Lipid Mediators in Health and Disease: Enzymes and Receptors as Therapeutic Targets for the Regulation of Immunity and Inflammation

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Key Words

phospholipase A2, prostaglandins, leukotrienes, platelet-activating factor, GPCR

Abstract

Prostaglandins, leukotrienes, platelet-activating factor, lysophosphatidic acid, sphingosine 1-phosphate, and endocannabinoids, collectively referred to as lipid mediators, play pivotal roles in immune regulation and self-defense, and in the maintenance of homeostasis in living systems. They are produced by multistep enzymatic pathways, which are initiated by the de-esterification of membrane phospholipids by phospholipase A2s or sphingomyelinase. Lipid mediators exert their biological effects by binding to cognate receptors, which are members of the G protein–coupled receptor superfamily. The synthesis of the lipid mediators and subsequent induction of receptor activity is tightly regulated under normal physiological conditions, and enzyme and/or receptor dysfunction can lead to a variety of disease conditions. Thus, the manipulation of lipid mediator signaling, through either enzyme inhibitors or receptor antagonists and agonists, has great potential as a therapeutic approach to disease. In this review, I summarize our current state of knowledge of the synthesis of lipid mediators and the function of their cognate receptors, and discuss the effects of genetic or pharmacological ablation of enzyme or receptor function on various pathophysiological processes.

INTRODUCTION—OVERVIEW OF LIPID MEDIATORS

Lipids are the major constituents of the cellular membranes of all organisms. Due to the amphipathic properties of lipid molecules, the structure of all cellular membranes is a bimolecular leaflet, termed the lipid bilayer (1, 2). Lipids are the most efficient source of energy for living organisms, and excess storage or decreased lipid usage results in energy imbalance and can lead to the development of metabolic syndromes (3). Certain lipid moieties are also covalently or noncovalently linked to proteins, a post-translational modification that facilitates many types of protein-protein or lipid-protein interactions (4). Lipid molecules also function directly as intercellular signaling molecules in immune self-defense and the maintenance of homeostasis (5, 6). Prostaglandins (PGs), leukotrienes (LTs), lipoxins (LXs), hydroxy fatty acids (eicosanoids), platelet-activating factor (PAF), lysophosphatidic acid (LPA), sphingosine 1-phosphate (S1P), and 2-arachidonoylglycerol, as well as others, are collectively referred to as lipid mediators. In concert with other types of signaling molecules, such as neurotransmitters, hormones, and cytokines, lipid mediators are shown to play important roles in the regulation of cell proliferation and differentiation, as well as the reproductive, gastrointestinal, and cardiovascular systems. They also play a fundamental role in inflammatory and immune responses. Lipid mediators have several characteristics that distinguish them from other signaling molecules. They are synthesized on demand in a calcium-dependent manner from precursor membrane lipids. With some exceptions, they have a relatively short half-life (seconds to minutes), and can be degraded both enzymatically and nonenzymatically. Finally, they act on class I G protein–coupled receptors (GPCRs). In this chapter, I summarize the production mechanisms of lipid mediators, and the biological effects of lipid mediator binding to cognate GPCRs. I also explore how disruption of the coordinated action of lipid mediators and their receptors can result in disease.

Table 1 presents a summary of the classification of lipid mediators and a brief description of their biological activities. **Figure 1** shows the structures of representative lipid mediators from each class. Because of space limitations, I focus this review primarily on the eicosanoids

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Table 1 Classification of lipid mediators

Class	Lipid mediators (abbreviated names) and biological functions
Fatty acids	Prostaglandins (PG); fever, pain, inflammation Thromoboxane (TX); platelet aggregation, vasoconstriction Leukotrienes (LT); lipoxins (LX); inflammation Resolvins (Res); anti-inflammation 12-Hydroxy-heptadecatrienoic acid (12-HHT); chemotaxis Annandamide; prostglanamide; analgesia and brain functions Isoprostanes; contraction of smooth muscle Short-chain fatty acids; insulin secretion
Phospholipids	Platelet-activating factor (PAF); inflammation, bone resorption Oxidized phospholipids (Ox-PL); vasoconstriction, chemotaxis Psychosine (Psy); sphingophosphorylcholine (SPC) ??
Lysophospholipids	Lysophosphatidic acid (LPA); sphingosine 1-phosphate (S1P); cell proliferation and development
Others	2-Arachidonoyl-glycerol (2-AG); brain function, immune regulation Ceramide; ceramide 1-phosphate (C1P); bile acid

The structures of representative compounds (underlined) are illustrated in **Figure 1**.

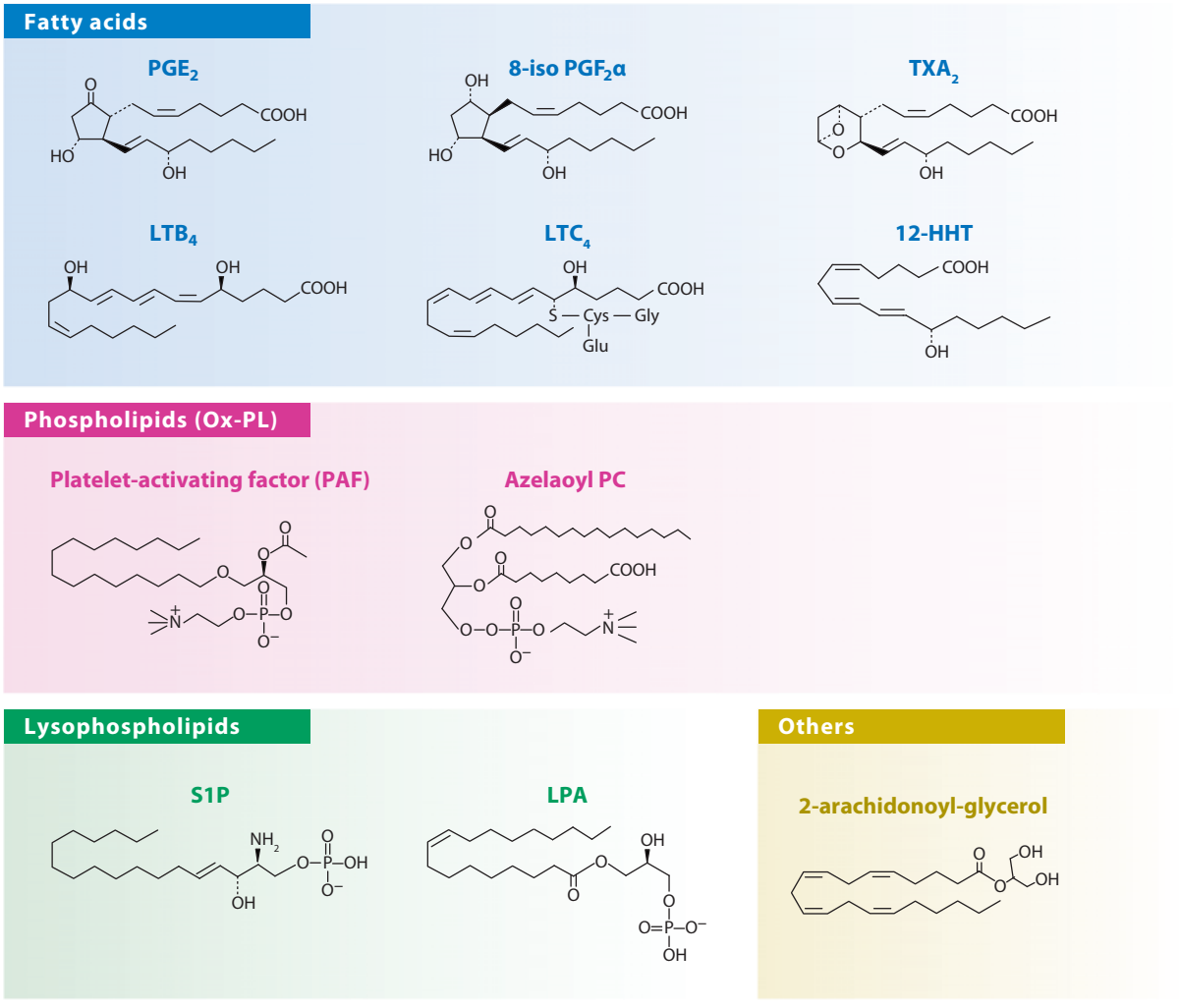

Figure 1

Structures of representative lipid mediators.

(e.g., PGs, LTs, HETEs and lipoxins) as well as PAF, but I briefly discuss, where relevant, other lipid mediators as well.

ENZYMES INVOLVED IN THE BIOSYNTHESIS OF EICOSANOIDS AND PAF

Phospholipase A2

When cells or tissues are exposed to physiological and pathological stimuli, glycerophospholipids are hydrolized and converted to two products: arachidonic acid (or polyunsaturated fatty acid) and lysophopholipids by the action of phospholipase A2 (PLA2) (7–9). Lysophopholipids function as signaling molecules, or serve as precursors of other lysophospholipid mediators (see below)

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(**Figure 2**). There are more than 20 different PLA2s in mammals. They are classified into four categories on the basis of their molecular structure and biochemical properties. Secretory PLA2s (sPLA2s) comprise proteins of approximately 20 kD that contain multiple disulfide (S-S) bonds (10, 11). These are stimulated by mM concentrations of Ca^{2+}, and their function is primarily extracellular. Cytosolic PLA2s (cPLA2s) are present in the cytosol, and are activated by μM concentrations of Ca^{2+} and protein serine/threonine kinases (12, 13). Calcium is not required for the catalytic activity of cPLA2s, but it is important for the interaction of this group of enzymes with the phospholipid membrane. There are six different cPLA2s (**Figure 3**), of which cPLA2α (Group IVA PLA2) has been shown to be a pivotal enzyme in the production of eicosanoids and PAF (14–16). The third category comprises the Ca-independent PLA2s (iPLA2s), which also localize to the cytosol (17–19). Although the precise function of the iPLA2s remains elusive, they are implicated in tissue developments and hormone secretion. The fourth category of PLA2s comprises the PAF acetylhydrolases, which inactivate PAF and oxidized phospholipids to yield lysophospholipids (20–22). There are at least three enzymes in this class: two are cytosolic enzymes and the third is present in plasma and tissue fluids. One of the cytosolic acetylhydrolases has a trimeric structure, consisting of α1, α2, and β subunits, and has been the subject of much recent attention, as the β subunit is identical to the human lissencephaly gene (LIS-1), the causative gene of Miller-Dieker syndrome (23).

Acyl-CoA:Lysophospholipid Acyltransferase

Lysophospholipids (derived from lysis) are cytotoxic at high concentrations, and if they are not secreted or metabolized into PAF or other types of lipid mediators, they are typically converted into glycerophospholipids. The incorporation of poly-unsaturated fatty acids at the *sn*-2 position of glycerophospholipids is catalyzed by acyl-CoA:lysophospholipid acyltransferases (simply termed acyltransferases). The asymmetrical distribution of saturated or monounsaturated fatty acids at the *sn*-1 position and polyunsaturated fatty acids at the *sn*-2 position is maintained in part by a deacylation-reacylation process first proposed by William E. Lands in the late 1950s (the Lands' cycle, or Kennedy independent cycle) (24–27). Several members of the acyltransferase family have been recently identified by our laboratory and others, and have been shown to possess broad and somewhat overlapped substrate specificities that are dependent on their tissue distribution and external stimuli (28–35). Thus, acyltransferases have emerged as a potential key to the diversity of membrane phospholipids. **Figure 4** shows a scheme of the putative acyltransferase family members (36). A more detailed discussion of the properties of individual enzymes can be found in several previously published reviews (29, 192).

Acetyl-CoA:lyso-PAF Acetyltransferase

As illustrated in **Figure 2**, lysophospholipids with an alkyl-ether bond at the *sn*-1 position are converted to PAF by the activity of an acetyltransferase. Although a number of pioneering efforts to characterize the acetyltransferase involved in this process have been reported, there has been limited success in cloning the gene, primarily because the protein is membrane-spanning and highly unstable (37–40). Shindou et al. (41, 42) examined the regulation mechanism of this acetyltransferase in 2005. They demonstrated that it is induced by lipopolysaccharide (LPS) at 10–20 hours after stimulation, and exhibits a biphasic pattern of activation, with activation on the order of minutes (min) by PAF, possibly through the mobilization of intracellular Ca^{2+} stores. At approximately 30 min, activation is regulated by LPS through p38 kinase (42). Our laboratory recently isolated a member of the acetyltransferase gene family (LysoPAFAT/LPCAT2) that

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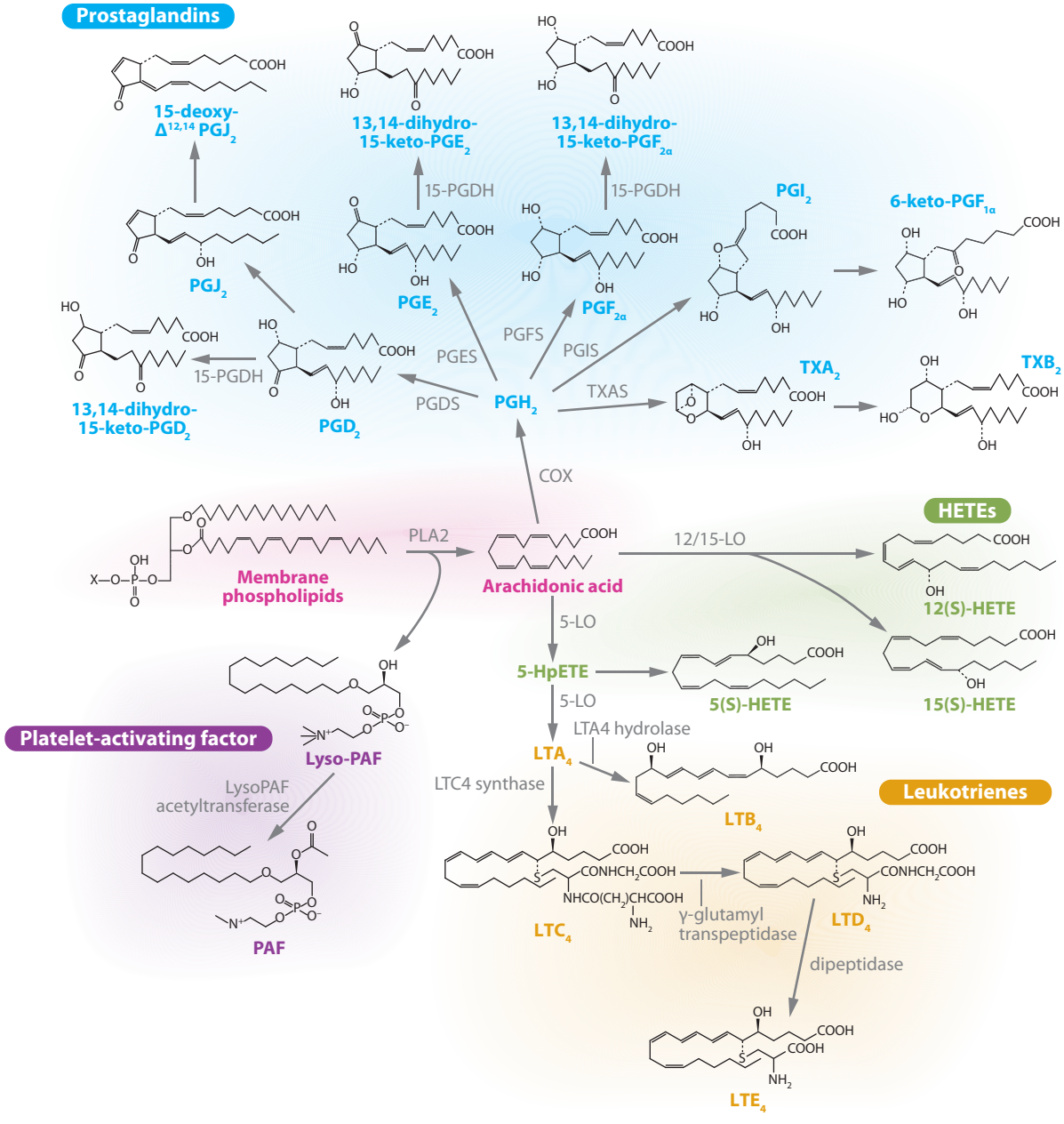

Figure 2

Biosynthetic pathways of eicosanoids and platelet-activating factor (PAF). Enzyme names are also described: 15-PGDH, 15-hydroxy-PG dehydrogenase; PGD(E, F, I)S, PGD (E, F, I) synthase; TXAS, TX synthase; 5-LO, 5-lipoxygenase; 12/15-LO, 12/15 lipoxygenase.

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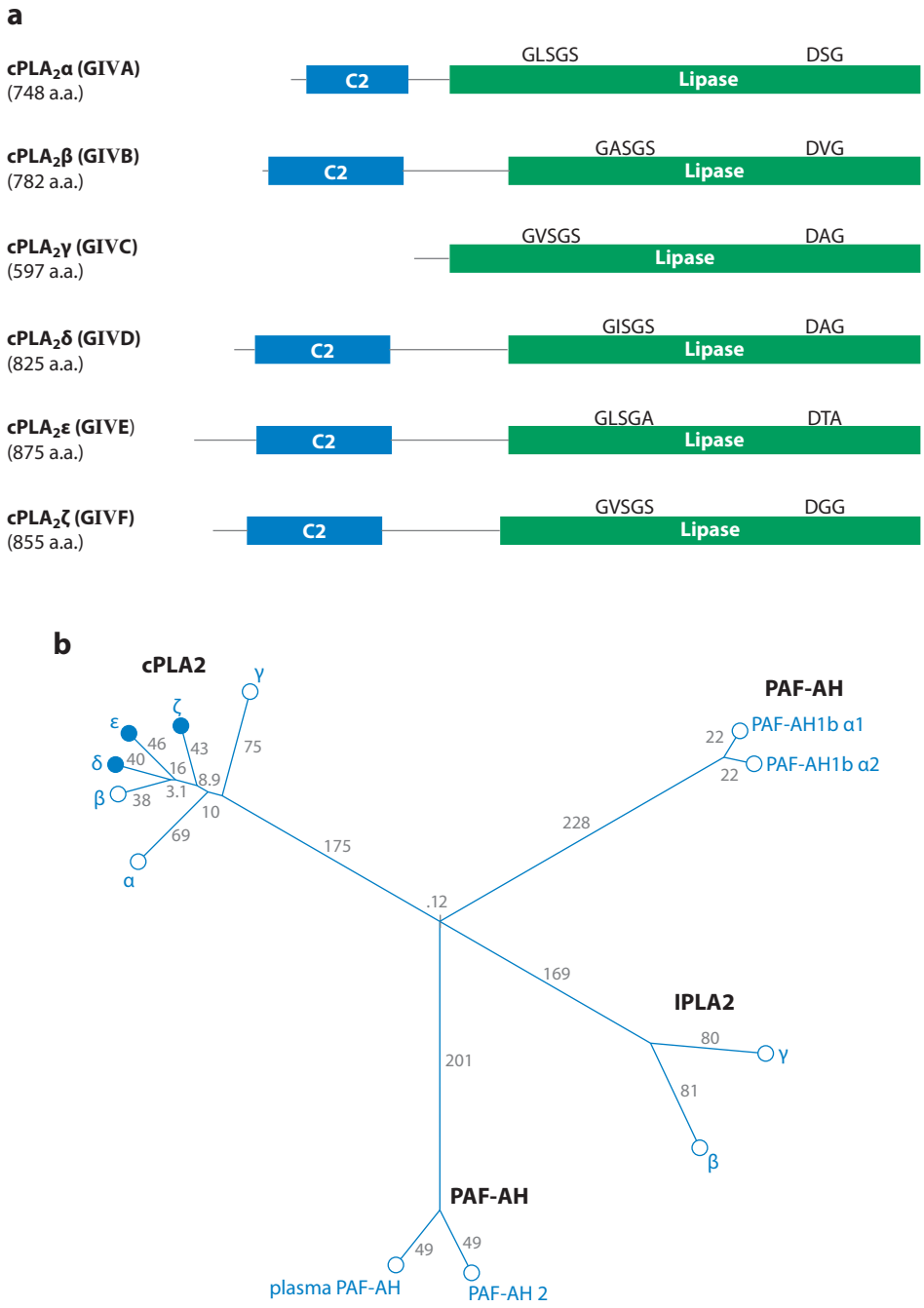

Figure 3

(*a*) Primary structure of six murine cytosolic PLA2 (cPLA2) enzymes. C2 is also referred to as Ca^{2+}-dependent lipid binding (CaLB). GIV, group IV. (*b*) Phylogenetic tree presentation of amino acid sequence similarity of cPLA2s and neighboring PLA2 enzymes. PAF-AH, platelet-activating factor acetylhydrolase.

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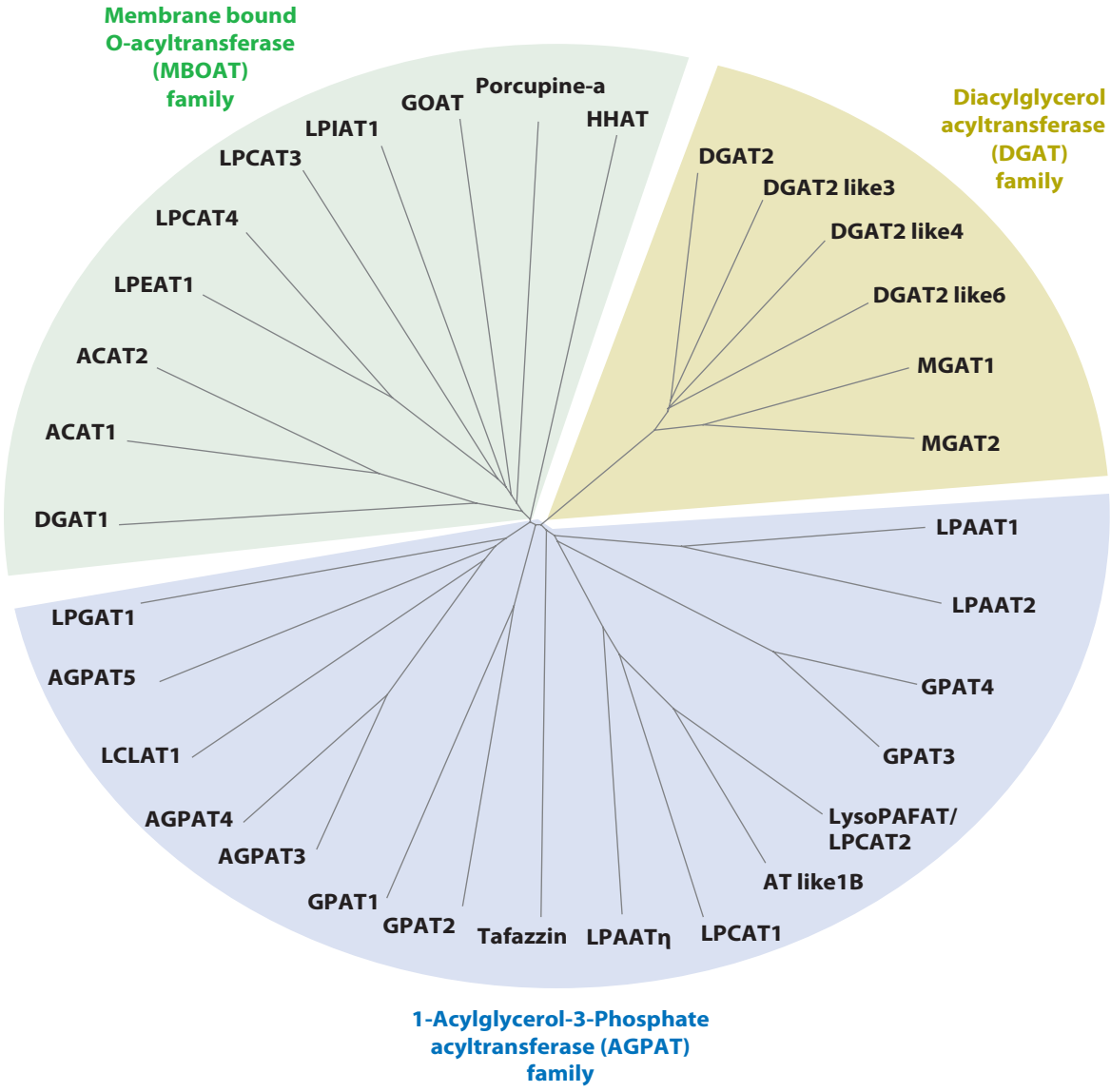

Figure 4

Lysophospholipid acyltransferase gene superfamily.

is highly expressed in inflammatory cells (43). The enzyme is a 60 kD protein with three putative membrane-spanning domains, several EF-hand motifs, and a C-terminal endoplasmic reticulum (ER) retention sequence. The properties of the enzyme (induction and activation) were similar to the previously published data of Shindou et al. (42). Quite unexpectedly, however, the enzyme also possessed lysophosphatidylcholine (LPC) acyltransferase activity, catalyzing the incorporation of arachidonoyl-CoA into LPC (or its alkyl ether analogue) to produce membrane PC derivatives (43). Thus, it appears that a single enzyme is responsible for the constitutive synthesis of membrane PCs under resting conditions, as well as the production of PAF in response to inflammatory signals

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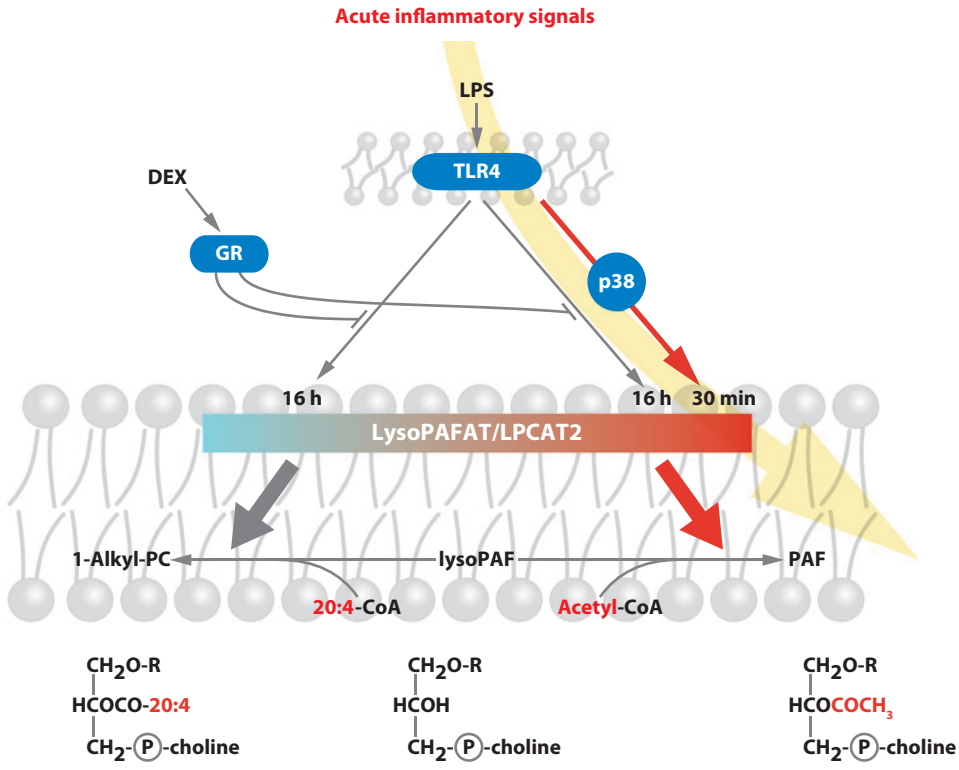

Figure 5

Dual functions of Lyso-PAF acetyltransferase 1/LysoPC acyltransferase 2 (LysoPAFAT/LPCAT2). Lipopolysaccharide (LPS) induces (~16 hours) and activates the enzyme by phosphorylation (~30 min) through TLR4 (Toll-like receptor 4). Dexamethasone (DEX) inhibits LPS-induced enzyme induction through glucocorticoid receptor (GR). Thus, a single enzyme catalyzes membrane biosynthesis under resting conditions, but produces PAF upon inflammatory signals (43).

(**Figure 5**). Other types of PAF biosynthetic enzymes display constitutive activity and do not require Ca^{2+}, such as LPCAT1 (44). As potential targets of anti-inflammatory drugs, the inducible lysoPAF acetyltransferases (such as LysoPAFAT/LPCAT2) are excellent candidates because the inhibition of this family of enzymes can potentially inhibit the proliferation of inflammatory cells through the disruption of membrane biogenesis without affecting the physiological function of PAF.

Cyclooxygenases and Lipoxygenases

Cyclooxygenase (Cox) catalyzes the incorporation of two oxygen molecules into arachidonic acid to form PG endoperoxides, which are converted into various types of PGs, depending on the type of cell or tissue, by terminal PG synthases (**Figure 2**). Two Cox isoenzymes have been identified: a constitutive form, Cox-1, and an inducible form, Cox-2 (45, 46). Aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit the activity of both enzymes through the acetylation of Ser residues near the catalytic pocket. Cox enzymes are present in the ER and nuclear membrane, and form homodimers. The induction of Cox-2 expression is regulated by at least three independent mechanisms: 1. transcriptional regulation through nuclear factor-kappa B (NF-κB) and

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cAMP response element binding protein (CREB); 2. mRNA stabilization through the proximal AT-rich sequence of the 3'-untranslated region; 3. the expression of microRNAs. Nonselective Cox inhibitors (such as the NSAIDs) are widely used in the treatment of inflammatory disorders and the prevention of thrombosis. They also have been explored as potential therapeutics in the prevention and treatment of colon cancer (47) and Alzheimer's disease (48). Cox-2 selective inhibitors, developed primarily by three pharmaceutical companies, have become globally distributed blockbuster drugs because of their decreased gastrointestinal side effects. However, the use of selective Cox-2 inhibitors has recently come under close scrutiny because of the association of these drugs with adverse cardiovascular effects (49), although the association remains controversial (50, 51). The cause of the cardiovascular side effects is not clear, but appears to involve the inhibition of prostacyclin (PGI2) formation and plaque destablization (52–54). The antipyretic and analgesic functions of acetoaminophene are not fully understood.

In mammals, the six different types of lipoxygenase are named according to the carbon position at which a single oxygen molecule is incorporated: 5-lipoxygenase, 8/12-lipoxygenase, two types of 12-lipoxygenase (12S-lipoxygease and 12R-lipoxygenase), and two types of 15-lipoxygenase (55). Among them, 5-lipoxygenase has been the most extensively studied (56). 5-Lipoxygenase catalyzes two sequential reactions, the incorporation of oxygen at the C-5 position and subsequent dehydration, to produce an allylic epoxide, LTA4 (57–61). LTA4 is then converted to LTB4 by LTA4 hydrolase (62–66), or to a cysteinyl-LT (such as LTC4, LTD4, or LTE4) by LTC4 synthase (67–69) (**Figure 2**). LTB4 is a potent chemoattractant and regulator of immune responses, whereas cysteinyl-LTs are a major component of the inflammatory agent slow reacting substance of anaphylaxis (SRS-A) (56, 70). The activity of 5-lipoxygenase is calcium dependent and regulated by multiple kinases. Like cPLA2α (71), 5-lipoxygenase translocates to the perinuclear membrane in response to changes in Ca^{2+} levels in the cytoplasm or nucleoplasm (72, 73). Five lipoxygenase activating proteins (FLAP) are present at the perinuclear membrane, and may be involved in the coupling of cPLA2α and 5-lipoxygenase activity by functioning as scaffolding proteins (74, 75). Scaffolding proteins such as FLAP have been proposed as mechanisms for the efficient production of eicosanoids and other lipid mediators by assembling multi-component signaling complexes. Although many enzymes are involved in the catalysis of lipid mediators, only 5-lipoxygenase inhibitors are currently approved for clinical use. Other inhibitors are in preclinical or clinical trials, and FLAP inhibitors appear promising. A group of alternative products of the lipoxygenase pathway, the lipoxins, resolvins, and neuroprotectins, has recently been described (76–80). The synthesis of these lipid mediators involves highly unsaturated fatty acids as substrates, such as eicosapentaenoic acid and docosahexaenoic acids, and utilizes more than two different lipoxygenases (81–83). Of note, members of this group of compounds appear to have anti-inflammatory or preresolution properties.

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Other Enzymes

LPA is produced from LPC by the action of lysophospholipase D (84–87). Lysophospholipase D is a secretory protein found in plasma (88) and is identical to autotaxin, a chemotactic protein (89). Sphingosine kinases (types 1, 2) convert sphingosine to S1P (90–92). In addition to the enzymes involved in synthesis, all lipid mediators are subject to enzymatic degradation. PG is converted to 15-keto-PG by 15-hydroxy-PG dehydrogenases in the presence of NAD+ or NADP+. LTB4 is inactivated by ω-oxidation or oxidation of the 12(R)-hydroxy moiety (93). The half-life of the eicosanoids is typically on the order of seconds (sec) to min, thus they are referred to as local mediators. By comparison, LPA and S1P have relatively long half-lives (min to hours) in vivo, and their levels are maintained in plasma in the μM and 10 nM concentration range, respectively.

G PROTEIN–COUPLED RECEPTORS FOR LIPID MEDIATORS

In humans, nearly 350 different nonolfactory GPCRs are identified (94). In 1991, our laboratory was the first to report the successful cloning of a lipid GPCR, the PAF receptor, using expression cloning in *Xenopus* oocytes and electrophysiology (95). Since that time, several lipid GPCRs have been identified and characterized; nine different PG receptors and four distinct LT receptors have been reported. The endothelial differentiation gene (Edg) family consists of three LPA receptors (LPA1-3) and five S1P receptors (S1P1-5). Members of this family are 40% homologous to the endocannabinoid receptors, CB1 and CB2. As of the writing of this review, more than 30 lipid GPCRs have been identified (**Figure 6**). Certain families of lipid GPCRs appear, because of the structural similarity of their ligands, to have evolved from the same ancestor (e.g., the PG and Edg receptor families), whereas other lipid GPCR families are evolved on the basis of functional similarity. For example, the LTB4 receptors BLT1 and BLT2 are evolutionarily distant from

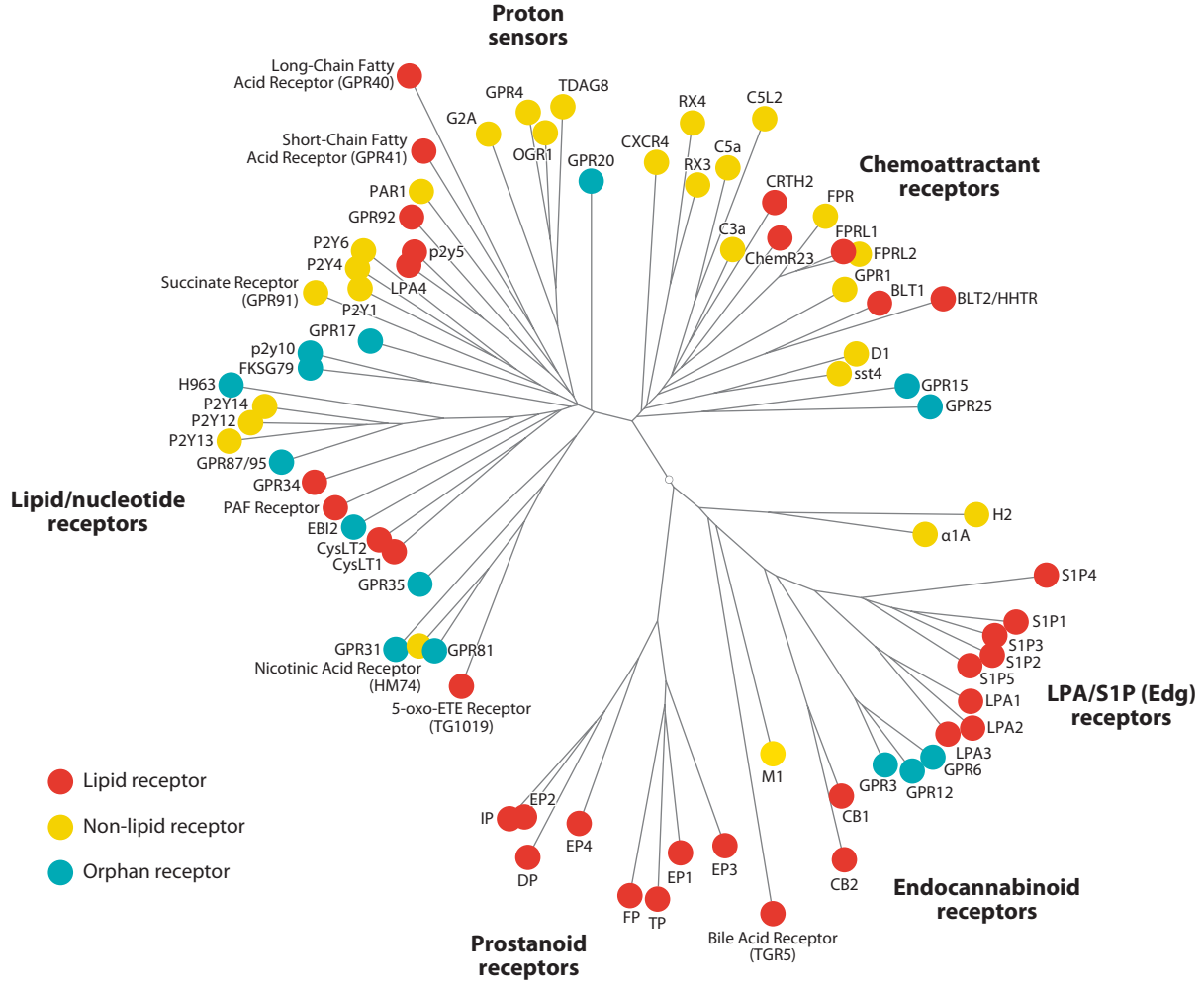

Figure 6

Phylogenetic tree of lipid G protein–coupled receptors (GPCRs) and orphan receptors.

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the PG or cysteinyl-LT receptors (CysLT1 and CysLT2), but in the same family as the Gi-coupled chemoattractant receptors, which also includes receptors for lipoxins, formyl-peptides, and complement (C5a, C3a). Most lipid GPCRs are relatively compact in size, comprising 300–400 amino acid residues.

Prostaglandin Receptors

PGs mediate a variety of biological functions in almost all tissues through the activation of their cognate receptors (96–98). The thromboxane (Tx) receptor (TP) was cloned in 1991 and has the structure of a typical GPCR. On the basis of homology with TP, seven additional PG receptors in various species have been identified. These include the PGD receptor (DP), PGE receptor (EP1-4), PGF receptor (FP) and PGI2 receptor (IP). These receptors have similar structural features, including PG receptor-specific consensus motifs in the seventh transmembrane domain and the second and third intracellular domains, as well as consensus amino acid sequences that are conserved in most class I GPCRs. Alternative splicing of human TP, EP3, and FP results in isoforms that have different C-terminal domains, but there are no significant differences in the ligand binding and intracellular signaling properties of the various isoforms. The eight PG receptors identified to date are classified into four subfamilies according to structural and functional similarity. The EP1-FP-TP subfamily mediates the mobilization of cytosolic Ca^{2+} (termed contractile receptors), and the EP2-EP4-IP-DP subfamily mediates the production of cyclic AMP (termed relaxant receptors) (96). The activation of EP3 results in a decrease in cyclic AMP production; hence, this receptor is classified an inhibitory receptor. A second type of PGD receptor, CRTH2 or DP2, belongs to the family of chemoattractant receptors like BLT1 and BLT2. In contrast to DP (DP1), CRTH2 exhibits broad specificity for a variety of ligands, including PGD2, 15-keto-PGD2 (inactive for DP1), and indomethacin (99). Similar to other chemoattractant receptors, CRTH2/DP2 mediates chemotaxis in eosinophils and lymphocytes through the activation of Gi.

Leukotriene Receptors

LTB4 is a one of the most potent chemotactic compounds for neutrophils, eosinophils and macrophages. In addition, recent studies have revealed that it plays a role in immune regulation. In our laboratory, we have cloned two types of LTB4 receptors, BLT1 and BLT2, using subtraction techniques and homology screening, respectively. BLT1 and BLT2 are members of the chemoattractant receptor family (100–102) (**Figure 6**). Originally, we reported that BLT2 is a low-affinity receptor for LTB4 and other hydroxy-eicosanoids (HETEs). However, we subsequently identified 12-hydroxy-heptadecatrienoic acid (12-HHT), a cyclooxygenase product, as a natural, high-affinity ligand for BLT2 by screening lipid fractions from mouse tissue extracts (103). 12-HHT is produced in equimolar amounts with TXA2 from PGH2 by thromboxane synthase. It can also be produced nonenzymatically from PGH2, particularly in the presence of heme and glutathione. In vivo, 12-HHT induced chemotaxis in mast cells through the activation of BLT2. On the basis of these results, we propose that BLT2 be now referred to as BLT2/HHTR. Additionally, two cysteinyl-LT receptors are members of the lipid/nucleotide receptor family: CysLT1, a high-affinity LTD4 receptor (104, 105), and CysLT2, a low-affinity receptor for LTC4 and LTD4 (106, 107) (**Figure 6**). The activation of CysLT1 results in bronchoconstriction and increased vascular permeability, however, the biological role and natural ligands for CysLT2 remain to be elucidated. BLT1, BLT2/HHTR, and CysLT1 mediate Ca^{2+} mobilization and the inhibition of adenylate cyclase, whereas CysLT2 mediates Ca^{2+} mobilization and increased cyclic AMP levels.

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PAF Receptor

PAF was originally identified as a hypotensive agent and platelet-activating mediator. It was subsequently shown to have versatile biological activities in almost all tissues. We have cloned PAF receptors from guinea pig, mouse, rat, and human (95, 108). Similar to PG and LT receptors, PAF receptors are relatively small-sized GPCRs. Two different PAF receptor mRNAs are generated by the use of alternative promoters. The expression of transcript I is regulated by estrogen and other hormones, whereas transcript II is regulated by the PKC-NFκB pathway. Transcripts I and II are expressed in different tissues, but their open reading frames are identical. The PAF receptor (PAFR) binds to PAF, as well as oxidized phospholipids, lysoPC, and lipopolysaccharide at higher concentrations, and is coupled to G proteins Gi, Gq, and G12/13. Activation of PAFR results in the inhibition of cyclic AMP formation, mobilization of intracellular Ca^{2+}, and the activation of mitogen-activated protein kinases. Thus, it appears that PAFR engages a variety of intracellular signaling pathways, which helps explain its wide range of biological functions. Additional information on PAFR activity and function can be found in reviews 109 and 110.

Lysophospholipid Receptors

Lysophospholipids, such as LPA and S1P, are membrane-derived lipid mediators. They are involved in the regulation of fundamental cellular responses, including cell proliferation, differentiation, migration, adhesion and morphogenesis. There are five different LPA receptors (LPA1-5) (111, 112), and a sixth, p2y5, a relative of p2y9/GPR23/LPA4 (56% homology), has recently been proposed. LPA1, LPA2 and LPA3 are Edg-family members, and are 50–60% homologous to each other, whereas LPA4 and LPA5, and possibly LPA6 (p2y5), form a distinct family (**Figure 6**). The two families of LPA receptors differ in their G protein specificity and tissue distribution, which might explain the broad range of functions of LPA. It is noteworthy that human genetic hair anomaly is associated with mutation of p2y5 (113, 114). At present, five different S1P receptors (S1P1-5) are identified, all of which are Edg-family members. In contrast to PG receptors, the lysophospholipid receptors appear to be less heterogenous, with similar G protein specificities and a ubiquitous pattern of expression. Additional information on lysophospholipid receptors is available in several other current reviews (115–119).

Other Lipid GPCRs

The cannabinoid receptors CB1 and CB2 are GPCRs that also interact with lipid ligands (120). CB1 is expressed primarily in the central nervous system and is involved in the regulation of GABAergic neurons (121, 122), whereas CB2 is expressed primarily in immune cells (123). Although not described in this review, several other lipid ligands (i.e., bile acids, short-chain fatty acids, lysophosphatidylserine, and lysophophatidylinositol) are reported to bind to cognate GPCRs (**Figure 6**). It also has been suggested that the proton-sensing GPCRs G2A, TDAG8, OGR1, and GPR4 interact either directly or indirectly with lipid molecules (124), but additional studies are needed to understand the significance of these lipid-GPCR interactions. In our laboratory, we are screening lipid ligands from tissue extracts in an effort to identify novel lipid mediators of orphan receptors.

Nuclear Receptors

There are reports that lipid mediators can bind to nuclear receptors (NRs), particularly the peroxisome-proliferator activator receptor (PPAR) family of NRs. PGJ2 derivatives (125, 126), PGI2, PAF, LPA (127), and long chain fatty acids have all been implicated in binding to PPARs.

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Although these observations are consistent with the production site of most lipid mediators, in the perinuclear or nuclear membrane, PPARs have spacious binding pockets and the in vivo biological significance of these interactions is yet to be determined.

LIPID MEDIATORS IN HEALTH AND DISEASES

Lipid mediators were historically considered terminal mediators, causing symptoms such as fever, pain, edema, smooth muscle contraction, and inflammation. However, recent studies using gene knockout mice (for both enzymes and receptors) have revealed that lipid mediators play a much more fundamental role in normal physiological processes and in disease. A discussion of the pathophysiological roles of all the various classes of lipid mediators is beyond the scope of a single review, and excellent review articles that describe the phenotypes of specific gene knockout mice, particularly the receptor knockout mice, are currently available (97, 116).

The remainder of this review is focused on the phenotype of mice carrying a deletion of cPLA2α, an enzyme that is either fully or partially involved in the production of various lipid mediators. I also examine the molecular mechanisms of cPLA2α function by comparing cPLA2α knockout mice to knockout mice in which downstream enzymes or individual receptors are targeted. Lastly, I explore how a comprehensive lipidomics approach to analyzing downstream lipid mediators using liquid chromatography–tandem mass spectrometry (LC-MS/MS) can offer valuable insight into the role of lipid mediators in health and human disease.

Phenotype of cPLA2α Knockout Mice

Subsequent to the discovery of cPLA2α in 1991 by Clark et al. (128) and Kramer et al. (12), we and others established cPLA2α knockout mice (129, 130), and backcrossed the knockout mice with a variety of mouse strains (C57/Bl6, C3HeN, C3HeJ, DBA, Balb/c, etc.). In our original report, we demonstrated that cPLA2α is essential for the production of various types of eicosanoids and PAF in peritoneal and alveolar macrophages (129). **Table 2** summarizes the phenotypes of cPLA2α

Table 2 Phenotype of cytosolic phospholipase A2α (cPLA2α)-null mice [modified from (131)]

Physiology and Pathology	References
Physiological abnormality	
Reproduction failure	(129, 130, 132)
Renal physiology failure	(175)
Change in brain lipid composition	(176)
Protection from disease models	
Ischemia-reperfusion injury	(130, 177)
MPTP-induced neurotoxicity	(178, 179)
Experimental allergic encephalomyelitis (EAE)	(180)
Ovalbumin-induced anaphylaxis and bronchial asthma	(129, 181, 182)
Thrombosis and platelet functions	(183)
Acute respiratory distress syndrome (ARDS)	(184, 185)
Bleomycin-induced lung fibrosis	(157)
Collagen-induced arthritis	(139)
Inflammatory bone resorption	(186)
Intestinal polyposis	(137, 187)
Parasite and bacterial infection	(188–190)
Type I diabetes mellitus	(191)

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knockout mice generated by others and us (131). cPLA2α-null mice are born in the expected Mendelian ratio, and appear to be healthy, with the exception of multiple small intestinal ulcers. This is consistent with a reported case of human cPLA2α-deficiency, in which individuals exhibit a bleeding tendency and intestinal ulcers (193). In cPLA2α knockout mice, routine biochemical and immunological parameters of the serum are in the normal range, and the life span appears normal. However, knockout mice exhibit abnormalities in synaptic plasticity and reproduction (implantation and labor), and exhibit much milder symptoms in a number of disease models. The mice are protected from anaphylactic symptoms, bronchial asthma, brain injury in response to multiple stimuli, thrombosis, collagen-induced arthritis (a model for rheumatoid arthritis), endotoxin-induced bone resorption, bleomycin-induced lung fibrosis, and experimental allergic encephalomyelitis (a model for multiple sclerosis).

Comparison with the Phenotypes of Other Knockout Mice

In this section, I compare the phenotypes of cPLA2α-deficient mice with other knockout mice, highlighting points of consensus and controversy.

Reproduction

When cPLA2α-null mice are born, they appear normal. However, when wild-type male mice are mated with cPLA2α-null female mice, there is an obvious reduction in the number of offspring (129). Implantation in knockout mice is severely impaired, and labor does not commence at term. Similar cPLA2α-null phenotypes have been reported by others (130, 132), who have proposed that the implantation defect of cPLA2α-null mice involves a deficiency of PGI2. Cox-1- or 5-lipoxygenase-null mice do not exhibit the implantation phenotype. Cox-2-null mice exhibit multiple abnormalities, including defects in ovulation, fertilization, implantation, and embryogenesis (133). It was recently reported that LPA3-null mice exhibit implantation failure owing to the lack of Cox-2 expression in the uterus, and that the defect is rescued by the administration of either a stable PGI2 analogue or PGE2 (134). However, the mechanism of implantation failure in cPLA2α-null mice remains controversial, as IP (PGI2 receptor)-null or EP-null mice do not exhibit this phenotype. Furthermore, several groups have reported that Cox-2-null mice do not exhibit such severe abnormalities in reproduction (135). Additional studies are needed to explore the nature of these apparent contradictions among various groups of mice.

The labor phenotype of cPLA2α knockout mice is due primarily to the loss of PGF2α. During periparturition, the expression of Cox-2 is induced, and the induction of luteolysis by PGF2α results in increased oxytocin receptor levels and decreased serum progesterone levels by luteolysis. FP-null mice exhibit a similar phenotype, and labor loss in both cPLA2α and FP knockout mice is rescued by the administration of a progesterone antagonist or ovariectomy at embryonic day 19 (136). Although the precise mechanism of the labor loss phenotype of cPLA2α-null mice remains obscure, cPLA2α inhibitors, such as NSAIDs, are contraindicated in pregnancy, and in women who can become pregnant.

Intestinal Polyposis

Deletion of Cox-2 or the use of Cox-2 selective inhibitors causes a reduction in polyp number and size in ΔAPC(716) mice, an animal model of familial adenomatous polyposis. A similar effect is observed in EP2-null mice. The expression of cPLA2α, Cox-2, and EP2 is upregulated in polyp tissue as compared with adjacent normal epithelium in ΔAPC(716) mice, on the basis of

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both Western blot and reverse-transcription PCR analysis. cPLA2α-null mice exhibit a relatively mild phenotype in terms of tumor size, particularly in the small intestine, which might be a result of the presence of other, compensatory, PLA2 isoforms (i.e., type X sPLA2) in the colon (137). The current hypothesis of tumor development in ΔAPC(716) mice is as follows: PGE2 is produced through the action of cPLA2α and Cox-2 (135), and possibly microsomal PGE synthase type 1. PGE2 binds to its cognate receptor, EP2, and induces cyclic AMP production and the activation of protein kinase A (138). Protein kinase A induces the expression of Cox-2 through binding of cyclic AMP response element (CRE) binding protein to CRE of the Cox-2 gene promoter, resulting in further elevation in PGE2 levels. The CRE-dependent induction of vascular endothelial growth factor (VEGF) and matrix metalloproteinases also promotes cell growth and cell invasion. Inhibitors of Cox-2, cPLA2α, or EP2 receptor antagonists effective in terminating polyp growth are the most likely candidates for preventing colon cancer.

Rheumatoid Arthritis

cPLA2α-null mice are resistant to collagen-induced arthritis, and their clinical scores are equally good (1 or lower during the experimental period of observation) as those of TNFα-null mice (139). Cox-2-null mice (140) and microsomal PGE synthase-null mice also exhibit a similar phenotype. Recently, genetic ablation of BLT1 (141, 142), DP, or IP (143) protected mice from developing arthritis, although the roles of individual mediators may be different. In most reports, the production of inflammatory cytokines, such as TNFα, interleukin 1, and interleukin 6, was decreased in synovial cells, and T-cell infiltration of the joint tissues was apparent. These observations are difficult to interpret, but it seems likely that the inhibition of cPLA2α would suppress all of these lipid mediators. However, the relationship between cytokines, chemokines, and lipid mediators certainly warrants further investigation.

Multiple Sclerosis

Experimental autoimmune (allergic) encephalomyelitis (EAE) is a Th1/Th17-type autoimmune disorder, characterized by demyelination and inflammation. cPLA2α-null mice are completely resistant to myelin oligodendrocyte glycoprotein (MOG)-induced EAE, an animal model of multiple sclerosis (MS). Using a comprehensive lipidomics approach, we have determined the various lipid mediators of the spinal cord in the disease processes are: naïve, induction, acute, and chronic. We found that Cox products are increased in early stages of the disease (induction phase to acute phase), whereas PAF is produced in the later, chronic phase. Genetic and pharmacological inhibition of Cox-2 prevent onset and progression of EAE (144, 145). The products of 5-lipoxygenase are decreased in all stages. These results are consistent with previous reports of PAFR-null mice, which exhibit milder symptoms during later phases of disease progression (the T-cell independent phase) (146), and 5-lipoxygensae-null mice, which exhibit a more severe phenotype than the littermate wild-type mice (147). It remains to be determined which Cox products and which receptors initiate disease onset and progression, and the mechanism of protection from disease by the products of 5-lipoxygenase.

Bronchial Asthma

Bronchial asthma is a Th2-type immune disorder, characterized by inflammatory cell migration, airway hypersensitivity, and remodeling of the bronchoalveolar structures (148). Experimentally, bronchial asthma can be induced in animals by sensitization with ovalbumin or house dust with adjuvants. When cPLA2-null mice are exposed to an antigen to induce bronchial asthma, the

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levels of various eicosanoids and PAF in bronchoalveolar lavage fluid are significantly decreased as compared with wild-type mice, as are the number of inflammatory cells. There is also decreased airway hypersensitivity (129). BLT1-null mice are also protected from these symptoms, through a mechanism involving the inhibition of early T-cell recruitment (both CD4+ and cytotoxic CD8+) and eosinophil infiltration (149–155). The levels of IgE, IgG1, and Th2 cytokines in plasma and bronchoalveolar lavage fluids differ depending on the immunization protocol. Compared with wild-type mice, LTC4 synthase-null mice or CysLT1-null mice exhibit attenuated airway hyperreactivity, decreased OVA-specific IgE production, and decreased eosinophil infiltration (156), whereas PAFR-null mice exhibit decreased airway hyperreactivity without corresponding changes in cellular infiltration and cytokine production (109). The roles of BLT2 and CysLT2 in this model remain elusive.

Pulmonary Fibrosis

Pulmonary fibrosis in cPLA2α-null mice was induced by multiple intraperitoneal injections of bleomycin. Cysteinyl-LTs were elevated in lung lavage fluid in the mice (157). In 5-lipoxygenase-null mice, the induction of pulmonary fibrosis resulted in milder lung pathology and lower hydroxyproline levels than wild-type mice, similar to cPLA2α-null mice (158). In Cox-2-null mice, pulmonary dysfunction was exacerbated, with no accompanying changes in collagen content or lung histology (159). Cox-1 overproduction in these mice was reported, which may partially compensate for the Cox-2 defect. In addition, the authors proposed that granulocyte-macrophage colony-stimulating factor (GM-CSF) enhances the production of PGE2, which has antifibrotic properties, whereas TGFβ induces profibrotic 5-lipoxygenase products (158). There have been several interesting and challenging reports of pulmonary fibrosis in CysLT1-, CysLT2- and LTC4 synthase-null mice. Although genetic ablation of LTC4 synthase attenuated lung fibrosis, CysLT1-null mice exhibited a more severe phenotype of fibrotic damage in the lung than the wild type (160). However, CysLT2-null mice were protected from fibrosis (161). The underlying mechanisms of these phenotypes are yet unknown, but the authors demonstrated that cysteinyl-LT was elevated in CysLT1-null mice, but not in CysLT2-null mice (160). Izumo et al. (162) reported that, on the bases of Aschcroft score, hydroxyproline content, and TGF-β levels, pharmacological inhibition of CysLT1 using Montelukast attenuates lung fibrosis. Additional studies and integrated analyses are needed to elucidate the roles of lipid mediators in the onset and development of pulmonary fibrosis. In this regard, it was recently reported that LPA1-null mice also exhibit attenuated lung fibrosis (163), and it was proposed that LPA1 antagonists may be of benefit in the prevention or treatment of lung fibrosis.

Others

Because of the scope of this review, I have omitted discussions of many other important findings related to lipid mediators in health and diseases. However, one prominent issue worth mentioning is the role of S1P in the immune response and embryogenesis. A potent S1P1 agonist (a functional antagonist because it elicits receptor internalization), FTY720 (a prodrug, only active after phosphorylation by sphingosine kinases), is currently undergoing clinical trials as an immunosuppressive drug for organ transplantation and MS. FTY720 dramatically decreases the number of circulating lymphocytes in the body by inhibiting the emigration of lymphocytes from local lymph nodes (164–168). The drug also shows adverse side effects including bradycardia. S1P1-null mice exhibit embryonic lethality at embryonic day 10 because of a lack of vasculogenesis (169), and LPA receptor double- and triple-knockout mice exhibit morphological and developmental abnormalities

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in the brain. The genetic ablation of autotaxin (lysophospholipase D) in mice results in similar phenotypes to S1P1-null mice (170, 171), and is also an embryonic lethal mutation. This is somewhat surprising, because lysophospholipase D is not involved in the production of S1P in vivo, and none of the double- or triple knockout mice of LPA receptors exhibit such lethal abnormality.

Comprehensive Analyses of Eicosanoids and PAF by LC-MS/MS

A potentially valuable and promising approach to understanding the molecular basis of the cPLA2α-null phenotypes in various disease states is the identification of specific downstream lipid mediators and the time course of their activity. Our laboratory is currently engaged in a systematic quantification of specific lipid mediators involved in human diseases, with the goal of using this information as a diagnostic and prognostic tool, and as a guide for future studies. Through a comprehensive, top-down profiling of lipid mediators, one can arrive at a better understanding of the roles of individual mediators. We have established a sensitive and highly selective method of measuring 14–20 different lipid mediators in a single sample within 10 min (172, 173). **Supplemental Figure 1*a*** (follow the **Supplemental Material link** from the Annual Reviews home page at **http://www.annualreviews.org**) is a typical chromatogram result, demonstrating that we can detect 14 different lipid mediators downstream of cPLA2α. Using peritoneal macrophages, it is clear that PGs are produced rapidly, whereas LTs are produced more slowly and are less labile. PAF is produced rapidly, but is also degraded quickly by PAF acetylhydrolases present in plasma and macrophages (**Supplemental Figure 1*b***).

Application of the Methods for Disease Models

I present the results of lipid profiling in two animal models of brain disease, kainate-induced seizures and experimental allergic encephalomyelitis, to illustrate the utility of this comprehensive lipidomics approach. In animals of various species, kainic acid (KA) induces seizures accompanied by the production of eicosanoids. In our laboratory, we profiled eicosanoids in the hippocampus and cerebral cortex at various times after the application of KA using LC-MS/MS (174). KA induced robust production of PGF2α and PGD2 in the hippocampus, and a relatively smaller level of production of other PGs and HETEs. LT was undetectable in the brain following KA stimulation. The production of eicosanoids was biphasic, with peaks of production within 30 min, and again at 16–24 hours, and was limited initially to the hippocampus. Cox-2 selective inhibitors abrogated the early production of mediators, with the exception of TXB2 and PGD2, which suggests that the latter two compounds are produced predominantly by Cox-1. The sustained production of PGs during the late phase in the brain appeared a result of notable induction of Cox-2, despite the limited supply of arachidonic acid by PLA2. The sustained production of PGs was not caused by cPLA2α, as cPLA2α-null mice exhibited a similar profile (Y. Kita & K. Yoshikawa, unpublished data). Although this study was not aimed at addressing the function of eicosanoids in epileptic seizures or neuronal degeneration, these results reveal that PG production is biphasic, which is an important consideration in developing treatments. Furthermore, the role of NSAIDs in the prevention or aggravation of seizure symptoms and neuronal cell death is controversial. In most studies that examine this issue, animals are pretreated with NSAIDs, likely inhibiting only the initial phase of PG production. However, the roles of the PGs produced in the early and late phases might be different, and further studies are necessary to determine the optimal type of Cox inhibitor(s) and time point for intervention to prevent cell death and promote cell recovery.

In a second study, researchers (Y. Kihara, Y. Kita, S. Ishii, unpublished data) measured multiple eicosanoids (PGs, LTs, and their metabolites) in animal models of MS. In the spinal cord, there

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was an obvious and dramatic shift from 5-lipoxygenase-dependent pathways to Cox-dependent pathways during the induction phase of disease progression. PAF was produced in the later phase of the disease. These results are in agreement with previous results demonstrating that PAF is not involved in the early T-cell dependent phase of the disease, but in the late phase, when macrophages or microglia are involved in disease progression (146). Thus, lipid profiling can be used to predict which eicosanoids will be involved in disease induction, and which ones will be involved in protecting against disease. This type of predictive power can be used to guide more definitive studies using knockout mice, and the use of specific inhibitors, receptor agonists, or antagonists at various stages of disease.

CONCLUSION

Herein I present a summary of the enzymes and receptors involved in lipid mediator synthesis and function, with an emphasis on eicosanoids and PAF. Eicosanoids and PAF are produced from membrane phospholipids by the action of PLA2, in particular, cPLA2α. Polyunsaturated fatty

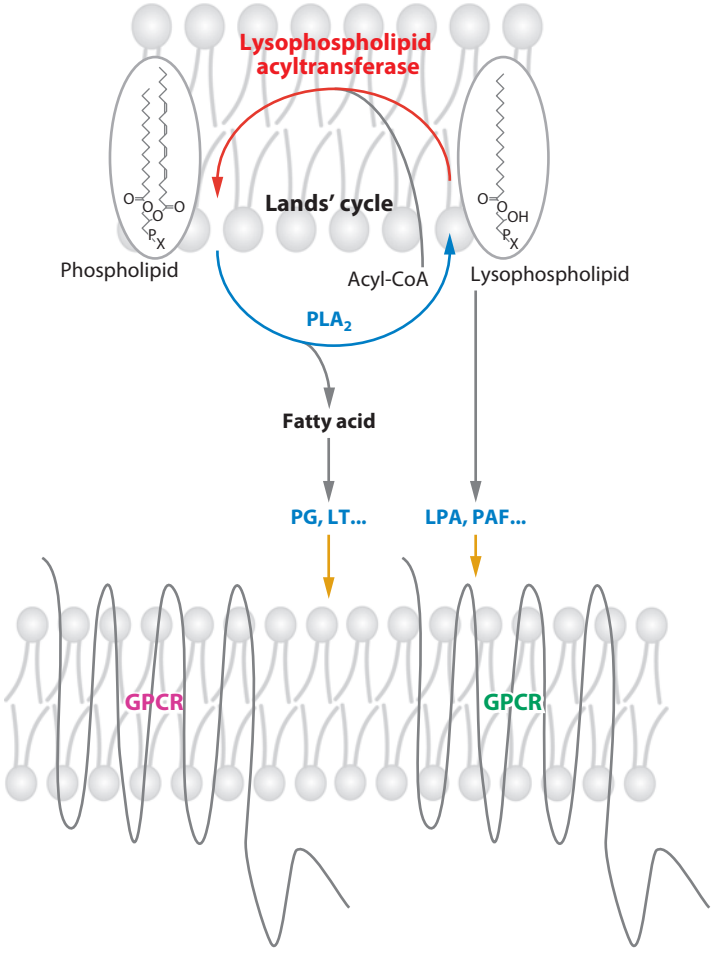

Figure 7

Production of lipid mediators during deacylation-reacylation cycle (Lands' cycle).

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acids and lysophospholipids are further metabolized into a variety of bioactive compounds, collectively termed lipid mediators. Unmetabolized lysophospholipids are incorporated into membrane phospholipids, by the actions of acyltransferases using polyunsaturated fatty acyl-CoA. Thus, the production of lipid mediators is tightly coupled to the Lands' cycle (**Figure 7**). Lipid mediators exert their functions through cognate GPCRs and the intracellular second messenger systems of individual cells and tissues. In concert with the actions of cytokines, neurotransmitters, hormones, and growth factors, lipid mediators play fundamental roles in maintaining healthy living systems. However, either excess or dysfunctions of the enzymes and/or receptors of lipid mediators can cause inflammatory and immune disorders, including bronchial asthma, rheumatoid arthritis, pulmonary fibrosis, MS, etc. Lipid mediators and their receptors are potentially important and novel therapeutic targets for various human diseases. Thus, although mouse models differ in many ways from human diseases, genetic knockout studies are a valuable tool for predicting both promising and adverse effects of drugs targeted for human use.

SUMMARY POINTS

1. Lipids have at least four major functions in the body: membrane components, an energy source, post-translational protein modification moieties, and autacoid lipid mediators.
2. Prostaglandins, leukotrienes (eicosanoids), and PAF are produced by enzymatic cascades initiated by Ca^{2+}-dependent cPLA2.
3. Lysophospholipids are converted into various lipid mediators, or transformed into phospholipids by the recently identified acyltransferase superfamily.
4. Lipid mediators bind and activate GPCRs, and some have been reported to act on nuclear receptors as well.
5. Knockout mice in which the enzyme(s) or receptor(s) of lipid mediator synthesis and function are targeted exhibit a variety of physiological and pathological phenotypes.
6. To understand the molecular mechanisms of the cPLA2α-null phenotypes, we have established a comprehensive high throughput method of quantifying lipid mediators using LC-MS/MS.
7. Enzymes and receptors are therapeutic targets for various inflammatory and immune diseases.

FUTURE ISSUES

1. It is necessary to determine the biological significance of membrane diversity and asymmetry (saturated at *sn*-1 versus unsaturated at *sn*-2).
2. The mechanism of coordinated regulation and intracellular coupling of multiple enzyme systems by the putative scaffold systems must be examined.
3. Identification of specific and nonspecific transporters of lipid mediators is necessary.
4. The link between second messenger systems (Ca^{2+}, cyclic nucleotide, etc.) and cell-specific functions and phenotypes needs to be established.
5. Researchers need to develop specific enzyme inhibitors and receptor agonists and antagonists.

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6. Lipid mediator production in animal disease models and human diseases should be monitored at various disease stages.

DISCLOSURE STATEMENT

The author is not aware of any biases that might be perceived as affecting the objectivity of the review.

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LITERATURE CITED

1. Vigh L, Escriba PV, Sonnleitner A, Sonnleitner M, Piotto S, et al. 2005. The significance of lipid composition for membrane activity: new concepts and ways of assessing function. *Prog. Lipid Res.* 44:303–44
2. Escriba PV. 2006. Membrane-lipid therapy: a new approach in molecular medicine. *Trends Mol. Med.* 12:34–43
3. Weiss R, Dziura J, Burgert TS, Tamborlane WV, Taksali SE, et al. 2004. Obesity and the metabolic syndrome in children and adolescents. *N. Engl. J. Med.* 350:2362–74
4. Mann RK, Beachy PA. 2004. Novel lipid modifications of secreted protein signals. *Annu. Rev. Biochem.* 73:891–923
5. Shimizu T, Wolfe LS. 1990. Arachidonic acid cascade and signal transduction. *J. Neurochem.* 55:1–15
6. Funk CD. 2001. Prostaglandins and leukotrienes: advances in eicosanoid biology. *Science* 294:1871–75
7. Dennis EA. 1997. The growing phospholipase A2 superfamily of signal transduction enzymes. *Trends Biochem. Sci.* 22:1–2
8. Kudo I, Murakami M. 2002. Phospholipase A2 enzymes. *Prostaglandins Other Lipid Mediat.* 68–69:3–58
9. Murakami M, Kudo I. 2002. Phospholipase A2. *J. Biochem. (Tokyo)* 131:285–92
10. Hanasaki K, Arita H. 2002. Phospholipase A2 receptor: a regulator of biological functions of secretory phospholipase A2. *Prostaglandins Other Lipid Mediat.* 68–69:71–82
11. Ni Z, Okeley NM, Smart BP, Gelb MH. 2006. Intracellular actions of group IIA secreted phospholipase A2 and group IVA cytosolic phospholipase A2 contribute to arachidonic acid release and prostaglandin production in rat gastric mucosal cells and transfected human embryonic kidney cells. *J. Biol. Chem.* 281:16245–55
12. Kramer RM, Roberts EF, Manetta J, Putnam JE. 1991. The $Ca^{2(+)}$-sensitive cytosolic phospholipase A2 is a 100-kDa protein in human monoblast U937 cells. *J. Biol. Chem.* 266:5268–72
13. Clark JD, Schievella AR, Nalefski EA, Lin LL. 1995. Cytosolic phospholipase A2. *J. Lipid Mediat. Cell Signal* 12:83–117
14. Bonventre JV, Sapirstein A. 2002. Group IV cytosolic phospholipase A2 (PLA2) function: insights from the knockout mouse. *Adv. Exp. Med. Biol.* 507:25–31
15. Ohto T, Uozumi N, Hirabayashi T, Shimizu T. 2005. Identification of novel cytosolic phospholipase A_2s, murine cPLA$_2$δ, ε, and ζ, which form a gene cluster with cPLA$_2$β. *J. Biol. Chem.* 280:24576–83
16. Ghosh M, Loper R, Gelb MH, Leslie CC. 2006. Identification of the expressed form of human cytosolic phospholipase A_2β (cPLA$_2$β): cPLA$_2$β3 is a novel variant localized to mitochondria and early endosomes. *J. Biol. Chem.* 281:16615–24

Annu. Rev. Pharmacol. Toxicol. 2009.49:123-150.

17. Gross RW. 1998. Activation of calcium-independent phospholipase A2 by depletion of internal calcium stores. *Biochem. Soc. Trans.* 26:345–9
18. Turk J, Ramanadham S. 2004. The expression and function of a group VIA calcium-independent phospholipase A2 (iPLA2beta) in beta-cells. *Can. J. Physiol. Pharmacol.* 82:824–32
19. Balsinde J, Balboa MA. 2005. Cellular regulation and proposed biological functions of group VIA calcium-independent phospholipase A2 in activated cells. *Cell Signal.* 17:1052–62
20. Tjoelker LW, Eberhardt C, Unger J, Trong HL, Zimmerman GA, et al. 1995. Plasma platelet-activating factor acetylhydrolase is a secreted phospholipase A2 with a catalytic triad. *J. Biol. Chem.* 270:25481–87
21. Hattori M, Adachi H, Aoki J, Tsujimoto M, Arai H, et al. 1995. Cloning and expression of a cDNA encoding the beta-subunit (30-kDa subunit) of bovine brain platelet-activating factor acetylhydrolase. *J. Biol. Chem.* 270:31345–52
22. Karasawa K, Kuge O, Kawasaki K, Nishijima M, Nakano Y, et al. 1996. Cloning, expression and characterization of plasma platelet-activating factor-acetylhydrolase from guinea pig. *J. Biochem.* 120:838–44
23. Hattori M, Adachi H, Tsujimoto M, Arai H, Inoue K. 1994. The catalytic subunit of bovine brain platelet-activating factor acetylhydrolase is a novel type of serine esterase. *J. Biol. Chem.* 269:23150–55
24. Lands WE. 1958. Metabolism of glycerolipides; a comparison of lecithin and triglyceride synthesis. *J. Biol. Chem.* 231:883–88
25. Lands WE. 1960. Metabolism of glycerolipids. 2. The enzymatic acylation of lysolecithin. *J. Biol. Chem.* 235:2233–37
26. Okuyama H, Lands WE. 1972. Variable selectivities of acyl coenzyme A:monoacylglycerophosphate acyltransferases in rat liver. *J. Biol. Chem.* 247:1414–23
27. Waku K, Lands WE. 1968. Acyl coenzyme A:1-alkenyl-glycero-3-phosphorylcholine acyltransferase action in plasmalogen biosynthesis. *J. Biol. Chem.* 243:2654–59
28. Coleman RA. 1992. Diacylglycerol acyltransferase and monoacylglycerol acyltransferase from liver and intestine. *Methods Enzymol.* 209:98–104
29. Lewin TM, Wang P, Coleman RA. 1999. Analysis of amino acid motifs diagnostic for the sn-glycerol-3-phosphate acyltransferase reaction. *Biochemistry* 38:5764–71
30. Cao J, Hawkins E, Brozinick J, Liu X, Zhang H, et al. 2004. A predominant role of acyl-CoA:monoacylglycerol acyltransferase-2 in dietary fat absorption implicated by tissue distribution, subcellular localization, and up-regulation by high fat diet. *J. Biol. Chem.* 279:18878–86
31. Cao J, Liu Y, Lockwood J, Burn P, Shi Y. 2004. A novel cardiolipin-remodeling pathway revealed by a gene encoding an endoplasmic reticulum-associated acyl-CoA:lysocardiolipin acyltransferase (ALCAT1) in mouse. *J. Biol. Chem.* 279:31727–34
32. Yang Y, Cao J, Shi Y. 2004. Identification and characterization of a gene encoding human LPGAT1, an endoplasmic reticulum-associated lysophosphatidylglycerol acyltransferase. *J. Biol. Chem.* 279:55866–74
33. Nakanishi H, Shindou H, Hishikawa D, Harayama T, Ogasawara R, et al. 2006. Cloning and characterization of mouse lung-type acyl-CoA:lysophosphatidylcholine acyltransferase 1 (LPCAT1). Expression in alveolar type II cells and possible involvement in surfactant production. *J. Biol. Chem.* 281:20140–47
34. Jain S, Stanford N, Bhagwat N, Seiler B, Costanzo M, et al. 2007. Identification of a novel lysophospholipid acyltransferase in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 282:30562–69
35. Soupene E, Fyrst H, Kuypers FA. 2008. Mammalian acyl-CoA:lysophosphatidylcholine acyltransferase enzymes. *Proc. Natl. Acad. Sci. USA* 105:88–93
36. Hishikawa D, Shindou H, Kobayashi S, Nakanishi H, Taguchi R, et al. 2008. Discovery of a lysophospholipid acyltransferase family essential for membrane asymmetry and diversity. *Proc. Natl. Acad. Sci. USA* 105:2830–35
37. Wykle RL, Malone B, Snyder F. 1980. Enzymatic synthesis of 1-alkyl-2-acetyl-sn-glycero-3-phosphocholine, a hypotensive and platelet-aggregating lipid. *J. Biol. Chem.* 255:10256–60
38. Ninio E, Mencia-Huerta JM, Benveniste J. 1983. Biosynthesis of platelet-activating factor (PAF-acether). V. Enhancement of acetyltransferase activity in murine peritoneal cells by calcium ionophore A23187. *Biochim. Biophys. Acta* 751:298–304
39. Snyder F, Lee TC, Blank M, Malone B, Woodard D, et al. 1985. Platelet-activating factor: alternate pathways of biosynthesis, mechanism of inactivation, and reacylation of lyso-PAF with arachidonate. *Adv. Prostaglandin Thromboxane Leukot. Res.* 15:693–96

Annu. Rev. Pharmacol. Toxicol. 2009.49:123-150.

40. Nixon AB, O'Flaherty JT, Salyer JK, Wykle RL. 1999. Acetyl-CoA:1-O-alkyl-2-lyso-sn-glycero-3-phosphocholine acetyltransferase is directly activated by p38 kinase. *J. Biol. Chem.* 274:5469–73
41. Shindou H, Ishii S, Uozumi N, Shimizu T. 2000. Roles of cytosolic phospholipase A_2 and platelet-activating factor receptor in the Ca-induced biosynthesis of PAF. *Biochem. Biophys. Res. Commun.* 271:812–17
42. Shindou H, Ishii S, Yamamoto M, Takeda K, Akira S, et al. 2005. Priming effect of lipopolysaccharide on acetyl-coenzyme A:lyso-platelet-activating factor acetyltransferase is MyD88 and TRIF independent. *J. Immunol.* 175:1177–83
43. Shindou H, Hishikawa D, Nakanishi H, Harayama T, Ishii S, et al. 2007. A single enzyme catalyzes both platelet-activating factor production and membrane biogenesis of inflammatory cells. Cloning and characterization of acetyl-CoA:LYSO-PAF acetyltransferase. *J. Biol. Chem.* 282:6532–39
44. Harayama T, Shindou H, Ogasawara R, Suwabe A, Shimizu T. 2008. Identification of a novel noninflammatory biosynthetic pathway of platelet-activating factor. *J. Biol. Chem.* 283:11097–106
45. Smith WL, Langenbach R. 2001. Why there are two cyclooxygenase isozymes. *J. Clin. Invest.* 107:1491–95
46. Thuresson ED, Lakkides KM, Rieke CJ, Sun Y, Wingerd BA, et al. 2001. Prostaglandin endoperoxide H synthase-1: the functions of cyclooxygenase active site residues in the binding, positioning, and oxygenation of arachidonic acid. *J. Biol. Chem.* 276:10347–57
47. Clevers H. 2006. Colon cancer–understanding how NSAIDs work. *N. Engl. J. Med.* 354:761–63
48. in 't Veld BA, Ruitenberg A, Hofman A, Launer LJ, van Duijn CM, et al. 2001. Nonsteroidal antiinflammatory drugs and the risk of Alzheimer's disease. *N. Engl. J. Med.* 345:1515–21
49. Bresalier RS, Sandler RS, Quan H, Bolognese JA, Oxenius B, et al. 2005. Cardiovascular events associated with rofecoxib in a colorectal adenoma chemoprevention trial. *N. Engl. J. Med.* 352:1092–102
50. Silverstein FE, Faich G, Goldstein JL, Simon LS, Pincus T, et al. 2000. Gastrointestinal toxicity with celecoxib vs nonsteroidal anti-inflammatory drugs for osteoarthritis and rheumatoid arthritis: the CLASS study: a randomized controlled trial. Celecoxib Long-term Arthritis Safety Study. *JAMA* 284:1247–55
51. Cannon CP, Curtis SP, FitzGerald GA, Krum H, Kaur A, et al. 2006. Cardiovascular outcomes with etoricoxib and diclofenac in patients with osteoarthritis and rheumatoid arthritis in the Multinational Etoricoxib and Diclofenac Arthritis Long-term (MEDAL) programme: a randomised comparison. *Lancet* 368:1771–81
52. Barry OP, Pratico D, Lawson JA, FitzGerald GA. 1997. Transcellular activation of platelets and endothelial cells by bioactive lipids in platelet microparticles. *J. Clin. Invest.* 99:2118–27
53. Smith LH, Boutaud O, Breyer M, Morrow JD, Oates JA, et al. 2002. Cyclooxygenase-2-dependent prostacyclin formation is regulated by low density lipoprotein cholesterol in vitro. *Arterioscler. Thromb. Vasc. Biol.* 22:983–88
54. Egan KM, Wang M, Fries S, Lucitt MB, Zukas AM, et al. 2005. Cyclooxygenases, thromboxane, and atherosclerosis: plaque destabilization by cyclooxygenase-2 inhibition combined with thromboxane receptor antagonism. *Circulation* 111:334–42
55. Kuhn H, Thiele BJ. 1999. The diversity of the lipoxygenase family. Many sequence data but little information on biological significance. *FEBS Lett.* 449:7–11
56. Samuelsson B, Dahlen SE, Lindgren JA, Rouzer CA, Serhan CN. 1987. Leukotrienes and lipoxins: structures, biosynthesis, and biological effects. *Science* 237:1171–76
57. Shimizu T, Radmark O, Samuelsson B. 1984. Enzyme with dual lipoxygenase activities catalyzes leukotriene A4 synthesis from arachidonic acid. *Proc. Natl. Acad. Sci. USA* 81:689–93
58. Shimizu T, Izumi T, Seyama Y, Tadokoro K, Radmark O, et al. 1986. Characterization of leukotriene A4 synthase from murine mast cells: evidence for its identity to arachidonate 5-lipoxygenase. *Proc. Natl. Acad. Sci. USA* 83:4175–79
59. Funk CD, Radmark O, Fu JY, Matsumoto T, Jornvall H, et al. 1987. Molecular cloning and amino acid sequence of leukotriene A4 hydrolase. *Proc. Natl. Acad. Sci. USA* 84:6677–81
60. Radmark O, Samuelsson B. 2005. Regulation of 5-lipoxygenase enzyme activity. *Biochem. Biophys. Res. Commun.* 338:102–10
61. Radmark O, Werz O, Steinhilber D, Samuelsson B. 2007. 5-Lipoxygenase: regulation of expression and enzyme activity. *Trends Biochem. Sci.* 32:332–41

Annu. Rev. Pharmacol. Toxicol. 2009.49:123-150.

62. Minami M, Ohno S, Kawasaki H, Radmark O, Samuelsson B, et al. 1987. Molecular cloning of a cDNA coding for human leukotriene A4 hydrolase. Complete primary structure of an enzyme involved in eicosanoid synthesis. *J. Biol. Chem.* 262:13873–76
63. Ohishi N, Minami M, Kobayashi J, Seyama Y, Hata J, et al. 1990. Immunological quantitation and immunohistochemical localization of leukotriene A4 hydrolase in guinea pig tissues. *J. Biol. Chem.* 265:7520–25
64. Haeggstrom JZ, Wetterholm A, Medina JF, Samuelsson B. 1993. Leukotriene A4 hydrolase: structural and functional properties of the active center. *J. Lipid Mediat.* 6:1–13
65. Haeggstrom JZ. 2004. Leukotriene A4 hydrolase/aminopeptidase, the gatekeeper of chemotactic leukotriene B4 biosynthesis. *J. Biol. Chem.* 279:50639–42
66. Martinez Molina D, Wetterholm A, Kohl A, McCarthy AA, Niegowski D, et al. 2007. Structural basis for synthesis of inflammatory mediators by human leukotriene C4 synthase. *Nature* 448:613–16
67. Welsch DJ, Creely DP, Hauser SD, Mathis KJ, Krivi GG, et al. 1994. Molecular cloning and expression of human leukotriene-C4 synthase. *Proc. Natl. Acad. Sci. USA* 91:9745–49
68. Lam BK, Penrose JF, Freeman GJ, Austen KF. 1994. Expression cloning of a cDNA for human leukotriene C4 synthase, an integral membrane protein conjugating reduced glutathione to leukotriene A4. *Proc. Natl. Acad. Sci. USA* 91:7663–67
69. Ago H, Kanaoka Y, Irikura D, Lam BK, Shimamura T, et al. 2007. Crystal structure of a human membrane protein involved in cysteinyl leukotriene biosynthesis. *Nature* 448:609–12
70. Austen KF. 2007. Additional functions for the cysteinyl leukotrienes recognized through studies of inflammatory processes in null strains. *Prostaglandins Other Lipid Mediat.* 83:182–87
71. Hirabayashi T, Kume K, Hirose K, Yokomizo T, Iino M, et al. 1999. Critical duration of intracellular Ca^{2+} response required for continuous translocation and activation of cytosolic phospholipase A2. *J. Biol. Chem.* 274:5163–69
72. Hanaka H, Shimizu T, Izumi T. 2002. Nuclear-localization-signal-dependent and nuclear-export-signal-dependent mechanisms determine the localization of 5-lipoxygenase. *Biochem. J.* 361:505–14
73. Hanaka H, Shimizu T, Izumi T. 2005. Stress-induced nuclear export of 5-lipoxygenase. *Biochem. Biophys. Res. Commun.* 338:111–16
74. Ferguson AD, McKeever BM, Xu S, Wisniewski D, Miller DK, et al. 2007. Crystal structure of inhibitor-bound human 5-lipoxygenase-activating protein. *Science* 317:510–12
75. Evans JF, Ferguson AD, Mosley RT, Hutchinson JH. 2008. What's all the FLAP about?: 5-lipoxygenase-activating protein inhibitors for inflammatory diseases. *Trends Pharmacol. Sci.* 29:72–78
76. Chiang N, Bermudez EA, Ridker PM, Hurwitz S, Serhan CN. 2004. Aspirin triggers antiinflammatory 15-epi-lipoxin A4 and inhibits thromboxane in a randomized human trial. *Proc. Natl. Acad. Sci. USA* 101:15178–83
77. Serhan CN. 2001. Lipoxins and aspirin-triggered 15-epi-lipoxins are endogenous components of anti-inflammation: emergence of the counterregulatory side. *Arch. Immunol. Ther. Exp. (Warsz.)* 49:177–88
78. Mukherjee PK, Marcheselli VL, Serhan CN, Bazan NG. 2004. Neuroprotectin D1: A docosahexaenoic acid-derived docosatriene protects human retinal pigment epithelial cells from oxidative stress. *Proc. Natl. Acad. Sci. USA* 101:8491–96
79. Arita M, Bianchini F, Aliberti J, Sher A, Chiang N, et al. 2005. Stereochemical assignment, antiinflammatory properties, and receptor for the omega-3 lipid mediator resolvin E1. *J. Exp. Med.* 201:713–22
80. Svensson CI, Zattoni M, Serhan CN. 2007. Lipoxins and aspirin-triggered lipoxin inhibit inflammatory pain processing. *J. Exp. Med.* 204:245–52
81. Serhan CN, Arita M, Hong S, Gotlinger K. 2004. Resolvins, docosatrienes, and neuroprotectins, novel omega-3-derived mediators, and their endogenous aspirin-triggered epimers. *Lipids* 39:1125–32
82. Serhan CN, Chiang N. 2008. Endogenous proresolving and anti-inflammatory lipid mediators: a new pharmacologic genus. *Br. J. Pharmacol.* 153(Suppl. 1):S200–15
83. Serhan CN, Gotlinger K, Hong S, Lu Y, Siegelman J, et al. 2006. Anti-inflammatory actions of neuroprotectin D1/protectin D1 and its natural stereoisomers: assignments of dihydroxy-containing docosatrienes. *J. Immunol.* 176:1848–59
84. Tokumura A, Harada K, Fukuzawa K, Tsukatani H. 1986. Involvement of lysophospholipase D in the production of lysophosphatidic acid in rat plasma. *Biochim. Biophys. Acta* 875:31–38

Annu. Rev. Pharmacol. Toxicol. 2009.49:123-150.

85. Tokumura A, Majima E, Kariya Y, Tominaga K, Kogure K, et al. 2002. Identification of human plasma lysophospholipase D, a lysophosphatidic acid-producing enzyme, as autotaxin, a multifunctional phosphodiesterase. *J. Biol. Chem.* 277:39436–42
86. Umezu-Goto M, Kishi Y, Taira A, Hama K, Dohmae N, et al. 2002. Autotaxin has lysophospholipase D activity leading to tumor cell growth and motility by lysophosphatidic acid production. *J. Cell Biol.* 158:227–33
87. Hama K, Aoki J, Fukaya M, Kishi Y, Sakai T, et al. 2004. Lysophosphatidic acid and autotaxin stimulate cell motility of neoplastic and non-neoplastic cells through LPA1. *J. Biol. Chem.* 279:17634–39
88. Nakamura K, Igarashi K, Ide K, Ohkawa R, Okubo S, et al. 2008. Validation of an autotaxin enzyme immunoassay in human serum samples and its application to hypoalbuminemia differentiation. *Clin. Chim. Acta* 388:51–58
89. Clair T, Lee HY, Liotta LA, Stracke ML. 1997. Autotaxin is an exoenzyme possessing 5′-nucleotide phosphodiesterase/ATP pyrophosphatase and ATPase activities. *J. Biol. Chem.* 272:996–1001
90. Olivera A, Kohama T, Tu Z, Milstien S, Spiegel S. 1998. Purification and characterization of rat kidney sphingosine kinase. *J. Biol. Chem.* 273:12576–83
91. Allende ML, Sasaki T, Kawai H, Olivera A, Mi Y, et al. 2004. Mice deficient in sphingosine kinase 1 are rendered lymphopenic by FTY720. *J. Biol. Chem.* 279:52487–92
92. Kihara A, Anada Y, Igarashi Y. 2006. Mouse sphingosine kinase isoforms SPHK1a and SPHK1b differ in enzymatic traits including stability, localization, modification, and oligomerization. *J. Biol. Chem.* 281:4532–39
93. Yokomizo T, Ogawa Y, Uozumi N, Kume K, Izumi T, et al. 1996. cDNA cloning, expression, and mutagenesis study of leukotriene B4 12-hydroxydehydrogenase. *J. Biol. Chem.* 271:2844–50
94. Bjarnadottir TK, Gloriam DE, Hellstrand SH, Kristiansson H, Fredriksson R, et al. 2006. Comprehensive repertoire and phylogenetic analysis of the G protein-coupled receptors in human and mouse. *Genomics* 88:263–73
95. Honda Z, Nakamura M, Miki I, Minami M, Watanabe T, et al. 1991. Cloning by functional expression of platelet-activating factor receptor from guinea-pig lung. *Nature* 349:342–46
96. Narumiya S, Sugimoto Y, Ushikubi F. 1999. Prostanoid receptors: structures, properties, and functions. *Physiol. Rev.* 79:1193–226
97. Narumiya S, FitzGerald GA. 2001. Genetic and pharmacological analysis of prostanoid receptor function. *J. Clin. Invest.* 108:25–30
98. Sugimoto Y, Narumiya S. 2007. Prostaglandin E receptors. *J. Biol. Chem.* 282:11613–17
99. Abe H, Takeshita T, Nagata K, Arita T, Endo Y, et al. 1999. Molecular cloning, chromosome mapping and characterization of the mouse CRTH2 gene, a putative member of the leukocyte chemoattractant receptor family. *Gene* 227:71–77
100. Yokomizo T, Izumi T, Chang K, Takuwa Y, Shimizu T. 1997. A G-protein-coupled receptor for leukotriene B4 that mediates chemotaxis. *Nature* 387:620–24
101. Yokomizo T, Kato K, Terawaki K, Izumi T, Shimizu T. 2000. A second leukotriene B_4 receptor, BLT2. A new therapeutic target in inflammation and immunological disorders. *J. Exp. Med.* 192:421–32
102. Iizuka Y, Yokomizo T, Terawaki K, Komine M, Tamaki K, et al. 2005. Characterization of a mouse second leukotriene B4 receptor, mBLT2: BLT2-dependent ERK activation and cell migration of primary mouse keratinocytes. *J. Biol. Chem.* 280:24816–23
103. Okuno T, Iizuka Y, Okazaki H, Yokomizo T, Taguchi R, et al. 2008. 12(S)-hydroxyheptadeca-5Z, 8E, 10E-trienoic acid is a natural ligand for leukotriene B4 receptor 2. *J. Exp. Med.* 205:759–66
104. Lynch KR, O'Neill GP, Liu Q, Im DS, Sawyer N, et al. 1999. Characterization of the human cysteinyl leukotriene CysLT1 receptor. *Nature* 399:789–93
105. Sarau HM, Ames RS, Chambers J, Ellis C, Elshourbagy N, et al. 1999. Identification, molecular cloning, expression, and characterization of a cysteinyl leukotriene receptor. *Mol. Pharmacol.* 56:657–63
106. Nothacker HP, Wang Z, Zhu Y, Reinscheid RK, Lin SH, et al. 2000. Molecular cloning and characterization of a second human cysteinyl leukotriene receptor: discovery of a subtype selective agonist. *Mol. Pharmacol.* 58:1601–8
107. Heise CE, O'Dowd BF, Figueroa DJ, Sawyer N, Nguyen T, et al. 2000. Characterization of the human cysteinyl leukotriene 2 receptor. *J. Biol. Chem.* 275:30531–36

Annu. Rev. Pharmacol. Toxicol. 2009.49:123-150.

108. Shimizu T, Honda Z, Nakamura M, Bito H, Izumi T. 1992. Platelet-activating factor receptor and signal transduction. *Biochem. Pharmacol.* 44:1001–8
109. Ishii S, Shimizu T. 2000. Platelet-activating factor (PAF) receptor and genetically engineered PAF receptor mutant mice. *Prog. Lipid Res.* 39:41–82
110. Prescott SM, Zimmerman GA, Stafforini DM, McIntyre TM. 2000. Platelet-activating factor and related lipid mediators. *Annu. Rev. Biochem.* 69:419–45
111. Lee CW, Rivera R, Gardell S, Dubin AE, Chun J. 2006. GPR92 as a new G12/13- and Gq-coupled lysophosphatidic acid receptor that increases cAMP, LPA5. *J. Biol. Chem.* 281:23589–97
112. Noguchi K, Ishii S, Shimizu T. 2003. Identification of p2y9/GPR23 as a novel G protein-coupled receptor for lysophosphatidic acid, structurally distant from the Edg family. *J. Biol. Chem.* 278:25600–6
113. Pasternack SM, von Kugelgen I, Aboud KA, Lee YA, Ruschendorf F, et al. 2008. G protein-coupled receptor P2Y5 and its ligand LPA are involved in maintenance of human hair growth. *Nat. Genet.* 40:329–34
114. Shimomura Y, Wajid M, Ishii Y, Shapiro L, Petukhova L, et al. 2008. Disruption of P2RY5, an orphan G protein-coupled receptor, underlies autosomal recessive woolly hair. *Nat. Genet.* 40:335–39
115. Hla T, Lee MJ, Ancellin N, Paik JH, Kluk MJ. 2001. Lysophospholipids–receptor revelations. *Science* 294:1875–78
116. Ishii I, Fukushima N, Ye X, Chun J. 2004. Lysophospholipid receptors: signaling and biology. *Annu. Rev. Biochem.* 73:321–54
117. Chun J, Rosen H. 2006. Lysophospholipid receptors as potential drug targets in tissue transplantation and autoimmune diseases. *Curr. Pharm. Des.* 12:161–71
118. Spiegel S, Milstien S. 2000. Functions of a new family of sphingosine-1-phosphate receptors. *Biochim. Biophys. Acta* 1484:107–16
119. Spiegel S, Milstien S. 2002. Sphingosine 1-phosphate, a key cell signaling molecule. *J. Biol. Chem.* 277:25851–54
120. Felder CC, Glass M. 1998. Cannabinoid receptors and their endogenous agonists. *Annu. Rev. Pharmacol. Toxicol.* 38:179–200
121. Maejima T, Hashimoto K, Yoshida T, Aiba A, Kano M. 2001. Presynaptic inhibition caused by retrograde signal from metabotropic glutamate to cannabinoid receptors. *Neuron* 31:463–75
122. Hashimotodani Y, Ohno-Shosaku T, Kano M. 2007. Endocannabinoids and synaptic function in the CNS. *Neuroscientist* 13:127–37
123. Sugiura T, Kondo S, Kishimoto S, Miyashita T, Nakane S, et al. 2000. Evidence that 2-arachidonoylglycerol but not N-palmitoylethanolamine or anandamide is the physiological ligand for the cannabinoid CB2 receptor. Comparison of the agonistic activities of various cannabinoid receptor ligands in HL-60 cells. *J. Biol. Chem.* 275:605–12
124. Murakami N, Yokomizo T, Okuno T, Shimizu T. 2004. G2A is a proton-sensing G-protein-coupled receptor antagonized by lysophosphatidylcholine. *J. Biol. Chem.* 279:42484–91
125. Forman BM, Tontonoz P, Chen J, Brun RP, Spiegelman BM, et al. 1995. 15-Deoxy-delta 12, 14-prostaglandin J2 is a ligand for the adipocyte determination factor PPAR gamma. *Cell* 83:803–12
126. Kliewer SA, Lenhard JM, Willson TM, Patel I, Morris DC, et al. 1995. A prostaglandin J2 metabolite binds peroxisome proliferator-activated receptor gamma and promotes adipocyte differentiation. *Cell* 83:813–19
127. McIntyre TM, Pontsler AV, Silva AR, St Hilaire A, Xu Y, et al. 2003. Identification of an intracellular receptor for lysophosphatidic acid (LPA): LPA is a transcellular PPARgamma agonist. *Proc. Natl. Acad. Sci. USA* 100:131–36
128. Clark JD, Lin LL, Kriz RW, Ramesha CS, Sultzman LA, et al. 1991. A novel arachidonic acid-selective cytosolic PLA2 contains a Ca^{2+}-dependent translocation domain with homology to PKC and GAP. *Cell* 65:1043–51
129. Uozumi N, Kume K, Nagase T, Nakatani N, Ishii S, et al. 1997. Role of cytosolic phospholipase A2 in allergic response and parturition. *Nature* 390:618–22
130. Bonventre JV, Huang Z, Taheri MR, O'Leary E, Li E, et al. 1997. Reduced fertility and postischaemic brain injury in mice deficient in cytosolic phospholipase A2. *Nature* 390:622–25

Annu. Rev. Pharmacol. Toxicol. 2009.49:123-150.

131. Shimizu T, Ohto T, Kita Y. 2006. Cytosolic phospholipase A2: biochemical properties and physiological roles. *IUBMB Life* 58:328–33
132. Song H, Lim H, Paria BC, Matsumoto H, Swift LL, et al. 2002. Cytosolic phospholipase A2alpha is crucial [correction of A2alpha deficiency is crucial] for 'on-time' embryo implantation that directs subsequent development. *Development* 129:2879–89
133. Lim H, Paria BC, Das SK, Dinchuk JE, Langenbach R, et al. 1997. Multiple female reproductive failures in cyclooxygenase 2-deficient mice. *Cell* 91:197–208
134. Ye X, Hama K, Contos JJ, Anliker B, Inoue A, et al. 2005. LPA3-mediated lysophosphatidic acid signalling in embryo implantation and spacing. *Nature* 435:104–8
135. Oshima M, Dinchuk JE, Kargman SL, Oshima H, Hancock B, et al. 1996. Suppression of intestinal polyposis in Apc delta716 knockout mice by inhibition of cyclooxygenase 2 (COX-2). *Cell* 87:803–9
136. Sugimoto Y, Segi E, Tsuboi K, Ichikawa A, Narumiya S. 1998. Female reproduction in mice lacking the prostaglandin F receptor. Roles of prostaglandin and oxytocin receptors in parturition. *Adv. Exp. Med. Biol.* 449:317–21
137. Takaku K, Sonoshita M, Sasaki N, Uozumi N, Doi Y, et al. 2000. Suppression of intestinal polyposis in Apc{delta 716} knockout mice by an additional mutation in the cytosolic phospholipase A_2 gene. *J. Biol. Chem.* 275:34013–16
138. Sonoshita M, Takaku K, Sasaki N, Sugimoto Y, Ushikubi F, et al. 2001. Acceleration of intestinal polyposis through prostaglandin receptor EP2 in Apc(Delta 716) knockout mice. *Nat. Med.* 7:1048–51
139. Hegen M, Sun L, Uozumi N, Kume K, Goad ME, et al. 2003. Cytosolic phospholipase A2alpha-deficient mice are resistant to collagen-induced arthritis. *J. Exp. Med.* 197:1297–302
140. Myers LK, Kang AH, Postlethwaite AE, Rosloniec EF, Morham SG, et al. 2000. The genetic ablation of cyclooxygenase 2 prevents the development of autoimmune arthritis. *Arthritis Rheum.* 43:2687–93
141. Shao WH, Del Prete A, Bock CB, Haribabu B. 2006. Targeted disruption of leukotriene B4 receptors BLT1 and BLT2: a critical role for BLT1 in collagen-induced arthritis in mice. *J. Immunol.* 176:6254–61
142. Kim ND, Chou RC, Seung E, Tager AM, Luster AD. 2006. A unique requirement for the leukotriene B4 receptor BLT1 for neutrophil recruitment in inflammatory arthritis. *J. Exp. Med.* 203:829–35
143. Honda T, Segi-Nishida E, Miyachi Y, Narumiya S. 2006. Prostacyclin-IP signaling and prostaglandin E2-EP2/EP4 signaling both mediate joint inflammation in mouse collagen-induced arthritis. *J. Exp. Med.* 203:325–35
144. Miyamoto K, Miyake S, Mizuno M, Oka N, Kusunoki S, et al. 2006. Selective COX-2 inhibitor celecoxib prevents experimental autoimmune encephalomyelitis through COX-2-independent pathway. *Brain* 129:1984–92
145. Ni J, Shu YY, Zhu YN, Fu YF, Tang W, et al. 2007. COX-2 inhibitors ameliorate experimental autoimmune encephalomyelitis through modulating IFN-gamma and IL-10 production by inhibiting T-bet expression. *J. Neuroimmunol.* 186:94–103
146. Kihara Y, Ishii S, Kita Y, Toda A, Shimada A, et al. 2005. Dual phase regulation of experimental allergic encephalomyelitis by platelet-activating factor. *J. Exp. Med.* 202:853–63
147. Emerson MR, LeVine SM. 2004. Experimental allergic encephalomyelitis is exacerbated in mice deficient for 12/15-lipoxygenase or 5-lipoxygenase. *Brain Res.* 1021:140–45
148. Austen KF. 2005. The mast cell and the cysteinyl leukotrienes. *Novartis Found. Symp.* 271:166–75; discussion 76–78, 98–99
149. Haribabu B, Verghese MW, Steeber DA, Sellars DD, Bock CB, et al. 2000. Targeted disruption of the leukotriene B_4 receptor in mice reveals its role in inflammation and platelet-activating factor-induced anaphylaxis. *J. Exp. Med.* 192:433–38
150. Goodarzi K, Goodarzi M, Tager AM, Luster AD, von Andrian UH. 2003. Leukotriene B4 and BLT1 control cytotoxic effector T cell recruitment to inflamed tissues. *Nat. Immunol.* 4:965–73
151. Ott VL, Cambier JC, Kappler J, Marrack P, Swanson BJ. 2003. Mast cell-dependent migration of effector CD8+ T cells through production of leukotriene B4. *Nat. Immunol.* 4:974–81
152. Tager AM, Bromley SK, Medoff BD, Islam SA, Bercury SD, et al. 2003. Leukotriene B4 receptor BLT1 mediates early effector T cell recruitment. *Nat. Immunol.* 4:982–90

Annu. Rev. Pharmacol. Toxicol. 2009.49:123-150.

153. Terawaki K, Yokomizo T, Nagase T, Toda A, Taniguchi M, et al. 2005. Absence of leukotriene B4 receptor 1 confers resistance to airway hyperresponsiveness and Th2-type immune responses. *J. Immunol.* 175:4217–25
154. Gelfand EW, Dakhama A. 2006. CD8+ T lymphocytes and leukotriene B4: novel interactions in the persistence and progression of asthma. *J. Allergy Clin. Immunol.* 117:577–82
155. Miyahara N, Miyahara S, Takeda K, Gelfand EW. 2006. Role of the LTB4/BLT1 pathway in allergen-induced airway hyperresponsiveness and inflammation. *Allergol. Int.* 55:91–97
156. Seymour ML, Rak S, Aberg D, Riise GC, Penrose JF, et al. 2001. Leukotriene and prostanoid pathway enzymes in bronchial biopsies of seasonal allergic asthmatics. *Am. J. Respir. Crit. Care Med.* 164:2051–56
157. Nagase T, Uozumi N, Ishii S, Kita Y, Yamamoto H, et al. 2002. A pivotal role of cytosolic phospholipase A_2 in bleomycin-induced pulmonary fibrosis. *Nat. Med.* 8:480–84
158. Peters-Golden M, Bailie M, Marshall T, Wilke C, Phan SH, et al. 2002. Protection from pulmonary fibrosis in leukotriene-deficient mice. *Am. J. Respir. Crit. Care Med.* 165:229–35
159. Card JW, Voltz JW, Carey MA, Bradbury JA, Degraff LM, et al. 2007. Cyclooxygenase-2 deficiency exacerbates bleomycin-induced lung dysfunction but not fibrosis. *Am. J. Respir. Cell Mol. Biol.* 37:300–8
160. Beller TC, Friend DS, Maekawa A, Lam BK, Austen KF, et al. 2004. Cysteinyl leukotriene 1 receptor controls the severity of chronic pulmonary inflammation and fibrosis. *Proc. Natl. Acad. Sci. USA* 101:3047–52
161. Beller TC, Maekawa A, Friend DS, Austen KF, Kanaoka Y. 2004. Targeted gene disruption reveals the role of the cysteinyl leukotriene 2 receptor in increased vascular permeability and in bleomycin-induced pulmonary fibrosis in mice. *J. Biol. Chem.* 279:46129–34
162. Izumo T, Kondo M, Nagai A. 2007. Cysteinyl-leukotriene 1 receptor antagonist attenuates bleomycin-induced pulmonary fibrosis in mice. *Life Sci.* 80:1882–86
163. Tager AM, LaCamera P, Shea BS, Campanella GS, Selman M, et al. 2008. The lysophosphatidic acid receptor LPA1 links pulmonary fibrosis to lung injury by mediating fibroblast recruitment and vascular leak. *Nat. Med.* 14:45–54
164. Matloubian M, Lo CG, Cinamon G, Lesneski MJ, Xu Y, et al. 2004. Lymphocyte egress from thymus and peripheral lymphoid organs is dependent on S1P receptor 1. *Nature* 427:355–60
165. Cinamon G, Matloubian M, Lesneski MJ, Xu Y, Low C, et al. 2004. Sphingosine 1-phosphate receptor 1 promotes B cell localization in the splenic marginal zone. *Nat. Immunol.* 5:713–20
166. Cinamon G, Zachariah MA, Lam OM, Foss FW Jr, Cyster JG. 2008. Follicular shuttling of marginal zone B cells facilitates antigen transport. *Nat. Immunol.* 9:54–62
167. Mandala S, Hajdu R, Bergstrom J, Quackenbush E, Xie J, et al. 2002. Alteration of lymphocyte trafficking by sphingosine-1-phosphate receptor agonists. *Science* 296:346–49
168. Schwab SR, Pereira JP, Matloubian M, Xu Y, Huang Y, et al. 2005. Lymphocyte sequestration through S1P lyase inhibition and disruption of S1P gradients. *Science* 309:1735–39
169. Liu Y, Wada R, Yamashita T, Mi Y, Deng CX, et al. 2000. Edg-1, the G protein-coupled receptor for sphingosine-1-phosphate, is essential for vascular maturation. *J. Clin. Invest.* 106:951–61
170. Tanaka M, Okudaira S, Kishi Y, Ohkawa R, Iseki S, et al. 2006. Autotaxin stabilizes blood vessels and is required for embryonic vasculature by producing lysophosphatidic acid. *J. Biol. Chem.* 281:25822–30
171. van Meeteren LA, Ruurs P, Stortelers C, Bouwman P, van Rooijen MA, et al. 2006. Autotaxin, a secreted lysophospholipase D, is essential for blood vessel formation during development. *Mol. Cell Biol.* 26:5015–22
172. Kita Y, Takahashi T, Uozumi N, Nallan L, Gelb MH, et al. 2005. Pathway-oriented profiling of lipid mediators in macrophages. *Biochem. Biophys. Res. Commun.* 330:898–906
173. Kita Y, Takahashi T, Uozumi N, Shimizu T. 2005. A multiplex quantitation method for eicosanoids and platelet-activating factor using column-switching reversed-phase liquid chromatography-tandem mass spectrometry. *Anal. Biochem.* 342:134–43
174. Yoshikawa S, Miyahara T, Reynolds SD, Stripp BR, Anghelescu M, et al. 2005. Clara cell secretory protein and phospholipase A2 activity modulate acute ventilator-induced lung injury in mice. *J. Appl. Physiol.* 98:1264–71
175. Downey P, Sapirstein A, O'Leary E, Sun TX, Brown D, et al. 2001. Renal concentrating defect in mice lacking group IV cytosolic phospholipase A_2. *Am. J. Physiol. Renal Physiol.* 280:F607–F618

Annu. Rev. Pharmacol. Toxicol. 2009.49:123-150.

176. Rosenberger TA, Villacreses NE, Contreras MA, Bonventre JV, Rapoport SI. 2003. Brain lipid metabolism in the cPLA2 knockout mouse. *J. Lipid. Res.* 44:109–17
177. Tabuchi S, Uozumi N, Ishii S, Shimizu Y, Watanabe T, et al. 2003. Mice deficient in cytosolic phospholipase A2 are less susceptible to cerebral ischemia/reperfusion injury. *Acta Neurochir. Suppl.* 86:169–72
178. Klivenyi P, Beal MF, Ferrante RJ, Andreassen OA, Wermer M, et al. 1998. Mice deficient in group IV cytosolic phospholipase A2 are resistant to MPTP neurotoxicity. *J. Neurochem.* 71:2634–37
179. Sapirstein A, Bonventre JV. 2000. Phospholipases A2 in ischemic and toxic brain injury. *Neurochem. Res.* 25:745–53
180. Marusic S, Leach MW, Pelker JW, Azoitei ML, Uozumi N, et al. 2005. Cytosolic phospholipase A2 alpha-deficient mice are resistant to experimental autoimmune encephalomyelitis. *J. Exp. Med.* 202:841–51
181. Nakatani N, Uozumi N, Kume K, Murakami M, Kudo I, et al. 2000. Role of cytosolic phospholipase A2 in the production of lipid mediators and histamine release in mouse bone-marrow-derived mast cells. *Biochem. J.* 352(Pt 2):311–17
182. Fujishima H, Sanchez Mejia RO, Bingham CO 3rd, Lam BK, Sapirstein A, et al. 1999. Cytosolic phospholipase A2 is essential for both the immediate and the delayed phases of eicosanoid generation in mouse bone marrow-derived mast cells. *Proc. Natl. Acad. Sci. USA* 96:4803–7
183. Wong DA, Kita Y, Uozumi N, Shimizu T. 2002. Discrete role for cytosolic phospholipase $A_2\alpha$ in platelets: studies using single and double mutant mice of cytosolic and group IIA secretory phospholipase A_2. *J. Exp. Med.* 196:349–57
184. Nagase T, Uozumi N, Ishii S, Kume K, Izumi T, et al. 2000. Acute lung injury by sepsis and acid aspiration: a key role for cytosolic phospholipase A2. *Nat. Immunol.* 1:42–46
185. Nagase T, Uozumi N, Aoki-Nagase T, Terawaki K, Ishii S, et al. 2003. A potent inhibitor of cytosolic phospholipase A_2, arachidonyl trifluoromethyl ketone, attenuates LPS-induced lung injury in mice. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 284:L720–726
186. Miyaura C, Inada M, Matsumoto C, Ohshiba T, Uozumi N, et al. 2003. An essential role of cytosolic phospholipase A2α in prostaglandin E2-mediated bone resorption associated with inflammation. *J. Exp. Med.* 197:1303–10
187. Hong KH, Bonventre JC, O'Leary E, Bonventre JV, Lander ES. 2001. Deletion of cytosolic phospholipase A_2 suppresses Apc(Min)-induced tumorigenesis. *Proc. Natl. Acad. Sci. USA* 98:3935–39
188. Das A, Asatryan L, Reddy MA, Wass CA, Stins MF, et al. 2001. Differential role of cytosolic phospholipase A2 in the invasion of brain microvascular endothelial cells by *Escherichia coli* and *Listeria monocytogenes*. *J. Infect. Dis.* 184:732–37
189. Sapirstein A. 2003. Implications of the cytosolic phospholipase A2 knockout mouse on perioperative therapeutics. *Int. Anesthesiol. Clin.* 41:79–90
190. Ishikawa S, Uozumi N, Shiibashi T, Izumi T, Fukayama M, et al. 2004. Short report: Lethal malaria in cytosolic phospholipase A_2- and phospholipase A_2IIA-deficient mice. *Am. J. Trop. Med. Hyg.* 70:645–50
191. Oikawa Y, Yamato E, Tashiro F, Yamamoto M, Uozumi N, et al. 2005. Protective role for cytosolic phospholipase A2α in autoimmune diabetes of mice. *FEBS Lett.* 579:3975–78
192. Shindou H, Shimizu T. 2008. Acyl-CoA:lysophospholipid acyltransferases (mini-review). *J. Biol. Chem.* 283: In press
193. Adler DH, Cogan JD, Phillips JA 3rd, Schnetz-Boutaud N, Milne GL, et al. 2008. Inherited human cPLA2α deficiency is associated with impaired eicosanoid biosynthesis, small intestinal ulceration and platelet dysfunction. *J. Clin. Invest.* 118:2121–31

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Errata

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