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Lipoxygenase and Leukotriene Pathways: Biochemistry, Biology, and Roles in Disease

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CONTENTS

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1. INTRODUCTION

Metabolism of arachidonic acid leads to several families of lipid mediators, including prostaglandins, thromboxane, leukotrienes, and lipoxins, along two major metabolic pathways, the cyclooxygenase (COX) and lipoxygenase (LO) pathways. These compounds, collectively known as eicosanoids, possess potent biological activities and are involved in maintenance of normal hemostasis, regulation of blood pressure, renal function, and reproduction as well as host defense. When formed in excess under pathological conditions, these molecules can elicit pain, fever, and inflammation and play roles in many acute and chronic endemic diseases.

In this article we will focus on the lipoxygenases and the leukotrienes (Figure 1). Although there are only two cyclooxygenases (COX-1 and COX-2) in man, there are no fewer than 6 lipoxygenases, one of which has been unequivocally linked to human disease, viz., 5-lipoxygenase (5-LO). Since the key discoveries in the mid 1970s, many comprehensive and highly cited review articles have appeared on the biochemistry and pathophysiological implications of lipoxygenases and leukotrienes.[1−4] It is now almost a decade since this topic was covered, and we believe that time is ripe to summarize and make an update of the most important knowledge that has evolved from the research field. We will walk the reader through basic lipoxygenase chemistry, describe 5-LO and leukotriene (LT) synthesis, including all key enzymes and the receptors that transduce leukotriene signaling, continue with the cellular and in vivo biology of lipoxygenases and leukotrienes, and finish with discussions of their roles in disease and possibilities for pharmacological interventions. In particular, we will integrate a basic biochemical understanding with recent developments in the field of structural biology and describe the most salient conclusions that can be drawn from work on transgenic mice, a powerful tool to define the in vivo biological and pathogenic roles of these enzymes. Extraction of results from more than three decades of research means that we cannot be complete, and we would therefore like to apologize to those distinguished colleagues whose work we have not found space to describe and cite.

2. CHEMISTRY OF THE LIPOXYGENASE REACTION

Lipoxygenase reactions are catalyzed by nonheme iron-containing dioxygenases, which introduce molecular oxygen into polyunsaturated fatty acids with one or more *cis,cis*-1,4-pentadiene moieties in the structure.[5−9] Please also consult previous reviews.[10,11] Lipoxygenase reactions can initiate synthesis of signaling molecules or contribute to structural or metabolic alterations within cells. Here, we will primarily consider the lipoxygenase reaction as part of a signaling pathway, which implicates formation of a fatty acid hydroperoxide.

2.1. Free Radical Mechanism of Lipoxygenases, Role of Iron

The lipoxygenase reaction is characterized by a lag phase, which can be abolished by the addition of equimolar amounts of hydroperoxides, presumably due to oxidation of the iron from Fe^{2+} (inactive, ferrous form) to Fe^{3+} (active, ferric form).[12,13] This means that, in intact cells, the levels of (fatty acid) hydroperoxides, which in turn are regulated by glutathione peroxidases, affect lipoxygenase activity and regulation.[14] In the most widely recognized reaction mechanism, i.e., the free radical mechanism,[15,16] catalysis is initiated by a stereospecific hydrogen abstraction at C-3 of the *cis,cis*-1,4-pentadiene, which leads to a carbon-centered radical (Figure 2). The hydrogen disintegrates into a proton and an electron, which reduces the iron to Fe^{2+}. The unpaired electron of the radical combines with one of the electrons of the neighboring double bond, forming a 1-*cis*, 3-*trans*-conjugated diene with migration of the radical to C-5, where it reacts with molecular oxygen to form a peroxy radical. The iron is subsequently oxidized by electron transfer to the peroxy radical, generating a peroxy-anion, which subsequently is protonated to give the hydroperoxide and active (Fe^{3+}) enzyme. The hydrogen abstraction and insertion of oxygen occur on opposite sides of the substrate, i.e., they have an antarafacial relation. It is important to note that nonenzymatic autoxidation can occur according to a similar mechanism without stereoselectivity.

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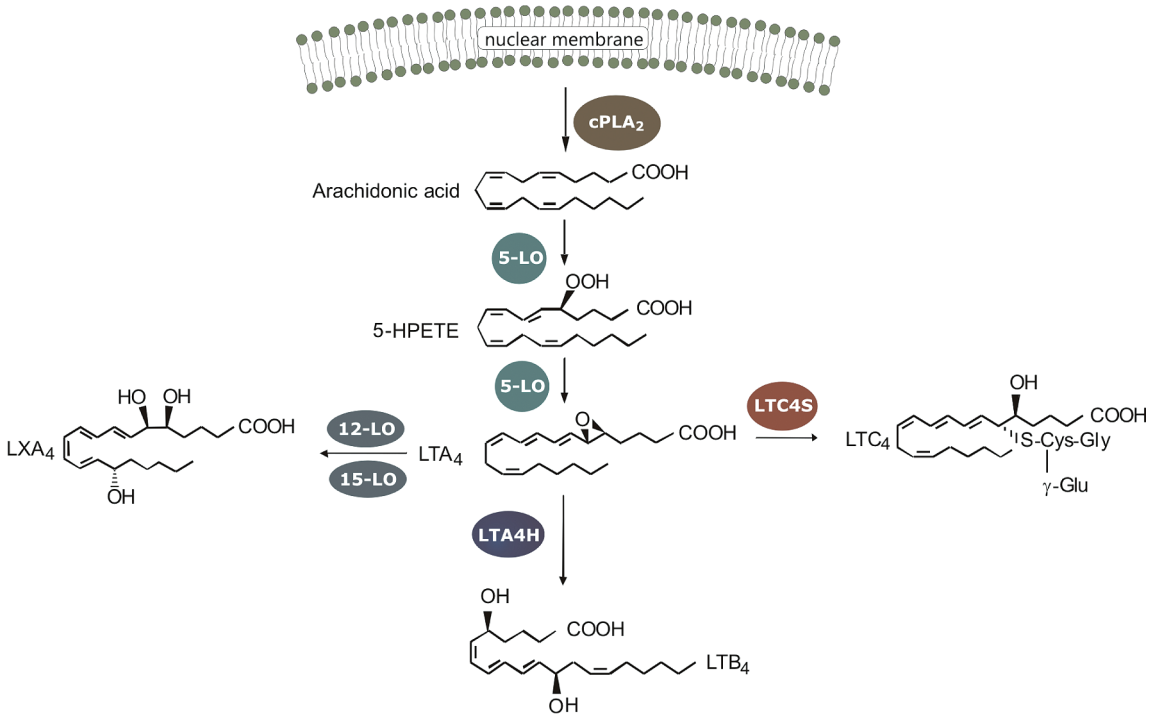

Figure 1. Biosynthesis of leukotrienes and lipoxins. Arachidonic acid is liberated by cPLA$_2\alpha$ and further converted by 5-LO into LTA$_4$, which in turn can be metabolized into LTB$_4$ and LTC$_4$ by the actions of LTA$_4$ hydrolase (LTA4H) and LTC$_4$ synthase (LTC4S), respectively. LTA$_4$ can also be converted into LXA$_4$ by 12-LO or 15-LO.

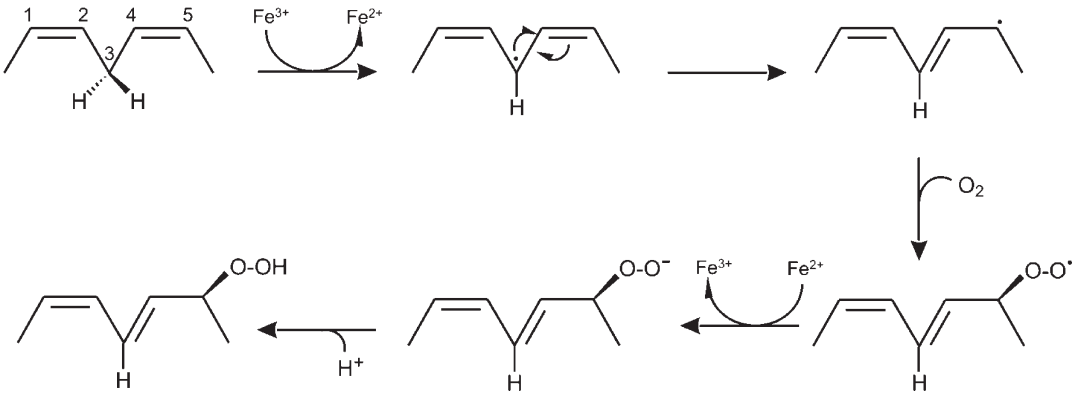

Figure 2. Mechanism for lipoxygenation of a *cis,cis*-1,4-pentadiene. The reaction is initiated by stereoselective hydrogen abstraction at C-3; the resulting radical migrates over the neighboring double bond and can react with molecular oxygen at C-5 to form a peroxy radical. Oxidation of the active site iron delivers one electron to form a peroxyanion, which is then protonated into a hydroperoxide. The hydrogen abstraction and insertion of oxygen occur on opposite sides of the substrate, i.e., an antarafacial relation.

3. LIPOXYGENASES

Lipoxygenases are found widely in plants, fungi, and animals and are classified according to their regioselectivity, i.e., the number of the carbon subjected to dioxygenation as well as their stereoselectivity that can be either "S" or "R", as defined by the Cahn–Ingold–Prelog criteria.[3,17]

3.1. Arachidonic Acid, A Prototype Substrate for Lipoxygenase

Plant lipoxygenases have been extensively studied, and soybean lipoxygenase was purified and crystallized already in 1947.[18] However, its catalytic properties were not thoroughly studied until Hamberg and Samuelsson in 1967 elucidated essential structural features of the substrates and the reaction mechanism.[7] For a polyunsaturated fatty acid, they found that the pro-S hydrogen of the methylene group at position ω8, in the center of a *cis,cis*-1,4-pentadiene, was removed and that molecular oxygen was inserted at ω6. Arachidonic acid, the prototype of a polyunsaturated fatty acid, is thus converted to 15(*S*)-hydroperoxy-5,8,11-*cis*-13-*trans*-eicosatetraenoic acid [15(*S*)-HpETE], which subsequently can be reduced to the corresponding 15(*S*)-hydroxyeicosatetraenoic acid [15(*S*)-HETE]. An S chirality of the hydroperoxide product was long believed to be a hallmark for a lipoxygenase-catalyzed reaction. However, it is now well established that lipoxygenases can also remove a pro-R hydrogen from the substrate with antarafacial insertion of oxygen to generate products with R chirality. In fact, the antarafacial relationship

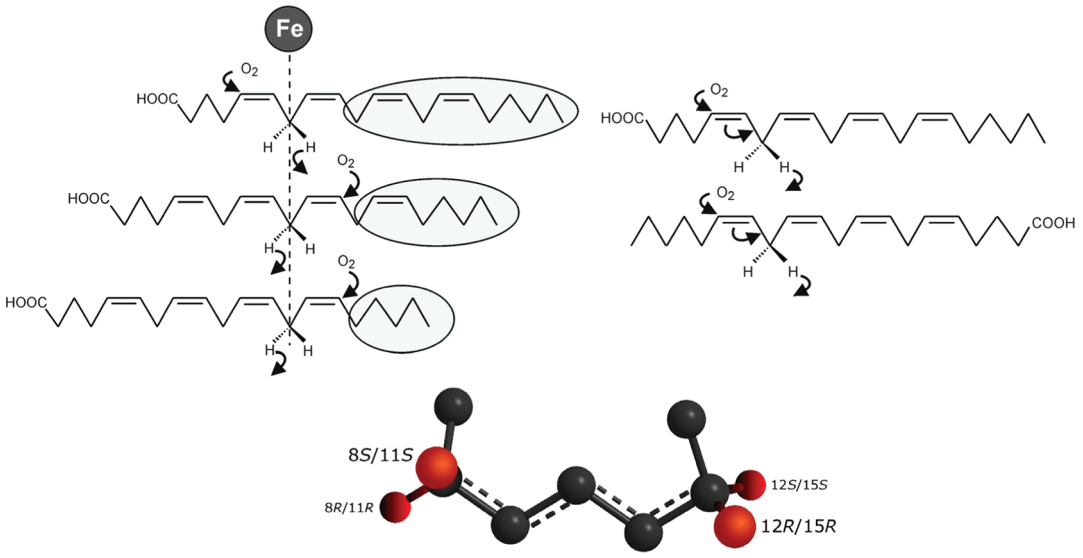

Figure 3. Control of regio- and stereospecificity of lipoxygenases. The proximity of the C-3 methylene group of the *cis,cis*-1,4-pentadiene to the catalytic iron will be a key determinant for the positional control. (Upper, left and right) Depending on the depth of the substrate-binding pocket (indicated in shaded gray) and the head-to-tail orientation of the incoming fatty acid substrate (carboxyl end or ω-end first), different CH_2 groups will be positioned for hydrogen abstraction and subsequent antarafacial oxygenation. (Lower) Considering arachidonic acid, S and R chirality will depend on the site of oxygenation, as depicted with oxygens pointing below the plane of the paper, which will be 8*R*/11*R* and 12*S*/15*S*, and oxygens above the plane in configurations 8*S*/11*S* and 12*R*/15*R*.

between hydrogen abstraction and oxygenation appears to be a universal mechanistic feature of lipoxygenase catalysis.

3.2. Control of Regioselectivity and Stereospecificity in Lipoxygenase Catalysis

Lipoxygenases are structurally conserved, and one may wonder how these enzymes can control the positional and chiral specificity of catalysis. Experiments with substrate analogues and site-directed mutagenesis of conserved residues along with structural information have shed light on these issues.[19−23] Thus, it is clear from sequence alignments and crystal structures that lipoxygenases contain a rather rigid catalytic machinery with an iron bound to a group of conserved amino acids, predominantly His residues and the C-terminal carboxyl group of the protein, which is usually an Ile. The necessary flexibility to achieve diversity in the positional and chiral specificity of lipoxygenases lies in the fatty acid substrates and their binding at the active sites.[24] Basically, the proximity of the C-3 methylene group of the *cis,cis*-1, 4-pentadiene to the catalytic iron will be a key determinant for the positional control. Depending on the depth of the substrate-binding pocket and the head-to-tail orientation of the incoming fatty acid substrate (carboxyl end or ω-end first), different CH_2 groups will be positioned for hydrogen abstraction and subsequent antarafacial oxygenation (Figure 3). Moreover, it was recently postulated that the stereocontrol, i.e., the R and S specificity, is determined by the presence of an Ala or Gly residue in a critical position of the lipoxygenase that can alter the site for oxygenation but still obey the rule of antarafaciality.[25]

4. BIOSYNTHESIS OF LEUKOTRIENES

Among all signaling molecules formed by lipoxygenases, the leukotrienes have an exceptional position in that their bioactions are of undisputed pathophysiological significance, particularly as mediators of inflammation. As indicated by their name, *leuko*trienes are primarily formed in various types of *leuko*cytes, especially granulocytes, monocytes/macrophages, mast cells, and dendritic cells.[26] Like other eicosanoids, leukotrienes are paracrine mediators exerting their actions in the local cellular milieu. The leukotrienes are divided into two major classes, the dihydroxy acid leukotriene B_4 (LTB_4), one of the most potent chemotactic agents known to date, and the cysteinyl-leukotrienes (cys-LTs), LTC_4, LTD_4, and LTE_4, powerful spasmogenic agents.[1]

4.1. Discovery of the Leukotriene Pathway and a Common Unstable Intermediate

The leukotrienes were discovered by Samuelsson and co-workers while investigating the lipoxygenase reactions in peripheral leukocytes that convert arachidonic acid into monohydroxylated products, in particular 5(*S*)-hydroxy-6-*trans*-8,11,14-*cis*-eicosatetraenoic (5(*S*)-HETE). They found that more polar compounds were also formed, and the five most abundant were structurally determined as 5(*S*),12(*R*)-dihydroxy-6,14-*cis*-8,10-*trans*-eicosatetraenoic acid (later termed LTB_4), together with two Δ^6-*trans* isomers of LTB_4, epimeric at C-12, and two 5, 6-dihydroxy acids.[27] From studies with $^{18}O_2$ and $H_2{}^{18}O$, it was demonstrated that the oxygen of the hydroxyl group at C-5 was derived from molecular oxygen, whereas the one at C-12 originated from water.[28] This finding, together with trapping experiments with methanol yielding 5-hydroxy-12-methoxy-eicosatetraenoic acids, indicated the presence of a common unstable epoxide intermediate in the formation of the dihydroxy compounds. On the basis of mechanistic and structural considerations, a tentative structure was proposed as 5(*S*)-*trans*-5,6-oxido-7, 9-*trans*-11,14-*cis*-eicosatetraenoic acid, i.e., LTA_4.

4.2. Conversion of Arachidonic Acid into Leukotriene A_4 (LTA_4) Is a Two-Step Concerted Reaction Catalyzed by a Single Lipoxygenase

The first step in the biosynthesis of LTA_4 is a classical lipoxygenation at C-5 of arachidonic acid generating 5(*S*)-hydroperoxy-6-*trans*-8,11,14-*cis*-eicosatetraenoic acid (5-HpETE),

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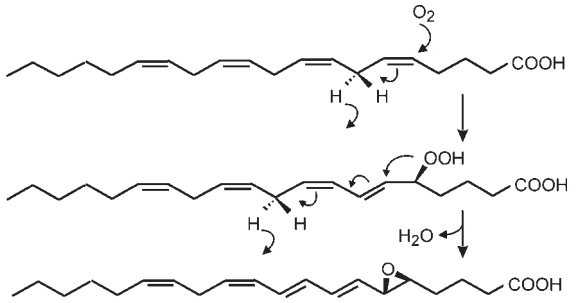

Figure 4. Biosynthesis of LTA_4 from arachidonic acid. A stereospecific abstraction of the pro-S hydrogen at C-7 is followed by radical migration to C-5 and formation of a Δ^6-*trans* double bond. Oxygen is introduced at C-5, in an antarafacial manner, to form 5(*S*)-hydroperoxy-6-*trans*-8,11,14-*cis*-eicosatetraenoic acid (5-HpETE). A subsequent abstraction of the pro-R hydrogen at C-10 of 5-HpETE is followed by radical migration to C-6 and rearrangements of the double bonds to $\Delta^{7,9}$-*trans* yielding a conjugated triene. Finally, the radical combines with the hydroperoxy group in a dehydration step to form the epoxide moiety of LTA_4.

whereas the second step, synthesis of LTA_4 from 5-HpETE, could be called a pseudolipoxygenation (Figure 4). It involves an initial abstraction of the pro-R hydrogen at C-10 of 5-HpETE followed by radical migration to C-6 and rearrangements of the double bonds to $\Delta^{7,9}$-trans yielding a conjugated triene. However, instead of a second oxygenation, the radical combines with the 5(*S*)-hydroperoxy group, which results in dehydration to form the epoxide moiety of LTA_4.[29,30] The origin of the LTA_4 synthase activity was not clear from the beginning, and it was not until purified 5-lipoxygenase was rigorously shown to produce both 5-HpETE and LTA_4 that one realized that 5-lipoxygenase and LTA_4 synthase activities reside in one and the same protein and are functionally coupled in a concerted reaction.[31,32]

4.3. Structural Elucidation of Slow-Reacting Substance of Anaphylaxis, A Mixture of Leukotrienes

The history of the leukotrienes as a bioactivity originates from the 1930s, when Harkavy reported that an alcohol-soluble substance from sputum of asthmatic patients caused spasm in smooth muscle cell preparations.[33] Later, a substance was described that could be released from perfusates of guinea pig lung, either by injections of cobra venom or by antigen challenge of sensitized animals.[34,35] The material induced a slowly developing and long-lasting contraction of guinea pig small intestine and was therefore given the name "slow-reacting substance" (SRS). In 1960, Brocklehurst demonstrated that antigen challenge of lungs from sensitized animals led to the release of a "slow-reacting substance of anaphylaxis" (SRS-A), which was clearly distinguishable from histamine.[36] SRS-A was characterized as an acidic, low molecular weight compound, which exhibited an ultraviolet (UV) spectrum indicating the presence of three conjugated double bonds in the structure.[37,38] Further studies showed that SRS-A incorporated both radiolabeled arachidonic acid and cysteine,[37,39] and that the biosynthesis of SRS-A was stimulated by the calcium ionophore A23187.[40,41]

During the work on leukotriene biosynthesis in polymorphonuclear leukocytes, Samuelsson and co-workers noted striking similarities with the biochemistry of SRS-A. The amounts of dihydroxy acids isolated from leukocytes increased after stimulation with A23187, and these compounds also displayed UV spectra characteristic for a conjugated triene structure (cf. the name leuko*triene*), which indicated that components of the leukotrienes could be shared with SRS-A. In fact, when the unstable epoxide intermediate, LTA_4, had been identified, it was proposed as a common intermediate in the biosynthesis of both the dihydroxy compound LTB_4 and SRS-A. Further structural elucidation of SRS-A demonstrated that it constituted a mixture of three compounds derived from LTA_4. LTC_4, 5(*S*)-hydroxy-6(*R*)-S-glutathionyl-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid, was a conjugate of LTA_4 with glutathione,[42,43] whereas the other two, LTD_4, 5(*S*)-hydroxy-6(*R*)-S-cysteinylglycyl-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid, and LTE_4, 5(*S*)-hydroxy-6(*R*)-*S*-cysteinyl-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid, were formed by successive cleavages of the tripeptide moiety of LTC_4 by γ-glutamyl transpeptidase and dipeptidase, respectively.[44,45]

5. ENZYMES AND PROTEINS IN LEUKOTRIENE BIOSYNTHESIS

5.1. Cytosolic Phospholipase $A_2\alpha$ ($cPLA_2\alpha$)

The majority of arachidonic acid in the cell is esterified in the *sn*-2 position of phospholipids. To increase the levels of free arachidonic acid available for leukotriene biosynthesis, a phospholipase, typically PLA_2, is required to release the fatty acid. Phospholipases A_2 comprise a large family of enzymes encompassing more than 15 isoforms that can be grouped based on primary structure, localization, and Ca^{2+} requirement.[46−48]

Enzymes within a few of these groups have been shown to catalyze arachidonic acid release in experimental models, but today it is widely accepted that cytosolic PLA_2 group IV ($cPLA_2$)α plays a major role in arachidonic acid release that leads to leukotriene production. Studies have shown, for example, that cells from transgenic mice, deficient in the $cPLA_2\alpha$ enzyme, have almost a complete inability to synthesize leukotrienes in response to a variety of stimuli.[49,50]

5.1.1. Molecular Properties and Regulation of $cPLA_2\alpha$.

$cPLA_2\alpha$ has a molecular mass of 85 kDa, becomes activated and translocates to membranes in response to submicromolar levels of Ca^{2+}, and selectively hydrolyses arachidonic acid esterified in the *sn*-2 position of phospholipids. Cytosolic $PLA_2\alpha$ was originally purified from U937 cells, and molecular cloning revealed a cDNA that predicts a protein of 748 amino acids (initial Met excluded).[51,52] A protein domain, 140 amino acids long and located in the N-terminal part of $cPLA_2\alpha$, was shown to associate with membrane vesicles in response to Ca^{2+}. This domain contains a segment of 45 amino acids with homology to the constant region 2 of protein kinase C (PKC), believed to be of importance for translocation of PKC. Thus, $cPLA_2\alpha$, in its amino terminal part, contained a putative Ca^{2+}-dependent phospholipid binding (CaLB) domain. Experiments with recombinant DNA technology verified the regulatory role of this protein segment for the Ca^{2+}-dependent binding of phospholipids and also demonstrated that the catalytic center was localized in the remaining C-terminal portion of the polypeptide chain.[53] The crystal structure of the C2 domain of $cPLA_2\alpha$ demonstrated that it is a β-sandwich structure that binds two Ca^{2+} ions and preferentially interacts with phosphocholine headgroups.[54] The Ca^{2+}-dependent translocation of $cPLA_2\alpha$ to ER and nuclear membranes with concomitant enzyme activation requires a functional C2 domain.[55−57]

Cytosolic $PLA_2\alpha$ has also been shown to bind with high affinity and specificity to phosphatidylinositol 4,5-bisphosphate (PIP_2) in a 1:1 stoichiometry.[58] This interaction results in a

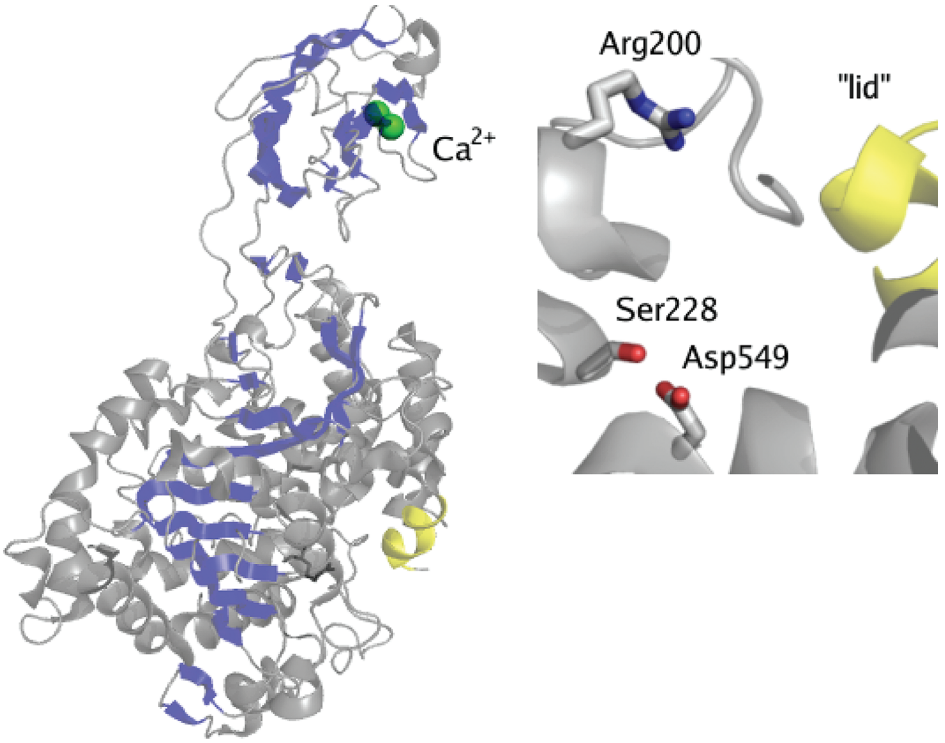

Figure 5. Structure of human cPLA$_2\alpha$. Crystal structure of cPLA$_2\alpha$ at 2.5 Å reveals an N-terminal Ca^{2+} binding (C2) domain and a catalytic domain (PDB id: 1CjY). Calcium ions are indicated as green spheres, and an amphipathic loop, acting as a flexible "lid" to the active site, is indicated in yellow. The catalytic residues, including the Ser-Asp dyad, and their relation to the "lid" are indicated to the right.

dramatic increase in catalytic activity and is probably mediated via binding of PIP_2 to a putative pleckstrin homology domain in cPLA$_2\alpha$, a structural motif that is also present in phospholipase Cδ_1. Furthermore, cPLA$_2\alpha$ is activated by ceramide-1-phosphate and by phosphorylation at Ser505.[57,59] This residue is located on the catalytic domain in close proximity to the C2 domain. Probably the phosphorylation of Ser505 changes the C2-catalytic domain interaction, improving the conformation of the catalytic site.

Cytosolic PLA$_2\alpha$ is constitutively expressed in most cells and tissues, but expression may also be increased by certain pro-inflammatory cytokines and growth factors and suppressed by glucocorticoids, as is the case for COX-2.[60] The human cPLA$_2\alpha$ gene is located on chromosome 1q25, next to the COX-2 gene.[61] The promoter has been cloned and partially characterized.[62] It has features typical of a housekeeping gene with no TATA or CAAT box although atypical in that it is not GC-rich and lacks Sp1/Egr-1 sites. Instead, it has a long stretch of CA repeats and a polypyrimidine tract. The 5′-flanking region also contains several interferon-c-response elements, a putative composite AP-1 site, and two glucocorticoid-response elements (GRE).

5.1.2. Crystal Structure and Catalytic Mechanism of cPLA$_2\alpha$. The complete crystal structure of cPLA$_2\alpha$ at 2.5 Å resolution has been determined and revealed an unusual catalytic Ser-Asp dyad, located in a deep cleft at the center of a hydrophobic funnel, which cleaves arachidonoyl phospholipids (Figure 5).[63] The mechanism by which cPLA$_2\alpha$ hydrolyzes the ester bond is thought to proceed through a modified so-called "serine protease" mechanism in which the Ser228-Asp549 dyad plays a major role and Arg200 plays a secondary role. Asp549 serves as a general base increasing the nucleophilicity of Ser228, allowing it to attack the carbonyl carbon of the *sn*-2 ester bond to form a serine−acyl intermediate. Arg200 stabilizes the phosphate group of the substrate. Compared to the conventional serine protease mechanism, cPLA$_2\alpha$ lacks the catalytically important His residue in the catalytic triad.

Interestingly, the structure also revealed an amphipathic loop acting as a flexible lid that must move to allow substrate access to the active site. This amphipathic loop is thought to move during substrate binding, exposing the hydrophobic surface in a similar way as seen in other lipases, which also contain flexible regions covering the hydrophobic active site. The cPLA$_2\alpha$ activity increases when enzyme is bound to the membrane, a so-called interfacial activation. This membrane-binding model is consistent with the observation of its inhibition by sphingomyelin-containing membranes, which have a more rigid structure.[64]

5.2. 5-Lipoxygenase (5-LO)

As described above, the first two steps in leukotriene biosynthesis are catalyzed by 5-LO in which arachidonic acid is converted into the unstable epoxide intermediate LTA_4.[32,65] In man, 5-LO is an *S*-lipoxygenase among 5 other known human lipoxygenases with different positional and stereochemical specificities.[3,66] Groundbreaking work by Samuelsson and co-workers, in parallel with other laboratories, allowed purification and molecular cloning of the cDNA as well as the gene of human 5-LO,[67−70] which in turn sparked intense research activity on the enzyme and its products.

5.2.1. Cellular Expression of 5-LO. Expression of 5-LO is essentially restricted to cells derived from the bone marrow such as granulocytes, monocytes/macrophages, mast cells, dendritic cells, and B-lymphocytes.[71] Cytokines and growth factors can increase enzyme expression as exemplified by the profound effects

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of TGF-β and vitamin D3 observed in HL-60 cells and Mono Mac 6 cells,[72] induction by IL-3 in mouse mast cells,[73] and by GM-CSF in mature neutrophils.[74,75] In contrast, the Th2 cytokine IL-4 reduces 5-LO expression in monocytes and maturing dendritic cells, a response paralleled by increased 15-LO expression.[76] The level of expression in a given leukocyte population seems to depend on DNA methylation, which limits transcription.[77] On the other hand, differentiation of leukocytes is associated with increased 5-LO expression, as demonstrated for monocytes differentiating into macrophages,[78,79] and this upregulation is apparently independent of DNA methylation.[71] Interestingly, cytomegalovirus infection of human vascular smooth muscle cells leads to a strong upregulation of 5-LO expression with concomitant LTB_4 synthesis, indicating that viral proteins can activate the 5-LO gene promoter in nonmyeloid cells.[80]

5.2.2. Regulation of 5-LO Gene (*ALOX5*) Transcription. Some clues to the understanding of 5-LO transcriptional regulation evolved from studies of the gene and its promoter. The gene encoding human 5-LO, known as *ALOX5*, is located on chromosome 10 and spans 71.9 kbp of DNA consisting of 14 exons divided by 13 introns.[70] The promoter region lacks a typical TATA or CCAT box but contains eight GC boxes, five of which are arranged in tandem and are recognized by the transcription factors Sp1 and Egr-1.[81,82] Thus, the promoter structure resembles those of so-called housekeeping genes, which are constitutively expressed in many cells and tissues. This was a surprising finding since 5-LO activity is detected almost exclusively in bone marrow-derived cells.

5.2.3. Naturally Occurring Mutations in the Gene Promoter of 5-LO. The *ALOX5* promoter has been found to contain naturally occurring mutations with respect to the number of GC boxes, resulting in either the deletion of one or two Sp1-binding sites, or the addition of one Sp1-binding site.[83] A pharmacogenetic association between *ALOX5* promoter genotype and the response to antiasthma treatment with a 5-LO inhibitor (ABT-761) has been demonstrated.[84] This finding suggests that the mutations lead to reduced 5-LO expression, in agreement with the recent observation that eosinophils from individuals with asthma carrying a mutated non5/non5 genotype express less 5-LO mRNA and produce less LTC_4.[85] However, the cellular and biochemical consequences of the variant GC boxes are not clear. Thus, with respect to another disease, atherosclerosis, mutations of the tandem GC boxes are linked to an increased intima-media thickness and higher plasma levels of C-reactive protein, suggesting that 5-LO expression is increased and accompanied by an elevated production of leukotrienes.[86] In light of the frequent use of mouse models, it is interesting to note that the tandem GC boxes are absent in the core part of the mouse *Alox5* promoter, which contains only one Sp1- or Sp3-binding site.[87]

5.2.4. Allosteric and Post-translational Regulation of 5-LO. Native 5-LO purified from human leukocytes is activated by Ca^{2+}, which is bound reversibly to the protein in a 2:1 stoichiometry.[88] Activation of 5-LO by Ca^{2+} requires the presence of phosphatidylcholine or coactosin-like protein, a 16 kDa F-actin binding protein that also promotes the LTA_4 synthase activity of 5-LO.[89,90] It appears that Ca^{2+} promotes optimal binding of 5-LO to these stimulatory factors. It has also been shown that Ca^{2+} decreases the Michaelis constant (K_m) for arachidonic acid and alters the reaction kinetics in favor of substrate inhibition. For maximal activity, 5-LO also requires ATP.[67,88] The mechanism of ATP activation is not clear but involves binding of the nucleotide to the protein without any apparent hydrolysis of phosphodiester bonds. Instead, ATP seems to have a stabilizing effect on 5-LO.

Identification of a phosphorylated 5-LO species in activated neutrophils was first demonstrated by Lepley and co-workers.[91] Further work revealed that 5-LO is phosphorylated in vitro at three sites: Ser-271 by MAPKAP kinase 2, Ser663 by ERK2, and Ser523 by PKA.[92−94] Phosphorylation at Ser271 and Ser663 is promoted by the presence of unsaturated fatty acids, including arachidonic acid, but these phosphorylation events do not seem to influence 5-LO enzyme activity.[95] On the other hand, cellular conditions that activate MAPKAPKs and ERKs (e.g., cell stress and phorbol esters) induce nuclear translocation of 5-LO and enhance leukotriene formation in intact cells.[96] In contrast, phosphorylation at Ser523 by PKA has a direct negative effect on 5-LO catalytic activity and prevents nuclear localization of the enzyme by interference with a nuclear import motif in the 5-LO primary structure,[94,97] mechanisms which together can explain the inhibitory actions of adenosine and increased cAMP on 5-LO and cellular leukotriene synthesis.[98] In this context it is interesting to note that neutrophils and monocytes from males have a significantly lower capacity to synthesize leukotrienes, as compared to cells from females.[99,100] This effect is due to androgen-induced ERK activation, which paradoxically results in reduced leukotriene synthesis.

5.2.5. Structure−function relationships in 5-LO. 5-Lipoxygenase is a soluble monomeric enzyme composed of 673 amino acids with a molecular mass of about 78 kDa.[69] The polypeptide chain is divided into two domains, an N-terminal regulatory domain (residues 1−120) and a catalytic C-terminal domain that harbors one atom of nonheme iron, involved in catalysis.[101] Earlier work with site-directed mutagenesis of conserved residues demonstrated that His372 and His550 are iron ligands, whereas the iron-binding function of His367 remained uncertain.[102,103] From the three-dimensional structure of soybean lipoxygenase-1,[104,105] it was discovered that the C-terminal isoleucine, Ile-673 in human 5-LO, folds back toward the active-site metal ion and functions as an iron ligand. Two other amino acids in 5-LO, viz., Glu376 and Gln558, are functionally important, because mutagenetic replacements of these residues resulted in complete loss of enzyme activity.[106,107]

Further structural and functional insights were uncovered when the first crystal structure of a mammalian lipoxygenase, viz., rabbit 15-LO, was determined.[23] Interestingly, the architecture of the substrate-binding site clarified the molecular mechanism for the positional specificity for oxygenation of arachidonic acid displayed by 5-, 12-, and 15-LO. Thus, the depth and width of the substrate-binding pocket appears to be critical such that a shallow pocket puts the catalytic iron close to C-15, whereas a deep pocket puts it close to C-5 (Figure 3). Furthermore, rabbit 15-LO contained an N-terminal, negatively charged, so-called β-barrel domain, a structure also found in the C-terminal domain of lipases. The role of this domain for lipoxygenases is presently unclear, but for 5-LO it has been shown to bind several regulatory factors such as Ca^{2+}, phosphatidyl choline, and coactosin-like protein, which suggests that this domain facilitates the association of 5-LO with membranes during catalysis.[108] In line with this notion, the β-barrel domain has also been shown to play an essential role for translocation of 5-LO to the nuclear membrane.[109]

5.2.6. Crystal Structure of 5-LO. The 5-LO protein resisted decades of intense crystallization efforts in several laboratories,

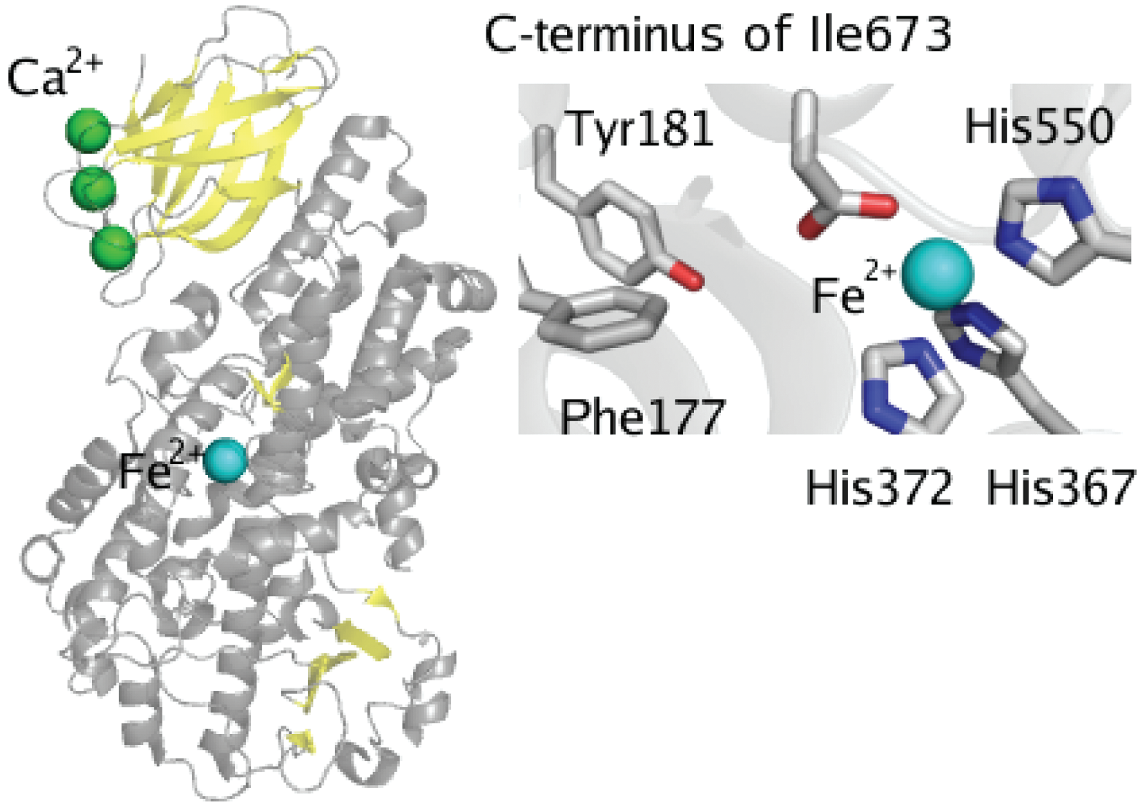

Figure 6. Structure of human 5-LO. Crystal structure of 5-LO at 2.4 Å resolution revealing a regulatory β-barrel domain in yellow and a catalytic α-helical domain in gray with a bound iron in magenta (PDB id: 3O8Y). Three bound Ca^{2+} have been modeled from the positions of Ca^{2+} binding ligands in the crystal structure of 8R-LO. The active site is depicted to the right. The side chains of Phe177 and Tyr181 form a cork that hinders entry of substrate to the catalytic center with iron bound by His372, His367, His550, and the carboxy terminal Ile673.

and for a long time, the only available structure was a model essentially demonstrating the presence of the regulatory β-barrel and the α-helical catalytic domain. However, in 2011, Newcomer and co-workers reported the first crystal structure of an engineered human 5-LO at 2.4 Å resolution (Figure 6).[110] On the basis of the crystal structures of rabbit 15S-LO and coral 8R-LO, they could identify a Lys-rich sequence close to the C-terminus that renders the protein unstable.[23,111] Replacement of Lys563-Lys-Lys565 with Glu-Gln-Leu, the corresponding sequence in coral 8R-LO, combined with removal of Ca^{2+} binding and putative membrane insertion amino acids (Δ40−44GlySer, Trp13Glu, Phe14His, Trp75Gly, and Leu76Ser) and exchange of two Cys for Ala (Cys240Ala and Cys561Ala) significantly increased the stability of the protein and made it suitable for crystallization. The crystal structure revealed an iron bound to His367, His372, and His550, in agreement with other lipoxygenase structures and previous mutagenesis data. The architecture of the active site, which is an elongated cavity, displays distinct characteristics and is lined with several residues unique to 5-LO (Tyr181, Thr364, His600, Ala603, Ala606). Surprisingly, the side-chains of two aromatic amino acids at the active center, viz., Phe177 and Tyr181, form a cork that seals off the active site and closes the cavity for substrate entry. Two mechanisms were suggested by which arachidonic acid could enter the active site and get access to the catalytic iron, one of which involves uncorking of the Phe-Tyr and the other of which involves a rotamer shift of Trp147 at the opposite end of the cavity. The latter mechanism suggests that arachidonic acid may enter 5-LO with its ω-end first from the opposite direction as compared to 15S-LO or 8R-LO, which lack the cork. Alternatively, the cork is somehow unplugged and the substrate enters with its carboxyl end first. Both mechanisms are consistent with the positional and stereochemical specificity of 5-LO.

5.3. 5-Lipoxygenase-Activating Protein (FLAP)

5.3.1. FLAP Is Critical for Cellular 5-LO Activity. In intact cells, 5-LO becomes activated and translocates to membranes in response to Ca^{2+}, a process accompanied by catalysis and enzyme inactivation.[112,113] Cellular 5-LO activity is dependent on a small membrane protein, five lipoxygenase-activating protein (FLAP), which was discovered through the inhibitory action of a drug, MK-886, on leukotriene biosynthesis in intact cells.[114] MK-886 binds to this membrane protein and can thereby prevent and revert translocation of 5-LO. FLAP was purified by means of an MK-886-based affinity chromatography, characterized, and cloned.[115,116] The deduced amino acid sequence showed that it is a unique 162 amino acid protein with three putative transmembrane domains. Experiments with osteosarcoma cells transfected with 5-LO cDNA alone or together with FLAP cDNA demonstrated that FLAP is essential for leukotriene biosynthesis in intact cells.[115]

5.3.2. Effects of FLAP on Leukotriene Production. Unlike other key proteins in the leukotriene cascade, FLAP is devoid of enzyme activity. FLAP is a homologue with LTC_4 synthase and several other microsomal GSH transferases but lacks a complete set of GSH-binding ligands and an Arg

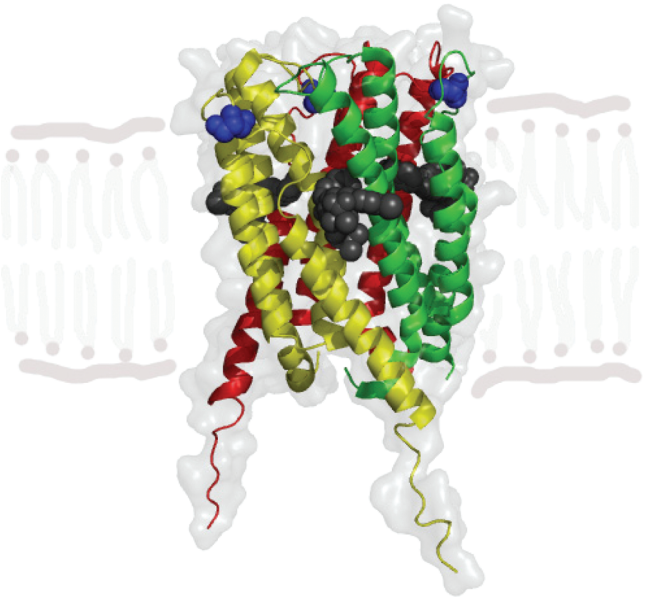

Figure 7. Structure of human FLAP. Crystal structure of FLAP at 4.0 Å resolution reveals a homotrimer (in red, yellow, and green) composed of four transmembrane helices connected by extending loops (PDB id: 2Q7R). Binding of MK591 is indicated in black balls embedded in membrane, whereas the positions at which GSH would bind, in analogy with LTC_4 synthase, are indicated as blue spheres located at the membrane/cytosol interphase.

residue assumed to be critical for catalysis in membrane GSH S-transferases.[117] Although the mechanism of action for FLAP is not fully understood, available data indicate that it is a scaffold protein for 5-LO. Thus, FLAP exerts some of its effects via allostery, and it has been suggested that FLAP presents or transfers arachidonic acid to 5-LO.[118] Indeed, FLAP was shown to be an arachidonate binding protein, and this binding could be competed by compounds such as MK-886.[119] FLAP also stimulates the utilization of arachidonic acid by 5-LO and increases the efficiency with which 5-LO converts 5-HpETE into LTA_4.[120]

5.3.3. FLAP Gene (*ALOX5AP*) and Regulation of Expression. The FLAP gene, *ALOX5AP*, has been cloned and spans >31 kb on chromosome 13.[121,122] It is divided into five small exons separated by four large introns, and the promoter region contains a possible TATA box as well as a potential GRE and AP-2 binding site. GM-CSF upregulates both FLAP and 5-LO in human neutrophils, suggesting a coordinated regulation of these two proteins.[74,123] Similarly, IL-5 upregulated FLAP in human eosinophils and stimulated 5-LO translocation without increasing 5-LO expression.[124] In the monocytic cell line THP-1, TNF-α induces FLAP expression via transcription factors C/EBPα, C/EBPδ, and C/EBPε, which bind between −25 and −12 in the gene promoter, and induction by lipopolysaccharide (LPS) in these cells involves the same transcription factors in combination with nuclear factor κB p50 binding at a specific site between −43 and −24.[125,126]

5.3.4. Crystal Structure of FLAP. The crystal structure of FLAP has been solved to 4.0 Å resolution.[127] The protein forms a homotrimer, and each monomer, which was devoid of bound GSH, is composed of four transmembrane helices connected by extended loops (Figure 7). The protein is homologous to human LTC_4 synthase with 33% sequence identity. In the crystal structure of FLAP in complex with MK591, one inhibitor molecule is bound between adjacent monomers in each of three grooves on the membrane-embedded surface of the trimer. Thus, unexpectedly the inhibitor binding site was located within the plane of the nuclear membrane, distant from the site where GSH would be expected to bind in analogy with GSH binding in the structure of LTC_4 synthase.[118] On the basis of mutagenesis studies, the previously identified binding sites for arachidonic acid and MK886 were found to overlap within the membrane, and this site was ideally positioned for FLAP to capture laterally diffusing arachidonic acid molecules to allow for presentation to 5-LO.[127] A distinct feature of the FLAP structure is an internal pocket at the bottom of the trimer open to the ER lumen with an internal diameter of 6 Å. Although LTC_4 synthase and FLAP are very similar in structure, this opening does not exist in LTC_4 synthase and its function is unknown.

5.4. LTA_4 Hydrolase

LTA_4 hydrolase catalyzes the final step in the biosynthesis of the proinflammatory compound LTB_4. The protein is widely distributed and has been detected in almost all mammalian cells, organs, and tissues examined, even in cells lacking 5-LO activity such as erythrocytes, fibroblasts, endothelial cells, keratinocytes, and airway epithelial cells.[26] Among the cellular elements of blood, neutrophils, monocytes, lymphocytes, and erythrocytes are rich sources of the enzyme, whereas eosinophils have low levels and basophils and platelets seem to lack LTA_4 hydrolase.

LTA_4 hydrolase has been purified and cloned from several mammalian sources, and cDNAs encode a 610 amino acid protein with a molecular mass of ∼69 kDa. Human LTA_4 hydrolase exists as a single copy gene with a size of >35 kbp on chromosome 12q22.[128] The coding sequence is divided into 19 exons ranging in size from 24 to 312 bp, whereas the 5′ upstream region has not been explored for promoter activity and identification of regulatory elements.

5.4.1. LTA_4 Hydrolase Is a Substrate-Selective and Suicide-Inactivated Epoxide Hydrolase. LTA_4 hydrolase is a highly substrate-specific epoxide hydrolase. Besides LTA_4, only the double-bond isomers LTA_5 and LTA_3 are turned over by LTA_4 hydrolase, albeit at a low rate.[129,130] LTA_4 hydrolase is unique among epoxide hydrolases, and the primary, secondary, and tertiary structures of the enzyme bear no resemblance to soluble xenobiotic epoxide hydrolase, although this enzyme also accepts LTA_4 as substrate.[131,132] Typically, LTA_4 hydrolase is inactivated and covalently modified by its substrate LTA_4 during catalysis, a process referred to as suicide inactivation.[129,133] During the inactivation process, LTA_4 binds to a 21-residue peptide, located in the middle of the polypeptide chain.[134] Mutational analysis has demonstrated that Tyr378, located within this peptide, is a major structural determinant for suicide inactivation.[135] Mutated proteins, carrying a Gln or Phe residue in position 378, were neither inactivated nor covalently modified by LTA_4. In addition, the mutated enzymes produced an isomer of LTB_4 (Δ^6-*trans*-Δ^8-*cis*- LTB_4), suggesting that Tyr378 may assist in the proper alignment of LTA_4 in the substrate-binding pocket to allow formation of the correct double-bond geometry in the enzymatic product LTB_4.

5.4.2. LTA_4 Hydrolase Is Bifunctional and Belongs to the M1 Family of Zinc Metallopeptidases. Sequence comparison with certain zinc metalloenzymes, e.g., aminopeptidase M and thermolysin, revealed the presence of a zinc-binding motif (**H**EXX**H**-X_{18}-**E**) in LTA_4 hydrolase.[136,137] Accordingly, LTA_4

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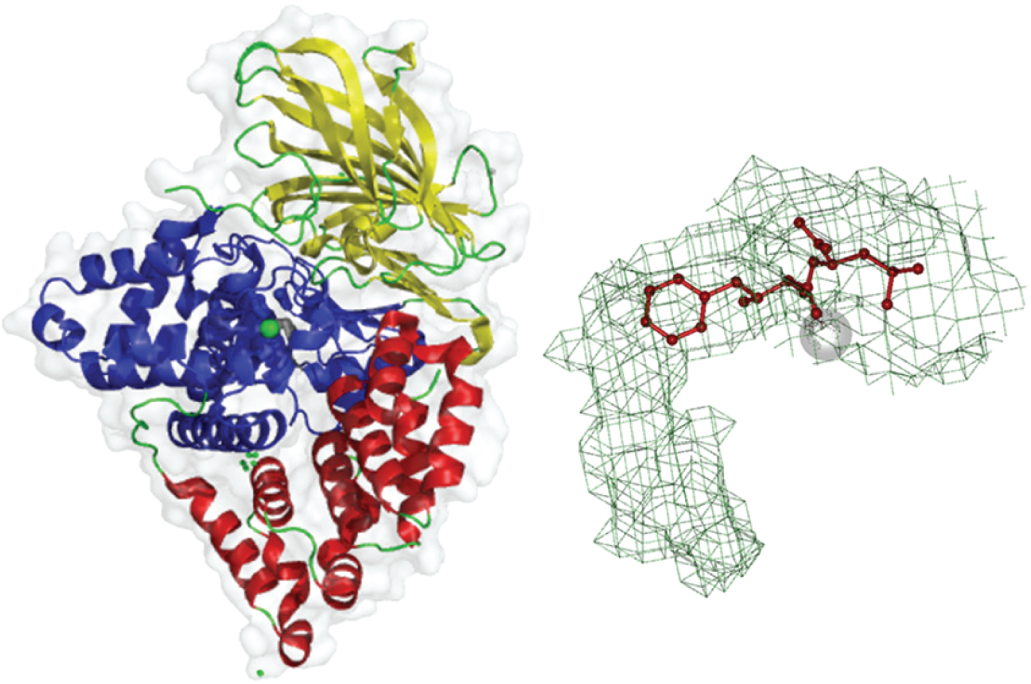

Figure 8. Structure of human LTA_4 hydrolase. Crystal structure of LTA_4 hydrolase at 1.95 Å resolution shows three domains, an N-terminal in yellow, a catalytic in blue, and a C-terminal domain in red, creating a deep cleft in between (PDB id: 1HS6). The catalytic zinc site, indicated as a green sphere, is located at the bottom of the interdomain cleft. The active site is depicted to the right (green mesh) and has an L-shaped architecture with a wide hydrophilic portion near the protein surface and a narrow hydrophobic tunnel penetrating deeper into the protein. A bound molecule of bestatin is indicated in red, and the catalytic zinc is indicated as a metal sphere.

hydrolase was found to contain one atom of zinc, bound to His295, His299, and Glu318, the primary function of which was catalytic.[138−140] Furthermore, the enzyme was found to exhibit a previously unknown peptidase activity that was selectively stimulated by monovalent anions, e.g., chloride ions, in a manner suggesting the presence of an anion binding site.[139,141,142] On the basis of its zinc signature, sequence homology, and aminopeptidase activity, LTA_4 hydrolase has been classified as a member of the M1 family of the MA clan of metallopeptidases.[143] Thus, LTA_4 hydrolase is distantly related to many other zinc proteases and aminopeptidases that are present in a variety of organisms from bacteria to mammals, including human enzymes such as aminopeptidase A (APA), aminopeptidase B (APB), aminopeptidase N (APN), and angiotensin-converting enzyme (ACE). From kinetic studies of synthetic substrates and crystal structures of enzyme−substrate complexes, LTA_4 hydrolase has been characterized as an Arg-specific tripeptidase.[144,145]

5.4.3. LTA_4 Hydrolase Cleaves the Chemotactic Pro-Gly-Pro, a Role for the Aminopeptidase Activity during Resolution of Inflammation. Ever since the discovery of the zinc aminopeptidase activity of LTA_4 hydrolase, endogenous physiological substrates have been sought. Some candidate substrates were identified, including certain opioid peptides and LTD_4, but were turned over with very low efficiency.[146,147] In search of an enzyme that degrades chemotactic peptides in the respiratory tract during acute influenza, Snelgrove and co-workers discovered that the tripeptide Pro-Gly-Pro (PGP) is a substrate for extracellular LTA_4 hydrolase.[148] Notably, this peptide does not contain an N-terminal Arg, as one would have expected from previous work in vitro.[144,145] PGP is chemotactic for neutrophils and is generated from the breakdown of collagen in the extracellular matrix by the action of matrix metalloprotease-8 (MMP-8) and/or MMP-9 followed by a secondary cleavage by prolyl endopeptidase. Amino terminal acetylation of PGP (AcPGP) can enhance its bioactivity and makes it resistant to cleavage by LTA_4 hydrolase. These results indicate that LTA_4 hydrolase displays dual and opposite functions during an inflammatory response. In the early phase, LTA_4 hydrolase will generate proinflammatory LTB_4, whereas inactivation of the chemotactic PGP will occur during the late resolution phase. It seems likely that inhibitors which block the epoxide hydrolase activity (LTB_4 synthesis), whereas sparing the aminopeptidase activity of LTA_4 hydrolase will have improved anti-inflammatory properties.

5.4.4. Crystal Structure of LTA_4 Hydrolase. The structure of LTA_4 hydrolase in complex with the competitive inhibitor bestatin has been determined at 1.95 Å resolution.[149] The protein molecule is folded into three domains, N-terminal, catalytic, and C-terminal, that are packed in a flat triangular arrangement with approximate dimensions 85 × 65 × 50 $Å^3$ creating a deep cleft in between (Figure 8). The N-terminal domain has a large concave and hydrophobic surface area and is structurally similar to bacteriochlorophyll *a*.[150] The fold of the catalytic domain is very similar to that of thermolysin, although the sequence identity is only ∼7% over the corresponding polypeptide chains. The C-terminal domain has structural features resembling a so-called armadillo repeat or HEAT region, which in turn suggests that it may take part in protein−protein interactions.[151]

The zinc site is located at the bottom of the interdomain cleft. As predicted, the metal is bound to His295, His299, and Glu318, with bestatin as the fourth ligand in a pentavalent coordination. In the vicinity of the prosthetic zinc, the catalytic residues Glu271, Glu296, and Tyr383 are located. Behind the pocket occupied by the phenyl ring of bestatin there is an L-shaped hydrophobic cavity ∼6−7 Å wide, which stretches 15 Å deeper

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into the protein (Figure 8). One patch of the cavity is hydrophilic, with Gln134, Asp375, and the hydroxyl of Tyr267 clustering together. This cavity was probed by structures of complexes between LTA_4 hydrolase and specific, active site-directed, inhibitors, some of which have been designed as LTA_4 mimics.[152] Indeed, the hydrophobic tail of the inhibitors, corresponding to the fatty acid backbone of LTA_4, is buried into the narrow hydrophobic pocket, strongly indicating that it functions as a substrate-binding cavity (Figure 8).

5.4.5. Mechanism of the Epoxide Hydrolase Reaction. The stereochemistry at C-12 and double-bond geometry are key structural determinants for the biological activity of LTB_4. Consequently, the role of LTA_4 hydrolase during enzymatic hydrolysis of LTA_4 into LTB_4 is to generate the 12*R* epimer of the hydroxyl group and to form the Δ^6-*cis*-Δ^8-*trans*-Δ^{10}-*trans* configuration of the conjugated triene. This reaction is unique and requires control of the stereospecific introduction of a hydroxyl group at a site (C-12) distant from the reactive epoxide moiety (C-5 to C-6). The crystal structure gives several important clues as to how LTA_4 hydrolase can execute its sophisticated chemistry.[153]

If LTA_4 is modeled into the hydrophobic pocket such that the 5,6-epoxide moiety is bound to Zn^{2+}, then C-7 to C-20 of the fatty acid backbone of LTA_4 fits snugly into the deeper cavity, adopting a bent conformation (Figure 9). Furthermore, the C-1 carboxylate can make direct electrostatic interactions with the positive charge of Arg563.[154] This crucial interaction will also ensure perfect substrate alignment required for catalysis, in agreement with the fact that the free carboxylic acid of LTA_4 is required for catalysis.[130,155] Furthermore, the catalytic zinc as well as Glu271 will be proximal to the labile allylic epoxide, suggesting that they will polarize a water molecule and promote an acid-induced activation and opening of the epoxide ring (Figure 9). A carbocation will be generated, according to an S_N1 reaction, whose charge is delocalized over the conjugated triene system (C-6 to C-12), leaving the planar sp^2 hybridized C-12 open for nucleophilic attack from either side of the molecule. In this model, Asp375 would direct a water molecule for attack at C-12 and thus control the positional and stereospecific insertion of the 12(*R*)-hydroxyl group in LTB_4, in agreement with mutational data.[156]

Moreover, the shape and curvature of the LTA_4 binding cavity also suggest the chemical strategy for creation of the 6-*cis* double bond in LTB_4. Because there is free rotation between C-6 and C-7 of LTA_4, the enzyme may keep this bond in a "pro-cis" configuration in the transition state, which would promote the formation of a cis double bond from the carbocation intermediate (Figure 9). The entire modeled LTA_4 molecule would then adopt a bent shape that fits very well with the architecture of the binding pocket. Hence, the critical double-bond geometry at Δ^6 in LTB_4 seems to be controlled by the exact binding conformation of LTA_4 at the active site.

5.4.6. Mechanism of the Aminopeptidase Activity. In agreement with what has been discussed for thermolysin, the peptide-cleaving activity of LTA_4 hydrolase proceeds according to a general base mechanism.[157] Thus, the catalytic zinc is complexed to its three amino acid ligands and an activated water molecule. The water is displaced from the zinc atom by the carbonyl oxygen of the substrate, which in turn is anchored to the active site via its N-terminal α-amino group binding to Glu271. In this role, Glu271 will stabilize the transition state and also contribute to the enzyme's exopeptidase specificity (Figure 9).

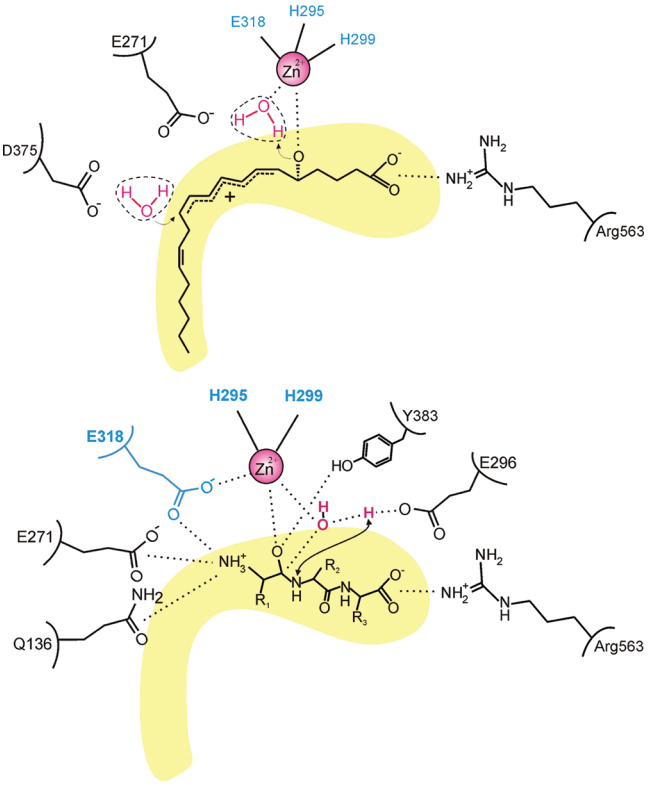

Figure 9. Catalytic mechanisms of LTA_4 hydrolase. (Upper) Mechanism of the epoxide hydrolase activity of LTA_4 hydrolase. The 5,6-epoxide moiety is bound to Zn^{2+}, and the C-1 carboxylate interacts with the positive charge of Arg563. The catalytic zinc as well as Glu271 polarize a water molecule to promote an acid-induced activation and opening of the epoxide ring. A carbocation is generated, according to an S_N1 reaction, whose charge is delocalized over the conjugated triene system (C-6 to C-12). Asp375 directs a water molecule for attack at C-12 to generate the 12(*R*)-hydroxyl group in LTB_4. Creation of the 6-*cis* double bond in LTB_4 is controlled by the exact binding conformation of LTA_4 in the L-shaped hydrophobic pocket. (Lower) The tripeptidase activity of LTA_4 hydrolase follows a general base mechanism. The catalytic zinc (in red) is complexed by His295, His299, Glu318 (in blue), and an activated water molecule (red). The N-terminus of the tripeptide is anchored to Glu271, assisted by the zinc ligand Glu318 and Gln 136, whereas the C-terminus is bound by Arg563. The water is displaced from the zinc atom by the carbonyl oxygen of the tripeptide and polarized by Glu296 to promote an attack on the carbonyl carbon of the scissile peptide bond. The oxyanion of the reaction intermediate is stabilized by Tyr383 and the zinc. In the final reaction step, Glu296 shuffles a proton from the hydrolytic water to the leaving group.

The water molecule is simultaneously polarized by the carboxylate of Glu296 to promote an attack on the carbonyl carbon of the scissile peptide bond. Further insights were obtained by crystal structures of enzyme in complex with Arg tripeptides and transition state analogues showing that the position of the substrate shifts during turnover with exchange of zinc coordinating groups, while maintaining the overall coordination geometry.[145] The oxyanion of the reaction intermediate is stabilized by Tyr383 and the zinc. In the final reaction step, Glu296 shuffles a proton from the hydrolytic water to the leaving group (Figure 9). Tripeptide substrates bind along the conserved GXMEN motif, precisely occupying the distance between Glu271 and Arg563, whereas the Arg specificity is governed by a narrow S1 pocket capped with Asp375.[145]

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5.4.7. Two Catalytic Activities Exerted via Specific but Overlapping Active Sites. Compilation of information from biochemical studies, mutational analysis, and X-ray crystallography leads to the conclusion that the two enzyme activities of LTA_4 hydrolase are exerted via distinct, yet overlapping active sites. Thus, certain residues are specifically required for the aminopeptidase reaction, i.e., Glu296 and Tyr383, whereas Asp375 is critical only for the epoxide hydrolase reaction. On the other hand, Glu271, Arg563, and the zinc atom are necessary for both catalyzes. In fact, Glu271 is a unique example of a residue that is shared between two catalytic machineries, yet carrying out a separate chemistry in each of the two enzyme reactions.

5.5. LTC_4 Synthase

LTC_4 synthase catalyzes the committed step in the biosynthesis of cys-LTs through conjugation of LTA_4 with glutathione. High levels of enzyme expression and capacity to synthesize LTC_4 are observed in eosinophils, mast cells, and monocytes.[158] Also platelets contain LTC_4 synthase, although these corpuscules cannot produce the substrate LTA_4.[159]

5.5.1. Molecular Properties of LTC_4 Synthase. LTC_4 synthase has a molecular mass of ~18 kDa and is stimulated by divalent cations, particularly Mg^{2+}, as well as phosphatidylcholine, whereas reduced glutathione is required for stability. This notoriously unstable enzyme was purified in the early 1990s, cloned, and sequenced, which revealed a 150-amino-acid protein with three predicted transmembrane spanning domains.[160−163] Two consensus sequences for PKC phosphorylation were found, and subsequent studies have shown that phosphorylation reduces the LTC_4 synthase activity.[164] Recently, a ribosomal protein S6 kinase (p70S6K) was suggested to play a key role in phosphoregulation of LTC_4 synthase in human macrophages.[165]

5.5.2. LTC_4 Synthase Is a Member of the MAPEG Superfamily of Integral Membrane Proteins. Sequence comparisons between LTC_4 synthase and FLAP demonstrated a 33% identity between the two proteins.[162,163] Further work identified two additional homologues microsomal GSH transferases (MGST2 and MGST3), which both possess LTC_4 synthase activity and peroxidase activity against lipid hydroperoxides.[166,167] MGST2 is 44% identical with LTC_4 synthase and may account for LTC_4 synthesis in certain cells and tissues such as endothelial cells and the testis.[168−170] Yet another homologue was subsequently found to catalyze isomerization of prostaglandin H_2 (PGH_2) into prostaglandin E_2 (PGE_2) and was denoted microsomal prostaglandin E synthase type 1 (mPGES-1). This enzyme was found to be induced by LPS and cytokines in tandem with COX-2 and appears to be the origin of PGE_2 synthesized during inflammation.[171,172] Several studies have connected mPGES-1 with inflammatory diseases and cancer, and therefore this enzyme is an attractive drug target, possibly with a better safety profile than COX-2.[173] LTC_4 synthase, FLAP, MGST1, MGST2, MGST3, and mPGES-1 are now recognized as members of a common superfamily of integral membrane proteins denoted MAPEG (membrane-associated proteins in eicosanoid and glutathione metabolism).[174]

5.5.3. Gene Structure and Regulation of LTC_4 Synthase Expression. The complete gene for human LTC_4 synthase (*LTC4S*) has also been cloned and sequenced.[175] This gene is located on chromosome 5q35, spans ~2.5 kb, and has a structure similar to *ALOX5AP*. Thus, it contains five exons and has exon−intron junctions that align identically. The promoter region of *LTC4S* contains several potential cis-elements including Sp1, AP-1, and AP-2. Further promoter characterization revealed that an Sp1 site and a putative initiator element (Inr) are involved in noncell-specific expression, whereas a Kruppel-like transcription factor and Sp1 are implied in cell-specific regulation of *LTC4S*.[176]

Studies in vitro have also demonstrated that LTC_4 synthase expression is induced by phorbol-12-myristate-13-acetate (PMA) in human erythroleukemia cells and in human eosinophils developed from cord blood progenitors treated with IL-3 and IL-5.[177,178] LTC_4 synthase is also induced in the monocytic cell line THP-1 treated with TGFβ, and this response seems to be mediated via Sp1 and Sp3.[179,180] On the other hand, TNF-α caused downregulation of LTC_4 synthase expression in THP-1 cells, a finding which contrasts with the in vivo induction of the enzyme observed in liver, heart, adrenal gland, and brain in rats after intraperitoneal injection of LPS.[181] In addition, the Th2 cytokine IL-4 induces a pronounced increase in the expression of LTC_4 synthase in cord blood-derived human mast cells and bone marrow-derived mouse mast cells, a response that may be signaled via a STAT-6 binding motif in the gene promoter.[182]

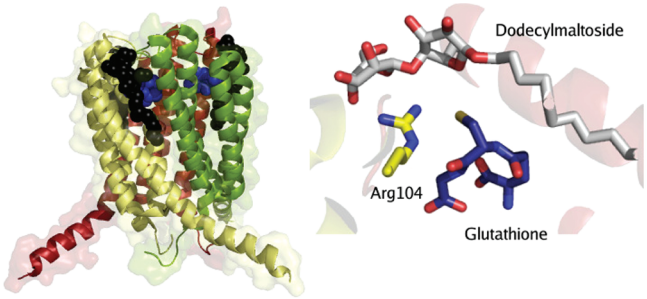

Figure 10. Structure of human LTC_4 synthase. Crystal structure of LTC_4 synthase at 2.0 Å resolution (PDB id: 2UUh) reveals a homotrimer composed of five α-helices, four of which go through the membrane. The lipid binding site, indicated by a bound dodecylmaltoside represented in black balls, is located in a hydrophobic crevice formed between two adjacent monomers. GSH, indicated in blue spheres, is bound by ligands from two adjacent monomers. The relationships between the critical Arg104, the juxtaposition of the thiol group of GSH, bound in its unique horseshoe conformation, and dodecylmaltoside are indicated to the right.

5.5.4. Crystal Structure of LTC_4 Synthase. In 2007, two research groups independently reported the crystal structure of human LTC_4 synthase, and one of the structures was solved at 2.0 Å resolution.[183,184] The enzyme is a trimer, and each monomer is composed of five α-helices, four of which traverse the membrane (Figure 10). As suggested by a bound molecule of the detergent dodecyl maltoside, the lipid (LTA_4) binding site is located in a hydrophobic crevice formed at the interface between two adjacent monomers. The crevice is lined with hydrophobic residues at both sides and at its deepest end becomes a protein pocket with Trp116 as the roof. A modeled LTA_4 molecule fits snugly into the hydrophobic crevice, and its C-20 ω-end is covered by Trp116, which presumably holds the lipid in place and locks it to the bottom of the hydrophobic cleft.

The GSH is bound deeper in the protein below the hydrophobic cleft and adopts a peculiar "horseshoe" shaped conformation, which is unique among GSH transferases. Residues from two monomers are involved in GSH binding; Arg104, Tyr97, Tyr93, Arg51, Asn55, Tyr59, and Glu58 from one monomer, and Arg30 and Gln53 from the neighboring monomer.

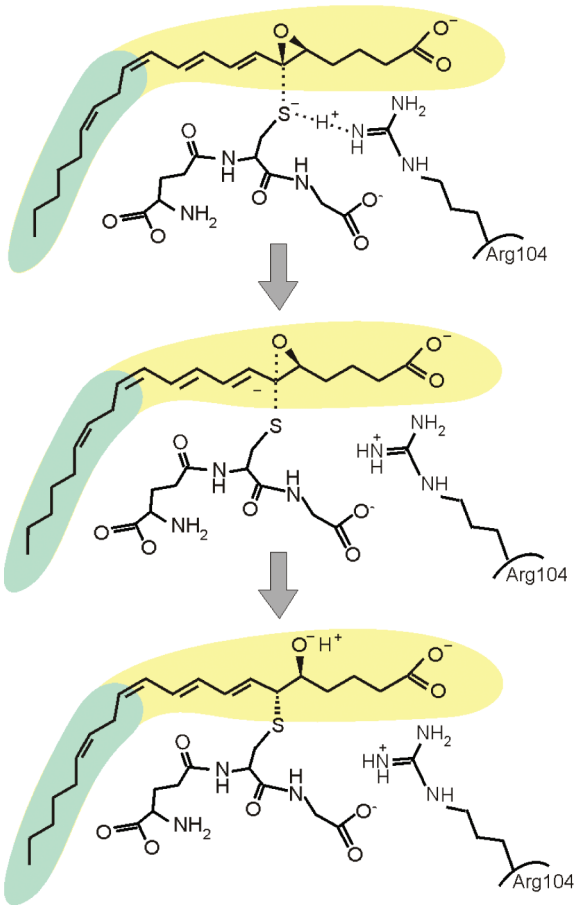

Figure 11. Catalytic mechanism of LTC_4 synthase. The GSH thiol is activated through deprotonation and stabilization by the neighboring Arg104. The resulting thiolate will attack the allylic C-6 of the oxirane ring of LTA_4 according to an S_N2 mechanism via an sp^2 hybridized transition state, which is stabilized by delocalization of the negative charge over the conjugated triene system. Finally, the epoxide opens, with an oxyanion as the leaving group, resulting in chiral inversion and formation of the 5(*S*)-hydroxy-6(*R*)-*S*-glutathionyl conjugate of LTA_4. Portions of the hydrophobic crevice facing the lipid bilayer are indicated in yellow, whereas green color depicts an all-protein pocket located deeper in the protein and covered at its end by Trp116.

From the structure, Arg104 was identified as a candidate residue for activation of the GSH thiol. Further studies with site-directed mutagenesis and analysis of the thiolate anion by UV spectrometry revealed that Arg104 rapidly activates the GSH thiol at three active sites per trimer.[117] Arg31 located near the active site has also been proposed to play a role in the catalytic mechanism, but structural and mutational data have been inconclusive.[117]

5.5.5. Catalytic Mechanism of LTC_4 Synthase. On the basis of structural, biochemical, and kinetic data, a mechanism for LTC_4 synthase can be deduced (Figure 11). In a first step, the cysteinyl thiol is activated through deprotonation and stabilization by the neighboring Arg104. The resulting thiolate will attack the allylic C-6 of the oxirane ring of LTA_4 according to an S_N2 mechanism via an sp^2 hybridized transition state, which is stabilized by delocalization of the negative charge over the conjugated triene system. Finally, the epoxide opens, with an oxyanion as the leaving group, resulting in chiral inversion and formation of the 5(*S*)-hydroxy-6(*R*)-*S*-glutathionyl conjugate of LTA_4 (Figure 11).

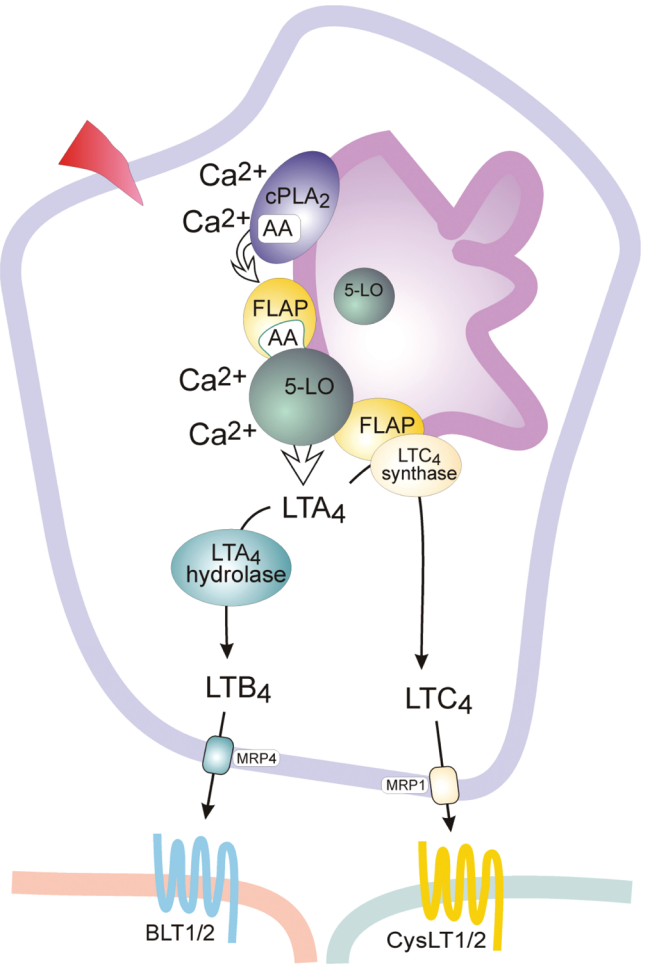

Figure 12. Cellular biosynthesis of leukotrienes. Upon cellular stimulation, intracellular $[Ca^{2+}]_i$ is increased, which leads to translocation of $cPLA_2\alpha$ and 5-LO to the nuclear membrane. Free arachidonic acid is presented to 5-LO by FLAP and further converted to LTA_4, which can be transformed into LTB_4 or LTC_4 by the soluble LTA_4 hydrolase or the membrane-bound LTC_4 synthase, respectively. FLAP can act as a protein scaffold for 5-LO and may form heterodimers with LTC_4 synthase. LTB_4 and LTC_4 are exported over the plasma membrane by MRP4 and MRP1 and will reach their cognate receptors on target cells.

6. INTRACELLULAR PROTEIN TRAFFICKING AND COMPARTMENTALIZATION OF LEUKOTRIENE BIOSYNTHESIS

6.1. Translocation of $cPLA_2\alpha$

A variety of cellular stimuli that increase $[Ca^{2+}]_i$ trigger $cPLA_2\alpha$-dependent release of arachidonic acid by promoting translocation of $cPLA_2\alpha$ from the cytosol to the perinuclear region including the nuclear envelope (Figure 12).[55,56] This is an important regulatory step, which is mediated by the $cPLA_2\alpha$ C2 domain and is necessary for the enzyme to access phospholipid substrates. The $cPLA_2\alpha$ C2 domain has a high affinity for Ca^{2+}, which binds to the calcium binding loops (CBLs).[54,185] Binding of Ca^{2+} reduces the electrostatic potential of the surface-exposed CBLs and is accompanied by membrane penetration by surrounding hydrophobic residues.[186]

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6.2. Translocation of 5-LO and Association with FLAP on the Nuclear Envelope

Early studies showed that, upon cell stimulation, 5-LO is activated, translocates to a membrane compartment, and gets rapidly inactivated in a Ca^{2+}-dependent manner.[113,187] Experiments with mouse mast cells stimulated with ionophore A23187 or anti-IgE showed that translocation may be reversible and that the strength and duration of the Ca^{2+} influx may determine the reversibility of translocation, as well as the extent of enzyme inactivation.[188] Furthermore, using the reversible 5-LO inhibitor zileuton, it was demonstrated that translocation and catalysis are not necessarily coupled and that translocation/membrane association can alter the substrate specificity of 5-LO and increase the efficiency of LTA_4 biosynthesis.[189]

Of particular interest was the discovery that FLAP is localized to the nuclear envelope of resting and activated neutrophils and that 5-LO, upon cell activation, translocates to the same compartment (Figure 12).[190] Translocation of 5-LO also occurs in cells that do not express FLAP and has, therefore, been suggested to be a FLAP-independent process.[191] In neutrophils, 5-LO translocation is associated with functional responses such as activation, adherence, and increased ability to synthesize LTB_4.[192] Furthermore, 5-LO has been shown to associate with growth factor receptor-binding protein 2 (Grb2), an "adaptor" protein for tyrosine kinase-mediated cell signaling, through Src homology 3 (SH3) domain interactions.[193] SH3 interactions regulate the assembly of protein complexes involved in cell signaling and cytoskeletal organization and may form the molecular basis for 5-LO translocation. Moreover, the *N*-terminal β-barrel domain in 5-LO plays a role in this process.[109]

6.2.1. 5-LO in the Nucleoplasm. Further analysis of the enzyme compartmentalization revealed that 5-LO can also be present in the nucleus of resting cells associated with the nuclear euchromatin, a site from which it translocates to the nuclear envelope (Figure 12).[194] Import of 5-LO to the nucleus seems to be regulated by multiple nuclear import sequences centered at Arg518, Arg112, and Lys158.[195,196] Nuclear localization of 5-LO is an important determinant for LTB_4 synthetic capacity, and import and export of 5-LO across the nuclear pores appear to be regulated by phosphorylation at Ser-271 and Ser-563.[97,197,198] On the other hand, adherence of eosinophils was accompanied by nuclear import of 5-LO from cytosol and reduced capacity to synthesize LTC_4.[199] This effect was explained by a resistance to activation of the nuclear pool of 5-LO. Interestingly, the soluble LTA_4 hydrolase has also been reported to reside in the nucleus of rat basophilic leukemia cells, rat alveolar macrophages, and human type II alveolar epithelial cells, whereas rat neutrophils only contained immunoreactive protein in the cytosol.[200,201]

6.2.2. Organization of a Leukotriene Biosynthetic Complex in the Nuclear Membrane. The master enzymes in leukotriene biosynthesis, viz., cPLA2α and 5-LO, apparently translocate to the nuclear membrane upon cell stimulation in a tightly regulated manner (Figure 12). At this site, FLAP and LTC_4 synthase are embedded in the membrane, ready to support biosynthesis of LTA_4 and its further conversion into LTC_4.[194,202,203] In fact, biophysical evidence has been presented indicating that FLAP and LTC_4 synthase form functional dimers and trimers within the membrane and that FLAP can act as a scaffold protein for association of 5-LO, thus creating a multiprotein biosynthetic complex on both the outer and inner nuclear membranes (Figure 12).[204,205] It is not clear how arachidonic acid, liberated by $cPLA_2\alpha$, can first reach its binding site in FLAP within the lipid bilayer, travel further to the active site of 5-LO, and, after conversion into LTA_4, reach the terminal LTA_4 hydrolase and LTC_4 synthase. To synchronize this machinery, it seems necessary that both $cPLA_2\alpha$ and 5-LO are juxtaposed in direct contact with the nuclear membrane and that all biosynthetic components are spatially interconnected. Because LTA_4 hydrolase is soluble and does not translocate to the nuclear membrane, transfer of LTA_4 from 5-LO to LTA_4 hydrolase seems to require a carrier, perhaps a soluble fatty acid binding protein.[206]

The compartmentalization of leukotriene biosynthesis at the outer as well as the inner nuclear membrane also suggests that the enzymes and/or their products may have additional intracellular/intranuclear functions, perhaps related to signal transduction or gene regulation. In line with this notion, it has been reported that LTB_4 is a natural ligand to the nuclear orphan receptor PPARα, suggesting that LTB_4 may have intranuclear functions possibly coupled to lipid homeostasis.[207,208]

In this context, it is also worth noticing that the receptor CysLT1 has been localized inside the nucleus of colorectal adenocarcinoma cells and both CysLT1 and CysLT2 were identified in the same compartment in a human mast cell line LAD2.[209,210] In addition, the BLT1 receptor has been found in intracellular granules of human endothelial cells colocalizing with CCL2 (MCP-1) and P-selectin.[211] Upon stimulation with LTB_4 or LPS, BLT1 was evenly distributed over the cytoplasm and in the nucleus.

6.3. Leukotriene Biosynthesis in Lipid Bodies and Actions on Extracellular Granules

Lipid bodies are nonmembrane-bound, lipid-rich cytoplasmic inclusions that are abundant in cells engaged in inflammatory and degenerative processes, and these organelles have been extensively studied as potential sources of eicosanoid and leukotriene synthesis, especially in eosinophils.[212,213] Several physiological stimuli, such as IgG, IL-5, platelet-activating factor, and the CCR3 agonists Eotaxin and CCL5 (RANTES), can elicit lipid body formation in human eosinophils, and these events are associated with increased formation of LTC_4.[214] Furthermore, all enzymes required for LTC_4 synthesis, i.e., cPLA2α, 5-LO, and LTC_4 synthase, are present within eosinophil lipid bodies.[215,216] The functional consequences of two sites for cys-LT production in human eosinophils, i.e., a perinuclear and a lipid body pool, are not fully understood but may reflect autocrine, paracrine, and intracrine roles for these mediators.[217]

Eosinophils can extrude membrane-bound granules extracellularly at sites associated with eosinophil infiltration, including allergic diseases and helminths, and functional capabilities of these organelles have been demonstrated.[218,219] Interestingly, receptors for cys-LTs, viz., CysLT1, CysLT2, and $P2Y_{12}$, are expressed on both extracellular and intracellular eosinophil granule membranes and can signal in response to their leukotriene agonists to elicit secretion.[220] Apparently, stimulation of granule secretion could be one intracrine function displayed by cys-LTs in human eosinophils.

7. TRANSCELLULAR BIOSYNTHESIS OF LEUKOTRIENES

Just like other eicosanoids such as prostaglandins and thromboxane, leukotrienes may be formed via transcellular biosynthesis. This concept involves a donor cell capable of generating the intermediate LTA_4, which is exported to a recipient cell equipped with enzymes for further metabolism into LTB_4 or cys-LTs. This

phenomenon has been well documented in vitro, using mixed incubations of neutrophils or other leukocytes rich in 5-LO activity, with, e.g., erythrocytes, endothelial cells, platelets, or smooth muscle cells, which contain downstream LTA_4 hydrolase and/or LTC_4 synthase.[221] The physiological role of this process has been under debate because LTA_4 is a highly unstable molecule ($t_{1/2} \approx 10$ s at neutral pH) that has to be shielded from hydrolytic attack by water.[222] However, several molecules, e.g., albumin, liposomes, and fatty acid binding protein, have been shown to act as chaperones against LTA_4 breakdown and could potentially facilitate transcellular trafficking of LTA_4 during cell−cell interactions.[206,222,223] In 2002, Fabre and co-workers could demonstrate that transcellular metabolism does indeed occur in vivo and that this process accounts for a significant portion of LTB_4 synthesized in a mouse model of cutaneous inflammation.[224] Similarly, substantial amounts of cys-LTs generated during mouse peritoneal inflammation are produced via transcellular routes.[225]

8. EXPORT OF LEUKOTRIENES AND THEIR COGNATE RECEPTORS

To elicit biological responses as paracrine mediators, leukotrienes are exported over the plasma membrane to reach neighboring cells (Figure 12). Release of LTB_4 and LTC_4 from human neutrophils and eosinophils, respectively, was described as saturable, carrier-mediated processes.[226,227] It was demonstrated that LTC_4 is transported over the membrane via a specific probenecid-sensitive export-carrier, later identified as the ATP-dependent efflux pump multidrug resistance protein ABCC1 (MRP1).[228,229] Another member of the same protein familiy, ABCC4 (MRP4) can mediate ATP-dependent LTB_4 efflux in the presence of glutathione, and the same pump can also transport LTC_4, e.g., in platelets.[230] Target cells are equipped with G protein-coupled 7-transmembrane-spanning receptors (GPCRs) that recognize specific leukotrienes and transduce intracellular signaling with different sets of G-proteins and second messengers in a ligand−receptor and cell-specific manner; for a review, see ref 231.

8.1. Receptors for LTB_4, BLT1, and BLT2

For LTB_4, two types of GPCR with different affinities and cellular expression profiles are known (BLT1 and BLT2). The BLT1-receptor was cloned and characterized as a 43 kDa GPCR distantly related to certain somatostatin receptors as well as some of the chemokine receptors, e.g., those which bind formyl-Met-Leu-Phe (fMLP), lipoxin A_4 (LXA_4), and C5a.[232,233] The receptor shows a high degree of specificity for LTB_4 with a K_d of 0.15−1 nM[233,234] and is expressed in a variety of inflammatory cells, including lymphocytes and mast cells.[235−239] Other LTB_4 analogues and lipoxygenase-derived products compete with LTB_4 for binding at BLT1 with a rank order of potency 20-OH-LTB_4 = 20-oxo-LTB_4 > 12(*R*)-HETE > 20-COOH-LTB_4.[231] In resting endothelial cells, BLT1 is expressed at low levels, but expression and function can be induced by LPS and LTB_4 itself, suggesting a functional role during the early vascular response to inflammation.[240]

A second GPCR for LTB_4, denoted BLT2, has also been identified.[234,241,242] This receptor is homologous to the BLT1 receptor but has a higher K_d value for LTB_4 (23 nM) and a broader ligand-specificity, including 12-HETE and 15-HETE, and exhibits a different binding profile for various BLT antagonists.[243] It was recently shown that 12(*S*)-hydroxy-5-*cis*-8,10-*trans*-heptadecatrienoic acid (12-HHT), a side-product during thromboxane synthesis from prostaglandin endoperoxide, is an endogenous high-affinity ligand for BLT2, thus creating a functional link between the leukotriene and prostaglandin pathways.[244] In contrast to the BLT1 receptor, which is predominantly found in leukocytes, BLT2 is ubiquitously expressed in various tissues. The physiological role of BLT2 is not yet established, but recent work in a mouse model of colitis suggests that BLT2 may signal anti-inflammatory functions.[245]

The genes encoding the LTB_4 receptors are located on chromosome 14.[241,246] Interestingly, the open reading frame of the gene encoding the BLT2 receptor is located within the promoter region of the BLT1 gene, an unusual gene structure previously not described among mammals.[235] On the basis of the crystal structure of rhodopsin, models of BLT1 structures have been generated, and residues implicated in catalysis and signaling have been identified by site-directed mutagenesis.[247,248]

8.2. Receptors for Cysteinyl Leukotrienes, CysLT1, CysLT2, and the related gpr17, $P2Y_{12}$, and "CysLTE"

The cys-LTs are recognized by two major GPCRs (CysLT1 and CysLT2), both of which have been cloned and characterized.[249−253] The CysLT1 receptor contains 336 amino acid residues and mRNA is found in, e.g., the spleen, peripheral blood leukocytes, lung tissue, smooth muscle cells, macrophages, and mast cells.[249,250,254,255] The preferred ligands for the CysLT1 receptor are LTD_4 followed by LTC_4 and LTE_4 in decreasing order of potency. It is generally believed that the classical bioactions elicited by cys-LTs, such as smooth muscle contraction, increased vascular permeability, and plasma leakage, emanate from CysLT1 signaling, and this receptor is targeted by antiasthma drugs such as montelukast. The gene encoding the receptor is located on the X chromosome.[249]

The CysLT2 receptor contains 345 amino acids with ~40% sequence identity to the CysLT1 receptor.[251−253] This receptor binds LTC_4 and LTD_4 equally well, whereas LTE_4 shows low affinity to the receptor. Studies on the tissue distribution of the CysLT2 receptor show high levels of mRNA in, e.g., heart, brain, peripheral blood leukocytes, spleen, placenta, and lymph nodes, whereas only small amounts are found in the lung. Human umbilical vein endothelial cells express almost exclusively CysLT2, and work on transgenic mouse models has indicated that CysLT2 is mostly expressed in small vessels of selected tissues and mediates increased vascular permeability by changes in transendothelial vesicle transport.[256−258] In a mouse model of endothelial overexpression of CysLT2, the receptor could be linked to myocardial ischemia−reperfusion injury, and CysLT2 has also been implicated in regulation of neuronal signaling in the gut.[259,260]

Cysteinyl leukotriene receptors are phylogenetically related to P2Y nucleotide receptors, and both CysLT1 and CysLT2 have been reported to signal in response to nucleotides in mast cells.[254,261] More recent work, however, has demonstrated that LTD_4 and uridine diphosphate signal through separate receptors in monocytes and U937 cells, thus disputing the earlier findings regarding CysLT1.[262,263] In 2006, gpr17 was deorphanized, revealing a GPCR capable of signaling with both cys-LTs and uracil nucleotides.[264,265] Gpr17 is expressed at high levels in brain, heart, and kidney, i.e., tissues often afflicted by ischemic damage, and gpr17 has been suggested to play a role as sensor of damage in spinal cord injury.[266] In line with this notion, it was

recently proposed that gpr17 governs the transition between immature and myelinating oligodendrocytes and may thus represent a potential therapeutic target for myelin repair in the central nervous system.[267] However, it is not clear how these data can be reconciled with a role for cys-LTs as agonists for gpr17. In fact, gpr17 has been identified as a ligand-independent negative regulator of CysLT1 signaling in response to LTD_4,[268] and data disputing the role of gpr17 as receptor for cys-LTs have been presented.[269]

Two additional receptors for LTE_4 have been discovered, one of which is identical with $P2Y_{12}$ and the other not yet characterized at the molecular level and here referred to as "CysLTE".[270,271] Both of these receptors possess interesting pharmacological properties; $P2Y_{12}$ is the receptor for the antithrombotic agent clopidogrel, and $P2Y_{12}$, as well as CysLTE, are poorly antagonized by the CysLT1 selective MK571, opening new avenues for therapeutic interventions. However, one should keep in mind that several P2Y receptors are sensitive to inhibition by CysLT1 antagonists, and further work is required to define the cys-LT related pharmacology of $P2Y_{12}$ and CysLTE.[263,272] Nevertheless, it is clear that cys-LT signaling is complex and involves multiple interconnected GPCRs in a cobweb of cross-talk and cross-desensitization between leukotrienes and nucleotides.[210,262,273−275]

9. TRANSGENIC MOUSE MODELS

Lipoxygenase products, particularly the leukotrienes, exhibit myriads of effects in various in vitro models of inflammation and immune modulation. In this article, it is not possible to cover all these biological activities, and instead we will focus on what has been learned from experiments with gene-disrupted mice, a powerful tool to delineate the biology of lipid mediators.

9.1. Deletion of cPLA$_2\alpha$

Targeted deletion of the cPLA$_2\alpha$ gene in mice leads to reduced fertility but is also protective against a number of inflammatory conditions.[49,50] For example, cPLA$_2\alpha$ knockout mice are less susceptible to allergic reactions, acute lung injury by sepsis and acid aspiration, inflammation-induced bone resorption, collagen-induced arthritis, experimental autoimmune encephalitis, and cognitive deficits in a mouse model of Alzheimer's disease.[276−280] In addition, cPLA$_2\alpha$ suppresses striated muscle growth and colon tumorigenesis.[281,282] Since cPLA$_2\alpha$ provides free arachidonic acid for both leukotriene and prostaglandin biosynthesis, it is difficult to determine to which extent deficits in specific lipoxygenase products contribute to these cPLA$_2\alpha$ knockout phenotypes.

9.2. 5-LO-Deficient Mice

The role of 5-LO and its products has been studied by gene targeting, and a steady stream of studies over the past 17 years with 5-LO-deficient mice has revealed a plethora of wide-ranging roles, primarily in pathophysiological settings.[283] 5-LO-deficient mice are more resistant to lethal effects of shock induced by platelet activating factor (PAF) and also show a marked reduction in the ear inflammatory response to exogenous arachidonic acid but not to phorbol ester, effects that appear to be mediated primarily via LTB_4.[283,284] Interestingly, the inflammatory response induced by arachidonic acid could be virtually eliminated by the prostaglandin synthase inhibitor indomethacin in 5-LO-deficient mice but not in normal animals, suggesting links between prostaglandins and 5-LO pathways during inflammatory reactions.[285] Of note, the inflammatory response was highly dependent on genetic factors since 5-LO-deficient mice on different backgrounds displayed markedly different reactions.[286] Use of these leukotriene-deficient mice enabled verification of the transcellular hypothesis that LTA_4 could be "passed" between different cell types in the settings of topical skin, as well as peritoneal, inflammation.[224,225]

In relation to the role of 5-LO products and host defense, 5-LO null mice are more susceptible to infections with *Klebsiella pnemoniae*, in line with a role for 5-LO and, in particular LTB_4, to augment neutrophil phagocytosis in antimicrobial host defense.[287,288] In agreement with these studies, it was recently demonstrated that 5-LO-deficient mice infected with *Borrelia burgdorferi* (carrier of Lyme disease) are more susceptible to development of persistent arthritis.[289] Interestingly, the adaptor protein MyD88, which mediates toll-like receptor signaling, is defective in activation of NF-κB in mice lacking 5-LO. This macrophage signaling anomaly was associated with lower basal and inducible expression of MyD88 and reflected impaired activation of STAT1 and overexpression of the STAT1 inhibitor SOCS1 via LTB_4 and its receptor BLT1.[290]

In contrast to the host defense roles of leukotrienes, there are occasions when 5-LO-deficient mice are either protected or are not affected positively or negatively when infected with pathogens. For instance, when infected with *Trypanosoma cruzi*, 5-LO null mice display reduced acute myocardial inflammation and mortality, and when infected with *Schistosoma mansoni*, the mice have reduced liver granulomas.[291,292]

In the respiratory system, 5-LO-deficient mice exhibit reduced airway reactivity in response to methacholine and lower levels of serum immunoglobulins using the ovalbumin model of airway inflammation.[293] Peters-Golden et al. showed that 5-LO null mice were protected from pulmonary fibrosis.[294] Interestingly, 5-LO deficiency prevents respiratory failure during ventilator-induced lung injury.[295]

With regards to the cardiovascular system, studies with 5-LO-deficient mice yielded some provocative data that the 5-LO pathway was directly involved in the atherogenic disease process,[296] but this was later tempered by negative results.[297−299] In contrast, 5-LO was found to be involved in the pathogenesis of hyperlipidemic abdominal aortic aneurysm (AAA).[297] However, related to AAA, the modality of aneurysm induction determines the extent of 5-LO pathway involvement.[298] Overall there are mixed data relating to the 5-LO/leukotriene pathway involvement in atherogenesis and AAA in mice, and this has been discussed in more detail elsewhere.[300]

Blockade of 5-LO in null mice did not affect cardiac ischemia−reperfusion injury, but the postischemic inflammatory response was actually elevated as measured by increased neutrophil influx and TNF-α synthesis in infarcted myocardium.[301]

There are many other recent examples of potential roles of 5-LO in various organ systems using 5-LO-deficient mice. Recently, it was demonstrated that hepatic inflammation and hepatocyte injury due to TNF-α were reduced in 5-LO deficient mice on a hyperlipidemic genetic background.[302] 5-LO expression is found in skin Langerhans cells, and a selective role of leukotrienes in the directed movement of dendritic cells following skin sensitization was determined using 5-LO deficient mice.[303] Finally, in very provocative data that will require further verification, 5-LO null mice exhibit impaired leukemia stem cells but not normal hematopoietic cells and do not develop the cardinal features of chronic myeloid leukemia.[304]

9.3. FLAP-Deficient Mice

The role of leukotrienes as inflammatory mediators has also been corroborated in studies of FLAP-deficient mice. Like the 5-LO-deficient mice, these animals showed a blunted response to topical arachidonic acid, had increased resistance to PAF-induced shock, and responded with less edema in zymosan-induced peritonitis.[305] Furthermore, the severity of collagen-induced arthritis was substantially reduced in FLAP null mice, indicating a role for leukotrienes in this model of inflammation.[306]

9.4. Mice Lacking LTA_4 Hydrolase

Mice deficient in LTA_4 hydrolase have been generated by targeted gene disruption.[284] These mice develop normally and are healthy. Analysis of their reactivity against various proinflammatory stimuli revealed that LTA_4 hydrolase is required for the formation of LTB_4 during an in vivo inflammatory reaction. Comparing the phenotype of these mice with that of 5-LO-deficient mice allowed a delineation of the relative contribution of LTB_4 and cys-LTs, respectively, to a specific inflammatory response. Thus, LTB_4 is responsible for the characteristic influx of neutrophils, which follows topical application of arachidonic acid and contributes to the vascular changes observed in this inflammatory model. In zymosan A-induced peritonitis, LTB_4 modulates only the cellular component of the response, whereas LTC_4 appears to be responsible for the plasma protein extravasation. Moreover, LTA_4 hydrolase was shown to be upregulated in the hearts of angiotensin II-induced hypertensive rats, thus providing further evidence for a role of LTA_4 hydrolase in inflammatory reactions in vivo.[307] Of note, LTA_4 hydrolase null mice are resistant to the lethal effects of systemic shock induced by PAF, thus identifying LTB_4 as a key mediator of this reaction.

9.5. LTC_4 Synthase-Deficient Mice

LTC_4 synthase-deficient animals have been generated by targeted gene disruption and were found to develop normally and without signs of impaired fertility.[170] In agreement with the results obtained with LTA_4 hydrolase-deficient mice, the LTC_4 synthase knockout mice displayed a reduced plasma protein extravasation in zymosan A-induced peritoneal inflammation. In addition, these mice exhibited an attenuated response in a passive cutaneous anaphylaxis model and were protected significantly against alveolar septal thickening by macrophages and fibroblasts, as well as collagen deposition in the bleomycin-induced lung fibrosis model.[308] Certainly, the LTC_4 synthase-deficient mice will be a useful tool for further studies of the biological role of LTC_4 in physiological as well as pathophysiological conditions. For instance, further insights may be gained regarding the described role of LTC_4 in the primary immune response involving mobilization of dendritic cells to lymph nodes,[309] the increased expression of LTC_4 synthase in human abdominal aortic aneurysm,[310] or the LTC_4 deficiency associated with pediatric neurodegenerative diseases.[311,312]

9.6. Mice Lacking BLT1 and/or BLT2

The role of the BLT1 receptor has been studied by targeted gene disruption.[313,314] The receptor was necessary to elicit the physiological effects of LTB_4 (e.g., chemotaxis, calcium mobilization, and adhesion to endothelium) and important for the recruitment of leukocytes in an in vivo model of peritonitis. As also observed in mice lacking 5-LO, FLAP, or LTA_4 hydrolase, BLT1-deficient mice were protected from the lethal effects of PAF-induced anaphylaxis. In a series of studies with knockout mice, BLT1 has been implicated in atherosclerosis, aortic aneurysm, asthma, and arthritis.[315−321]

9.7. Deletions of CysLT1, CysLT2, and gpr17

CysLT1 deficient mice display no overt phenotype under normal nonstressed conditions. However, when inflammation is induced either with zymosan or by means of IgE-mediated passive cutaneous anaphylaxis, plasma protein extravasation is significantly reduced in the null mice.[322] These data support a role for cys-LTs generated by monocytes/macrophages/mast cells via CysLT1 to regulate the microvasculature in acute inflammation.

CysLT1 receptor knockout mice were examined in the same bleomycin-induced lung fibrosis model mentioned above for LTC_4 synthase-deficient mice.[308] These mice displayed elevated levels of cys-LTs in bronchoalveolar lavage fluid and enhanced alveolar septal thickening in the bleomycin-induced lung fibrosis model, at variance with the well-established anti-inflammatory effects of clinically used CysLT1 antagonists. Moreover, these results were unexpectedly opposite to those observed in the LTC_4 synthase-deficient mice and indicate that cys-LTs promote chronic fibrotic via the CysLT2 receptor.[323]

Two different strains of CysLT2 deficient mice have been generated and studied in a variety of inflammatory settings.[258,323] Neither of these strains showed any overt abnormalities. Analogous to the CysLT1 receptor-deficient mice, the increased vascular permeability associated with IgE-dependent passive cutaneous anaphylaxis was attenuated in CysLT2 receptor-deficient mice, but unlike the previously mentioned studies, the plasma protein extravasation response to zymosan in peritoneal inflammation was not altered. Moos et al. revealed that the microvascular leakage mediated via CysLT2 involves a transcytotic vesicular transport of proteins across the endothelial layer.[258] CysLT2-mediated leakage has been demonstrated in the cremaster muscle and ear microvasculature.[258,324] In the pulmonary bleomycin-induced fibrosis model, there was a reduction in the cardinal features of pathophysiology in the null mice indicating the ability of CysLT2 to promote chronic pulmonary inflammation with fibrosis.[323] CysLT2 was also shown to play a role in myocardial ischemia−reperfusion injury, at least in settings where the receptor is overexpressed via enhanced microvascular permeability, upregulation of gene expression for leukocyte adhesion molecules, and recruitment of inflammatory leukocytes into the infarcted myocardial tissue.[259]

As mentioned above, there exists some controversy as to whether gpr17 is a bona fide leukotriene receptor. gpr17 was found predominantly in oligodendrocytes, and gpr17 knockout mice showed early onset of oligodendrocyte myelination, indicating that this GPCR orchestrates the transition between immature and myelinating oligodendrocytes.[267] No studies have been reported on any leukotriene-dependent functions in these gpr17-deficient mice. Therefore, further studies will be necessary to support a putative leukotriene function of this receptor.

10. OTHER MAMMALIAN LIPOXYGENASE FAMILY MEMBERS

Besides 5-LO, there are five other LO family members in humans and six in mice. These will be briefly described in the following sections and in Table 1.

Table 1. Human and Mouse Lipoxygenases

lipoxygenase (human; mouse) [common abbreviations]	gene name (human; mouse)	main substrate(s)	main product(s)	biological (pathological) functions
5-lipoxygenase[a] [5-LO, 5-LOX]	*ALOX5; Alox5*	arachidonic acid	5(*S*)-HpETE, LTA_4	leukotriene products, inflammatory mediators
"platelet-type" 12-lipoxygenase[a] [P-12LO, 12-LOX]	*ALOX12; Alox12*	arachidonic acid	12(*S*)-HpETE	modulation of platelet aggregation
12*R*-lipoxygenase[a] [12R-LO]	*ALOX12B; Alox12b*	linoleoyl-ω-hydroxy- ceramide	9(*R*)-hydroperoxylinoleoyl-ω-hydroxyceramide	maintenance of epidermal permeability barrier
15-lipoxygenase type 1;	*ALOX15; Alox15*	arachidonic acid	15(*S*)-HpETE, 12(*S*)-HpETE	oxidative modification of lipids in foam cells;
"leukocyte-type"12-lipoxygenase [15-LO, 15-LOX-1; L-12LO, 12/15-LO, 12/15-LOX]		linoleic acid esterified 20:4, 18:2	13(*S*)-HpODE esterified HpETE, HpODE	inflammation resolving lipid mediators
15-lipoxygenase type 2; 8-lipoxygenase [15-LO-2; 8-LO, 8-LOX]	*ALOX15B; Alox15b*	arachidonic acid	15(*S*)-HpETE (human), 8(*S*)-HpETE (mouse)	negative cell cycle regulator/ tumor suppressor
epidermis-type lipoxygenase-3[a] [e-LOX3]	*ALOXE3; Aloxe3*	9(*R*)-hydroperoxy-linoleoyl -ω-hydroxyceramide	9(*R*),10(*R*)-*trans*-epoxy-11*E*-13(*R*)-hydroxylinoleoyl-ω-hydroxyceramide	maintenance of epidermal permeability barrier
nonexpressed[¥]; "epidermal-type" 12-lipoxygenase [nonexpressed[¥]; e-12LO, e-LOX1)	*ALOX12E*[¥]*; Aloxe*	(methyl) arachidonate	12(*S*)-HpETE, 15(*S*)-HpETE (6:1 ratio)	murine hair follicle function?

[a] The human and mouse enzymes have similar product profiles.

10.1. Human 15-Lipoxygenase-1 (15-LO-1) and Mouse "Leukocyte-Type" 12-LO

The enzymes now known as 12/15-LO include the 15-LO originally discovered in reticulocytes characteristically found in species such as rabbits and humans, as well as 12-LO originally found in porcine leukocytes and subsequently in mouse and rat tissues. 12/15-LO enzymes catalyze the regio- and stereospecific insertion of molecular oxygen into a variety of free and esterified polyunsaturated fatty acids. Depending on the species, there is usually a distinct polarity toward one regioisomer, but both are often formed simultaneously. For instance, the rabbit and human 12/15-LO enzymes form approximately 9:1 15-HpETE/12-HpETE, whereas the corresponding ratio in mice is about 1:4 when considering arachidonic acid as the primary substrate. A hydrogen atom is abstracted at either position 13 or 10 prior to oxygen addition in the *S*-stereoconfiguration.

In humans, the 12/15-LO is found in eosinophils and airway respiratory epithelium, whereas in mice the highest expression of 12/15-LO is present in peritoneal macrophages.[325] From early studies, a role for the enzyme in rabbit reticulocytes was proposed whereby oxygenation of intracellular organelle membranes would lead to their degradation prior to maturation into erythrocytes devoid of these organelles.[326] Deciphering the roles of 12/15-LO has, in fact, turned out to be much more difficult than anticipated because of significant species variation in expression profiles and the lack of an overt phenotype in 12/15-LO knockout mice. Because in mice the 12/15-LO is most avidly expressed in peritoneal macrophages, various macrophage functions were tested.[325] However, initial experiments failed to show any real impact on phagocytosis and host defense to several pathogens,[292,325] but later on it was discovered that a subset of 12/15-LO deficient mice were susceptible to death when infected with *T. gondii*.[327]

Macrophages turn to foam cells in atherosclerotic lesions and are known to participate in the oxidative modification of lipoproteins, which is a key initiating event in atherogenesis. The majority of studies have revealed a role for macrophage 12/15-LO in promoting atherosclerotic lesions,[328−333] but subsequent studies have shown a protective role.[334] Therefore, it remains to be determined why there are divergent results in the setting of atherosclerosis. One point that seems clear is that 12/15-LO can regulate interleukin-12 and Th1 lymphocyte polarized responses, and several of the proposed activities of the enzyme are likely mediated through this mechanism.[327,331]

The original postulated role of 12/15-LO in reticulocyte maturation in rabbits was not supported in mice, and there were no abnormalities in hematopoietic cells in young knockout mice.[325] A more recent study found that 12/15-LO was necessary for hematopoietic stem cell function.[335] Also, 12/15-LO was identified as a suppressor of myeloproliferative disease as some aging 12/15-LO mice were found to have enlarged spleens.[336] We and other investigators have failed to find this dramatic phenotype in our colonies of 12/15-LO-deficient mice.[299] Much further work is required to precisely delineate the roles of 12/15-LO in mammalian biology given the many perplexing and apparently contradictory findings.

Lipoxygenases are also involved in the generation of lipoxins and related lipid mediators. The central intermediate in the 5-LO pathway, viz., LTA_4, may be converted by 12-LO (see below) or 15-LO into lipoxins, often via transcellular routes involving neutrophils and platelets (Figure 1). In the presence of aspirin, COX-2 is acetylated and capable of converting arachidonic acid

into 15(*R*)-HETE, which in turn is the substrate for production of 15-epi-lipoxin A_4 (15-epi-LXA_4), also termed aspirin-triggered lipoxin.[337] Lipoxins and aspirin-triggered lipoxins have anti-inflammatory and proresolving properties in several settings and may be regarded as lead structures for development of antiphlogistic drugs. Several other classes of molecules derived from eicosapentaenoic and docosahexaenoic acid have been described that possess potent bioactivity promoting the resolution of inflammation.[338]

10.2. Human and Mouse "Platelet-Type" 12-LO

The lipoxygenase found in human platelets in 1974 was the first mammalian form to be characterized and is now known as "platelet-type" 12-lipoxygenase (12-LO).[339] Molecular oxygen was inserted at C-12 of arachidonic acid to form 12(*S*)-hydroperoxy-5,8,10,14-eicosatetraenoic acid (12-HpETE), and the reaction was inhibited by 5,8,11,14-eicosatetraynoic acid (ETYA). It was nearly 20 years later that the molecular tools for this platelet pathway were isolated; the cDNA was found to encode a 663-residue polypeptide of molecular mass 75 kDa.[340,341] The recombinant cytosolic enzyme made almost exclusively one regio- and stereospecific isomer that was previously identified in platelets, i.e., 12(*S*)-HETE, and it was devoid of any hydroperoxidase activity.[342] Besides being present in platelets and their precursor megakaryocyte cells, the 12-LO enzyme was identified in keratinocytes in the germinal layer and was overexpressed in the setting of psoriasis.[343] Platelet 12-LO is also found in some types of cancer cells (e.g., A431 epidermoid carcinoma and Lewis lung carcinoma).

Efforts have been underway for some time to crystallize the 12-LO, but this has been problematic. Low-resolution data have been acquired, and there is reason to believe the active enzyme may form a homodimer.[344] The enzyme appears to have two domains like other lipoxygenases, and removal of the N-terminal beta-barrel domain yields an enzyme that still retains catalytic activity (~20% of the native form).

The role of this enzyme in human and murine platelet biology has been difficult to decipher, compared to the well-known function of promoting platelet aggregation by the other main enzyme (thromboxane synthase) that converts arachidonate to thromboxane A_2. Knockout mice for the platelet-type 12-LO were specifically generated for this purpose.[345] However, convincing roles, other than a minor affect in modulating ADP-induced platelet aggregation, were not unraveled.[345] In skin, this 12-LO may play a minor role in maintenance of the water permeability barrier under basal conditions.[346]

10.3. Human and Mouse 12R-LO

A 12R-LO in skin was the first mammalian lipoxygenase found to introduce molecular oxygen in the opposite stereoconfiguration to all other mammalian lipoxygenases. The cDNAs for human and mouse 12R-LOs were cloned in 1998, encoding enzymes 701 amino acids in length with 86% amino acid identity between the species.[347−349] These enzymes have weak enzymatic activity toward arachidonic acid compared to other lipoxygenases, but the little product that is formed is of the *R*-stereoconfiguration.[348] They also convert other polyunsaturated fatty acids with oxygenation occurring at the ω9 position.[348] The molecular basis for *R* versus *S* stereocontrol of oxygen insertion was subsequently determined to reside in a simple Gly residue near the active site.[25]

Expression of 12R-LO, based on mRNA, was found in skin and hair follicles, and a very profound expression pattern in the developing mouse embryo gave credence to an important role in epidermal function.[348] Expression occurred abruptly in epithelial layers between embryonic days 14.5 and 15.5 throughout the epidermis, nasal passageways, and surface of the tongue. Subsequently, knockout mice were generated and shown to have a striking phenotype, whereby newborn mouse skin would dry out within hours after birth and result in neonatal mortality.[350] Interestingly, null mutations in the human gene *ALOX12B*, which encodes 12R-LO, give rise also to a striking scaly skin phenotype characterized as autosomal recessive congenital ichthyosis (ARCI)[351] The mouse phenotype appears to be more severe as expression of the *Alox12b* gene is indispensable for life.

Recently, a putative biochemical pathway for the epidermal barrier was proposed.[352] In this scheme, 12R-LO forms 9(*R*)-hydroperoxylinoleoyl-ω-hydroxyceramide from the lipid substrate linoleoyl-ω-hydroxyceramide present in stratum corneum. Further conversion by downstream enzymes (eLOX3; see next section), followed by hydrolysis of the oxygenated fatty acyl moiety allows for the attachment of ω-hydroxyceramide to protein to form a lipid−protein scaffold that maintains the epidermal permeability barrier.

10.4. Human and Mouse Epidermal LO-Type 3

Kinzig and colleagues isolated a cDNA encoding a lipoxygenase from mouse skin by polymerase chain reaction (PCR) using primers based on other lipoxygenase sequences and termed it epidermis-type lipoxygenase-3 (eLOX3)[353] The full-length cDNA encodes a protein of 711 amino acids with a calculated molecular mass of 80.6 kDa and displays 54% sequence identity with the 12R-LO cloned a year earlier by similar strategies. This lipoxygenase was expressed in the stratified epithelia of skin, tongue, and forestomach, and the gene (*Aloxe3*) and its location were found to be similar in structure to other lipoxygenase genes on the central region of mouse chromosome 11. eLOX3 showed no enzyme activity when incubated under standard conditions with arachidonic acid.[353]

A few years after the description of eLOX3, a genetic research study examining families with ARCI found evidence of mutations in the orthologous human gene *ALOXE3*, as well as *ALOX12B*, that would lead to premature stop codons in transcripts.[351] Shortly thereafter, Brash and colleagues put together three important findings: (i) colocalized expression of 12R-LO and eLOX3 in skin; (ii) mutations in both corresponding genes giving rise to skin pathologies; and (iii) the lack of traditional oxygenase activity in eLOX3, to define a novel biochemical linear pathway with 12R-LO converting arachidonic acid to 12(*R*)-HpETE and eLOX3, taking this hydroperoxide and converting it to 8(*R*)-hydroxy-11(*R*),12(*R*)-epoxyeicosa-5*Z*,9*E*,14*Z*-trienoic acid, one of the known isomers of hepoxilin A_3, and to 12-ketoeicosatetraenoic acid in a 2:1 ratio.[354] Thus, eLOX3 was found to be a hydroperoxide isomerase that incorporates both atoms of oxygen from the hydroperoxide into the epoxyalcohol, and the enzyme was postulated to be active in the ferrous form, unlike all other known lipoxygenases.[354] Human and mouse eLOX3 enzymes have somewhat different substrate specificities. Thus, while both enzymes prefer hydroperoxides of *R*-stereochemistry (vs *S*-configuration) as substrates, the human enzyme prefers 12(*R*)-HpETE, while the mouse enzyme preferentially metabolizes 8(*R*)-HpETE and converts 8(*S*)-HpETE into 10(*R*)-hydroxy-8(*S*),9(*S*)-epoxyeicosa-5*Z*,11*Z*,14*Z*-trienoic acid.[355]

Subsequent to the studies identifying eLOX3 as a hydroperoxide isomerase, it was recently found that an atypical synthetic fatty acid, 9*E*,11*Z*,14*Z*-20:3ω6, could be oxygenated by this

enzyme specifically to the 9(*S*)-hydroperoxide. However, the reaction showed a pronounced lag phase, which could be overcome with high concentrations of hydroperoxide activator. Under these conditions, dioxygenase activity with arachidonic acid was observed that gave rise to a mixture of HpETEs.[356] It was suggested that eLOX3, because of its inefficient activation, favors hydroperoxide isomerase cycling rather than dioxygenase activity.[356]

As mentioned above, both 12R-LO and eLOX3 participate in a linear biochemical pathway whereby both lipoxygenases contribute to the epidermal permeability barrier. The 12R-LO metabolite, 9(*R*)-hydroperoxylinoleoyl-ω-hydroxyceramide, is acted upon by eLOX3 to form the specific C-18 epoxyalcohol [9(*R*),10(*R*)-*trans*-epoxy-11*E*,13(*R*)-hydroxy] and 9-keto-10*E*,12*Z*-esters of the ceramide.[352] Therefore, it appears that oxygenation of O-linoleoyl-ω-hydroxyceramide by the two lipoxygenases is required to facilitate the ester hydrolysis and subsequent bonding of the ω-hydroxyceramide to protein to form the appropriate epidermal barrier function that is critical to life.

10.5. Human 15-LO-2 and Mouse 8-LO

In mouse skin, application of tumor promoters typically induces an 8*S*-lipoxygenase activity. A cDNA encoding this activity was isolated from a mouse epidermal library.[357] The predicted protein of 677 amino acids with a calculated molecular mass of 76 kDa forms almost exclusively 8(*S*)-HpETE from arachidonic acid and 9(*S*)-hydroperoxyoctadecadienoic acid from linoleic acid. The mRNA for 8-LO was found primarily in the stratum granulosum layer of skin but also to some extent in brain. It turned out that the mouse 8-LO was very much related in size and amino acid composition to a second form of 15-LO (76% identity) that was also cloned by the same group around the same time, and they have indeed been classified as species orthologues.[358] Whereas in the mouse enzyme oxygenation occurs at position 8 of arachidonic acid, the human orthologue forms exclusively 15(*S*)-HpETE.[358] This 15-lipoxygenase type 2 or second isoform was expressed in hair roots, prostate, lung, and cornea but was absent in most other tissues.

The functions of the 8S-LO and 15-LO-2 have been difficult to decipher due in part to their disparate tissue expression patterns and oxygenation specificity. However, in human prostate glands, 15-LO-2 was found primarily in benign prostatic epithelium with reduced expression in prostate adenocarcinoma,[359] and this led to findings indicating that the enzyme may act as a negative cell cycle regulator/tumor suppressor within prostatic epithelial cells.[360,361] In support of these observations, when either 15-LO-2 or 8S-LO were inducibly expressed in keratinocytes, activation of product synthesis from the two pathways led to cell growth suppression with an associated inhibition of DNA synthesis, which suggested common signaling pathways despite the formation of differing positional isomers.[362]

10.6. Mouse Epidermal 12-LO

A cDNA encoding a 662 amino acid lipoxygenase was isolated from RNA obtained from epidermis of young newborn mice (3−6 days old). The enzyme, when expressed in HEK293 cells or baculovirus-infected insect cells, forms predominantly 12(*S*)-HETE from arachidonic acid, with small amounts of 15-HETE detected in a ratio of 6:1.[363,364] The enzyme was noted to have much lower catalytic efficiency compared to 15-LO or platelet 12-LO with relatively modest conversion of substrate and was later found to prefer methyl arachidonate as substrate.[363,365] The gene is linked with several other lipoxygenase genes on mouse chromosome 11. However, in humans the gene, on the short arm of chromosome 17, is a pseudogene that gives rise to transcripts containing premature stop codons.[366] In mice the unique expression pattern of this gene in early embryos, differentiated keratinocytes, defined regions of the root sheath and bulb of hair follicles; within the conjunctiva of the eyelid and in distinct sebaceous glands, implies very specific roles of this epidermal 12-lipoxygenase in murine biology, while it appears that humans have lost a specific function for the enzyme.[363,364,366] Because the enzyme is not apparently expressed as active enzyme in humans, this particular murine epidermal 12-lipoxygenase isoform has remained the least well characterized of the lipoxygenase family members.

11. LIPOXYGENASES IN HUMAN DISEASES

The identification of lipoxygenases and lipoxygenase products and search for their biological roles in man have been ongoing ever since the first discoveries of 12-HETE, 5-HETE, and 15-HETE in humans in the mid-1970s.[339,367,368] Thus far, only 5-LO has been shown to possess indisputable roles in human pathology by virtue of its central role in leukotriene biosynthesis.[28]

11.1. Acute and Chronic Inflammation

Ever since their discovery, the leukotrienes have attracted much attention because of their powerful biological effects in vitro and in vivo.[1] These lipid mediators are active in the low nanomolar range and elicit a plethora of cellular proinflammatory and immune modulatory responses. For example, LTB_4 is one of the most potent chemotactic agents known to date, comparable with IL-8 and C5a, and cys-LTs are at least three log-orders more potent than histamine in provoking smooth muscle constriction in the airways.[369,370] Consequently, 5-LO and the leukotrienes have been implicated in the pathogenesis of many human acute and chronic inflammatory diseases such as asthma, rheumatoid arthritis, inflammatory bowel disease, psoriasis, dermatitis, nephritis, atherosclerosis, and cancer (Figure 13).[371,372] It is not within the scope of this article to cover each of these diseases, and here we will focus on those that are currently in the focus of the field's attention.

11.2. Asthma and Rhinitis

The structural elucidation of a slow-reacting substance of anaphylaxis as a mixture of leukotrienes connected the 5-LO pathway with a bioactivity that had been studied for decades and was known to have actions in allergic inflammation.[36,43] The interest in LTC_4, LTD_4, and LTE_4 was heightened even further when it became clear that they are at least 1000 times more active on a molar basis than histamine as bronchoconstrictors and could be isolated from human asthmatic lung tissue challenged with allergen in amounts commensurate with their spasmogenic potency.[370,373] Low nanomolar concentrations of cys-LTs cause plasma leakage from postcapillary venules, actions that could be translated into inflammatory edema in the respiratory tract.[374] All these early data pointed to a role for cys-LTs in the pathogenesis of bronchial asthma, and subsequent work on enzymes and receptors responsible for synthesis and signaling of leukotrienes added a large body of evidence corroborating this notion.[375] The final proof was not obtained until antileukotriene

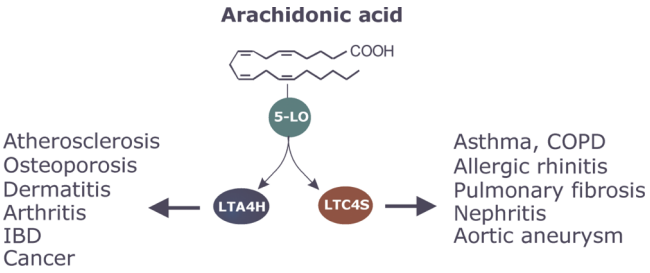

Figure 13. Diseases associated with leukotrienes. Several human chronic inflammatory diseases have been associated with an overproduction of leukotrienes. Certain diseases are more linked to LTB_4, downstream of LTA_4 hydrolase (listed to the left), whereas others are linked to cys-LT production, downstream of LTC_4 synthase (listed to the right).

drugs were shown to be efficacious against human asthma.[376] A special form of asthma, aspirin-intolerant asthma, is characterized by nasal polyps and severe exacerbations upon challenge with aspirin or other nonsteroidal anti-inflammatory drugs (NSAIDs). This type of asthma is associated with a chronic overproduction of cys-LTs, presumably due to overexpression of LTC_4 synthase, and is believed to be more leukotriene-dependent than allergic asthma.[377,378] Interestingly, COX-2 inhibitors seem to be well tolerated by aspirin-intolerant asthmatics.[379−381]

Several other allergic and inflammatory diseases of the upper respiratory tract are also driven by leukotrienes, such as allergic rhinitis and bronchial hyperreactivity induced by exercise and cold air. In fact, release of SRS-A from human nasal polyps was demonstrated long before its structure was elucidated.[382] After the discovery of the leukotrienes, several lines of evidence soon connected the cys-LTs with immediate hypersensitivity reactions, e.g., preferential synthesis of cys-LTs in human eosinophils and mast cells challenged with immunological stimuli,[383,384] release of cys-LTs upon nasal allergen provocation of patients sensitive to ragweed,[385] and detection of cys-LTs in tear fluid of subjects challenged with allergen.[386] As was the case for asthma, the final proof of the involvement of leukotrienes in human allergic rhinitis appeared when an orally active 5-LO inhibitor, zileuton, was shown to attenuate nasal congestion in patients subjected to allergen challenge.[387] Interestingly, two other reports on the ability of zileuton and a CysLT1 antagonist, MK-571, to alleviate asthmatic responses to cold air and exercise were published at the same time, demonstrating the involvement of cys-LTs in certain types of airway hyperreactivity.[388,389]

11.3. Cardiovascular Diseases

Lipoxygenases, in particular human 15-LO-1, have for long been implicated in atherosclerosis. The enzyme was believed to participate in oxidation of LDL, conversion of macrophages into foam cells, and promotion of the atherogenic process.[390−392] Several studies using 12/15-LO deficient mice, as alluded to in section 10.1, have supported a role for 12/15-LO in promoting atherosclerotic lesion development.[328−333] However, the role of 12/15-LO in atherosclerosis is controversial. Thus, overexpression of 15-LO in macrophages protected against atherosclerosis in the rabbit, and in one study with 12/15-LO ApoE double deficient mice, it was concluded that LO activity in the local milieu afforded protection against atherosclerosis, via formation of proresolving lipid mediators.[334,393] Recently, the profile of lipoxygenase expression was assessed in human atherosclerotic plaque.[394] In addition to 5-LO, the only significantly expressed enzyme was 15-LO-2, and 15-LO-1 could not be detected.[394]

Already in 1988, formation of LTB_4 in human atherosclerotic plaque was demonstrated and was followed by a report on increased urinary levels of LTE_4 in patients during episodes of myocardial ischemia.[395,396] In the context of cell−cell interactions and transcellular metabolism, cys-LTs were found to constrict coronary arteries in the rabbit.[397,398] Furthermore, human atherosclerotic coronary arteries were shown to express 5-LO, FLAP, and LTA_4 hydrolase, associated with macrophages, and exhibited a contractile response when challenged with LTC_4 and LTD_4.[399] In retrospect, these early data on the potential role of leukotrienes in cardiovascular diseases were quite convincing but still did not receive much attention. The observations that 5-LO deficiency or a BLT1 antagonist were found to have a significant protective effect against atherosclerosis in ApoE- and/or LDLR-deficient mice triggered new interest in the field and was soon followed by morphological as well as genetic evidence further supporting a role for 5-LO and leukotrienes, in particular LTB_4, in human atherosclerosis, myocardial infarction, and stroke.[400] High levels of expression of all enzymes in leukotriene biosynthesis were detected in samples of human atherosclerotic plaque, 5-LO mRNA levels correlated with clinical stage of disease, 5-LO as well as LTA_4 hydrolase colocalized in intimal macrophages, and mRNA levels correlated with symptoms of plaque instability.[401,402] From molecular genetic studies of the Icelandic population, variant 5-LO genotypes were found to be associated with increased atherosclerosis, and dietary $\omega 6$ polyunsaturated fatty acids promoted, whereas marine $\omega 3$ fatty acids inhibited, this effect.[86] Similarly, variants of *ALOX5AP* were associated with increased risk of both myocardial infarction and stroke, and variants of the LTA_4 hydrolase gene were found to confer an increased risk of myocardial infarction in an ethnicity-dependent manner.[403,404] Together, these reports centered around LTB_4 as the culprit molecule, and all key cellular components, i.e., macrophages, T-cells, fibroblasts, smooth muscle cells, and endothelial cells, express BLT receptors and can either produce or respond to LTB_4 with migration or release of mediators.[237,405−408] Subsequent work on mouse models of atherosclerosis have yielded variable and inconclusive results, probably reflecting differences in etiology between the murine and human diseases and other factors that are intrinsic to a particular animal model.[300] For example, in an extended investigation of 5-LO ApoE double deficient mice, only a minor atheroprotective effect was observed in the leukotriene-deficient animals whereas deletion of the receptor BLT1 diminished lesion formation during early, but not late, stages of atherosclerosis development and during intermittent hypoxia-induced atherogenesis.[297,315,316]

Similar discrepancies between animal models have been observed also for development of aortic aneurysms where deletion of 5-LO in ApoE knockout mice fed a hyperlipidemic diet protected against aortic aneurysm.[297] In contrast, genetic or pharmacological ablation of the 5-LO pathway did not afford protection against angiotensin II-induced aneurysm formation in Apo E knockout mice fed a normal chow diet.[409] On the other hand, deletion of BLT1 inhibited aortic aneurysm formation in the angiotensin II/Apo E double deficient model, and a selective BLT1 antagonist protected

against the early phase of aneurysm development in the same model.[317,410] In studies of human abdominal aortic aneurysms, it was suggested that LTB_4 plays a role as a chemotactic factor.[411] A broader analysis of vessel wall tissue as well as the intraluminal thrombus from abdominal aortic aneurysms revealed that the expression of 5-LO, FLAP, and LTC_4 synthase, rather than the expected LTA_4 hydrolase, are upregulated, and the expression levels are mirrored by a predominant formation of cys-LTs associated with increased release of MMP-2, which potentially could contribute to extracellular matrix degradation.[310] Hence, a large body of evidence indicates that leukotrienes are involved in various stages and types of cardiovascular disease. Better animal models and clinical trials are required to assess the therapeutic potential of antileukotrienes in treatment and prevention of atherosclerosis, aortic aneurysms, myocardial infarction, and stroke.

11.4. Arthritis

Early identification of LTB_4 in synovial fluid from patients with rheumatoid arthritis, spondyloarthritis, and gouty effusions suggested the involvement of 5-LO and leukotrienes in inflammatory arthropathies.[412−414] Further clinical studies in patients with rheumatoid arthritis revealed positive effects of 5-LO inhibition on clinical disease measures which were not, however, comparable to those of traditional nonsteroidal anti-inflammatory drugs.[415,416] Through pharmacological blockade of BLT1 or deletion of the *ALOX5AP* gene, Griffiths et al. could demonstrate a critical role of 5-LO and LTB_4 in collagen-induced arthritis.[306,417] More recently, simultaneous disruption of BLT1 and BLT2 abolished all disease pathology, i.e., loss of joint architecture, inflammatory cell infiltration, fibrosis, pannus formation, and bone erosion, in collagen-induced arthritis in mice.[418] In an antibody-induced mouse model of arthritis, LTB_4 and BLT1 were required for neutrophils to deliver IL-1 into the joint, via a sequential relationship and contributions from CCR1 and CXCR2 signaling.[321] Moreover, selective deletion of BLT2, which signals in response to the cyclooxygenase metabolite 12-HHT as well as LTB_4, reduced incidence and severity of bone and cartilage loss in an autoantibody-induced inflammatory arthritis model.[419] A nonredundant role for LTB_4 and BLT1 in neutrophil recruitment into the joints was also demonstrated in the K/BxN mouse model of inflammatory arthritis.[319] Further studies of this animal model revealed that fibroblast-like synoviocytes could synthesize LTB_4 and also respond to this mediator with expression of a more aggressive phenotype with increased migration and invasiveness.[320] Hence, a large body of preclinical and clinical data indicates that 5-LO and leukotrienes contribute to arthritis.

11.5. Cancer

Lipoxygenases and their products have been implicated in several aspects of carcinogenesis such as tumor cell proliferation, differentiation, and apoptosis, as well as migration, invasion of carcinoma cells, and angiogenesis.[420] Early studies on melanoma and prostate cancer cells indicated a role of 12-HETE in tumor cell adhesion to endothelium via increased surface expression of integrins and rearrangement of cytoskeletal proteins in a PKC-dependent manner.[421,422] Further work showed that platelet-type 12-LO in human prostate cancer cells stimulated angiogenesis and tumor growth, and overexpression of 12-LO in prostate cancer cells increased their metastatic potential.[423,424] More recently, it was demonstrated that 12-HETE, produced by 15-LO-1, promotes invasion of breast cancer into lymphatic vessels and formation of lymph node metastasis.[425] On the other hand, increased expression of 15-LO-1 and particularly 15-LO-2 with a corresponding increase in product formation have been associated with increased apoptosis and antitumorigenic activities in colon and prostate cancer.[360,426−428]

Several lines of evidence have also suggested a role for 5-LO and leukotrienes in various forms of cancer, in line with the proinflammatory properties of these mediators. Increased expression of 5-LO and the BLT1 receptor was observed in pancreatic cancer, and 5-LO expression was suggested to be an indicator of early neoplastic lesions.[429,430] Furthermore, LTB_4 stimulates growth of pancreatic cancer cells, and a BLT antagonist LY293111 was shown to inhibit proliferation and induce apoptosis in human pancreatic cancer cells.[431] LTB_4 has also been shown to promote stem cell proliferation[432] and, interestingly, deletion of 5-LO impaired differentiation, cell division, and survival of leukemia stem cells, thus preventing chronic myeloid leukemia.[304] LTA_4 hydrolase was also shown to be overexpressed in esophageal adenocarcinomas, and LTA_4 hydrolase inhibitors have been shown to suppress both esophageal and colon cancer cells.[433,434] LTB_4 has also been shown to possess proangiogenic properties, in part by signaling via BLT2,[435] enhance formation of reactive oxygen species and leukocyte adherence in response to hypoxia,[436,437] and increase vascular permeability in a neutrophil-dependent manner via secretion of heparin-binding protein.[437,438] Colon and prostate cancer have also been associated with elevated levels of CysLT1 expression and a negative correlation with patient survival.[439−441] On the other hand, an increased CysLT2 expression was associated with apoptosis signaling and a high degree of differentiation in colorectal adenocarcinomas.[441] Moreover, expression of enzymes and receptors of the leukotriene cascade was observed in human neuroblastoma and was associated with tumor survival.[442]

11.6. Psoriasis and Hereditary Ichthyosis

Soon after the discovery of 12-LO, it was shown that 12-HETE is present in psoriatic lesions, suggesting a role for this mediator in psoriasis.[443] When the stereochemistry of 12-HETE derived from platelets was compared to 12-HETE from psoriatic lesions, the latter was found to carry an *R*-hydroxyl group instead of the *S*-enantiomer expected from a traditional lipoxygenase.[444] It was generally assumed that a cytochrome P450 was active in psoriatic skin and responsible for the unusual chirality of the metabolite. It was not until 1998 that human and mouse R-lipoxygenases were discovered and cloned from skin.[347,348] The biochemical role of 12R-LO in formation and maintenance of the epidermal permeability barrier is supported by the dramatic fragile skin phenotype observed after disruption of the gene encoding 12R-LO, *Alox12b*, in mice that leads to death shortly after birth.[350] Furthermore, an increasing body of genetic evidence has strongly associated 12R-LO, alone or together with human epidermal LO (eLOX3), with various forms of congenital ichthyosis.[351,445−447] Hence, with these discoveries, the circle starting with 12-HETE in psoriatic lesions has been closed.

12. ANTILEUKOTRIENE DRUGS

Inasmuch as leukotrienes possess potent biological activities, the pharmaceutical industry has made considerable efforts to produce enzyme inhibitors and receptor antagonists to block the synthesis and actions of these lipid mediators. In fact, all components of the leukotriene cascade depicted in Figure 12,

with the exception of LTC_4 synthase, have been targeted by small molecules. $cPLA_2\alpha$ is included in this scheme, and potent and selective inhibitors of this enzyme have recently been presented.[448,449] Because $cPLA_2\alpha$ also provides substrate for prostaglandin formation and other PLA_2 isoforms, e.g., $sPLA_2$ and $iPLA_2$, contribute to lipoxygenase product formation, inhibitors of PLA_2 will not be further discussed here.[47] Given later is a summary of the most successful antileukotrienes developed thus far along with comments on new directions and therapeutic opportunities.

12.1. Inhibitors of 5-LO and FLAP

Given its position at the top of the pathway, 5-LO would seem a logical therapeutic target to block leukotriene-driven diseases. Already at an early stage, a long line of compounds, claimed to be 5-LO inhibitors, were developed including (a) *redox inhibitors* such as phenidone, BW-755C (Burroughs-Wellcome), and ICI 2207968 (ICI Pharmaceuticals); (b) *iron-binding inhibitors*, many of which utilize a hydroxamic acid moiety as the metal ligand and are exemplified by the *N*-hydroxyurea molecule zileuton (Abbott) and the aryl hydroxymate BWA4C (Wellcome); and (c) *active site-directed inhibitors* designed from simplistic models of the enzyme's catalytic site and resulting in compounds such as the methoxyalkyl thiazoles ICI211965 and ICID2138.[450] Members of these three inhibitor classes suffered from lack of selectivity, structure−activity relationships, and enantioselectivity, caused methemoglobin formation, and displayed poor efficiency and oral availability. Nonetheless, in clinical trials zileuton showed beneficial effects in rheumatoid arthritis, inflammatory bowel disease, psoriasis, allergic rhinitis, and asthma. In spite of a short half-life requiring a large dose four times a day, zileuton made it to the clinic and is today marketed in the United States for treatment of asthma.[376,451] Development of 5-LO inhibitors will now enter a new phase with the crystal structure of the enzyme at hand.[110] This advance will allow structure-based drug design to tailor-make selective 5-LO inhibitors and fully evaluate their therapeutic potential.

Several inhibitors of FLAP have also been developed over the years, including the classical prototype MK-886, which today is a most valuable laboratory tool for potent inhibition of cellular leukotriene biosynthesis, as well as MK-0591.[452,453] Another early compound was Bay-X1005, which was resurrected by deCode Genetics as DG-031 and tested in clinical trials for treatment of myocardial infarction.[403,454] More recently, a second generation of FLAP inhibitors was developed from the novel lead molecule 3-[3-*tert*-butylsulfanyl-1-[4-(6-methoxypyridin-3-yl)-benzyl]-5-(pyridin-2-ylmethoxy)-1*H*-indol-2-yl]-2,2-dimethylpropionic acid (AM103), and these molecules are primarily intended for use as an antiasthma medication but also potentially for treatment of cardiovascular diseases.[118,455,456]

12.2. LTA_4 Hydrolase Inhibitors

Development of inhibitors targeting LTA_4 hydrolase began when its zinc content and aminopeptidase activity were discovered, offering a simple model of the active site.[153] Thus, scientists in academia identified bestatin, captopril, and kelatorphan as LTA_4 hydrolase inhibitors,[457−459] and these drugs were followed by ω-[(ω-arylalkyl)aryl]alkanoic acids,[460] α-keto-β-amino esters, thioamines, and inverted hydroxamic acids, some of which were developed as potent and selective transition state mimics.[461−463] Efforts by scientists at Santen Parmaceutical identified dipeptidic thiols exemplified with SA-6541, which displayed anti-inflammatory properties in several preclinical models.[464,465] Searle/Pharmacia also ran an ambitious drug development program and reported a series of nonpeptidic, nonzinc-chelating phenoxyethylamino analogues including imidazopyridines, amidines, and cyclic and acyclic amino acid derivatives, ultimately resulting in the identification of the clinical candidate SC-57461A.[466] When given orally, this compound was efficient in the mouse ex vivo assay of LTB_4 synthesis in blood, the rat model of ionophore-induced peritoneal eicosanoid formation, and in cotton-top tamarins, a model of inflammatory bowel disease. Because of a long-lived metabolite and toxicity issues, SC-5761A was not developed further. The crystal structure of LTA_4 hydrolase triggered new interest in development of LTA_4 hydrolase inhibitors, and several companies started programs on structure-based drug design. Thus, deCode Genetics developed a 4-[(2*S*)-2-{[4-(4-chlorophenoxy)phenoxy]methyl}-1-pyrrolidinyl]butanoic acid (DG-51) for treatment of myocardial infarction and stroke, Schering chemists made glutamic acid analogues, and Johnson & Johnson developed (1-[4-(benzothiazol-2-yloxy)benzyl]piperidine)-4-carboxylic acid (JNJ-26993135) for treatment of inflammatory bowel disease and allergic airway inflammation.[467−471]

In this context, it is interesting to note that inhibitors of LTA_4 hydrolase block LTB_4 synthesis, while sparing LTA_4 synthesis to allow shunting into lipoxin synthesis (Figure 1). Thus, inhibitors of LTA_4 hydrolase may alleviate inflammation by two mechanisms, reduced LTB_4 synthesis and enhanced formation of anti-inflammatory lipoxin.[469,471] Recently, the chemotactic tripeptide Pro-Gly-Pro was identified as an endogenous substrate for the aminopeptidase activity of LTA_4 hydrolase, which in turn indicates that LTA_4 hydrolase plays an anti-inflammatory role during the resolution phase of inflammation.[148] Hence, it appears likely that inhibitors that spare the aminopeptidase activity of LTA_4 hydrolase have a greater therapeutic potential as compared to all previously developed molecules, which block both LTB_4 synthesis and peptide cleavage.

12.3. Inhibitors of LTC_4 Synthase

For unclear reasons, very few, if any, inhibitors of LTC_4 synthase have been developed thus far. When LTC_4 synthase was cloned and found to be a homologue with FLAP, it was also shown that micromolar concentrations of MK-886 could inhibit LTC_4 synthase, indicating that significant structural similarities exist between the arachidonic acid-binding site in FLAP and the catalytic center of LTC_4 synthase.[163] Further work at Merck led to the identification of a phenylpyridine compound, L-699,333, that was a low micromolar inhibitor of LTC_4 synthase and the most potent inhibitor at the time.[472] However, this molecule was unselective and had previously been identified as a nanomolar inhibitor of 5-LO.

Recent work has uncovered new receptors for cys-LTs, which in turn has made the biosynthetic upstream enzyme, LTC_4 synthase, an attractive target for pharmacological inhibition of cys-LT signaling. Because high-quality crystals (2 Å) of human LTC_4 synthase can be generated and are stable, it is now possible to carry out structure-based design of inhibitors.[184] This is most likely the first time a human integral membrane protein has become amenable to this sophisticated technique.

12.4. BLT Receptor Antagonists

Initially, BLT antagonists were developed under the assumption that only one receptor played a significant role in mediating LTB_4 responses and with the aim of treating nonallergic inflammatory diseases. However, LTB_4 signals via two receptors, BLT1 and BLT2, and while BLT1 has established roles in immune

modulation and chemotaxis of a variety of cells, in particular neutrophils and T-lymphocyte subsets, the biological function of BLT2 is still unclear.

Over the years, a range of BLT antagonists has been developed, e.g., amelubant (BIIL-284, Boehringer-Ingelheim), etalocib sodium (LY-293111, Eli Lilly), moxilubant maleate (CGS-25019C, Novartis), and the highly BLT1 selective biphenylyl-substituted chroman carboxylic acid CP-105696 (Pfizer).[243,473−476] On the basis of data from BLT1- and/or BLT2-deficient mice, other preclinical models, and clinical trials, BLT antagonists could be of potential use in rheumatoid arthritis, asthma and chronic obstructive pulmonary disease, inflammatory bowel disease, psoriasis, osteoporosis, atherosclerotic vascular diseases, and cancer.[477] However, BLT antagonists have not demonstrated significant benefits in clinical trials, and development programs have been discontinued. Poor in vivo efficacy and clinical activity may be explained by an insignificant role of LTB_4 in tested pathologies, opposing roles of BLT1 and BLT2 in inflammation, or choice of patient population and/or clinical end points in clinical trials.

12.5. Cys-LT Receptor Antagonists

This class of compounds represents the best example of clinically useful antileukotriene drugs and has already been on the market for more than a decade. These pharmacological agents, also known as "lukasts", are used in the clinical management of asthma and allergic rhinitis.[376] Three compounds are available, montelukast (Merck), zafirlukast (AstraZeneca), and pranlukast (ONO), the latter only sold in Japan and other Asian countries.[478−480] Lukasts are generally well-tolerated and associated with few side effects. However, a certain number of patients (∼40%) do not respond to these agents, and in several clinical trials, they have not fully matched the potency of inhaled corticosteroids.[481] On the other hand, lukasts certainly play an important role in asthma treatment, either as first-line or add-on agents. For instance, aspirin-intolerant asthma, a condition associated with high production of cys-LTs, is normally treated with a CysLT1 antagonist as a first-line therapy, and the same is true for patients suffering from exercise-induced asthma.[482] These drugs are also the first choice for treatment of asthma in children or patients that have trouble handling an inhaler. The combination of lukasts and corticosteroids can also further suppress airway inflammation beyond the effects of either agent alone.[375] Interestingly, results obtained in a mouse model of chronic allergic asthma have indicated that lukasts, unlike corticosteroids, can prevent or even revert airway remodeling.[483,484] Moreover, two recent parallel, multicenter, pragmatic trials suggested that after 2 months a lukast was equivalent to an inhaled glucocorticoid as first-line controller therapy and equivalent to a long-acting β-agonist as add-on therapy for diverse primary care patients.[485]

The biological roles of the CysLT2 receptor are not yet defined, although several lines of evidence suggest that the receptor signals in the vasculature to increase permeability as a part of an inflammatory response.[258] A potent and selective CysLT2 antagonist has recently been described and will aid in the characterization of the receptor biology.[486,487] The family of receptors for cys-LTs has expanded and now includes CysLT1, CysLT2, gpr17, $P2Y_{12}$, and CysLTE, which are functionally interconnected, allowing complex signaling patterns.[488] This receptor complexity may explain the poor responsiveness of certain patients to treatment with lukasts, and in the future it may be possible to develop isoreceptor-specific antagonists, e.g., to block $P2Y_{12}$, and CysLTE signaling, which are poorly antagonized by MK571. As discussed above, complete inhibition of cys-LT synthesis by blocking LTC_4 synthase is also a feasible strategy to circumvent the heterogeneity of CysLT receptor signaling pathways.

13. FUTURE PERSPECTIVES

The area of lipoxygenase and leukotriene research has received significant attention over many decades and, as a consequence, has reached a significantly advanced state. Essentially all proteins have been biochemically characterized, cloned, and knocked out in mice. Entire genes and their promoters have been characterized and crystal structures of the corresponding gene products have been solved, even including integral membrane proteins. Nevertheless, the field of lipoxygenases and leukotrienes continues to generate exciting new findings and novel paths for exploration as is often the case with physiologically relevant research areas.

Looking into the crystal ball, we see further work on the biosynthetic complex, located at the nuclear membrane, which regulates leukotriene synthesis. In spite of structural information on all key proteins, i.e., $cPLA_2\alpha$, 5-LO, FLAP, LTA_4 hydrolase, and LTC_4 synthase, it is still unknown how they assemble and interact both physically and functionally. Work on this subject will likely uncover new possibilities for interference with leukotriene biosynthesis. In these efforts, crystallography will be an important tool, for instance, to solve the structure of protein complexes and gain further insights to the mechanism of 5-LO.

Another area of future interest is the family of GPCRs, which govern leukotriene signaling across cell membranes and levels of intracellular second messengers. New receptors for LTE_4 have recently been discovered, and their roles in cys-LT signaling and related diseases such as asthma need to be elucidated.[270,271] Furthermore, leukotriene receptors are clearly interconnected at multiple levels, and deciphering this apparent cobweb of cross-regulatory events will be a major future challenge. Unexpected new data suggest that some of the GPCRs are located in the cell nucleus and further work will tell whether these receptors are involved in intranuclear signaling events. The structure of a second GPCR, i.e., the β2-adrenergic receptor, was recently solved, which increases the possibility that the structure of a BLT and/or CysLT receptor will soon follow.[489]

The lipid mediators generated by lipoxygenases elicit biological responses in their own capacity. However, rather few studies have addressed the issue of cross-talk and synergies with other lipid, peptide, or carbohydrate mediators. We believe that the full battery of "omics" technologies (genomics, proteomics, lipidomics, metabolomics, etc.) will identify new partners and competitors to the proteins and metabolites of the lipoxygenase cascades. Finally, translational work involving new animal models and clinical studies will be required to transfer all basic discoveries into novel insights to pathogenic processes and opportunities for treatment of human disease.

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Jesper Z. Haeggström received his M.D. and completed a B.A. at Uppsala University in 1981. After practice as a general physician, he moved to Stockholm, Sweden, and began his postgraduate training under the supervision of Nobel Laureate, Professor Bengt Samuelsson. He received his Ph.D. in 1988, carried out his postdoctoral training in molecular biology and protein chemistry, and became associate professor at Karolinska Institutet in 1991. In 1999, Dr. Haeggström received a position as "*Karolinska Institutet Senior Investigator*", and in the following year he became full professor, head of Division Physiological Chemistry 2, and vice chairman of the Department of Medical Biochemistry and Biophysics. In 2007 Jesper Z. Haeggström was awarded the *Bert L. Valle Visiting Professorship* at Harvard Medical School, Boston, in 2008 he was elected member of the Nobel Assembly, and in 2009 he received a *Distinguished Professor Award* from Karolinska Institutet.

Colin D. Funk holds a Tier I Canada Research Chair in Molecular, Cellular and Physiological Medicine and a Heart and Stroke Foundation of Ontario Career Investigator Award, and he is currently a Professor in the Department of Biomedical and Molecular Sciences at Queen's University. Dr. Funk received his undergraduate training in Biochemistry at Queen's and doctorate training in Experimental Medicine at the Royal Victoria Hospital/Mc Gill University. From there he worked three years with Nobel Laureate Professor Bengt Samuelsson at the Karolinska Institute in Stockholm, Sweden. Dr. Funk spent 6 years at Vanderbilt University in the Division of Clinical Pharmacology, Department of Pharmacology, as Assistant/Associate Professor and 9 years at University of Pennsylvania in the Center for Experimental Therapeutics as Professor of Pharmacology. In 2004, he moved to his present position at Queen's, where he works with induced mutant mouse models and lipid mediators of inflammation in the cardiovascular system.

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