins (wide range of chemokines and cytokines), and reactive oxygen species (ROS).



Are Natural Chemical Mediators in Control of Resolution ?

Illustration 2.

both considerable clinical and commercial successes[1,16], but are not without significant unwanted side effects[17,18,19]. 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 relatively unappreciated sides of human biology[20,21], but also provides an opportunity to explore new therapeutic approaches based on these novel endogenous mechanisms that may reduce the possibilities for unwanted toxic side effects and help control inflammation with a high degree of precision.

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[*] **Note to reader:** These illustrations/figures were prepared to run in parallel to the text with seven tables to help facilitate the use of this material. For the animated version of these illustrations, please go to our Web site, http://serhan.bwh.harvard.edu.

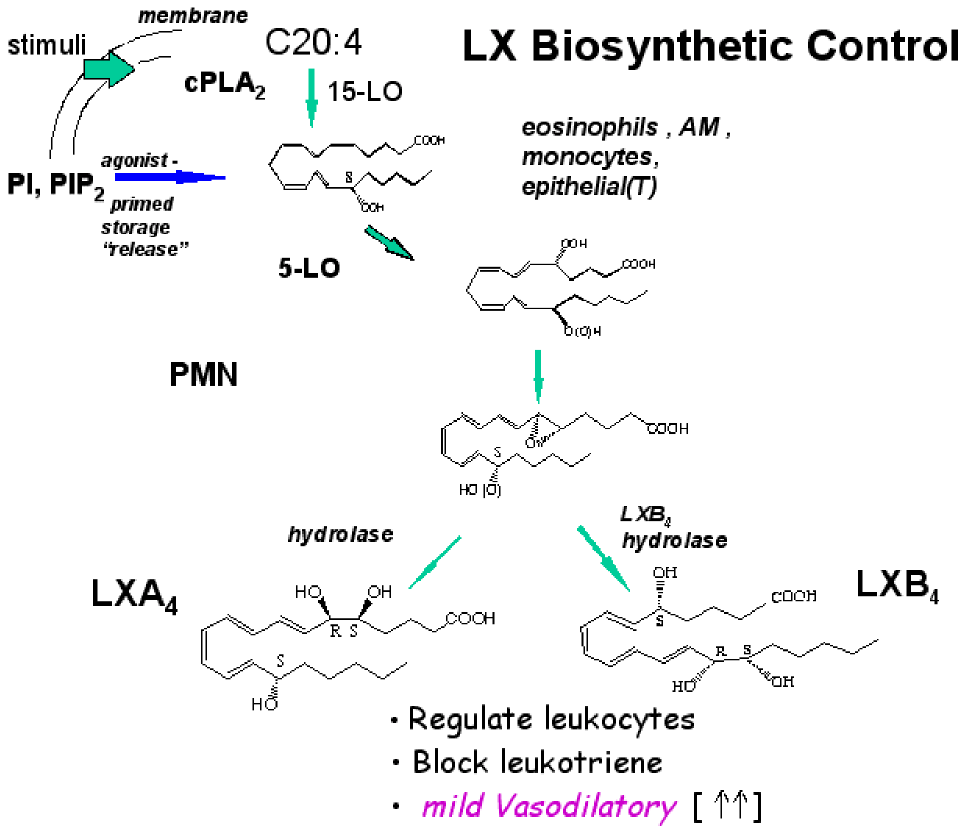
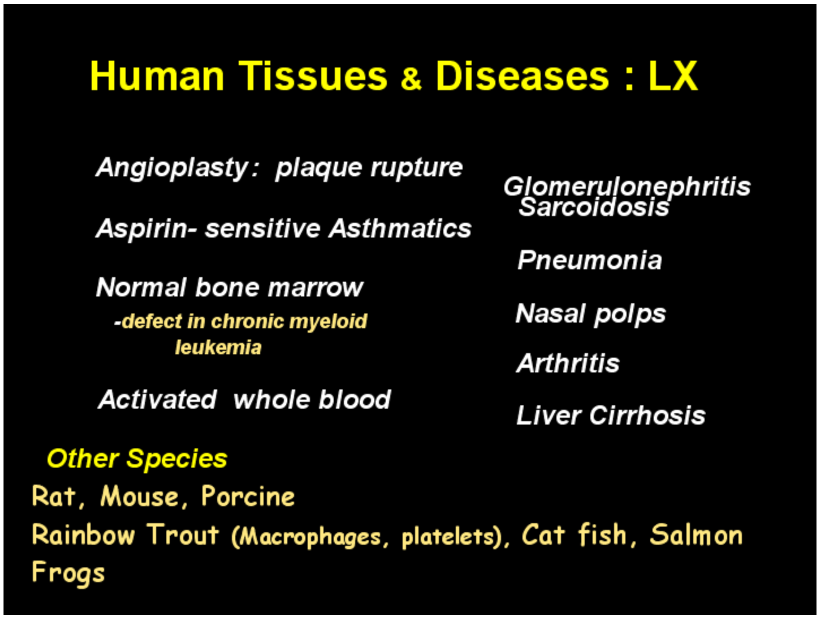


Illustration 3. LXA_4 and a positional isomer, LXB_4, are generated during transcellular biosynthesis initiated by 15-LO. This event also blocks LT formation and therefore regulates leukocytes.



Human Tissues & Diseases : LX

Angioplasty: plaque rupture	*Glomerulonephritis*
	Sarcoidosis
Aspirin- sensitive Asthmatics	*Pneumonia*
Normal bone marrow *-defect in chronic myeloid leukemia*	*Nasal polps*
	Arthritis
Activated whole blood	*Liver Cirrhosis*

Other Species
Rat, Mouse, Porcine
Rainbow Trout (Macrophages, platelets), Cat fish, Salmon
Frogs

Illustration 4. Alterations in LX levels are associated to the pathophysiology of several human diseases. The LX structure is conserved in evolution and is produced by several other species.

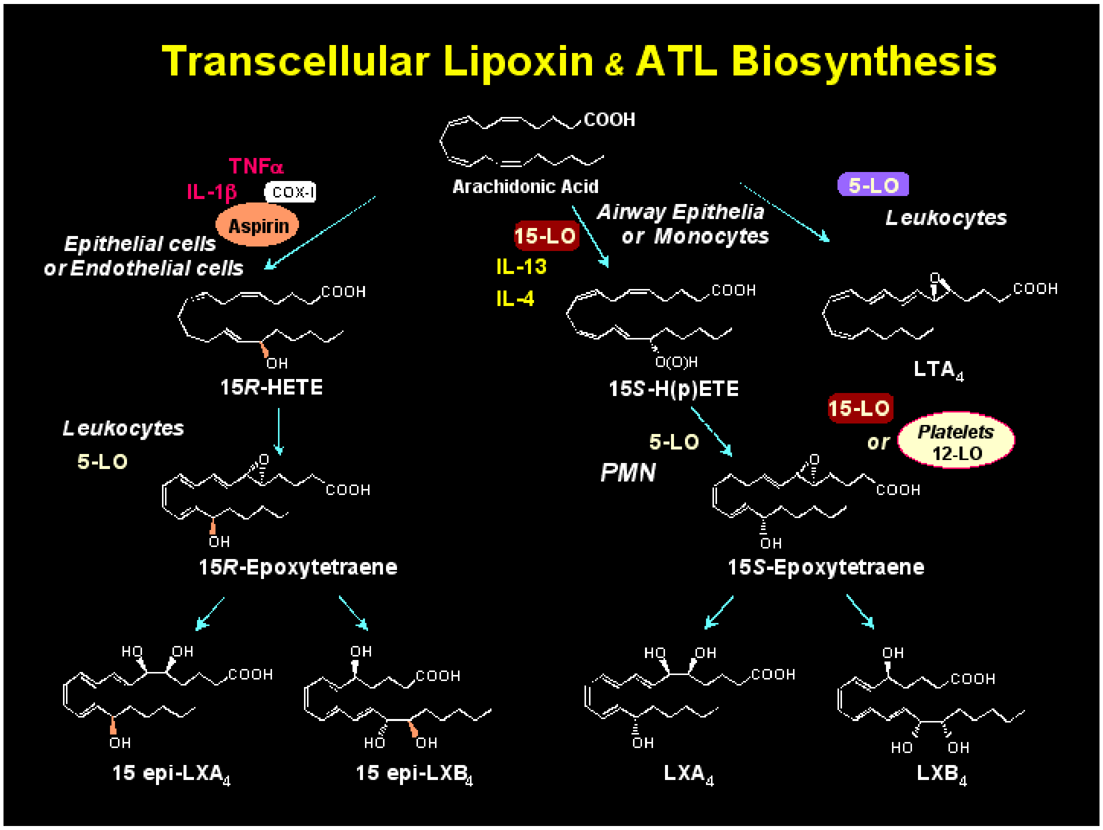


Illustration 5. Two main pathways (right and middle) appear to be used in human cells and tissues to generate LXs. 15-epi-LXs are generated when COX-2 is upregulated after acetylation by ASA (left).

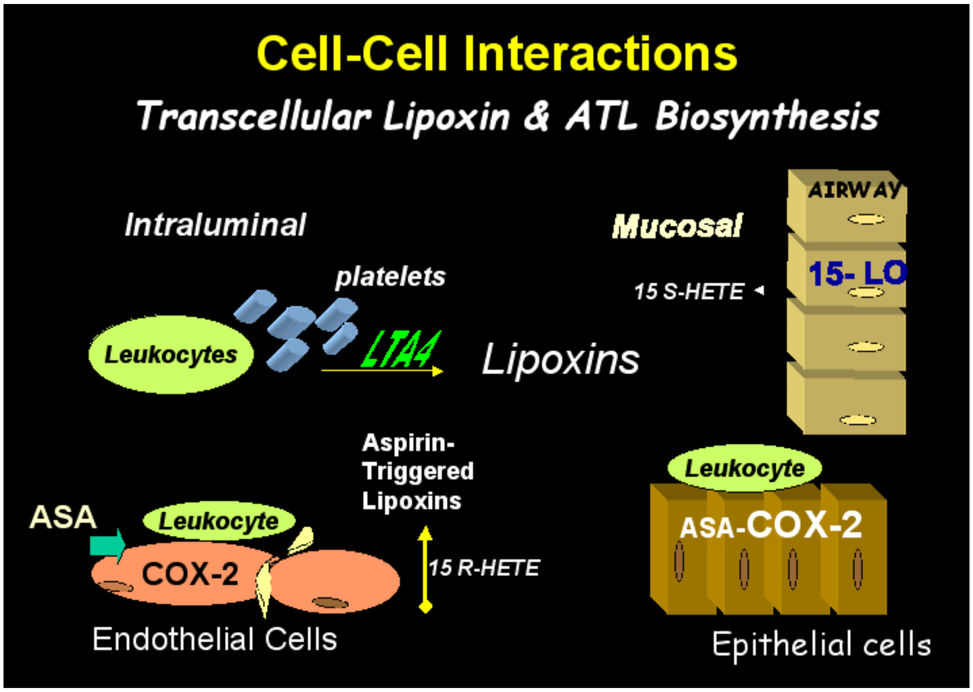
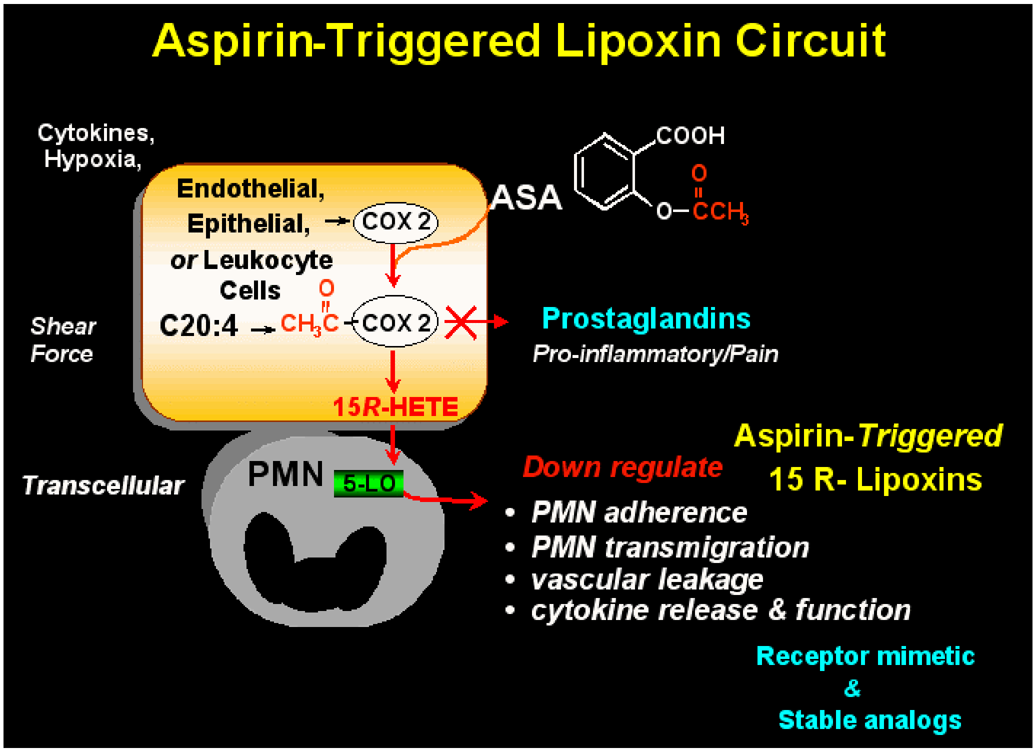


Illustration 6. During cell-cell interactions, LX and their endogenous carbon-15 position epimers ATL can be amplified by transcellular biosynthesis via the interactions of two or more cell types.



Aspirin-Triggered Lipoxin Circuit

Cytokines, Hypoxia,

Shear Force

Transcellular

Endothelial, Epithelial, or Leukocyte Cells → COX 2

C20:4 → $CH_3\overset{O}{\overset{\|}{C}}$-COX 2 ✗→ Prostaglandins

Pro-inflammatory/Pain

15R-HETE

PMN 5-LO

Down regulate

Aspirin-Triggered 15 R- Lipoxins

- PMN adherence
- PMN transmigration
- vascular leakage
- cytokine release & function

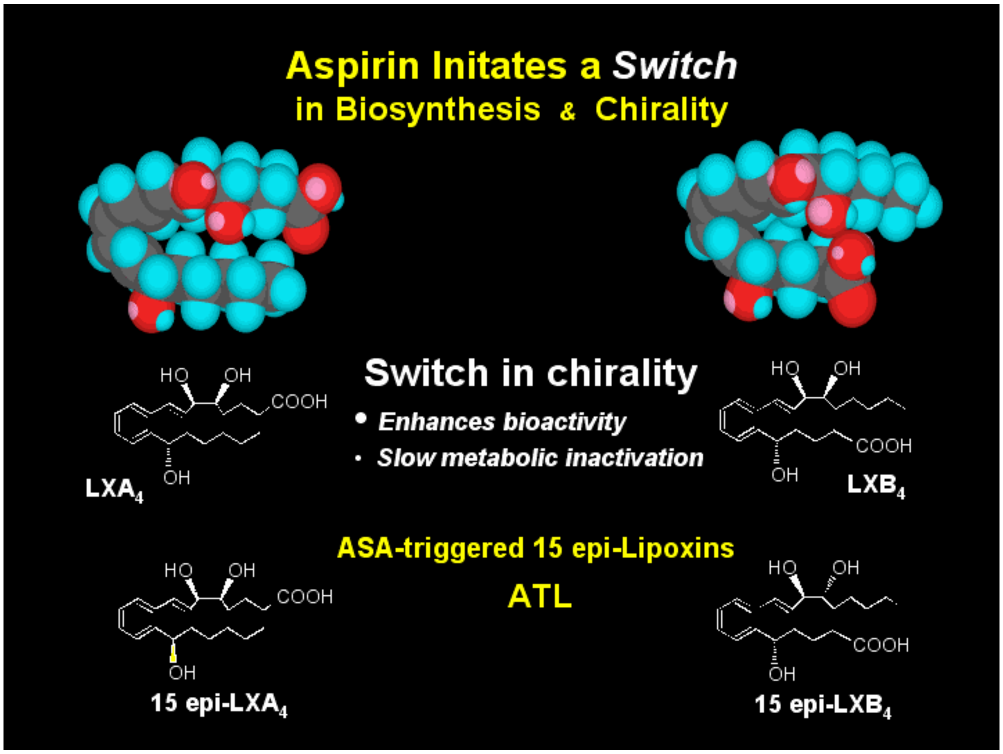
Receptor mimetic & Stable analogs

Illustration 7. Irreversible acetylation of COX-2 by ASA changes the enzyme's product from PG intermediate to precursors of ATL. The acetylated COX-2 remains catalytically active to generate 15R-HETE (see text).

As a class, LX and their analogs possess physiologic, pathophysiologic, and pharmacological actions in several target tissues. On their discovery, it was not initially clear what the role of these new compounds was since they did not carry bioactions like LT or PG. Each action of LX is stereoselective in that changes in potencies accompany double bond isomerization and change in alcohol chirality (*R* or *S*) at key positions, as well as selective dehydrogenation of alcohols and reduction of double bonds (Illustrations 8, 9, and 10). The self-limited impact of LX in the local microenvironment suggests that they contribute to resolution of injury sites and/or resolve inflammatory loci by regulating further recruitment of PMN and stimulating monocyte migration to promote healing and remodeling. The human PMN and monocyte responses with LXA_4 were examined in further detail and will be presented later in our review (*vide infra in silico).*

LXA_4 stimulates rapid lipid remodeling within seconds and releases arachidonic acid within PMN but without oxygenation, which is sensitive to pertussis toxin (PTX) treatment[22,23], findings that pointed to the involvement of a G protein-coupled receptor (GPCR) in the actions of LX on human leukocytes. This specific GPCR was identified and cloned in human and mouse, and denoted LXA_4 receptor (ALXR). Together, they were identified as the first cloned LO-derived eicosanoid receptors. More recently, using a similar approach as for ALXR enabled identification of the long sought-after human LTB_4 receptor (denoted as BLT)[24] and its murine homolog[25].

It is of interest that the BLT displays some sequence homology with ALXR, which was originally cloned as a purinergic receptor[26]. Both ALXR and BLT are more akin to early notions regarding the exclusivity of chemokine receptors and their structures rather than PG receptors (*vide infra;* Illustration 11). Their similarity likely reflects the structures of LT and LX, which contain thermal- and UV-sensitive conjugated double bond systems, whereas PG contain a more chemically stable and less flexible cyclopentane-containing structure. BLT was suggested



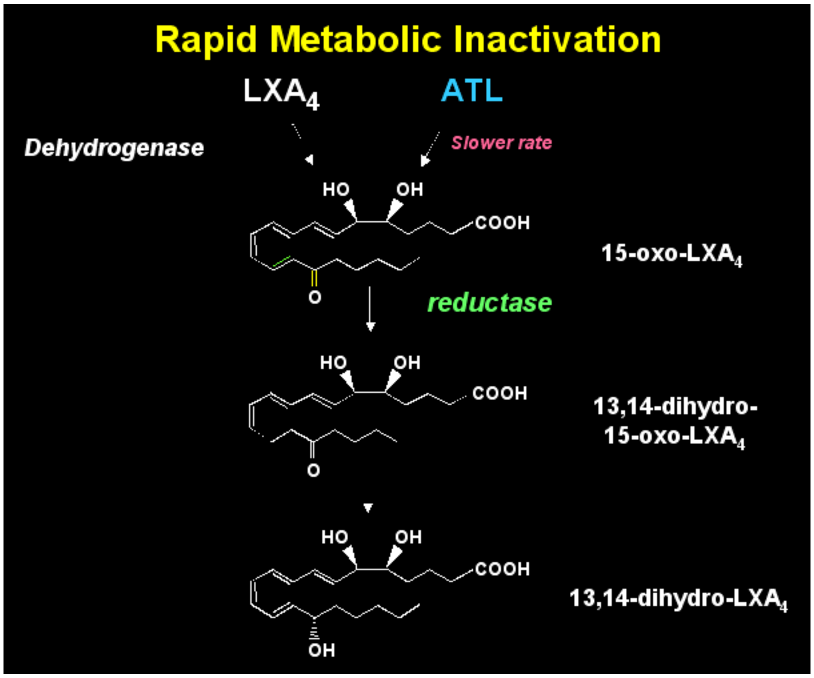
Aspirin Initates a *Switch*
in Biosynthesis & Chirality

Switch in chirality

- *Enhances bioactivity*
- *Slow metabolic inactivation*

ASA-triggered 15 epi-Lipoxins
ATL

Illustration 8. ASA initiates generation of 15-epi-LX that carry their 15 position alcohol in the R configuration, which show enhanced bioactivity and slower metabolic inactivation than 15S native LX. The dehydrogenase is stereospecific and prefers the S rather than R 15 epimer while the ALXR is more accommodating in handling both epimers.



Rapid Metabolic Inactivation

Illustration 9. 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 to 15-PGDH, which catalyzes the reduction of the C15 oxo-group to give 13,14-dihydro-LXA_4. These metabolites appear to be inactive as inhibitors of PMN.

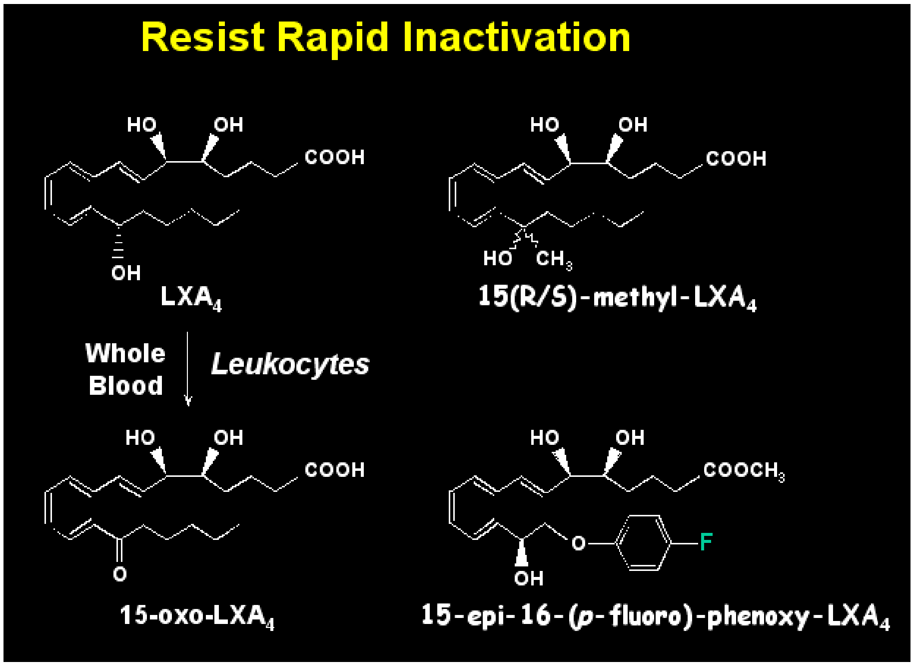


Illustration 10. Metabolic stable analogs of both LXA$_4$ and 15-epi-LXA$_4$ (shown on the right) were designed to resist rapid inactivation at carbon-15 and the ω-end of the molecule[20,53]. For LXB$_4$ analogs that resist rapid inactivation, see Serhan et al.[20] and Maddox et al.[52]

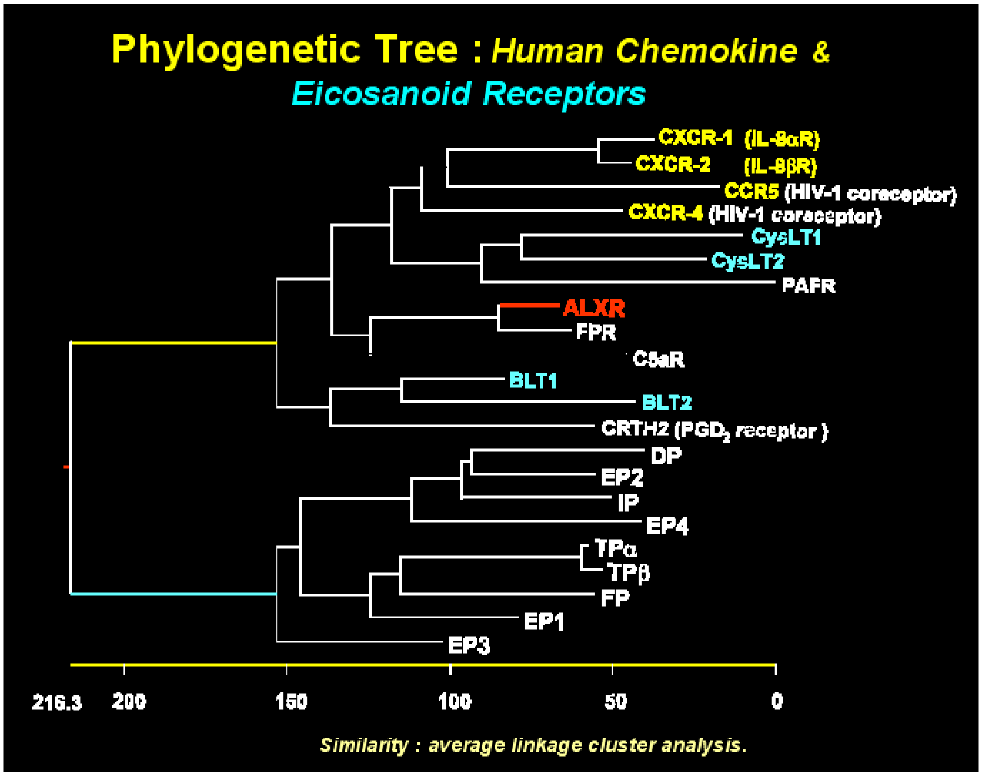


Illustration 11. Structural similarity of deduced amino acid sequences of human eicosanoid and chemokine receptors is determined by the average linkage cluster analysis. Abbreviations: TP (thromboxane A$_2$ receptor), EP$_1$, EP$_2$, EP$_3$, EP$_4$ (subtypes of PGE$_2$ receptor), FP (PGF$_2$ receptor), IP (prostacyclin receptor), ALXR (lipoxin A$_4$ receptor), and BLT (LTB$_4$ receptor). BLT, CXCR-4, and CCR-5 were identified as coreceptors for HIV-1 entry.

as a novel coreceptor mediating HIV-1 entry into CD4-positive cells[27]. Along these lines, ALXR was also recently shown to interact with specific peptide and protein ligands[28]. These observations suggest that receptors of this class can interact with both small endogenous lipophilic ligands of the host as well as larger exogenous protein structures, an interesting aspect of these receptors that might relate to their potential multifunctional roles as sensing receptors in host defense. Here, we provide an update and overview of current knowledge of the actions of LXA_4 and the characterization of one of its seven transmembrane receptors, namely ALXR, that is involved in regulating PMN, monocyte, and epithelial responses.

BIOSYNTHESIS OF LIPOXINS

Transcellular Biosynthesis of LX and 15-epi-LX: The Role of Cell-Cell Interaction

Platelet-leukocyte interactions and/or platelet-leukocyte microaggregates[29] promote the formation of LX by transcellular conversion of the leukocyte (Illustration 5, right side) 5-LO epoxide product LTA_4. Once thought to be solely an intracellular intermediate in LT production, it is now clear that LTA_4 released by activated leukocytes is available for enzymatic conversion by neighboring cell types[5,30]. When platelets are adherent, their 12-LO converts LTA_4 to LXA_4 and LXB_4. For a review and mechanistic details with recombinant 12-LO, see Serhan[21]. Hence it is important to note that human platelets, which do not produce LX on their own, become a major source of LX, given their abundance *in vivo* and their highly active 12-LO.

15-LO-initiated LX production is illustrated (Illustration 5, middle right) by airway epithelial cells, monocytes, or eosinophils, which upregulate their 15-LO when exposed to cytokines such as IL-4 or IL-13[31,32]. The 15-LO, by definition, inserts molecular oxygen at the carbon-15 position of, for example, arachidonic acid, in the "*S*" configuration. When these cell types are activated, they generate and release 15*S*-HETE[7,14], which is rapidly taken up and converted by PMN to LX via the action of their 5-LO. This event not only leads to LX biosynthesis, but also "turns off" LT formation. 5-LO conversion of 15*R*-HETE also results in inhibition of LT biosynthesis[15]. 15*R*-HETE is a major product of arachidonic acid in several cell types when COX-2 is upregulated after acetylation by ASA (Illustration 5, left side). Thus, it is possible that ASA can regulate the *in vivo* production of LT by 15*R*-HETE conversion to 15-epi-LX, and 15-epi-LX can in turn also regulate the cellular actions of LT.

We sought evidence for alternate explanations for ASA 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 Levy[33]), and prevention of cardiovascular diseases[34]. Inhibition of COX and biosynthesis of PG can account for many of ASA therapeutic properties[35]; however, its ability to regulate PMN-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 (Illustration 6 and 7). Hence, inflammatory stimuli (i.e., TNFα, LPS, etc.) induce COX-2 to generate 15*R*-HETE when ASA is administered[14]. This intermediate carries a carbon-15 alcohol in the *R* configuration that is rapidly converted by activated PMN to 15-epi-LX, or LX that carry their 15 position alcohol in the *R* configuration[21] rather than 15*S* native LX, which in humans can result from LO:LO interaction, the molecular oxygen inserted mainly in the *S* configuration.

LXB_4 is a positional isomer of LXA_4, carrying alcohol groups at carbon 5*S*, 14*R*, and 15*S* positions, instead of the C-5*S*, 6*R*, and 15*S* positions present in LXA_4. ASA-triggered LXB_4 carries a 15*R* alcohol, hence 15-epi-LXB_4 (Illustrations 5 and 8). Although LXA_4 and LXB_4 show similar activities in some biologic systems[36], in many others each shows distinct actions([37]

and reviewed in [21]). 15-epi-LXB_4, for example, is a more potent inhibitor of cell proliferation than LXA_4 or 15-epi-LXA_4[21]. Next in this review we shall focus on findings indicating that 15-epi-LXA_4 is generated in inflammatory exudates in an ASA-dependent manner and that ASA-triggered LXA_4 and novel fluorinated LXA_4 as well as LXB_4 stable analogues are potent, topically active inhibitors of PMN-directed actions *in vivo*.

Aspirin (ASA)-Dependent Generation of 15-epi-LXA_4 *In Vivo*

To determine whether 15-epi-LXA_4 could be detected in animal experimental models, experiments were first carried out with a mouse peritonitis model[13]. In this model of inflammation, COX-2 protein levels were upregulated by intraperitoneal injection of lipopolysaccharide (LPS). Peritonitis was induced by intraperitoneal injection of casein. To test whether ASA treatment of the mice results in the generation of 15-epi-LXA_4 during an inflammatory event, ASA was administrated by intraperitoneal injection. 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 system[13].

Given one or two doses of ASA[13], the mean values for 15-epi-LXA_4 production were ~1.5 and 1.8 ng/5 ml peritoneal lavage per mouse. Without administration of ASA, approximately 0.5 ng of 15-epi-LXA_4 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 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-LXA_4. The physiological relevance of these values obtained in the absence of experimental challenge is currently not clear. These results demonstrated that ASA administration in murine peritonitis gives inflammatory exudates that generate 15-epi-LXA_4 in appreciable levels from endogenous substrate within these inflammatory cells, thus establishing a biosynthetic circuit for ATL/15-epi-LX generation *in vivo*.

LX AND HUMAN DISEASES

These methods (e.g., LC/MS/MS and ELISA) were recently used to evaluate ATL and LXA_4 formation in ASA-tolerant and -intolerant asthmatics and their relation to LTC_4. Of interest, 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 challenge[38]. 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 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 disease[39] and chronic myelogenous leukemia[40,41,42,43,44]. These diseases contrast with recent findings that LXA_4 production is up-regulated in localized juvenile periodontitis[45, see Illustration 18] as well as following atherosclerotic plaque rupture[46], and with nasal polyps[47]. Together, this result indicates that alterations in LX levels may be linked to the pathophysiology of several human diseases and may display local organ-specific functions that stand apart from their roles in inflammation and within local inflammatory lesions (Table 1). In this context, the ability of both LXA_4 and LXB_4 and their stable analogs to lower intraocular pressure may underlie their role in the physiology of ocular pressure regulation within the eye[48,49]. In human eye tissues, the receptor ALXR is indeed present and appears to be associated with corneal epithelial cells[50]. LX are evolutionarily conserved in several species of fish and frogs (see Illustration 4 and review in [7,21]), findings that again raise the possibility that the function of LX in humans is fundamental; yet, our knowledge of their range of actions in humans is still evolving.

TABLE 1
Lipoxins and Human Diseases

Organ/System	Impact in vivo	Reference
Hematologic and Oncologic	Defect in LX production with cells from chronic myeloid leukemia patients in blast crisis	Stenke et al., 1991 [41]
	LX stimulate nuclear form of PKC in erythroleukemia cells	Beckman et al., 1992 [98]
	Formation of LX by granulocytes from eosinophilic donors	Serhan et al., 1987 [99]
Vascular	Angioplasty-induced plaque rupture triggers LX formation	Brezinski et al., 1992 [46]
Renal	LX trigger renal hemodynamic changes generated in experimental glomerular nephritis	Katoh et al., 1992 [100]
	Increased LX excretion in rat kidney transfected with rh15-LO	Munger et al., 1999 [101]
Dermatologic	LXA_4 regulates delayed hypersensitive reactions in skin	Feng et al., 1996 [102]
	LX inhibit PMN infiltration and vascular permeability	Takano et al., 1997 [55]
Pulmonary	LXA_4 detected in bronchoalveolar lavage fluids from patients with pulmonary disease and asthma	Chavis et al., 1995 [103]
	Production of LX by nasal polyps and bronchial tissue	Edenius et al., 1990 [47]
	LXA_4 inhalation shifts and reduces LTC_4-induced contraction is asthmatic patients	Christie et al., 1992 [104]
	Aspirin-intolerant asthmatics display a lower biosynthetic capacity than aspirin-tolerant patients	Sanak et al., 2000 [38]
Hepatic	LX generation decreased in cirrhotic patients	Claria et al., 1998 [39]
Rheumatoid arthritis	LX levels increase with recovery	Thomas et al., 1995 [105]
Ocular	LXA_4 reduces intraocular pressure	Cotran et al., 1995 [48, 49]
Localized juvenile periodontitis	LXA_4 production is upregulated	Pouliot et al., 2000 [45]

DESIGN OF STABLE ANALOGS OF LXA_4 AND 15-EPI-LXA_4: LX AND ATL ANALOGS

Mechanism of LXA_4 Inactivation

LX, as other autacoids, are rapidly biosynthesized in response to stimuli, act locally, and then are rapidly enzymatically inactivated. The major route of LXA_4 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[20] (Illustrations 9 and 10). 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[51]. 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[52]. It has recently been shown that 15-oxo-LXA_4 is also produced from LXA_4 in mouse whole blood[53] suggesting that the mouse shares with the human a common pathway for LXA_4 inactivation (Illustration 10).

STRUCTURE REQUIREMENTS FOR LXA_4 ANTI-INFLAMMATORY ACTIONS

As a class, LX possess physiologic, pathophysiologic, and pharmacological actions in several target tissues. Each action of LX is stereoselective in that changes in potencies accompany double bond isomerization and change in alcohol chirality (R or S) at key positions as well as selective dehydrogenation of alcohols and reduction of double bonds (Table 2). For example, the 15-hydroxyl group is important for anti-inflammatory properties since ASA-triggered LXA_4 (15R-LXA_4) with the 15-hydroxyl group in the R-configuration as well as 15(R/S)-methyl-LXA_4 have been established in several experimental settings to be more potent than native LXA_4 (15S-LXA_4) *in vitro* and in *vivo*[54,55]. Also, both 15-oxo-LXA_4[51] and 15-deoxy-LXA_4[20] are biologically inactive in inhibiting superoxide anion generation and transmigration in PMN, respectively. The 13,14-double bond is important since 13,14-dihydro-LXA_4 proved to be inactive in inhibiting superoxide anion generation[51]. These pharmacophores for LX anti-inflammatory action (Table 2) are also required for their interaction with ALXR since these biologically inactive isomers (e.g., 15-oxo-LXA_4, 15-deoxy-LXA_4, and 13,14-dihydro-LXA_4) did not bind to ALXR, whereas the active ones (e.g., 15R-LXA_4 and 15[R/S]-methyl-LXA_4) give specific binding to ALXR, as demonstrated by specific [^{3}H]-LXA_4 binding (see Illustration 13).

Metabolic Stable Aanalogs of LXA_4 and 15-epi-LXA_4

In view of the rapid transformation and inactivation of the LX by monocytes, and, potentially, other cells *in vivo*, it was highly desirable to design LX analogs that could resist this form of metabolism, maintain their structural integrity, and potentially enhance beneficial bioactions. LX analogs were constructed with specific modifications of the native structures of LXA_4 and LXB_4, such as the addition of methyl groups on carbon-15 and carbon-5 of LXA_4 and LXB_4 structures, respectively, to block dehydrogenation by 15-PGDH. For example, 15(R/S)-methyl-LXA_4 is a racemic stable analog of both LXA_4 and 15-epi-LXA_4 (Illustration 10). Additional analogs of LXA_4 were synthesized with a phenoxy group bonded 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 make 16-(para-fluoro)-phenoxy-LXA_4 to hinder degradation of the phenoxy ring. The

TABLE 2
Pharmacophores of LXA_4's Anti-Inflammatory Actions

Functional group	*compound*	*property*
15-hydroxyl group	LXA_4 15(R)-LXA_4 15(R/S)-methyl-LXA_4 15-deoxy-LXA_4 15-oxo-LXA_4	Inhibit PMN invitro and in vivo More potent than LXA_4 More potent than LXA_4 Inactive in inhibiting PMN transmigration Inactive in inhibiting superoxide generation
11,12-cis double bond	11-trans-LXA_4	
13, 14-double bond	13,14-dihydro-LXA_4	Inactive in inhibiting superoxide generation

ASA-triggered 15-epi counterpart of 16-(para-fluoro)-phenoxy-LXA_4, 15-epi-16-(para-fluoro)-phenoxy-LXA_4, was also synthesized (Illustration 10). These modifications not only prolong the half-life of the compounds in blood but also enhance their bioavailabilities as well as bioactivities[53].

The ATL are less effectively converted *in vitro* to their 15-oxo-metabolite than LXA_4[20]. This indicates that the dehydrogenation step is highly stereospecific and suggests that, when ATL are generated *in vivo*, their biologic half-life is increased by about twofold greater than that of native LXA_4 (Illustration 8), thereby enhancing their ability to evoke bioactions. Hence, biologically stable analogs of LX and ATL can be engineered to enhance their bioactions, which suggests that they are useful tools, and offers leads for developing novel therapeutic modalities. These analogs proved to be active and also to act via competition at ALXR (Illustration 13).

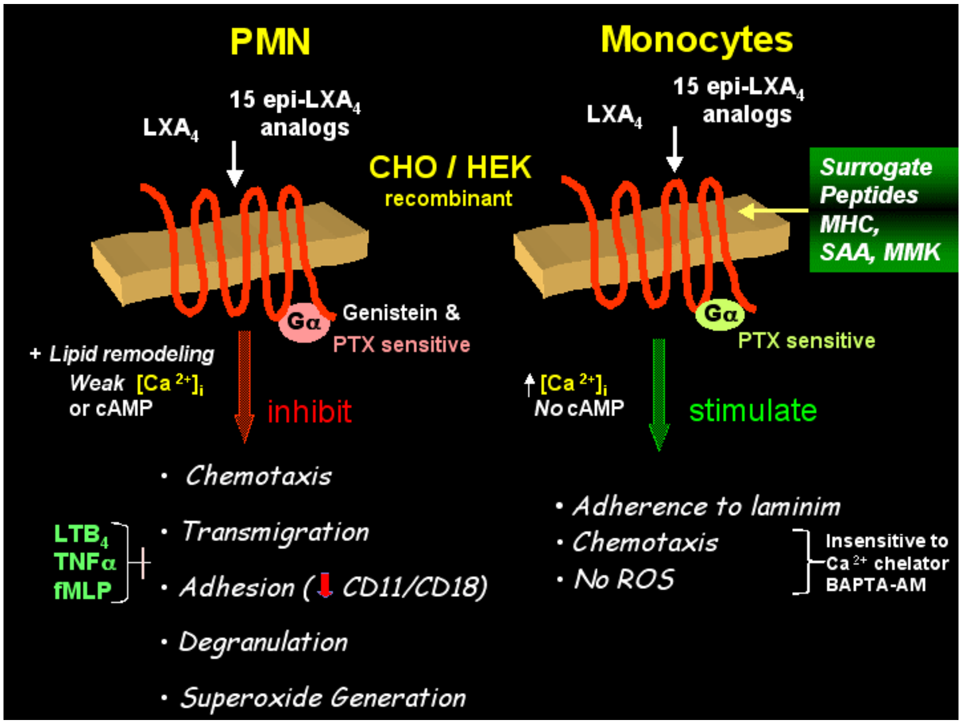
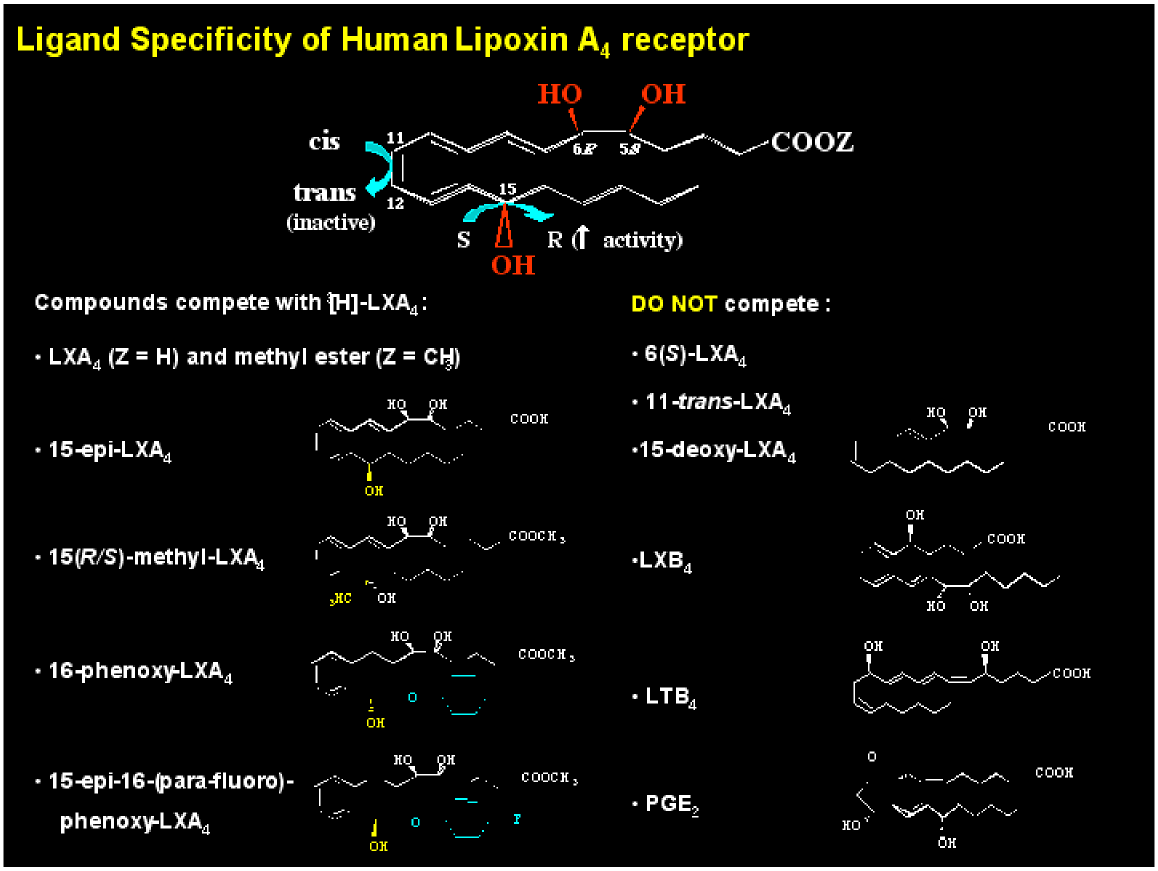


Illustration 12. ALXR inhibits PMN and stimulates monocyte functions via PTX-sensitive G proteins (Gα) upon activation by LXA_4 and 15-epi-LXA_4 as well as LX analogs. In PMN, neither intracellular calcium ($[Ca^{2+}]_i$) nor cAMP was increased in response to LX. In monocytes, LXA_4 induced an increase of $[Ca^{2+}]_i$, which is not the second messenger for LXA_4-stimulated adherence or chemotaxis since these responses were unaffected by BAPTA-AM (a Ca^{2+} chelator). See text for details.



Ligand Specificity of Human Lipoxin A_4 receptor

Compounds compete with $[^3H]$-LXA_4:

- LXA_4 (Z = H) and methyl ester (Z = CH_3)
- 15-epi-LXA_4
- 15(*R*/*S*)-methyl-LXA_4
- 16-phenoxy-LXA_4
- 15-epi-16-(para-fluoro)-phenoxy-LXA_4

DO NOT compete:

- 6(*S*)-LXA_4
- 11-*trans*-LXA_4
- 15-deoxy-LXA_4
- LXB_4
- LTB_4
- PGE_2

Illustration 13. LXA_4 interaction with ALXR is highly stereospecific, that is the 5S, 6R-orientation of the two hydroxyl groups as well as 11-cis double bond conformation are essential for bioactions. 15-epi-LXA_4 (an ASA-triggered lipoxin, ATL) carries a carbon-15 alcohol at the R configuration, opposite to the S configuration in native LXA_4, and was shown to have higher potency than native LXA_4 in certain bioassays. In 15(R/S)-methyl-LXA_4, hydrogen at carbon -15 was replaced by a methyl group at a racemate at carbon -15. 16-phenoxy-LXA_4 has a phenoxyl group at carbon -16. These compounds, which are more resistant to rapid dehydrogenation by 15-hydroxy PG dehydrogenase (15-PGDH) than native LXA_4, compete with $[^3H]$-LXA_4 specific binding on PMN and are potent inhibitors for PMN functions *in vitro* and *in vivo*.

LX BIOACTIONS *IN VITRO* AND *IN VIVO*

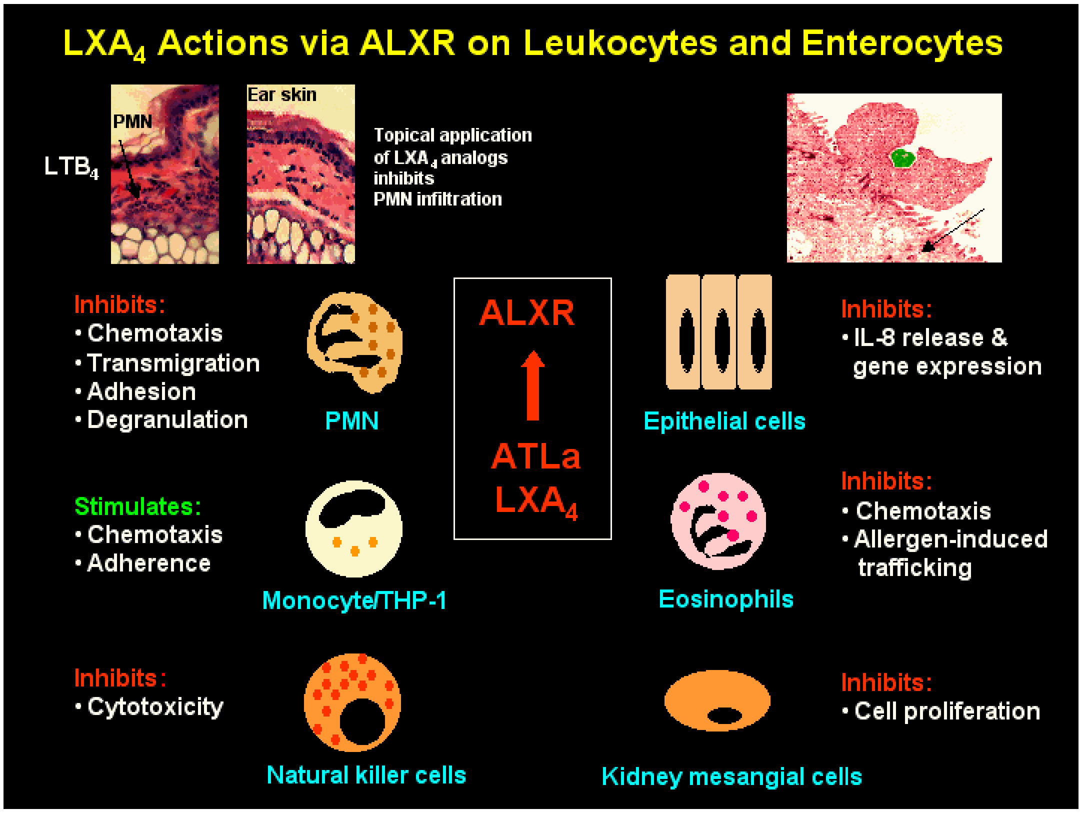
LXA_4 evokes mild vasodilatory and counter-regulatory roles in both *in vivo* (Table 3) and *in vitro* models. These counter-regulatory actions are initiated via unique cell surface receptors on leukocytes and enterocytes (Illustration 14). With other cell types such as endothelium and mesangial cells (Illustration 20), LXA_4 evokes bioactions and interacts with a subclass of peptido-LT receptors ($CysLT_1$)[21]. The leukocyte receptors are physiologically and pharmacologically distinct and evoke selective actions on each type of leukocyte tested to date. With human peripheral blood leukocytes, LXA_4 inhibits both isolated PMN and eosinophil chemotaxis *in vitro* in the nanomolar range[56,57] and blocks human natural killer (NK) cell cytotoxicity in a stereoselective fashion[21]. In cell-cell interaction systems, LXA_4 inhibits PMN transmigration across both endothelial and epithelial monolayers[36,58] via actions on both cell types (i.e., PMN and endothelial cells, PMN and epithelial cells). These responses are also evident *in vivo* with murine receptors[54,55].

When applied topically to mouse ears, these LX stable analogues inhibit both PMN infiltration and vascular permeability changes in a concentration-dependent fashion (Illustration 15)[54,55]. At 130 nmol per ear, the degree of inhibition of PMN infiltration was more than 90%

TABLE 3
Biological Actions of Lipoxins

Cell type/Tissue	Action	Reference
Neutrophils	Inhibit chemotaxis, adherence and transmigration	Lee et al., 1989 [56]; Serhan et al., 1995 [20]
	Inhibit PMN-epithelial and endothelial cell interactions	Colgan et al., 1993 [58]; Papayianni et al., 1996 [36]
	Block superoxide anion generation	Levy et al., 1999 [82]
	Inhibit CD11b/CD18 expression and IP$_3$ formation	Fiore & Serhan, 1995 [71]; Grandordy et al., 1990 [22]
	Modulate L-selectin expression	Filep et al., 1999 [106]
Monocytes	Stimulate chemotaxis and adhesion to laminin without increase in cytotoxicity	Maddox et al., 1997 [66]
Eosinophils	Inhibit migration/chemotaxis	Bandeira-Melo et al., 2000 [107]
NK cells	Block cytotoxicity	Ramstedt et al., 1987 [108]
Myeloid progenitors	Stimulate myeloid bone marrow-derived progenitors	Stenke et al., 1994 [44]
Enterocytes	Inhibit TNF-α-induced IL-8 expression and release	Gronert et al., 1998 [50]
	Inhibit Salmonella typhimurium-induced IL-8	Gewirtz et al., 1998 [79]
Fibroblasts	Inhibit IL-1β-induced IL-6, IL-8 and MMP-3 production	Sodin-Semrl et al., 2000 [67]
Endothelia (HUVEC)	Stimulate protein kinase C-dependent prostacyclin formation	Leszczynski & Ustinov, 1990 [109]
	Block P-selectin expression	Scalia et al., 1997 [110]
Mesangial cells	Inhibit LTD$_4$-induced proliferation	McMahon et al., 2000 [78]
Pulmonary artery	Induce relaxation and reverses pre-contraction by PGF$_2$ or endothelin-1	Dahlen & Serhan, 1991 [111]
Bronchi	Relaxation after pre-contraction by peptido-leukotrienes	Christie et al., 1992 [104]

for both analogues, with apparent IC$_{50}$s noted at ~13 to 26 nmol per ear range for each analogue. In the same concentration range, these two LXA$_4$ stable analogues also inhibited the vascular permeability, namely, extravasation of Evans blue. At 130 nmol per ear, the inhibition of vascular permeability change was >98% for 15(*R/S*)-methyl-LXA$_4$, and ~87% for 16-phenoxy-LXA$_4$, respectively, and their impact was noted visually. The inhibition of vascular permeability changes paralleled inhibition of PMN infiltration with both the ATL and LX analogues. Also, the fluorinated analog of ATL, denoted ATLa, at levels as low as ~24 nmol per mouse, potently inhibited TNF-α-induced leukocyte recruitment into the dorsal air-pouch (Illustration 16)[50]. Inhibition was evident by either local intra-air-pouch delivery (~77% inhibition) or via systemic delivery by intravenous injection (~85% inhibition) and proved more potent than local delivery of ASA. Rank order for inhibiting PMN infiltration was: ATLa (10 µg, i.v.) ≈ ATLa (10 µg, local) ≈ dexamethasone (10 µg, local) > ASA (1.0 mg, local) (Illustration 17). Applied topically to mouse ear skin, ATLa also inhibited PMN infiltration induced by LTB$_4$ (~78% inhibition) or phorbol ester (~49% inhibition), which initiates endogenous chemokine production. Our results indicate that this fluorinated analog of the natural ATL is bioavailable by both local or systemic delivery routes and is a more potent and precise inhibitor of PMN accumulation than ASA *in vivo*[50,54].



LXA_4 Actions via ALXR on Leukocytes and Enterocytes

Topical application of LXA_4 analogs inhibits PMN infiltration

Inhibits:
- Chemotaxis
- Transmigration
- Adhesion
- Degranulation

PMN

Epithelial cells

Inhibits:
- IL-8 release & gene expression

Stimulates:
- Chemotaxis
- Adherence

Monocyte/THP-1

Eosinophils

Inhibits:
- Chemotaxis
- Allergen-induced trafficking

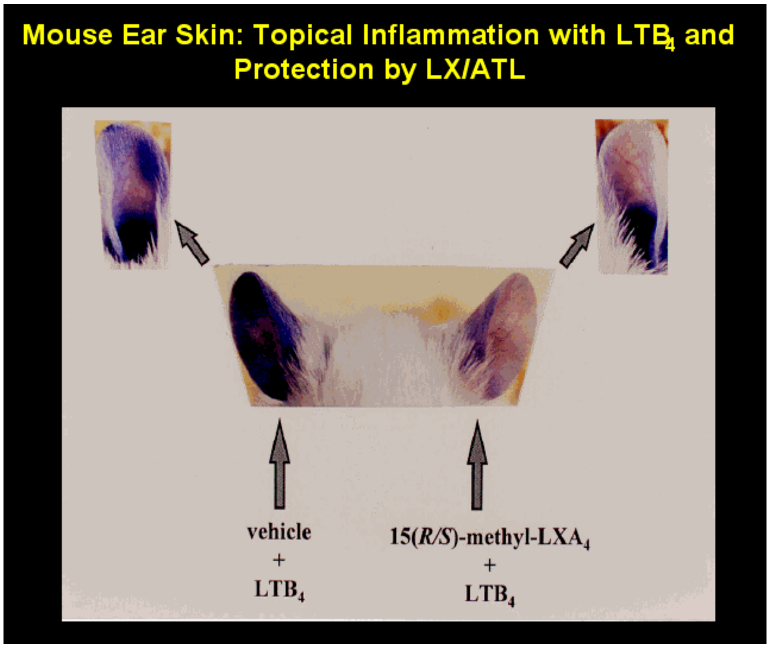
Inhibits:
- Cytotoxicity

Natural killer cells

Kidney mesangial cells

Inhibits:
- Cell proliferation

Illustration 14. Actions of LXA_4 in leukocytes[21] and human epithelial cells[50,79] (upper left panel). Ear biopsies: Inhibition of LTB_4-induced PMN infiltration into mouse ear by topical application of LXA_4 analogs in acute skin inflammation[55]. PMN is indicated by an arrow (upper right panel). Photomicrograph: Internalization of Salmonella typhimurium (shown in green) by intestinal epithelium (indicated by an arrow). In response to this gastrointestinal pathogen, intestinal epithelium secretes chemokines, which promote PMN infiltration. This chemokine (IL-8) secretion can be downregulated by LXA_4 analogs.



Mouse Ear Skin: Topical Inflammation with LTB_4 and Protection by LX/ATL

Illustration 15. When applied topically to mouse ears, LXA_4 stable analogue 15(R/S)-methyl-LXA_4 inhibits vascular permeability, visualized by extravasation of Evans blue.

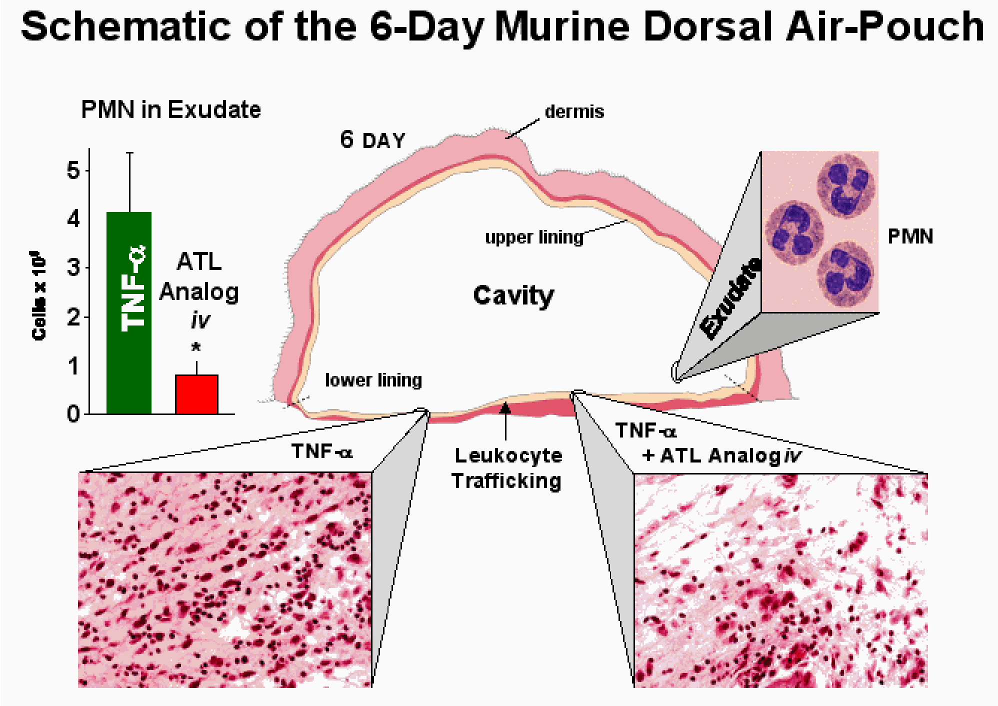


Illustration 16. The 6-day murine dorsal air-pouch is characterized by the presence of a nascent lining that encloses the air cavity. TNF-α induces leukocyte infiltration, predominantly PMN, which is inhibited by i.v. injection of ATL analog (see insets).

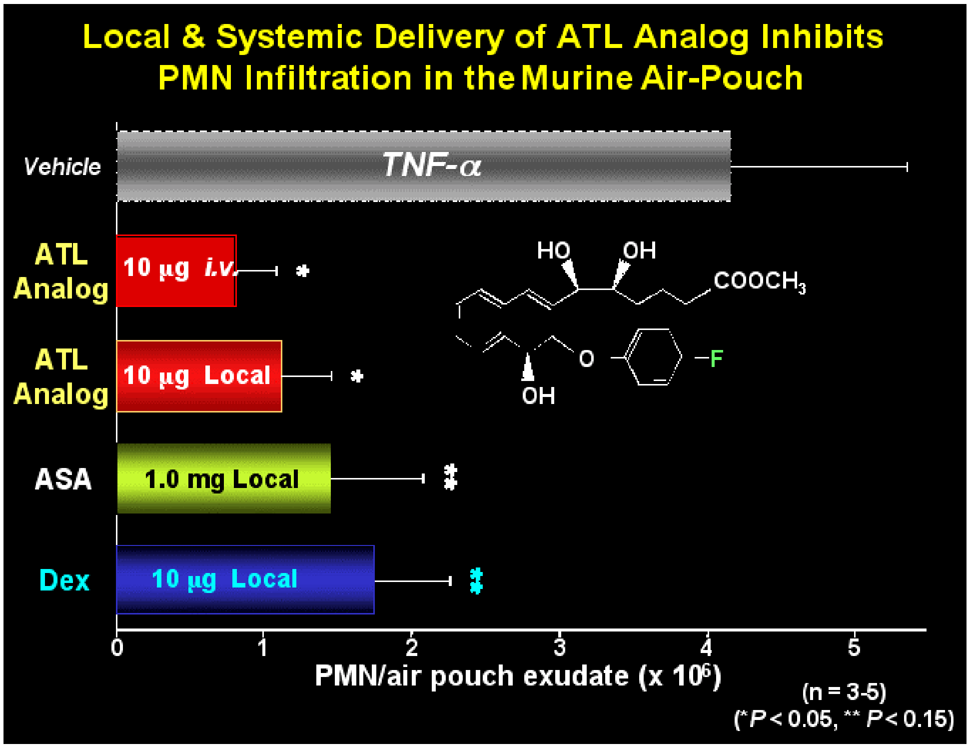


Illustration 17. Direct comparison of LX/ATL analogs with dexamethasone and ASA treatment. Note that the analog is >100 times more potent than ASA in preventing PMN entry[53].

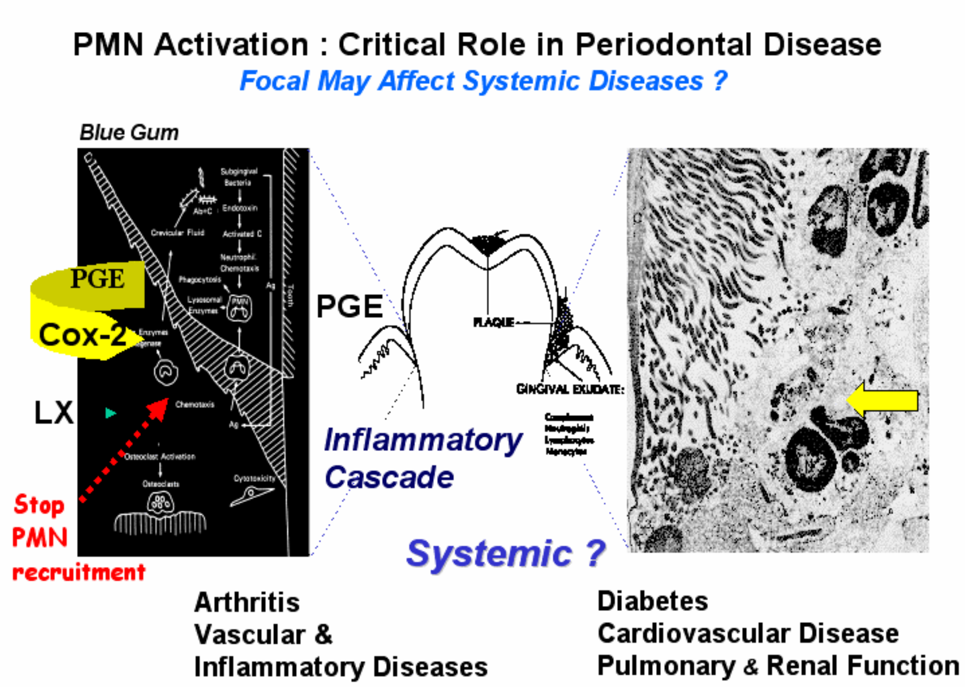


Illustration 18. Recruitment of PMN (right) followed by aberrant release of inflammatory mediators (left) contributes to the onset of periodontal disease and is associated with tissue destruction. The periodontal pathogen P. gingivalis-inflamed periodontal tissues display an increased PGE_2, a potent stimulator of bone loss, as well as upregulated COX-2 expression. LX stable analogs blocked P. gingivalis-elicited PMN infiltration and PGE_2 level when introduced into murine air-pouch cavity, suggesting that LX plays a novel protective role in periodontitis, limiting further PMN recruitment and PMN-mediated tissue injury.

LXA_4 RECEPTORS

Molecular Cloning and Receptor Expression

The synthesis of the radiolabeled [11,12-^{3}H]-LXA_4[59] enabled the first direct characterization of specific LXA_4 binding sites present on PMN that are likely to mediate many of its selective actions on these cells[60]. Intact PMN demonstrate specific and reversible [11,12-^{3}H]-LXA_4 binding (Kd ~0.5 nM and B_{max} ~1830 sites per PMN), which is modulated by guanosine stable analogs. These LXA_4 binding sites are inducible in promyelocytic lineage (HL-60) cells exposed to differentiating agents (e.g., retinoic acid, DMSO, and PMA) and confer LXA_4-stimulated phospholipase activation[61]. Together, these findings provided further evidence that LXA_4 interacts with specific membrane-associated receptors on human leukocytes that belong to the classical GPCR.

Based on our finding that functional ALXR are inducible in HL-60 cells, several putative receptor cDNAs that are also induced within this temporal frame, cloned earlier from myeloid lineages and designated orphans[62,63], were systematically examined for their ability to specifically bind and signal with LXA_4. Chinese hamster ovary (CHO) cells transfected with one of the orphans (denoted previously as pINF114, also known as FPRL1 and FPR2) display specific [^{3}H]-LXA_4 binding with high affinity (Kd = 1.7 nM) and demonstrated selectivity when compared to LXB_4, LTB_4, LTD_4, and PGE_2 (Illustration 13 and Table 4)[64]. These transfected CHO cells transmit signal with LXA_4, activating both GTPase and the release of arachidonic acid (C20:4) from membrane phospholipid, indicating that this cDNA encodes a functional receptor for LXA_4 in myeloid cells. The mouse ALXR cDNA was cloned from a spleen cDNA library and displays specific [^{3}H]-LXA_4 binding and LXA_4-initiated GTPase activity when transfected into CHO cells[55]. The human and mouse ALXR represented the first cloned LO-derived eicosanoid receptors. Both human[64] and mouse[55] ALXR cDNA contain an open reading frame of 1051 nucleotides, which encode a protein of 351 amino acids. Northern blot analysis demonstrated that ALXR mRNA is ~1.4 Kb in both human and mouse[55]. Chromosome mapping revealed that the gene encoding ALXR[64] is located on chromosome 19q[65], denoted as FPRH1 in this early report of the orphan receptor.

Northern blot analysis of multiple murine tissues demonstrated that ALXR mRNA is most abundant in PMN, spleen, and lung with lesser amounts in heart and liver[55]. In the absence of disease, the pattern is similar in human tissues. In humans, ALXR mRNA is also abundant in PMN followed by spleen, lung, placenta, and liver[55,64]. To date, ALXR is identified by function and direct actions, and cloned in both human and mouse PMN[55,64], human monocytes[66], and human enterocytes[50], as well as synovial fibroblasts[67]. In human PMN, results of subcellular fractionation experiments revealed that [^{3}H]-LXA$_4$ binding sites are associated with plasma membrane and endoplasmic reticulum (42.1%) and granule (34.5%) as well as nuclear-enriched fractions (23.3%), a distribution distinct from [^{3}H]-LTB$_4$ binding[60]. The finding that LXA$_4$ blocks both PAF and fMLP-stimulated eosinophil chemotaxis[57] suggests that functional ALXR is also present on eosinophils. In human enterocytes, ALXR is present in crypt and brush border colonic epithelial cells[50].

Retinoic acid, PMA and DMSO, which lead to granulocytic phenotypes in HL-60 cells, induce a approximate three- to fivefold increase in the expression of ALXR as monitored by specific [^{3}H]-LXA$_4$ binding[61] (see Table 7). Transcription of ALXR is dramatically upregulated by cytokines in human enterocytes, with lymphocyte-derived interleukin (IL)-13 and interferon-γ being most potent, followed by IL-4 and IL-6. IL-1β and LPS also showed moderate induction of ALXR mRNA[50]. In view of the cytokine regulation of ALXR, it is likely that the expression of these receptors will change dramatically in disease states, which in turn, might attenuate mucosal inflammatory and allergic responses.

Structure-Function Relationships

Deduced amino acid sequence places ALXR within the GPCR superfamily characterized by seven putative transmembrane segments (TMS) with N-terminus on the extracellular side of the membrane and C-terminus on the intracellular side[68]. The overall homology between human and mouse ALXR is 76% in nucleotide sequence and 73% in deduced amino acid[55]. An especially high homology is evident for their second intracellular loop (100%) and between their sixth TMS (97%) followed by the second, third, and seventh TMS as well as the first extracellular loop (87 to 89%), suggesting essential roles for these regions in ligand recognition and G protein coupling. Molecular evolution analysis suggests that ALXR is only distantly related to prostanoid receptors and belongs to the cluster of chemoattractic peptide receptors exemplified by fMLP, C5a, and IL-8 receptors[69] and now known to also include BLT as well as the recently cloned cysteinyl-LT receptors (Illustration 11). BLT was obtained from human HL-60 cells[24] and mouse eosinophils[25] and found to share an overall ~30% homology with ALXR in deduced amino acid sequences. High homologous region (~46%) is present within the second transmembrane segments in both ALXR and BLT with the amino acid sequence LNLALAD. Prostanoids interact with their receptors via COO$^-$ interacting with an arginine residue within the seventh transmembrane segments[70]. Neither ALXR nor BLT share this Arg (in seventh transmembrane segments) requirement[24,64], yet both ligands contain COOH, which at physiological pH could present as a counter-anion. Together, these findings further provide evidence that the origin of receptors for LT and LX is distinct from that of receptors for prostanoids.

TABLE 4
Competitive Binding of ^{3}H-LXA_4 with Structure-Related Eicosanoids

	PMN	*HL-60*	*CHO-ALXR (human)*	*HEK-ALXR (human)*	*HUVEC*
competition	LXA_4 (Kd= 0.5 nM) LXA_4-methyl ester 15(R/S)-methyl-LXA_4 16-phenoxy-LXA_4 15-epi-LXA_4	LXA_4	LXA_4 (Kd= 1.7 nM)	LXA_4 LXA_4-methyl ester 15(R/S)-methyl-LXA_4 15-epi-16-(para-fluoro)-phenoxy-LXA_4	LXA_4 (Kd = 11 nM) LTD_4 SKF104353 ($CysLT_1$ antagonist)
partial competition	LTC_4 (IC_{50}= 62 nM) LTD_4 (IC_{50}= 56 nM) fMLP (IC_{50} ~ 1,000 fold higher than LXA_4)	LTC_4	LTD_4 (Ki= 80 nM) fMLP	15-deoxy-LXA_4 (IC_{50} ~ 1,000 fold higher than LXA_4)	
no competition	LTB_4 LXB_4 6S-LXA_4 11-trans-LXA_4 SKF 104353 (also see Illustration #13)	LTB_4 ONO-4057 LXB_4 SKF 104353	LTB_4 LXB_4 PGE_2	LTB_4	ONO-4057 (LTB_4 antagonist)

Ligand Binding Specificity: Peptide and Lipid Ligands

ALXR is stereoselective for its eicosanoid-based ligands. Table 4 summarizes the present knowledge of related lipid ligand affinity and specificity for ALXR. Intact human PMN and retinoic acid-differentiated HL-60 cells demonstrate specific and reversible [^{3}H]-LXA_4 binding with Kds ~0.5 and ~0.6 nM, respectively[60,61]. Several isomers of LXA_4 tested, namely 11-trans-LXA_4, 6S-LXA_4, and LXB_4, did not compete for these recognition sites, consistent with their functional responses in these systems. Results from Scatchard analyses indicate that [^{3}H]-LXA_4 binds PMN granule membrane-enriched fractions with comparable Kd (0.8 nM) but with a larger Bmax (4.1×10^{-11} M) than plasma membrane fractions (Kd = 0.7 nM, Bmax = 2.1×10^{-11} M)[64]. Hence, it appears that additional receptors can be mobilized by granule fusion to the plasma membrane of PMN. [^{3}H]-LXA_4 specific binding is stereoselective, since LTB_4, LXB_4, 6S-LXA_4, 11-trans-LXA_4, or SKF104353 (a $CysLT_1$ antagonist) do not compete for [^{3}H]-LXA_4 in human PMN (Table 4 and Illustration 13).

ALXR/FPRL Displays Multirecognition with Unrelated Peptide Ligands: A Possible Dual Function for the Receptor?

Human and mouse ALXR cDNA, each transfected into CHO cells, display specific binding with [^{3}H]-LXA_4, the human Kd 1.7 nM[64] and mouse Kd 1.5 nM[55], respectively. Human ALXR-transfected CHO cells were also tested for binding with other eicosanoids, including LXB_4, LTD_4, LTB_4, and PGE_2. Only LTD_4 shows competition with [^{3}H]-LXA_4 binding, giving a K_i of 80 nM (Table 4)[64]. It is of interest to note that, although ALXR shares ~70% homology with FPR, ALXR binds [^{3}H]-fMLP with only low affinity (Kd ~5 µM) and proves to be selective for LXA_4 by 3 log orders of magnitude[71]. More recently, it was reported that certain peptides/proteins in the µM range can also interact with ALXR (as indicated above, is also known as FPRL-1) in *in vitro* model systems. These findings are summarized in Tables 5 and 6. The functional role(s) of these peptides in human biology, pertaining to their ability to activate FPRL-1, albeit at µM levels, remains of interest. The apparent EC_{50} value for receptor activation (determined by mobilization of $[Ca^{2+}]_i$) by the best synthetic rogue peptide (e.g. MMK-1) of this synthetic series is approximately 2 nM[72], whereas LXA_4 and its analogs stimulate monocyte adherence via ALXR at concentrations less than 1 nM (EC_{50} for analogs ~8×10^{-11} M, EC_{50} for LXA_4 ~8×10^{-10} M)[66] or inhibit PMN transmigration and adhesion at 10^{-10} M[20]. These new findings suggest that small peptides as well as bioactive lipids can function as ligands for the same receptor, however with different affinity and/or distinct interaction sites within the receptor and separate intracellular signaling depending on the cell type and model system. Hence, it appears likely that the intracellular protein interactions following ligand-receptor binding are different for peptide vs. lipid ligands of this receptor because different conformations of the ligand-receptors are likely to be formed. Taken together, the finding that specific LXA_4-related structures (Illustration 13) and certain peptides interact with this receptor may reflect the need for mutlirecognition and receptor redundancies in the immune system in that the lipid ligands appear as endogenous ligands, while many of the peptides capable of activating ALXR/FPRL-1 *in vitro* are of exogenous origins, presumably microbial-derived peptides (Tables 5 and 6).

Among the related eicosanoid heteroligands tested in the HL-60 cell system, only LTC_4 at ~3-log molar excess competes for [^{3}H]-LXA_4 specific binding[58]. The cross competition of LTC_4 and LTD_4 observed with LXA_4 in several systems suggests that the “true” peptido-LT receptors may also be of this class of receptors. In several tissues and cell types other than leukocytes, results from pharmacological experiments indicate that LXA_4 acts via interacting with a subclass of peptido-LT receptors ($CysLT_1$) as a partial agonist to mediate its actions[60,73]. 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 that is sensitive to $CysLT_1$-receptor antagonists[74], which is not likely to be a physiologic action of LXA_4. In certain cell types, LXA_4 (in the nanomolar range) blocks LTD_4 actions, and in this regard blocks specific [^{3}H]-LTD_4 binding to mesangial cells[73] and human umbilical vein endothelial cells (HUVEC)[55,61]. HUVEC specifically bind [^{3}H]-LXA_4 at a Kd of 11 nM, which can be inhibited by LTD_4 and SKF104353[61]. Therefore, it appears that LXA_4 interacts with at least two classes of cell surface receptors, one specific for LXA_4, which is present on leukocytes and enterocytes (ALXR) (see Illustration 14) the other shared by LTD_4, which is present on HUVEC and mesangial cells ($CysLT_1$) (Illustration 19 and 20). Along these lines, an inducible $CysLT_1$ was recently identified and cloned from HUVEC[75].

Recombinant $CysLT_1$ receptor gave stereospecific binding with both [^{3}H]-LTD_4 and a novel labeled mimetic of ATL ([^{3}H]-ATLa) that was displaced with LTD_4 and ATLa (~IC_{50} 0.2 to 0.9 nM), and not with a biologically inactive ATL/LX isomer. In sharp contrast, LTD_4 was an ineffective competitive ligand for recombinant ALXR with [^{3}H]-ATLa. Endogenous murine $CysLT_1$ receptors also gave specific [^{3}H]-ATLa binding that was displaced with essentially equal affinity by LTD_4 or ATLa. Systemic ATLa proved to be a potent inhibitor (>50%) of $CysLT_1$-mediated vascular leakage in murine skin (200 µg/kg) in addition to its ability to block PMN recruitment to dorsal air-pouch (4 µg/kg). These results indicate that ATL and LTD_4 bind and compete with equal affinity at $CysLT_1$, providing a molecular basis for ATL serving as a local damper of both vascular $CysLT_1$ signals as well as ALXR-regulated PMN traffic[75].

TABLE 5
Comparison of Lipid vs. Peptide Ligands for Human ALXR in Phagocytic Cells

Ligand	*PMN*		*MONOCYTE*	
	Ligand binding / Signaling	*Concentration*	*Ligand binding / Signaling*	*Concentration*
LXA_4 and ATLa	- [^{3}H]-LXA_4 binding - Inhibit transmigration and adhesion - Inhibit chemotaxis	- Kd ~ 0.5 x10^{-9} M - ~10^{-10} M - peaked at 10^{-8} M	- Induces adherence	- 10^{-11} - 10^{-10} M
MMK-1	- Induces chemotaxis - Displace [^{3}H]-LXA_4 binding	- ~3 x 10^{-9} M - IC50 ~10^{-11} M		
MHC peptide	- Induces chemotaxis - Displace [^{3}H]-LXA_4 binding	- ~3 x 10^{-9} M - IC50 ~10^{-11} M		
SAA [112]	- [^{125}I]-SAA binding - Induces chemotaxis - Ca^{2+} mobilization	- Kd ~ 45 x10^{-9} M - 0.8 – 4.0 x 10^{-6} M - 0.8 – 4.0 x 10^{-6} M	- Induces chemotaxis - Ca^{2+} mobilization - Increase CCR5 phosphorylation -	- 0.8 – 4.0 x 10^{-6} M - 0.8 – 4.0 x 10^{-6} M - 1 – 20 µg/ml
F-peptide (gp120 peptide) [113]	- Induces chemotaxis - Ca^{2+} mobilization	- – 5.0 x 10^{-6} M - 2.5 – 10.0 x 10^{-6} M	- Induces chemotaxis - Ca^{2+} mobilization - Inhibits CCR5 and CXCR4 - binding and chemotaxis	- 1.0 – 5.0 x 10^{-6} M - 1.0 – 10.0 x 10^{-6} M - 1.0 – 5.0 x 10^{-5} M
V3 peptide (gp120 peptide) [114]	- Induces chemotaxis - Ca^{2+} mobilization	- 1 – 6 x 10^{-6} M - 1 – 3 x 10^{-6} M	- Induces chemotaxis - Ca^{2+} mobilization - Increase CCR5 phosphorylation	- 1 – 6 x 10^{-6} M - 1 – 3 x 10^{-6} M
T21/DP107 (gp41 peptide) [115]	- Induces chemotaxis - Ca^{2+} mobilization	- 10^{-8} - 10^{-4} M - 10^{-7} - 10^{-5} M	- Induces chemotaxis - Ca^{2+} mobilization	- 10^{-7} - 10^{-5} M
N36 (gp41 peptide) [116]	- Induces chemotaxis - Ca^{2+} mobilization	- 10^{-6} - 10^{-4} M - 2.5 – 5 x 10^{-6} M	- Induces chemotaxis - Ca^{2+} mobilization	- 10^{-6} - 10^{-4} M - 10^{-6} - 10^{-5} M
WKYMVm [117]	- Induces chemotaxis - Ca^{2+} mobilization - Stimulate NADPH-oxidase activity - Induce CD11b/CD18 mobilization	- 10^{-13} - 10^{-6} M - 10^{-12} - 10^{-9} M - EC50 ~ 2 x 10^{-9} M - EC50 ~ 5 x 10^{-11} M	- Induces chemotaxis - Ca^{2+} mobilization	- 10^{-12} - 10^{-7} M - 10^{-12} - 10^{-8} M
LL-37 [118]	- Induces chemotaxis	- 10^{-6} - 10^{-5} M	- Ca^{2+} mobilization - Induces chemotaxis	- 10^{-6} - 10^{-5} M - 10^{-6} - 10^{-5} M
Amyloid β_{42} [119]			- Ca^{2+} mobilization - Induces chemotaxis	- 5 x 10^{-6} M - 10^{-6} - 10^{-4} M
PrP106-126 (Neurotoxic prion peptide) [120]			- Induces chemotaxis - Production of pro-inflammatory cytokines	- 10^{-5} – 10^{-4} M - 3 x 10^{-5} M

TABLE 6
Comparison of Lipid vs. Peptide Ligands for Recombinant Human ALXR

Ligand	*Cell type expressing recombinant human ALXR*	*LIGAND BINDING/SIGNALING*	*CONCENTRATION*
LXA_4 and ATLa	CHO HEK293	Induces chemotaxis $[^3H]$-LXA_4 binding	10^{-7} M Kd ~ 1.5 nM
MMK-1	CHO HEK293	Induces chemotaxis Displace $[^3H]$-LXA_4 binding	10^{-9} M IC50 ~10^{-11} M
MHC peptide	HEK293	Displace $[^3H]$-LXA_4 binding	IC50 ~10^{-11} M
SAA [112]	HEK293	Partially displace $[^3H]$-LXA_4 binding	IC50 ~10^{-8} - 10^{-7} M
F-peptide (gp120 peptide) [113]			
V3 peptide (gp120 peptide) [114]	HEK293	Induces chemotaxis Ca^{2+} mobilization	1 – 3 x 10^{-6} M
T21/DP107 (gp41 peptide) [115]	HEK293	Induces chemotaxis Ca^{2+} mobilization	10^{-8} - 10^{-5} M 10^{-8} - 10^{-6} M
N36 (gp41 peptide) [116]	HEK293	Induces chemotaxis Ca^{2+} mobilization	10^{-6} - 10^{-5} M 10^{-6} - 10^{-5} M
WKYMVm [117]	HEK293 HL-60 (undifferentiated)	Induces chemotaxis Ca^{2+} mobilization	10^{-13} - 10^{-6} M EC50 ~7.5 x 10^{-11} M
Amyloid β_{42} [119]	HEK293	Induces chemotaxis Ca^{2+} mobilization	10^{-6} - 10^{-5} M 10^{-6} - 10^{-5} M
LL-37 [118]	HEK293	Induces chemotaxis Ca^{2+} mobilization	10^{-6} - 10^{-5} M 10^{-6} - 10^{-5} M

Signal Transduction

The cytoplasmic signaling cascade of ALXR appears to be highly cell type-specific. For example, in human PMN LXA_4 stimulates rapid lipid remodeling (within seconds) with release of arachidonic acid that is evoked via PTX-sensitive G proteins[23] without formation of either LT or PG. Only a modest Ca^{2+} mobilization was observed (Illustration 12). Also, LXA_4 was reported to block intracellular generation of IP_3[22] as well as Ca^{2+} mobilization in response to other stimuli[56]. In human peripheral blood monocytes and cultured THP-1 cells, LXA_4 triggers intracellular Ca^{2+} release and adherence to laminin[66,76]. Thus, different intracellular signaling pathways are present in PMN vs. monocytes despite identical receptor sequences (see Table 7 and Illustration 12). It is of interest that Ca^{2+} is not the second messenger for LX actions in monocytes, since LXA_4-stimulated monocyte adherence to laminin is not dependent on a LX-stimulated increase in $[Ca^{2+}]_i$. The EC_{50} value for LXA_4-stimulated increase in $[Ca^{2+}]_i$ is >100 nM in monocytes, which is more than 2 log orders of magnitude higher than that required for

TABLE 7
Signal Transduction of Human and Mouse ALXR

cell type	*LXA$_4$ and ATLa-evoked signal transduction*	*kinase associated*	*Gene expression*	*up-regulated by*
Human HL-60 (differentiated)	• PLD activation (lipid remodeling)	protein kinase C (staurosporine sensitive)		retinoic acid, DMSO, PMA
Human PMN	• PLD activation • GTPase activity • C20:4 release • PIPP signal (↑PSDP accumulation) (with second signal) • no increase of cAMP, proton efflux and very weak $[Ca^{2+}]_i$	tyrosine kinase (genistein sensitive)		
Human monocyte	• increase of $[Ca^{2+}]_i$ (PTX sensitive) • no increase of cAMP and proton efflux			
Human enterocyte	• no proton efflux		Reduce IL-8 mRNA level	IL-13, IL-4, interferon-γ
Human synovial fibroblast [67]	• PLD activation • Inhibit NF-κB binding		Stimulate TIMP transcription	
CHO expressing human receptor	• GTPase activity • arachidonic acid release (PTX sensitive) • no increase of cAMP and $[Ca^{2+}]_i$			
CHO expressing mouse receptor	• GTPase activity			

LXA$_4$-stimulated adherence (EC$_{50}$ <1 nM). In view of G-protein coupling events in monocytes, both Ca^{2+} mobilization and adherence are PTX-sensitive. This indicates that receptor coupling in monocytes and PMN is similar to this point, although there could be different PTX-sensitive G-protein subtypes that couple to the intracellular domains of the receptors and diverge downstream in the signal transduction pathways leading to chemotaxis of monocytes and inhibition of PMN. The characteristics of ALXR in various cell types are briefly summarized in Table 6. Also, LXA$_4$ modulates MAP kinase activities on mesangial cells in a PTX-insensitive manner[77], suggesting the presence of additional novel ALXR subtypes and/or signaling pathways in these cells (Table 7).

In retinoic acid–differentiated HL-60 cells, LXA$_4$ stimulated PLD activation that is staurosporine sensitive, suggesting the involvement of PKC in signal transduction in these cells[61]. It was also demonstrated that LXA$_4$ blocks LTB$_4$ or fMLP-stimulated PMN transmigration or adhesion by regulation of β2 integrin–dependent PMN adhesion[71]. This modulatory action is partially reversed by prior exposure to genistein, a tyrosine kinase inhibitor[36]. In human renal mesangial cells, LXA$_4$ stimulates MAP kinase superfamily via two distinct receptors: one via a PTX-sensitive G protein, leading to p38 activation, and the other via a PTX-insensitive G protein, leading to ERK activation[78]. In human enterocytes (T84), ALXR activation by LXA$_4$ and LX analogs diminishes *Salmonella typhimurium*–induced IL-8 transcription[79]. The reduction of IL-8 mRNA level parallels decrements in IL-8 secretion, indicating that in these cells ALXR mechanism of action for blocking this chemokine is at the gene transcriptional level. In addition, LXA_4 induces tissue factor activity by increasing its mRNA level in EC304 cells (nonendothelial parenchymal cells) via a PTX-sensitive and PKC-dependent mechanism[80]. The ability of LXA_4 to induce tissue factor is an intriguing result. Its physiological role remains to be established in relation to LX generation and proximity to tissue factor releasing cells *in vivo*.

Molecular Mechanism in Anti-Inflammation: Are Lipoxins and ATL Unique?

LXA_4, ATL, and their stable analogs activate ALXR, which then modulates PMN responses *in vitro*, such as chemotaxis, transmigration, adhesion, degranulation, cytokine release and functions, as well as inhibits PMN recruitment in several murine models. For example, ATL analog inhibits TNF-α–initiated PMN infiltration in murine dorsal air-pouch[53] and LTB_4-induced PMN influx during dermal inflammation[55] as well as PMN-mediated second organ injury[81]. Recently, additional results from this laboratory indicated that, with PMN, ALXR interaction with LX and ATL analogs regulates a newly described polyisoprenyl phosphate (PIPP) signaling pathway[82] (Illustration 19). ALXR activation reverses LTB_4-initiated polyisoprenyl phosphate remodeling, leading to accumulation of presqualene diphosphate (PSDP), a potent negative intracellular signal in PMN which inhibits recombinant PLD and superoxide anion generation. When compared to other eicosanoids of COX, LO, and p450 products reported in the literature to display potential anti-inflammatory properties (see Table 9), LX and ATL stand apart both in mechanism and amount range for action. For example, PGE_2 reduced the antigen response[83] and inhibited macrophage phagocytosis[84], presumably via increasing intracellular cAMP levels, which in turn inhibits MAPK activation by stimulating PKA-dependent phosphorylation of Raf-1[85]. In contrast, LXA_4 does not give significant increase of cAMP levels in PMN[76]. In addition, cyclopenteneone PG such as 15-deoxy-$\Delta^{12,14}$-PGJ_2**,** in relatively high amounts, give anti-inflammatory action in adjuvant-induced arthritis in rats[86].

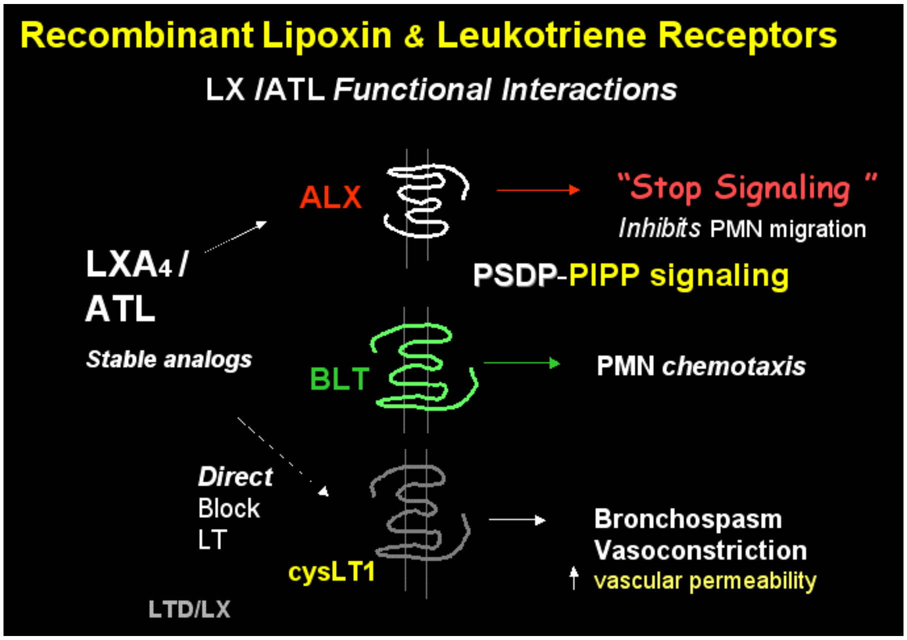
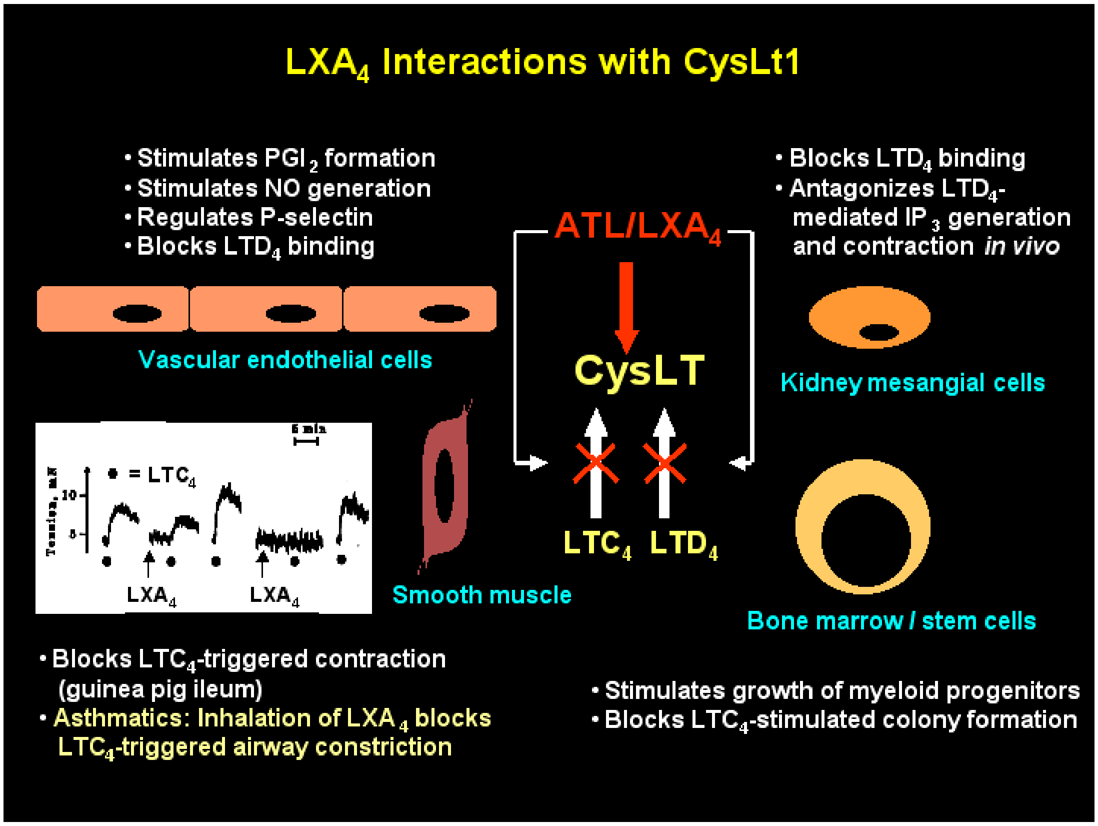


Illustration 19. LXA_4/ATL elicits bioactions with ALXR and a shared $CysLT_1$ subtype via direct ligand-receptor interaction and inhibits BLT bioactions via regulating intracellular PSDP-PIPP signaling.



LXA$_4$ Interactions with CysLt1

- Stimulates PGI$_2$ formation
- Stimulates NO generation
- Regulates P-selectin
- Blocks LTD$_4$ binding

Vascular endothelial cells

- Blocks LTD$_4$ binding
- Antagonizes LTD$_4$-mediated IP$_3$ generation and contraction *in vivo*

Kidney mesangial cells

ATL/LXA$_4$

CysLT

LTC$_4$ LTD$_4$

Smooth muscle

Bone marrow / stem cells

- Blocks LTC$_4$-triggered contraction (guinea pig ileum)
- Asthmatics: Inhalation of LXA$_4$ blocks LTC$_4$-triggered airway constriction

- Stimulates growth of myeloid progenitors
- Blocks LTC$_4$-stimulated colony formation

Illustration 20. Regulatory actions of LXA$_4$ in vascular endothelial cells, smooth muscle contraction and rat glomerular mesangial cells as well as in bone marrow via a subclass of peptido-LT receptors (CysLT$_1$).

TABLE 8
LXA$_4$-Induced Signal Transduction via CysLT$_1$ or Other Receptors

cell type	*LXA$_4$ and ATLa-evoked signal transduction*	*kinase associated*	*Gene expression*
Human endothelium	• prostacyclin generation • nitric oxide generation • no increase of $[Ca^{2+}]_i$ and proton efflux		
Human renal mesangial cells		ERK (PTX-insensitive) P38 (PTX-sensitive)	
Parenchymal cells		protein kinase C	Increase tissue factor mRNA level (PTX and GF-109203X sensitive)

TABLE 9
Lipoxins and Other Potential Anti-Inflammatory Eicosanoids

Lipid	*Bioaction*		***Proposed mechanism***
	Inhibition	Stimulation	
COX product **PGE$_2$**	• Macrophage phagocytosis (Rossi et al., 1998) [84] • Acute allergic inflammation in hamster cheek pouches (Raud et al., 1988) [83]	• Potentiates LTB$_4$-induced vascular permeability change	↑cAMP
PGI$_2$	• *LPS-induced TNF-α production (Guyton et al., 2001) [121]* • *Human PMN chemotaxis (Nicolini et al., 1990) [122]*	• Inflammatory swelling and acetic acid writhing (Murata et al., 1997) [123] • Enhances LTB$_4$-induced PMN infiltration in skin (Ekerdt et al., 1992) [124]	↑cAMP
PGD$_2$	• granulocyte infiltration in colitis (Ajuebor et al., 2000) [125] • Langerhans cell migration (Angeli et al., 2001) [126]	• eosinophil infiltration and airway hyperreactivity (Matsuoka et al., 2000) [127]	↑cAMP (DP-dependent)
Cyclopentenone prostaglandins			
15-deoxy-$\Delta^{12,14}$-PGJ$_2$	• *adjuvant-induced arthritis in rats (Kawahito et al., 2000) [86]* • *MCP-1 expression (Rovin et al., 2001) [128]* • *LPS-induced TNF-α and NO production (Guyton et al., 2001) [121]*		↓NFkB and IkB kinase (GPCR-independent) (Rossi et al., 2000) [88]
PGA$_2$	• Viral replication (Santoro et al., 1988) [129]		↓NFkB and IkB kinase (GPCR-independent)
LOX product **LXA$_4$** **LXB$_4$**	• PMN • *allergic pleural eosinophil influx (Bandeira-Melo et al., 2000) [107] and duration of pleural exudation (Bandeira-Melo et al., 2000) [89]*	• Monocyte chemotaxis • Uptake of apoptotic PMN by macrophages	↑PSDP (in PMN) (ALXR-dependent in both PMN and monocytes)
COX-LO product **ATL**	• PMN • *allergic pleural eosinophil influx (Bandeira-Melo et al., 2000) [107] and duration of pleural exudation (Bandeira-Melo et al., 2000) [89]*	• Monocyte chemoptaxis • Uptake of apoptotic PMN by macrophages	↑PSDP (in PMN) (ALXR-dependent in both PMN and monocytes)
P450 product **11,12-epoxy eicosatrienoic acid**	• PMN adhesion and VCAM-1 expression (Node et al., 1999) [130]		↓NFkB and IkB kinase ↑cAMP

However, it appears that 15-deoxy-$\Delta^{12,14}$-PGJ_2 acts via mechanisms that are independent of cell surface GPCRs[87,88]. To date, LXA_4 and ATL are the only lipid mediators that possess anti-inflammatory and proresolution properties acting in the nanomolar range, since they regulate leukocyte trafficking and contribute to the early resolution of allergic pleural edema[89].

SUMMARY

LX are the trihydroxytetraene-containing class of eicosanoids primarily generated by cell-cell interactions via transcellular biosynthesis that serve 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 toward monocytes (in a nonphlogistic fashion) that could lead to resolution of the inflammatory response and/or promotion of repair and healing. ASA impinges on this homeostatic system and evokes the endogenous biosynthesis of the carbon-15 epimers of LX, namely ATL, that mimic the bioactions of native LX in several biological systems (see Tables) and can thus modulate in part the beneficial actions of ASA in humans.

LXA_4 elicits biological actions via at least two main classes of receptor systems known to date: (1) ALXR on leukocytes and enterocytes and (2) a shared $CysLT_1$ subtype on endothelial and mesangial cells. ALXR belongs to the classical GPCR and was identified in mammalian tissues and characterized using direct evidence obtained with specific [^{3}H]-LXA_4 binding and activation of functional responses with LXA_4. ALXR is the first cloned LO-derived eicosanoid receptor; ALXR and BLT are more akin to chemokine receptors in their deduced amino acid sequences than the currently known prostanoid receptors. The cytoplasmic signaling pathways and bioactions of ALXR are cell type-specific. In human PMN, LXA_4 stimulates rapid lipid remodeling with release of arachidonic acid in a PTX-sensitive fashion, but does not trigger significant increases in intracellular Ca^{2+}. LXA_4 inhibits PMN adhesion, chemotaxis, and transmigration as well as degranulation and was implicated as an endogenous “stop signal” acting on PMN. In human monocytes, LX/ATL are potent chemoattractants and, in THP-1 cells and monocytes, LX/ATL initiate intracellular Ca^{2+} release via ALXR, but neither Ca^{2+} nor cAMP proved to be the required second messengers of LX actions in these cell types, indicating different intracellular signaling pathways despite identical receptor cDNA sequences. LXA_4 stimulates chemotaxis and adherence in monocytes but no apparent “proinflammatory” responses of these cells *in vitro or in vivo*, findings that may relate to the recruitment of monocytes to sites of wound healing and clearance. Indeed, LX and ATL stimulate the uptake of apoptotic PMN by macrophages in a nonphlogistic fashion[90].

The activation of a LX biosynthetic circuit *in vivo* requires upregulation of key enzymes by cytokines such as IL-4 and IL-13 (Illustration 5) that also control the expression of the receptor ALXR[50]. Moreover, both the temporal and spacial components in LX formation and actions are important determinants in their bioimpact during an acute inflammatory reaction[91]. LX and ATL appear to be the first recognized members of a new mediator class; namely, endogenous mediators of anti-inflammation. PGE_2 may display anti-inflammation in certain settings[92], but in most it enhances inflammation *in vivo*[54]. This is likely the result of numerous receptor isoforms and differential coupled mechanisms for PGE_2 and its diverse role in human physiology. Hence, the ability of PGE_2 to stimulate expression of 15-LO and set in place LX biosynthesis suggests that inhibition of PG can delay the onset of LX biosynthesis and prolong resolution[91].

The relationship between LX generation and current NSAID therapies is more intertwined than currently appreciated[93] in that ASA inhibits COX-1 and converts COX-2 into an ASA-triggered lipid mediator-generating system that produces an array of novel compounds from polyunsaturated fatty acids including arachidonic acid and eicosapentaenoic acid (EPA for example), some of which display potent anti-inflammatory or anti-PMN recruitment activity[94] as well as impinge on the role of these compounds in resolution. Results with ATL and LX analogues reviewed here show highly potent stereoselective actions in the sub- to nanomolar range sustaining LX and ATL actions in several *in vivo* models, indicating that these pathways (Illustrations 5, 6, 7) are likely to be important *in vivo* in human host defense. They join the many mediators that govern this process *in vivo* such as select cytokines (IL-10, IL-4, IL-13), proteins of interest in resolution[9]. In this regard, LX and ATL receptor activation not only inhibits proinflammatory events such as IL-6 gene expression[67] but stimulates IL-4 generation *in vivo*[95] and stimulates the phagocytosis of apoptotic PMN by macrophages[90]. 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. Hence, it is not surprising that others have recently found a protective action for COX-2 in cardiovascular disease[96]. 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 its natural resolution. In addition, it appears that LX also display organ-specific actions in addition to host defense and immune roles such as the eye, kidney, lung, oral and GI tract and within bone marrow progenitors, possibly involving stem cells (Tables 1 and 3).

In this context, the words of Francis Bacon (1561–1620) tell of this dilemma: *"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."*

In agreement with *in vitro* results, ALXR agonists, namely LXA_4 and 15-epi-LXA_4 as well as their stable analogs, are topically active in inhibiting PMN infiltration as well as vascular permeability in murine skin inflammation. The development of these relatively few synthetic stable analogs has already provided valuable tools to evaluate the biological roles, significance, and pharmacological actions of ALXR as well as provided a novel means to selective therapies for inflammatory diseases. Along these lines, we recently reported that ASA and other NSAIDs together with dietary omega-3 polyunsaturated fatty acid[94] (ω-3 PUFA) supplementation stimulate the generation of a novel array of bioactive compounds, i.e., 5,12,18*R*-tri HEPE derived from the interactions of ω-3 with COX-2 and ASA (Illustration 21). This uncovers entirely new biochemical pathways such as the 18R series from eicosapentanoic acid and might provide a basis to explain the beneficial actions of fish oil-based treatment reported for many human diseases[97]. These novel ATL mediators from cell-cell interactions with ω-3 conversion by COX-2 give novel ligands and biotemplates to further explore the receptors and critical pathways in endogenous anti-inflammation and expediting resolution. They also underscore the important role of the vascular endothelium in generating biogenetic intermediates and as a vast metabolic organ in and of itself[94] (Illustrations 6, 7, and 21). Together with the LX and 15-epi-LX, the identification of these novel endogenous anti-inflammatory lipid mediators[94] gives us new avenues of approach in considering therapeutics for inflammation, cardiovascular diseases, and cancer (http://letheon.bwh.harvard.edu/research/overview/cet+ri.phtml).

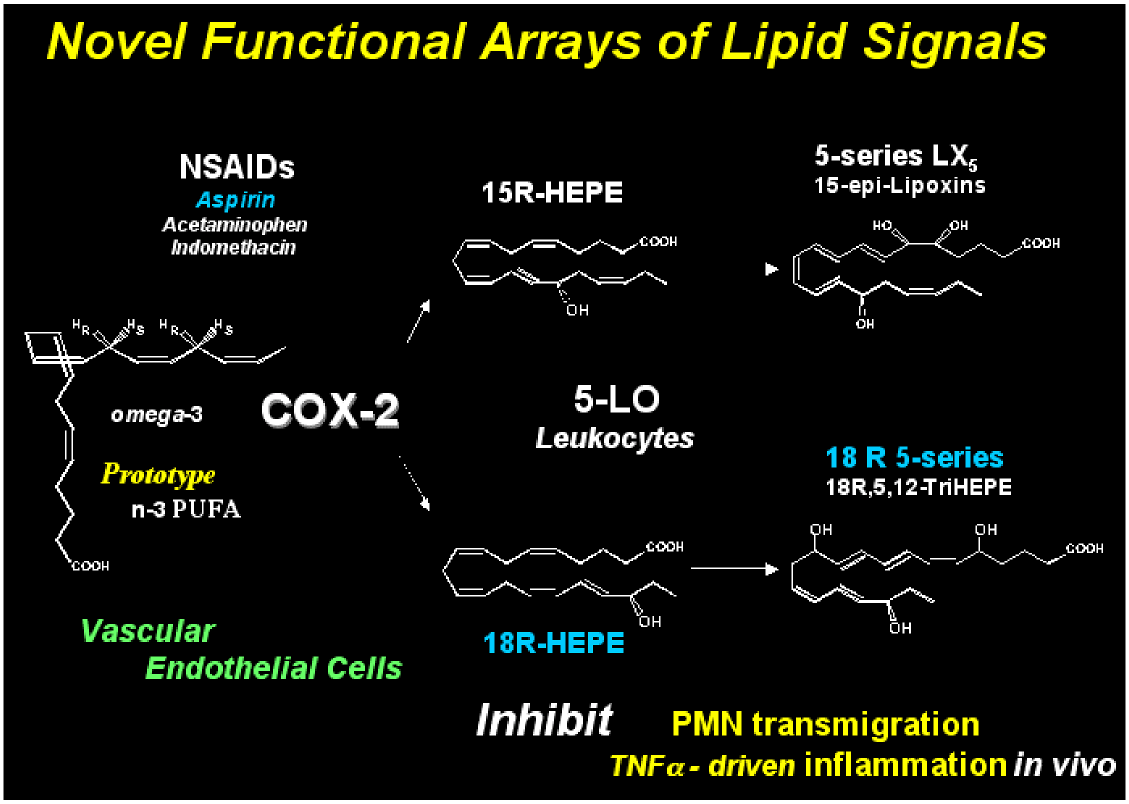


Illustration 21. Interactions of omega–3 polyunsaturated fatty acids (ω-3 PUFA) with ASA-acetylated COX-2, which generate novel arrays of bioactive compounds, such as 5,12,18R-tri HEPE or 15R-LX/ATL, each of which inhibits PMN transmigration in vitro and inflammation in vivo. A prototypic oxygenation with EPA is depicted as an ω-3-containing fatty acid[94].

ACKNOWLEDGMENTS

The author's laboratory is supported in part by National Institutes of Health grant nos. GM38765, DK50305, and P01-DE13499. The authors thank Mary Halm Small for expert assistance in manuscript preparation.

REFERENCES

1. Gallin, J.I., Snyderman, R., Fearon, D.T., Haynes, B.F., and Nathan, C., Eds. (1999) *Inflammation: Basic Principles and Clinical Correlates*. Lippincott Williams & Wilkins, Philadelphia.
2. Samuelsson, B., Dahlén, S.E., Lindgren, J.Å., Rouzer, C.A., and Serhan, C.N. (1987) Leukotrienes and lipoxins: structures, biosynthesis, and biological effects. *Science* **237**, 1171–1176.
3. Cronstein, B.N., Montesinos, M.C., and Weissmann, G. (1999) Sites of action for future therapy: an adenosine-dependent mechanism by which aspirin retains its antiinflammatory activity in cyclooxygenase-2 and NFkB knockout mice. *Osteoarthritis Cartilage* **7**, 361–363.
4. Cronstein, B.N., Montesinos, M.C., and Weissmann, G. (1999) Salicylates and sulfasalazine, but not glucocorticoids, inhibit leukocyte accumulation by an adenosine-dependent mechanism that is independent of inhibition of prostaglandin synthesis and p105 of NFkB. *Proc. Natl. Acad. Sci. U.S.A.* **96**, 6377–6381.
5. Krump, E., Picard, S., Mancini, J., and Borgeat, P. (1997) Suppression of leukotriene B_4 biosynthesis by endogenous adenosine in ligand-activated human neutrophils. *J. Exp. Med.* **186**, 1401–1406.
6. Qiu, F.-H., Wada, K., Stahl, G.L., and Serhan, C.N. (2000) IMP and AMP deaminase in reperfusion injury down-regulates neutrophil recruitment. *Proc. Natl. Acad. Sci. U.S.A.* **97**, 4267–4272.
7. Serhan, C.N. (1994) Lipoxin biosynthesis and its impact in inflammatory and vascular events. *Biochim. Biophys. Acta* **1212**, 1–25.

8. Serhan, C.N., Haeggström, J.Z., and Leslie, C.C. (1996) Lipid mediator networks in cell signaling: update and impact of cytokines. *FASEB J.* **10**, 1147–1158.
9. de Waal Malefyt, R. (1999) Role of interleukin-10, interleukin-4, and interleukin-13 in resolving inflammatory responses. In *Inflammation: Basic Principles and Clinical Correlates.* Gallin, J.I., Snyderman, R., Fearon, D.T., Haynes, B.F., and Nathan, C., Eds. Lippincott Williams & Wilkins, Philadephia. pp. 837–849.
10. Diamond, P., McGinty, A., Sugrue, D., Brady, H.R., and Godson, C. (1999) Regulation of leukocyte trafficking by lipoxins. *Clin. Chem. Lab. Med.* **37**, 293–297.
11. Marcus, A.J. (1995) Aspirin as prophylaxis against colorectal cancer. *N. Engl. J. Med.* **333**, 656–658.
12. Vane, J.R. (1982) Adventures and excursions in bioassay: the stepping stones to prostacyclin. In *Les Prix Nobel: Nobel Prizes, Presentations, Biographies and Lectures.* Almqvist & Wiksell, Stockholm. pp. 181–206.
13. Chiang, N., Takano, T., Clish, C.B., Petasis, N.A., Tai, H.-H., and Serhan, C.N. (1998) Aspirin-triggered 15-epi-lipoxin A_4 (ATL) generation by human leukocytes and murine peritonitis exudates: development of a specific 15-epi-LXA_4 ELISA. *J. Pharmacol. Exp. Ther.* **287**, 779–790.
14. Clària, J. and Serhan, C.N. (1995) Aspirin triggers previously undescribed bioactive eicosanoids by human endothelial cell-leukocyte interactions. *Proc. Natl. Acad. Sci. U.S.A.* **92**, 9475–9479.
15. Clària, J., Lee, M.H., and Serhan, C.N. (1996) Aspirin-triggered lipoxins (15-epi-LX) are generated by the human lung adenocarcinoma cell line (A549)-neutrophil interactions and are potent inhibitors of cell proliferation. *Mol. Med.* **2**, 583–596.
16. Showell, H.J. and Cooper, K. (1999) Inhibitors and antagonists of cyclooxygenase, 5-lipoxygenase, and platelet activating factor. In *Inflammation: Basic Principles and Clinical Correlates.* Gallin, J.I., Snyderman, R., Fearon, D.T., Haynes, B.F., and Nathan, C., Eds. Lippincott Williams & Wilkins, Philadelphia. pp. 1177–1193.
17. Malaviya, R. and Abraham, S.N. (2000) Role of mast cell leukotrienes in neutrophil recruitment and bacterial clearance in infectious peritonitis. *J. Leukoc. Biol.* **67**, 841–846.
18. Swan, S.K., Rudy, D.W., Lasseter, K.C., Ryan, C.F., Buechel, K.L., Lambrecht, L.J., Pinto, M.B., Dilzer, S.C., Obrda, O., Sundblad, K.J., Gumbs, C.P., Ebel, D., Quan, H., Larson, P.J., Schwartz, J.I., Musliner, T., Gertz, B.J., Brater, D.C., and Yao, S.L. (2000) Effect of cyclooxygenase-2 inhibition on renal function in elderly persons receiving a low-salt diet: a randomized, controlled trial. *Ann. Intern. Med.* **133**, 1–9.
19. Cannon, G.W., Caldwell, J.R., Holt, P., McLean, B., Seidenberg, B., Bolognese, J., Ehrich, E., Mukhopadhyay, S., and Daniels, B. (2000) Rofecoxib, a specific inhibitor of cyclooxygenase 2, with clinical efficacy comparable with that of diclofenac sodium: results of a one-year, randomized, clinical trial in patients with osteoarthritis of the knee and hip. Rofecoxib Phase III Protocol 035 Study Group. *Arthritis Rheum.* **43**, 978–987.
20. Serhan, C.N., Maddox, J.F., Petasis, N.A., Akritopoulou-Zanze, I., Papayianni, A., Brady, H.R., Colgan, S.P., and Madara, J.L. (1995) Design of lipoxin A_4 stable analogs that block transmigration and adhesion of human neutrophils. *Biochemistry* **34**, 14609–14615.
21. Serhan, C.N. (1997) Lipoxins and novel aspirin-triggered 15-epi-lipoxins (ATL): a jungle of cell-cell interactions or a therapeutic opportunity? *Prostaglandins* **53**, 107–137.
22. Grandordy, B.M., Lacroix, H., Mavoungou, E., Krilis, S., Crea, A.E., Spur, B.W., and Lee, T.H. (1990) Lipoxin A_4 inhibits phosphoinositide hydrolysis in human neutrophils. *Biochem. Biophys. Res. Commun.* **167**, 1022–1029.
23. Nigam, S., Fiore, S., Luscinskas, F.W., and Serhan, C.N. (1990) Lipoxin A_4 and lipoxin B_4 stimulate the release but not the oxygenation of arachidonic acid in human neutrophils: dissociation between lipid remodeling and adhesion. *J. Cell. Physiol.* **143**, 512–523.
24. Yokomizo, T., Izumi, T., Chang, K., Takuwa, T., and Shimizu, T. (1997) A G-protein-coupled receptor for leukotriene B_4 that mediates chemotaxis. *Nature* **387**, 620–624.
25. Huang, W.-W., Garcia-Zepeda, E.A., Sauty, A., Oettgen, H.C., Rothenberg, M.E., and Luster, A.D. (1998) Molecular and biological characterization of the murine leukotriene B_4 receptor expressed on eosinophils. *J. Exp. Med.* **188**, 1063–1074.
26. Akbar, G.K.M., Dasari, V.R., Webb, T.E., Ayyanathan, K., Pillarisetti, K., Sandhu, A.K., Athwar, R.S., Daniel, J.L., Ashby, B., Barnard, E.A., and Kunapuli, S.P. (1996) Molecular cloning of a novel P2 purinoreceptor from human erythroleukemia cells. *J. Biol. Chem.* **271**, 18363–18367.
27. Owman, C., Garzino-Demo, A., Cocchi, F., Popovic, M., Sabirsh, A., and Gallo, R.C. (1998) The leukotriene B_4 receptor functions as a novel type of coreceptor mediating entry of primary HIV-1 isolates into CD4-positive cells. *Proc. Natl. Acad. Sci. U.S.A.* **95**, 9530–9534.
28. Chiang, N., Fierro, I.M., Gronert, K., and Serhan, C.N. (2000) Activation of lipoxin A_4 receptors by aspirin-triggered lipoxins and select peptides evokes ligand-specific responses in inflammation. *J. Exp. Med.* **191**, 1197–1207.
29. Lehr, H.-A., Frei, B., and Arfors, K.-E. (1994) Vitamin C prevents cigarette smoke-induced leukocyte aggregation and adhesion to endothelium in vivo. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 7688–7692.
30. Fiore, S. and Serhan, C.N. (1989) Phospholipid bilayers enhance the stability of leukotriene A_4 and epoxytetraenes: stabilization of eicosanoids by liposomes. *Biochem. Biophys. Res. Commun.* **159**, 477–481.

31. Nassar, G.M., Morrow, J.D., Roberts, L.J., II, Lakkis, F.G., and Badr, K.F. (1994) Induction of 15-lipoxygenase by interleukin-13 in human blood monocytes. *J. Biol. Chem.* **269**, 27631–27634.
32. Levy, B.D., Romano, M., Chapman, H.A., Reilly, J.J., Drazen, J., and Serhan, C.N. (1993) Human alveolar macrophages have 15-lipoxygenase and generate 15(S)-hydroxy-5,8,11-cis-13-trans-eicosatetraenoic acid and lipoxins. *J. Clin. Invest.* **92**, 1572–1579.
33. Levy, G.N. (1997) Prostaglandin H synthases, nonsteroidal anti-inflammatory drugs, and colon cancer. *FASEB J.* **11**, 234–247.
34. Savage, M.P., Goldberg, S., Bove, A.A., Deutsch, E., Vetrovec, G., Macdonald, R.G., Bass, T., Margolis, J.R., Whitworth, H.B., Taussig, A., Hirshfeld, J.W., Cowley, M., Hill, J.A., Marks, R.G., Fischman, D.L., Handberg, E., Herrmann, H., and Pepine, C.J. (1995) Effect of thromboxane A_2 blockade on clinical outcome and restenosis after successful coronary angioplasty. *Circulation* **92**, 3194–3200.
35. Samuelsson, B. (1982) From studies of biochemical mechanisms to novel biological mediators: prostaglandin endoperoxides, thromboxanes and leukotrienes. In *Les Prix Nobel: Nobel Prizes, Presentations, Biographies and Lectures.* Almqvist & Wiksell, Stockholm. pp. 153–174.
36. Papayianni, A., Serhan, C.N., and Brady, H.R. (1996) Lipoxin A_4 and B_4 inhibit leukotriene-stimulated interactions of human neutrophils and endothelial cells. *J. Immunol.* **156**, 2264–2272.
37. Tamaoki, J., Tagaya, E., Yamawaki, I., and Konno, K. (1995) Lipoxin A_4 inhibits cholinergic neurotransmission through nitric oxide generation in the rabbit trachea. *Eur. J. Pharmacol.* **287**, 233–238.
38. Sanak, M., Levy, B.D., Clish, C.B., Chiang, N., Gronert, K., Mastalerz, L., Serhan, C.N., and Szczeklik, A. (2000) Aspirin-tolerant asthmatics generate more lipoxins than aspirin-intolerant asthmatics. *Eur. Respir. J.* **16**, 44–49.
39. Clària, J., Titos, E., Jiménez, W., Ros, J., Ginès, P., Arroyo, V., Rivera, F., and Rodés, J. (1998) Altered biosynthesis of leukotrienes and lipoxins and host defense disorders in patients with cirrhosis and ascites. *Gastroenterology* **115**, 147–156.
40. Stenke, L., Näsman-Glaser, B., Edenius, C., Samuelsson, J., Palmblad, J., and Lindgren, J.Å. (1990) Lipoxygenase products in myeloproliferative disorders: increased leukotriene C_4 and decreased lipoxin formation in chronic myeloid leukemia. *Adv. Prostaglandin Thromboxane Leukot. Res.* **21B**, 883–886.
41. Stenke, L., Edenius, C., Samuelsson, J., and Lindgren, J.A. (1991) Deficient lipoxin synthesis: a novel platelet dysfunction in myeloproliferative disorders with special reference to blastic crisis of chronic myelogenous leukemia. *Blood* **78**, 2989–2995.
42. Stenke, L., Nasman-Glaser, B., Edenius, C., Samuelsson, J., Palmblad, J., and Lindgren, J.A. (1991) Lipoxygenase products in myeloproliferative disorders: increased leukotriene C_4 and decreased lipoxin formation in chronic myeloid leukemia. *Adv. Prostaglandin Thromboxane Leukot. Res.* **21B**, 883–886.
43. Stenke, L., Mansour, M., Edenius, C., Reizenstein, P., and Lindgren, J.A. (1991) Formation and proliferative effects of lipoxins in human bone marrow. *Biochem. Biophys. Res. Commun.* **180**, 255–261.
44. Stenke, L., Reizenstein, P., and Lindgren, J.A. (1994) Leukotrienes and lipoxins--new potential performers in the regulation of human myelopoiesis. *Leukemia Res.* **18**, 727-732.
45. Pouliot, M., Clish, C.B., Petasis, N.A., Van Dyke, T.E., and Serhan, C.N. (2000) Lipoxin A_4 analogues inhibit leukocyte recruitment to *Porphyromonas gingivalis*: a role for cyclooxygenase-2 and lipoxins in periodontal disease. *Biochemistry* **39**, 4761–4768.
46. Brezinski, D.A., Nesto, R.W., and Serhan, C.N. (1992) Angioplasty triggers intracoronary leukotrienes and lipoxin A_4. Impact of aspirin therapy. *Circulation* **86**, 56–63.
47. Edenius, C., Kumlin, M., Björk, T., Anggard, A., and Lindgren, J.A. (1990) Lipoxin formation in human nasal polyps and bronchial tissue. *FEBS Lett.* **272**, 25–28.
48. Cotran, P.R., Hsu, C., Fokin, V.V., Petasis, N.A., and Serhan, C.N. (1995) Lipoxin analogs reduce intraocular pressure in rabbits. In International Symposium on Experimental and Clinical Ocular Pharmacology and Pharmaceutics, Geneva, Switzerland, Sept. 28-Oct. 1, 1995.
49. Cotran, P.R., Hsu, C., and Serhan, C.N. (1995) Lipoxin derivatives reduce intraocular pressure in rabbits. In Association for Research in Vision and Ophthalmology.
50. Gronert, K., Gewirtz, A., Madara, J.L., and Serhan, C.N. (1998) Identification of a human enterocyte lipoxin A_4 receptor that is regulated by IL-13 and IFN-g and inhibits TNF-a-induced IL-8 release. *J. Exp. Med.* **187**, 1285–1294.
51. Clish, C.B., Levy, B.D., Chiang, N., Tai, H.-H., and Serhan, C.N. (2000) Oxidoreductases in lipoxin A_4 metabolic inactivation. *J. Biol. Chem.* **275**, 25372–25380.
52. Maddox, J.F., Colgan, S.P., Clish, C.B., Petasis, N.A., Fokin, V.V., and Serhan, C.N. (1998) Lipoxin B_4 regulates human monocyte/neutrophil adherence and motility: design of stable lipoxin B_4 analogs with increased biologic activity. *FASEB J.* **12**, 487–494.
53. Clish, C.B., O'Brien, J.A., Gronert, K., Stahl, G.L., Petasis, N.A., and Serhan, C.N. (1999) Local and systemic delivery of a stable aspirin-triggered lipoxin prevents neutrophil recruitment *in vivo*. *Proc. Natl. Acad. Sci. U.S.A.* **96**, 8247–8252.
54. Takano, T., Clish, C.B., Gronert, K., Petasis, N., and Serhan, C.N. (1998) Neutrophil-mediated changes in vascular permeability are inhibited by topical application of aspirin-triggered 15-epi-lipoxin A_4 and novel lipoxin B_4 stable analogues. *J. Clin. Invest.* **101**, 819–826.

55. Takano, T., Fiore, S., Maddox, J.F., Brady, H.R., Petasis, N.A., and Serhan, C.N. (1997) Aspirin-triggered 15-epi-lipoxin A_4 and LXA_4 stable analogs are potent inhibitors of acute inflammation: evidence for anti-inflammatory receptors. *J. Exp. Med.* **185**, 1693–1704.
56. Lee, T.H., Horton, C.E., Kyan-Aung, U., Haskard, D., Crea, A.E., and Spur, B.W. (1989) Lipoxin A_4 and lipoxin B_4 inhibit chemotactic responses of human neutrophils stimulated by leukotriene B_4 and *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine. *Clin. Sci.* **77**, 195–203.
57. Soyombo, O., Spur, B.W., and Lee, T.H. (1994) Effects of lipoxin A_4 on chemotaxis and degranulation of human eosinophils stimulated by platelet-activating factor and N-formyl-L-methionyl-L-leucyl-L-phenylalanine. *Allergy* **49**, 230–234.
58. Colgan, S.P., Serhan, C.N., Parkos, C.A., Delp-Archer, C., and Madara, J.L. (1993) Lipoxin A_4 modulates transmigration of human neutrophils across intestinal epithelial monolayers. *J. Clin. Invest.* **92**, 75–82.
59. Brezinski, D.A. and Serhan, C.N. (1991) Characterization of lipoxins by combined gas chromatography and electron-capture negative ion chemical ionization mass spectrometry: formation of lipoxin A_4 by stimulated human whole blood. *Biol. Mass Spectrom.* **20**, 45–52.
60. Fiore, S., Ryeom, S.W., Weller, P.F., and Serhan, C.N. (1992) Lipoxin recognition sites. Specific binding of labeled lipoxin A_4 with human neutrophils. *J. Biol. Chem.* **267**, 16168–16176.
61. Fiore, S., Romano, M., Reardon, E.M., and Serhan, C.N. (1993) Induction of functional lipoxin A_4 receptors in HL-60 cells. *Blood* **81**, 3395–3403.
62. Nomura, H., Nielsen, B.W., and Matsushima, K. (1993) Molecular cloning of cDNAs encoding a LD78 receptor and putative leukocyte chemotactic peptide receptors. *Int. Immunol.* **5**, 1239.
63. Perez, H.D., Holmes, R., Kelly, E., McClary, J., and Andrews, W.H. (1992) Cloning of a cDNA encoding a receptor related to the formyl peptide receptor of human neutrophils. *Gene* **118**, 303–304.
64. Fiore, S., Maddox, J.F., Perez, H.D., and Serhan, C.N. (1994) Identification of a human cDNA encoding a functional high affinity lipoxin A_4 receptor. *J. Exp. Med.* **180**, 253–260.
65. Bao, L., Gerard, N.P., Eddy, R.L., Jr., Shows, T.B., and Gerard, C. (1992) Mapping of genes for the human C5a receptor (C5AR), human FMLP receptor (FPR), and two FMLP receptor homologue orphan rceptors (FPRH1, FPRH2) to chromosome 19. *Genomics* **13**, 437–440.
66. Maddox, J.F., Hachicha, M., Takano, T., Petasis, N.A., Fokin, V.V., and Serhan, C.N. (1997) Lipoxin A_4 stable analogs are potent mimetics that stimulate human monocytes and THP-1 cells via a G-protein linked lipoxin A_4 receptor. *J. Biol. Chem.* **272**, 6972–6978.
67. Sodin-Semrl, S., Taddeo, B., Tseng, D., Varga, J., and Fiore, S. (2000) Lipoxin A_4 inhibits IL-1 beta-induced IL-6, IL-8, and matrix metalloproteinase-3 production in human synovial fibroblasts and enhances synthesis of tissue inhibitors of metalloproteinases. *J. Immunol.* **164**, 2660–2666.
68. Baldwin, J.M. (1993) The probable arrangement of the helices in G protein-coupled receptors. *EMBO J.* **12**, 1693–1703.
69. Toh, H., Ichikawa, A., and Narumiya, S. (1995) Molecular evolution of receptors for eicosanoids. *FEBS Lett.* **361**, 17–21.
70. Ushikubi, F., Hirata, M., and Narumiya, S. (1995) Molecular biology of prostanoid receptors; an overview. *J. Lipid Mediators Cell Signalling* **12**, 343–359.
71. Fiore, S. and Serhan, C.N. (1995) Lipoxin A_4 receptor activation is distinct from that of the formyl peptide receptor in myeloid cells: inhibition of CD11/18 expression by lipoxin A_4-lipoxin A_4 receptor interaction. *Biochemistry* **34**, 16678–16686.
72. Klein, C., Paul, J.I., Sauvé, K., Schmidt, M.M., Arcangeli, L., Ransom, J., Trueheart, J., Manfredi, J.P., Broach, J.R., and Murphy, A.J. (1998) Identification of surrogate agonists for the human FPRL-1 receptor by autocrine selection in yeast. *Nat. Biotechnol.* **16**, 1334–1337.
73. Badr, K.F., DeBoer, D.K., Schwartzberg, M., and Serhan, C.N. (1989) Lipoxin A_4 antagonizes cellular and in vivo actions of leukotriene D_4 in rat glomerular mesangial cells: evidence for competition at a common receptor. *Proc. Natl. Acad. Sci. U.S.A.* **86**, 3438–3442.
74. Wikström Jonsson, E. (1998) Functional characterization of receptors for cysteinyl leukotrienes in muscle. Doctoral Thesis, Karolinska Institute, Stockholm.
75. Gronert, K., Martinsson-Niskanen, T., Ravasi, S., Chiang, N., and Serhan, C.N. (2001) Selectivity of recombinant human leukotriene D_4, leukotriene B_4, and lipoxin A_4 receptors with aspirin-triggered 15-epi-LXA_4 and regulation of vascular and inflammatory responses. *Am. J. Pathol.* **158**, 3–9.
76. Romano, M., Maddox, J.F., and Serhan, C.N. (1996) Activation of human monocytes and the acute monocytic leukemia cell line (THP-1) by lipoxins involves unique signaling pathways for lipoxin A_4 versus lipoxin B_4. *J. Immunol.* **157**, 2149–2154.
77. McMahon, B., McPhilips, F., Fanning, A., Brady, H.R., and Godson, C. (1998) Modulation of mesangial cell MAP kinase activities by leukotriene D_4 and lipoxin A_4. *J. Am. Soc. Nephrol.* **9**, 355A.
78. McMahon, B., Stenson, C., McPhillips, F., Fanning, A., Brady, H.R., and Godson, C. (2000) Lipoxin A_4 antagonizes the mitogenic effects of leukotriene D_4 in human renal mesangial cells: differential activation of MAP kinases through distinct receptors. *J. Biol. Chem.* **275**, 27566–27575.

79. Gewirtz, A.T., McCormick, B., Neish, A.S., Petasis, N.A., Gronert, K., Serhan, C.N., and Madara, J.L. (1998) Pathogen-induced chemokine secretion from model intestinal epithelium is inhibited by lipoxin A_4 analogs. *J. Clin. Invest.* **101**, 1860–1869.
80. Maderna, P., Godson, C., Hannify, G., Murphy, M., and Brady, H.R. (2000) Influence of lipoxin A(4) and other lipoxygenase-derived eicosanoids on tissue factor expression. *Am. J. Physiol. Cell Physiol.* **279**, C945–C953.
81. Chiang, N., Gronert, K., Clish, C.B., O'Brien, J.A., Freeman, M.W., and Serhan, C.N. (1999) Leukotriene B_4 receptor transgenic mice reveal novel protective roles for lipoxins and aspirin-triggered lipoxins in reperfusion. *J. Clin. Invest.* **104**, 309–316.
82. Levy, B.D., Fokin, V.V., Clark, J.M., Wakelam, M.J.O., Petasis, N.A., and Serhan, C.N. (1999) Polyisoprenyl phosphate (PIPP) signaling regulates phospholipase D activity: a "stop" signaling switch for aspirin-triggered lipoxin A_4. *FASEB J.* **13**, 903–911.
83. Raud, J., Dahlén, S.-E., Sydbom, A., Lindbom, L., and Hedqvist, P. (1988) Enhancement of acute allergic inflammation by indomethacin is reversed by prostaglandin E2: apparent correlation with in vivo modulation of mediator release. *Proc. Natl. Acad. Sci. U.S.A.* **85**, 2315–2319.
84. Rossi, A.G., McCutcheon, J.C., Roy, N., Chilvers, E.R., Haslett, C., and Dransfield, I. (1998) Regulation of macrophage phagocytosis of apoptotic cells by cAMP. *J. Immunol.* **160**, 3562–3568.
85. Pillinger, M.H., Capodici, C., Han, G., and Weissmann, G. (1997) Inflammation and anti-inflammation: gating of cell/cell adhesion at the level of mitogen-activated protein kinases. *Ann. N. Y. Acad. Sci.* **832**, 1–12.
86. Kawahito, Y., Kondo, M., Tsubouchi, Y., Hashiramoto, A., Bishop-Bailey, D., Inoue, K.-i., Kohno, M., Yamada, R., Hla, T., and Sano, H. (2000) 15-deoxy-$D^{12,14}$-PGJ_2 induces synoviocyte apoptosis and suppresses adjuvant-induced arthritis in rats. *J. Clin. Invest.* **106**, 189–197.
87. Serhan, C.N. and Devchand, P.R. (2001) Novel antiinflammatory targets for asthma: A role for PPARg? *Am. J. Respir. Cell Mol. Biol.* **24**, 658–661.
88. Rossi, A., Kapahi, P., Natoli, G., Takahashi, T., Chen, Y., Karin, M., and Santoro, M.G. (2000) Anti-inflammatory cyclopentenone prostaglandins are direct inhibitors of IkappaB kinase. *Nature* **403**, 103–108.
89. Bandeira-Melo, C., Serra, M.F., Diaz, B.L., Cordeiro, R.S.B., Silva, P.M.R., Lenzi, H.L., Bakhle, Y.S., Serhan, C.N., and Martins, M.A. (2000) Cyclooxygenase-2-derived prostaglandin E_2 and lipoxin A_4 accelerate resolution of allergic edema in *Angiostrongylus costaricensis*-infected rats: relationship with concurrent eosinophilia. *J. Immunol.* **164**, 1029–1036.
90. Godson, C., Mitchell, S., Harvey, K., Petasis, N.A., Hogg, N., and Brady, H.R. (2000) Cutting edge: lipoxins rapidly stimulate nonphlogistic phagocytosis of apoptotic neutrophils by monocyte-derived macrophages. *J. Immunol.* **164**, 1663–1667.
91. Levy, B.D., Clish, C.B., Schmidt, B., Gronert, K., and Serhan, C.N. (2001) Lipid mediator class switching during acute inflammation: signals in resolution. *Nat. Immunol.* **2**, 612–619.
92. Dahlén, B. (1993) Leukotrienes as mediators of asthma induced by aspirin and allergen. Thesis, Department of Thoracic Medicine, Karolinska Institutet, Stockholm, p.68.
93. FitzGerald, G.A. and Patrono, C. (2001) The coxibs, selective inhibitors of cyclooxygenase-2. *N. Engl. J. Med.* **345**, 433–442.
94. Serhan, C.N., Clish, C.B., Brannon, J., Colgan, S.P., Chiang, N., and Gronert, K. (2000) Novel functional sets of lipid-derived mediators with antiinflammatory actions generated from omega-3 fatty acids via cyclooxygenase 2-nonsteroidal antiinflammatory drugs and transcellular processing. *J. Exp. Med.* **192**, 1197–1204.
95. Hachicha, M., Pouliot, M., Petasis, N.A., and Serhan, C.N. (1999) Lipoxin (LX)A_4 and aspirin-triggered 15-epi-LXA_4 inhibit tumor necrosis factor 1a-initiated neutrophil responses and trafficking: regulators of a cytokine-chemokine axis. *J. Exp. Med.* **189**, 1923–1929.
96. Shinmura, K., Tang, X.-L., Wang, Y., Xuan, Y.-T., Liu, S.-Q., Takano, H., Bhatnagar, A., and Bolli, R. (2000) Cyclooxygenase-2 mediates the cardioprotective effects of the late phase of ischemic preconditioning in conscious rabbits. *Proc. Natl. Acad. Sci. U.S.A.* **97**, 10197–10202.
97. Bracco, U. and Deckelbaum, R.J., Eds. (1992) *Polyunsaturated Fatty Acids in Human Nutrition*. Raven Press, New York.
98. Beckman, B.S., Despinasse, B.P., and Spriggs, L. (1992) Actions of lipoxins A_4 and B_4 on signal transduction events in Friend erythroleukemia cells. *Proc. Soc. Exp. Biol. Med.* **201**, 169–173.
99. Serhan, C.N., Hirsch, U., Palmblad, J., and Samuelsson, B. (1987) Formation of lipoxin A by granulocytes from eosinophilic donors. *FEBS Lett.* **217**, 242–246.
100. Katoh, T., Takahashi, K., DeBoer, D.K., Serhan, C.N., and Badr, K.F. (1992) Renal hemodynamic actions of lipoxins in rats: a comparative physiological study. *Am. J. Physiol.* **263**, F436–442.
101. Munger, K.A., Montero, A., Fukunaga, M., Uda, S., Yura, T., Imai, E., Kaneda, Y., Valdivielso, J.M., and Badr, K.F. (1999) Transfection of rat kidney with human 15-lipoxygenase suppresses inflammation and preserves function in experimental glomerulonephritis. *Proc. Natl. Acad. Sci. U.S.A.* **96**, 13375–13380.
102. Feng, Z., Godfrey, H.P., Mandy, S., Strudwick, S., Lin, K.-T., Heilman, E., and Wong, P.Y.-K. (1996) Leukotriene B_4 modulates *in vivo* expression of delayed-type hypersensitivity by a receptor-mediated mechanism: regulation by lipoxin A_4. *J. Pharmacol. Exp. Ther.* **278**, 950–956.

103. Chavis, C., Chanez, P., Vachier, I., Bousquet, J., Michel, F.B., and Godard, P. (1995) 5,15-diHETE and lipoxins generated by neutrophils from endogenous arachidonic acid as asthma biomarkers. *Biochem. Biophys. Res. Commun.* **207**, 273–279.
104. Christie, P.E., Spur, B.W., and Lee, T.H. (1992) The effects of lipoxin A_4 on airway responses in asthmatic subjects. *Am. Rev. Respir. Dis.* **145**, 1281–1284.
105. Thomas, E., Leroux, J.L., Blotman, F., and Chavis, C. (1995) Conversion of endogenous arachidonic acid to 5,15-diHETE and lipoxins by polymorphonuclear cells from patients with rheumatoid arthritis. *Inflamm. Res.* **44**, 121–124.
106. Filep, J.G., Zouki, C., Petasis, N.A., Hachicha, M., and Serhan, C.N. (1999) Anti-inflammatory actions of lipoxin A_4 stable analogs are demonstrable in human whole blood: modulation of leukocyte adhesion molecules and inhibition of neutrophil-endothelial interactions. *Blood* **94**, 4132–4142.
107. Bandeira-Melo, C., Bozza, P.T., Diaz, B.L., Cordeiro, R.S.B., Jose, P.J., Martins, M.A., and Serhan, C.N. (2000) Cutting edge: lipoxin (LX) A_4 and aspirin-triggered 15-epi-LXA_4 block allergen-induced eosinophil trafficking. *J. Immunol.* **164**, 2267–2271.
108. Ramstedt, U., Serhan, C.N., Nicolaou, K.C., Webber, S.E., Wigzell, H., and Samuelsson, B. (1987) Lipoxin A-induced inhibition of human natural killer cell cytotoxicity: studies on stereospecificity of inhibition and mode of action. *J. Immunol.* **138**, 266–270.
109. Leszczynski, D. and Ustinov, J. (1990) Protein kinase C-regulated production of prostacyclin by rat endothelium is increased in the presence of lipoxin A4. *FEBS Lett.* **263**, 117–120.
110. Scalia, R., Gefen, J., Petasis, N.A., Serhan, C.N., and Lefer, A.M. (1997) Lipoxin A_4 stable analogs inhibit leukocyte rolling and adherence in the rat mesenteric microvasculature: role of P-selectin. *Proc. Natl. Acad. Sci. U.S.A.* **94**, 9967–9972.
111. Dahlén, S.-E. and Serhan, C.N. (1991) Lipoxins: bioactive lipoxygenase interaction products. In *Lipoxygenases and Their Products.* Wong, A. and Crooke, S.T., Eds. Academic Press, San Diego. pp. 235–276.
112. Su, S.B., Gong, W., Gao, J.-L., Shen, W., Murphy, P.M., Oppenheim, J.J., and Wang, J.M. (1999) A seven-transmembrane, G protein-coupled receptor, FPRL1, mediates the chemotactic activity of serum amyloid A for human phagocytic cells. *J. Exp. Med.* **189**, 395–402.
113. Deng, X., Ueda, H., Su, S.B., Gong, W., Dunlop, N.M., Gao, J.-L., Murphy, P.M., and Wang, J.M. (1999) A synthetic peptide derived from human immunodeficiency virus type 1 gp120 downregulates the expression and function of chemokine receptors CCR5 and CXCR4 in monocytes by activating the 7-transmembrane G-protein-coupled receptor FPRL1/LXA4R. *Blood* **94**, 1165–1173.
114. Shen, W., Proost, P., Li, B., Gong, W., Le, Y., Sargeant, R., Murphy, P.M., Van Damme, J., and Wang, J.M. (2000) Activation of the chemotactic peptide receptor FPRL1 in monocytes phosphorylates the chemokine receptor CCR5 and attenuates cell responses to selected chemokines. *Biochem. Biophys. Res. Commun.* **272**, 276–283.
115. Su, S.B., Gao, J.I., Gong, W., Dunlop, N.M., Murphy, P.M., Oppenheim, J.J., and Wang, J.M. (1999) T21/DP107, a synthetic leucine zipper-like domain of the HIV-1 envelope gp41, attracts and activates human phagocytes by using G-protein-coupled formyl peptide receptors. *J. Immunol.* **162**, 5924–5930.
116. Le, Y., Jiang, S., Hu, J., Gong, W., Su, S., Dunlop, N.M., Shen, W., Li, B., and Wang, J.M. (2000) N36, a synthetic N-terminal heptad repeat domain of the HIV-1 envelope protein gp41, is an activator of human phagocytes. *Clin. Immunol.* **96**, 236–242.
117. Dahlgren, C., Christophe, T., Boulay, F., Madianos, P.N., Rabiet, M.J., and Karlsson, A. (2000) The synthetic chemoattractant Trp-Lys-Tyr-Met-Val-DMet activates neutrophils preferentially through the lipoxin A_4 receptor. *Blood* **95**, 1810–1818.
118. Yang, D., Chen, Q., Schmidt, A.P., Anderson, G.M., Wang, J.M., Wooters, J., Oppenheim, J.J., and Chertov, O. (2000) LL-37, the neutrophil granule- and epithelial cell-derived cathelicidin, utilizes formyl peptide receptor-like 1 (FPRL1) as a receptor to chemoattract human peripheral blood neutrophils, monocytes, and T cells. *J. Exp. Med.* **192**, 1069–1074.
119. Le, Y., Gong, W., Tiffany, H.L., Tumanov, A., Nedospasov, S., Shen, W., Dunlop, N.M., Gao, J.-L., Murphy, P.M., Oppenheim, J.J., and Wang, J.M. (2001) Amyloid b_{42} activates a G-protein-coupled chemoattractant receptor, FPR-like-1. *J. Neuroscience* **21(RC123),** 1–5.
120. Le, Y., Yazawa, H., Gong, W., Yu, Z., Ferrans, V.J., Murphy, P.M., and Wang, J.M. (2001) The neurotoxic prion peptide fragment PrP(106-126) is a chemotactic agonist for the G protein-coupled receptor formyl peptide receptor-like 1. *J. Immunol.* **166**, 1448–1451.
121. Guyton, K., Bond, R., Reilly, C., Gilkeson, G., Halushka, P., and Cook, J. (2001) Differential effects of 15-deoxy-delta(12,14)-prostaglandin J2 and a peroxisome proliferator-activated receptor gamma agonist on macrophage activation. *J. Leukocyte Biol.* **69**, 631–638.
122. Nicolini, F.A., Mehta, P., Lawson, D., and Mehta, J.L. (1990) Reduction in human neutrophil chemotaxis by the prostacyclin analogue iloprost. *Thromb. Res.* **59**, 669–674.
123. Murata, T., Ushikubi, F., Matsuoka, T., Hirata, M., Yamasaki, A., Sugimoto, Y., Ichikawa, A., Aze, Y., Tanaka, T., Yoshida, N., Ueno, A., Oh-ishi, S., and Narumiya, S. (1997) Altered pain perception and inflammatory response in mice lacking prostacyclin receptor. *Nature* **388**, 678–682.

124. Ekerdt, R. and Müller, B. (1992) Role of prostanoids in the inflammatory reaction and their therapeutic potential in the skin. *Arch. Dermatol. Res.* **284**, S18–21.
125. Ajuebor, M.N., Singh, A., and Wallace, J.L. (2000) Cyclooxygenase-2-derived prostaglandin D(2) is an early anti-inflammatory signal in experimental colitis. *Am. J. Physiol. Gastrointest. Liver Physiol.* **279**, G238–G244.
126. Angeli, V., Faveeuw, C., Roye, O., Fontaine, J., Teissier, E., Capron, A., Wolowczuk, I., Capron, M., and Trottein, F. (2001) Role of the parasite-derived prostaglandin D2 in the inhibition of epidermal Langerhans cell migration during schistosomiasis infection. *J. Exp. Med.* **193**, 1135–1147.
127. Matsuoka, T., Hirata, M., Tanaka, H., Takahashi, Y., Murata, T., Kabashima, K., Sugimoto, Y., Kobayashi, T., Ushikubi, F., Aze, Y., Eguchi, N., Urade, Y., Yoshida, N., Kimura, K., Mizoguchi, A., Honda, Y., Nagai, H., and Narumiya, S. (2000) Prostaglandin D2 as a mediator of allergic asthma. *Science* **287**, 2013–2017.
128. Rovin, B.H., Lu, L., and Cosio, A. (2001) Cyclopentenone prostaglandins inhibit cytokine-induced nf-kappab activation and chemokine production by human mesangial cells. *J. Am. Soc. Nephrol.* **12**, 1659–1667.
129. Santoro, M.G., Favalli, C., Mastino, A., Jaffe, B.M., Esteban, M., and Garaci, E. (1988) Antiviral activity of a synthetic analog of prostaglandin A in mice infected with influenza A virus. *Arch. Virol.* **99**, 89–100.
130. Node, K., Huo, Y., Ruan, X., Yang, B., Spiecker, M., Ley, K., Zeldin, D.C., and Liao, J.K. (1999) Anti-inflammatory properties of cytochrome P450 epoxygenase-derived eicosanoids. *Science* **285**, 1276–1279.

This article should be referenced as follows:

Serhan, C.N. and Chiang, N. (2002) Lipid-derived mediators in endogenous anti-inflammation and resolution: lipoxins and aspirin-triggered 15-epi-lipoxins. *TheScientificWorldJOURNAL* **2**, 169–204.

Handling Editor:

Edward J. Goetzl, Principal Editor for *Inflammation* – a domain of TheScientificWorld.