Cytosolic phospholipase A<sub>2</sub>: Physiological function and role in disease

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Abbreviations: COX, cyclooxygenase; cPLA<sub>2</sub>, cytosolic PLA<sub>2</sub>; DSS, dextran sodium sulfate; EAE, experimental autoimmune encephalomyelitis; ER, endoplasmic reticulum; FLAP, 5-LO activating protein; GI, gastrointestinal; iPLA<sub>2</sub>, calcium-independent PLA<sub>2</sub>; 5-LO, 5-lipoxygenase; LTB<sub>4</sub>, leukotriene B<sub>4</sub>; PG<sub>E</sub><sub>2</sub>, prostaglandin E<sub>2</sub>; MAPK, mitogen-activated protein kinases; mPGES-1, microsomal PGE synthase-1; PLA<sub>2</sub>, phospholipase A<sub>2</sub>; sPLA<sub>2</sub>, secreted PLA<sub>2</sub>; TXA<sub>2</sub>, thromboxane A<sub>2</sub>. 
Abstract

The Group IV phospholipase A₂ family is comprised of six intracellular enzymes (GIVA, B, C, D, E, F) commonly referred to as cytosolic phospholipase A₂ (cPLA₂) α, β, γ, δ, ε and ζ. They contain a Ser-Asp catalytic dyad and all except cPLA₂γ have a C2 domain, but differences in their catalytic activities and subcellular localization suggest unique regulation and function. With the exception of cPLA₂α, the focus of this review, little is known about the in vivo function of Group IV enzymes. cPLA₂α catalyzes the hydrolysis of phospholipids to arachidonic acid and lysophospholipids that are precursors of numerous bioactive lipids. The regulation of cPLA₂α is complex involving transcriptional and post-translational processes particularly increases in calcium and phosphorylation. cPLA₂α is a highly conserved, widely expressed enzyme that promotes lipid mediator production in human and rodent cells from a variety of tissues. The diverse bioactive lipids produced as a result of cPLA₂α activation regulate normal physiological processes and disease pathogenesis in many organ systems as shown using cPLA₂α knockout mice. However, humans recently identified with cPLA₂α deficiency exhibit more pronounced effects on health than observed in mice lacking cPLA₂α indicating that much remains to be learned about this interesting enzyme.

Key words: Arachidonic acid, calcium, Golgi apparatus, inflammation, cyclooxygenase, leukotrienes, lipoxygenase, phospholipases/A2, prostaglandins, protein kinases/MAP kinase.
OVERVIEW OF THE MAMMALIAN PLA₂ FAMILY

The mammalian PLA₂ family is comprised of six types of diverse enzymes: GIV PLA₂ (cytosolic PLA₂, cPLA₂), GVI PLA₂ (calcium-independent PLA₂, iPLA₂), GVII and GVIII PLA₂ (platelet-activating factor-acetylhydrolases, PAF-AH), GXV PLA₂ (lysosomal PLA₂, LPLA₂), GXVI PLA₂ (adipose PLA₂, AdPLA) and several groups of secreted PLA₂ (sPLA₂). Several reviews have described in detail the structure, activities and known functions of the different groups of PLA₂ enzymes many of which contain several subtypes (1-8). Most of these enzymes have no structural similarity, and have different catalytic mechanisms and regulation. cPLA₂s are most similar to iPLA₂s (also classified as patatin-like enzymes), since they utilize an active site Ser-Asp dyad for catalysis unlike the catalytic mechanism of other PLA₂s that involves a His residue (2-4). It has been shown that the active site of the plant lipid acylhydrolase patatin contains a Ser-Asp dyad with structural similarity to cPLA₂α and has homology with the iPLA₂ family (4,9). Although they have only one active site, cPLA₂s and iPLA₂s exhibit multiple enzymatic activities including PLAr, PLAr, lysophospholipase and transacylase activities, and some iPLA₂s are lipases (2,10). Many types of mammalian PLA₂ enzymes can release arachidonic acid for eicosanoid production (2). An intriguing observation is that different types of PLA₂s can cooperate in cells (sPLA₂s with either cPLA₂α or iPLA₂β) in promoting arachidonic acid release although the mechanism is not understood (1). Recent studies have shown specialized roles of several sPLA₂ enzymes in mediating production of eicosanoids that regulate mast cell maturation, asthma, host defense and metabolic disorders as detailed in a recent review (11). However, cPLA₂α is the only PLA₂ that exhibits preference for hydrolysis of arachidonic acid from phospholipid substrates that occurs in cells stimulated with diverse agonists (4). Most cells contain several types of PLA₂ enzymes and studies have suggested that they have distinct functions. For example, a comparison of the function of cPLA₂α and iPLA₂β in mast cells and macrophages found that cPLA₂α, but not iPLA₂β, selectively hydrolyzes arachidonic acid containing phospholipids in response to cell stimulation (12,13).
GROUP IV PLA₂ FAMILY

The six members of the cPLA₂ family (cPLA₂α, β, γ, δ, ε and ζ) share only about 30% homology, and have differences in enzymatic properties, tissue expression and subcellular localization suggesting that they are not redundant (4,14). Analysis of the interfacial kinetic and binding properties of the cPLA₂ family showed that they exhibit very different relative lysophospholipase, PLA₂ and PLA₁ activities, inhibitor sensitivities, calcium dependence and activation by anionic phospholipids highlighting potential differences in regulation and function (10). With the exception of the established role of cPLA₂α in initiating production of lipid mediators, little is known about the in vivo function of other cPLA₂ isoforms. However, there is evidence emerging from studies using cultured cells that members of the cPLA₂ family, including cPLA₂α, play a role in regulating membrane trafficking that does not involve production of oxygenated metabolites of arachidonic acid. PLA₂ (and PLA₁) enzymes are implicated in regulating intracellular membrane trafficking by participating in the formation of transport carriers from donor membrane (15). Membrane budding requires alterations in membrane curvature that can occur by formation of lysophospholipids. Four cytoplasmic PLAs including cPLA₂α, PAF-AH lb, iPLA₂β and iPLA₁γ are implicated in the formation of tubules from the endoplasmic reticulum-Golgi intermediate compartment and the Golgi apparatus, maintenance of Golgi structure and transport through the Golgi. The details of the role of these enzymes have recently been reviewed (15,16). cPLA₂α regulates intra-Golgi transport by mediating the formation of tubules that connect the Golgi stacks (17). The localization of cPLA₂α at the Golgi in endothelial cells has also been shown to regulate the transport of junction proteins (VE-cadherin, occludin, claudin-5) from the Golgi to cell-cell junctions (18). In these studies it was shown that the enzymatic activity of cPLA₂α is required for regulating transport but oxygenated metabolites of arachidonic acid are not involved. Considering that these are fundamental processes important for cell function it is surprising that the cPLA₂α knockout mouse does not exhibit any major phenotype. Golgi transport was found to be normal in cells deficient in cPLA₂α but results of an siRNA
screen suggested that GVIII PLA₂ (PAF-AH) compensates for the loss of cPLA₂α in regulating Golgi transport (17). Other Group IV PLA₂s have been shown to localize to endocytic vesicles suggesting a possible role in regulating transport (14,19,20). This is supported by a recent study showing that cPLA₂ε regulates formation of tubules involved in clathrin-independent endocytic trafficking (21). Therefore in studies designed to understand the role of cPLA₂α in regulating cellular processes it is important to consider that it has a functional role independent of eicosanoid production.

GROUP IVA cPLA₂ (cPLA₂α)

It has been over 25 years since a soluble, calcium-regulated, high molecular weight PLA₂ that exhibited arachidonic acid selectivity was identified in cells and purified (22-29). Cloning of PLA₂α revealed the C2 domain for calcium regulation (30,31). The identification of the active site residues, characterization of stimulus-dependent phosphorylation by mitogen-activated protein kinases (MAPK) and structural elucidation then followed (32-36). Over the years we have learned a great deal about the regulation of cPLA₂α and its role in initiating the production bioactive lipid mediators. cPLA₂α activation results in the production of diverse lipid mediators derived from its products arachidonic acid and lysophospholipids. Leukotrienes are potent pro-inflammatory mediators produced by cells involved in the inflammatory response particularly neutrophils, macrophages and mast cells (37). Prostaglandins are made by many cell types and have very diverse functions. In addition to their role in regulating homeostatic processes, they can promote all the cardinal signs of acute inflammation and participate in the maintenance of chronic inflammation (38,39). However they facilitate the resolution of inflammation by altering the balance of pro-inflammatory and anti-inflammatory cytokines, and by enhancing clearance of apoptotic neutrophils (38,40-43). cPLA₂α is also implicated in the production of the pro-resolving lipid mediators (lipoxins) and bioactive lysophospholipids (or derived from lysophospholipids) such as platelet-activating factor and lyso-phosphatidylinositol (44-46). The generation of mice deficient in
cPLA₂α has revealed its role in regulating normal physiological processes and disease pathogenesis in rodents (47,48). More recently the identification of humans with cPLA₂α deficiency has emphasized its important role in human health (49-51). This review will provide a brief overview of the regulation of cPLA₂α and primarily focus on reviewing its function in specific organs of mice and humans.

REGULATION OF cPLA₂α

cPLA₂α is widely expressed in cells throughout all tissues in mice and humans. It is a highly conserved enzyme with mouse and human sharing 95% amino acid identity suggesting similarities in regulation and function (31). In addition to PLA₂ activity, cPLA₂α catalyzes other enzymatic reactions through the Ser-Asp catalytic dyad including PLA₁, lysophospholipase and transacylase activity although the physiological relevance of these activities in vivo is unknown (2,4,32,35). The intense focus on cPLA₂α stems from its preferential hydrolysis of sn-2 arachidonic acid, and its well-established role in initiating the release of arachidonic acid for the production of lipid mediators (52). cPLA₂α KO mice have provided a source for cells, including (but not limited to) mast cells, neutrophils, macrophages, platelets, endothelial cells and lung fibroblasts, to establish a role for cPLA₂α in mediating arachidonic acid release and production of lipid mediators (47,53-59). cPLA₂α is regulated at the transcriptional level and by post-translational mechanisms. It is basally expressed in many cells due in part to TFIID binding to the TATA-less promoter (60-62). cPLA₂α expression is also induced transcriptionally through a number of signaling pathways involving Ras and MAPKs, and transcriptional activators NF-κB, Krüppel-like factor, hypoxia-inducible factor, Sp1 and c-Jun have been described (63-70). Pro-inflammatory cytokines induce expression of human cPLA₂α mRNA that is blocked by glucocorticoids (71). However, glucocorticoids are not universally suppressive since they paradoxically enhance cPLA₂α expression in human amnion fibroblasts (see Reproduction section below) (72). Recently the first cytokine-dependent enhancer element has been identified by DNase I HS site analysis of the human cPLA₂α gene (73). This
IL-1β-responsive distal regulatory element contains an AP-1 site, which regulates both basal and IL-1β induced expression of cPLA₂α. Binding of c-Jun to this site acts as a repressor in the basal state and an activator in response to IL-1β, and an important role for C/EBPβ as a transcriptional activator of cPLA₂α was demonstrated.

cPLA₂α is rapidly activated in cells by post-translational processes including increases in intracellular calcium and phosphorylation by MAPK (Fig. 1). These signaling pathways are activated in cells through engagement of many types of receptors indicating that cPLA₂α activation and arachidonic acid release occur commonly in response to cell stimulation. There have been a number of reviews that detail the regulation of cPLA₂α by posttranslational processes (2-4,74). In brief, increases in intracellular calcium promote the translocation of cPLA₂α from the cytosol to intracellular membrane (75-78). Calcium binds to the N-terminal C2 domain of cPLA₂α that increases the hydrophobicity of the calcium binding loops (CBL), which penetrate the membrane bilayer (79-83) (Fig. 2). Differences in phospholipid binding specificity of the C2 domains of protein kinase C (anionic phospholipids) and cPLA₂α (phosphatidylcholine) play an important role in determining their distinct subcellular targeting to the plasma membrane and intracellular membranes, respectively (84-86). The catalytic domain is then positioned on the membrane by calcium-independent mechanisms in part through a tryptophan residue (W464) that stabilize membrane binding (87-90). cPLA₂α preferentially translocates to the Golgi apparatus and, at higher intracellular calcium concentrations, to the endoplasmic reticulum (ER) and nuclear envelope (76-78,91,92) (Fig. 1). Binding of cPLA₂α to the Golgi may in part involve interaction of the C2 domain with sphingolipids including ceramide-1-P and lactosylceramide (93-96). Translocation of cPLA₂α to membrane is necessary but not sufficient for cPLA₂α to release arachidonic acid (92). The catalytic activity of cPLA₂α is enhanced by phosphorylation of S505 by the MAPKs, extracellular regulated protein kinases and p38 (33,34,97,98). More recent studies suggest that cPLA₂α is also activated by c-jun N-terminal kinases (99,100). Activation of cPLA₂α in vascular smooth muscle cells by calcium/calmodulin-dependent kinase II that phosphorylates S515 has been described (101).
Phosphorylation of cPLA$_2$α on S727 by MAPK-interacting kinase does not enhance catalytic activity per se but blocks its binding to an inhibitory complex in the cytosol composed of p11(S100A10)/Annexin A2 (74,102-105) (Fig. 1). In addition to phosphorylation, the activity of cPLA$_2$α is regulated by basic residues in the catalytic domain that are the site for activation by polyphosphoinositides (89,106-108). The basic residues are required for the ability of cPLA$_2$α to release arachidonic acid in cells but are not required for translocation of cPLA$_2$α to the Golgi (92). Collectively the results show that the ability of cPLA$_2$α to release arachidonic acid in cells involves calcium-dependent membrane binding and optimization of catalytic activity by phosphorylation and interaction of basic residues in the catalytic domain with anionic components, perhaps polyphosphoinositides, in the membrane (92).

The translocation of cPLA$_2$α to intracellular membranes raises the interesting question of how cPLA$_2$α-derived arachidonic acid couples to the downstream enzymes 5-lipoxygenase (5-LO) and cyclooxygenases (COX)-1 and -2 (Fig. 1). There are general similarities in the regulation of cPLA$_2$α and 5-LO involving both calcium and phosphorylation (109). 5-LO, a soluble enzyme (cytoplasmic or intra-nuclear) in resting cells, translocates to the nuclear envelope in response to calcium, which binds to an N-terminal C2-like domain (110-115). Leukotriene production requires interaction of 5-LO with 5-LO activating protein (FLAP), an integral nuclear envelope protein that functions as an arachidonic acid binding protein (116). It has recently been shown that leukotriene synthesis involves activation-dependent formation of supramolecular complexes on the nuclear envelope composed of FLAP with 5-LO, and FLAP with leukotriene C$_4$ synthase (117,118). In addition cPLA$_2$α-derived arachidonic acid was shown to affect the conformation of FLAP and enhance its ability to recruit 5-LO for complex formation (119). It is not known if cPLA$_2$α physically associates with these complexes or if arachidonic acid released in their vicinity diffuses through the membrane to initiate leukotriene production.

COX-1 and -2 are also found in internal membranes but unlike cPLA$_2$α and 5-LO they are integral membrane proteins primarily localized in the ER (120). However, it was recently shown that COX-2 localizes to the Golgi in cancer cell lines detected using a COX-2 specific fluorescent probe.
Another study investigating the post-translational processing and degradation of COX-2, found catalytically active COX-2 (but not COX-1) localized to Golgi as a result of anterograde trafficking from the ER, a step required for post-translational modification prior to COX-2 degradation through the ER-associated degradation pathway (122). It is interesting that microsomal PGE synthase-1 (mPGES-1) was also found at the Golgi suggesting that this site has a system for prostaglandin E₂ (PGE₂) production involving cPLA₂α/COX-2/mPGES-1 (122). Another report showed that COX-1, but not COX-2, is constitutively found at the Golgi in airway epithelial cells where it co-localizes with cPLA₂α (123). The localization of cPLA₂α, COX, mPGES-1 and leukotriene synthetic enzymes at intracellular membranes including the ER, nuclear envelope and Golgi may efficiently coordinate the production of certain eicosanoids. However, several downstream enzymes involved in prostaglandin and leukotriene production are cytosolic and not apparently subject to stimulus-dependent translocation although the sub-cellular organization of enzymes involved in eicosanoid production is only beginning to be elucidated.

ROLE OF cPLA₂α IN NORMAL PHYSIOLOGICAL PROCESSES AND DISEASE

The first characterization of mice with cPLA₂α gene disruption published in 1997 was followed by a large body of work that provides a comprehensive view of cPLA₂α function in normal processes and disease pathogenesis in mice (47,48). The cPLA₂α KO mouse develops normally and lives a normal life span with no overt adverse health effects. Considering the evolutionary conservation of cPLA₂α and role in eicosanoid production similarities in the regulation and function of cPLA₂α in rodents and humans is expected. However, as discussed in this review there are deleterious effects on the health of humans with cPLA₂α deficiency suggesting differences in the role of cPLA₂α and eicosanoids in mouse models and humans. This could be due to a number of factors including differences in expression of other types of PLA₂ enzymes that could compensate, and differences in the tissue specific expression of receptors for lipid mediators that could influence their function. In many cases the mechanisms responsible for
observed phenotypes in human and mouse cPLA$_2$α deficiencies is not well understood. This is understandable considering that cPLA$_2$α is the first regulatory enzyme that releases the lipid mediators arachidonic acid and lysophospholipids, which are precursors for an enormous number of diverse bioactive lipid metabolites that can have opposing effects.

*Gastrointestinal tract*

The consequences of inherited cPLA$_2$α deficiency in several human patients have now been described (49-51). In all of these patients a common clinical finding are abnormalities in the gastrointestinal (GI) tract. However there are differences in the clinical presentations and severity of disease. In one subject two rare heterozygous single base pair mutations in PLA2G4A were found; one mutation (S111P) occurred in the C2 domain of cPLA$_2$α and the other allele had a mutation in the catalytic domain (R485H) (Fig. 2) (49). The expression of cPLA$_2$α protein and PLA$_2$ activity in platelet lysates was reduced compared to normals. The patient exhibited a global reduction in eicosanoid production with reduced urinary metabolites of thromboxane A$_2$ (TXA$_2$), prostaglandin I$_2$, prostaglandin D$_2$, and PGE$_2$, and undetectable leukotriene E$_4$ in urine. The production of LTB$_4$ from leukocytes in A23187-stimulated blood was reduced by 97%. The patient had occult GI blood loss and anemia in childhood with more acute GI bleeding in his fourth decade with recurrent ulcerations in the ileum and jejunum of the small intestine. The beneficial effect of misoprostol suggests that cPLA$_2$α-mediated PGE$_2$ production is important for function of the small intestine. Unlike the effect of non-steroid anti-inflammatory drug toxicity, the patient did not exhibit gastroduodenal ulcerations, and the colon was normal. The results support a role for cPLA$_2$α in providing arachidonic acid for a large proportion of eicosanoid production in humans. Other PLA$_2$s may contribute to the residual eicosanoids produced, or the patient may retain a low level of cPLA$_2$α activity. To investigate the effect of the mutations on cPLA$_2$α function in more detail, we compared the catalytic activity of wild type and mutant forms of purified cPLA$_2$α *in vitro*, and compared their cellular function by expression in cells lacking endogenous
cPLA₂α (124). cPLA₂α with the S111P mutation showed slightly reduced catalytic activity and interfacial binding *in vitro* at saturating calcium. When expressed in cells there was less translocation of cPLA₂α with the S111P to Golgi in response to serum stimulation and reduced arachidonic acid release. In contrast, cPLA₂α with the R485H mutation translocated to Golgi in serum-stimulated cells but failed to release arachidonic acid, and was inactive *in vitro*. Consequently, the mutations in the C2 and catalytic domains of cPLA₂α compromise function but do not result in a complete loss of protein or activity.

Another patient, and his twin sister, with inherited cPLA₂α deficiency also exhibited GI abnormalities from early childhood characterized by duodenal ulcers that were corrected by misoprostol treatment (51). The incidence of small intestinal ulcers was not evaluated. Homozygous base pair mutations (D575H) in the cPLA₂α catalytic domain were identified (Fig. 2). cPLA₂α protein was not detected in lysates from patients with the homozygous mutations. Unlike the patients described above the mutations resulted in global diathesis (see Hemostasis section below).

The most severe clinical manifestation of inherited cPLA₂α deficiency occurred in siblings diagnosed with cryptogenic multifocal ulcerating stenosing enteritis due to homozygous deletion mutations in *PLA2G4A* (50). The affected male had severe peptic ulcers and bleeding beginning at an early age (4 yr) then over the next several years developed more severe disease involving the ileum, stomach, esophagus, duodenum, biliary system and liver, although the colon remained normal. He developed type 2 diabetes, peripheral neuropathy and osteoporosis. His sister exhibited similar severe GI disease beginning at age 2 yr that worsened over time. She experienced infections with *Campylobacter enteritis* and *Salmonella enteriditis*, and developed Candida septicemia, staphylococcus lung infection and acute respiratory distress syndrome. Other extra-intestinal disease included acute renal failure, endometriosis, left ventricular concentric hypertrophy, fibrotic bladder with stones and infertility. The siblings contained homozygous 4 bp deletions in the *PLA2G4A* gene predicted to result in a frameshift and premature stop codon with the loss of 43 amino acids at the C-terminus of cPLA₂α (from V707) (Fig. 2). cPLA₂α was undetectable by immunohistochemical analysis of small bowel biopsy and could not be
detected in peripheral blood mononuclear cells by western blot. Many factors could contribute to the individual phenotypic differences in disease severity associated with inherited cPLA₂α deficiency but the studies show a critical role for cPLA₂α and eicosanoids in maintaining the integrity of the gut particularly the small intestine.

The small intestine of cPLA₂α knockout mouse has numerous small ulcers but there appears to be little affect of the lesions on the health of mice (125). However, there is a role for cPLA₂α in protecting the GI tract of mice from chemical toxicity induced by COX inhibitors and dextran sodium sulfate (DSS) (126,127). Injury induced by COX inhibitors in cPLA₂α KO mice did not occur in the colon but was extensive in the small intestine, the site of greatest drug absorption (126). Differences in toxicity of cPLA₂α WT and KO mice did not correlate with PGE₂ levels but may have been due to greater mitochondrial toxicity in KO mice. In the DSS model, there was extensive damage in the colons of cPLA₂α KO mice that correlated with lower levels of prostaglandins in colon tissue compared to DSS treated WT mice (127). Mice deficient in mPGES-1 also exhibited greater DSS toxicity in the colon suggesting an important protective role for PGE₂. In contrast to cPLA₂α KO mice, which recovered from DSS toxicity to a similar extent as WT mice, the recovery of mPGES-1 KO mice was compromised. The studies in mice and humans show an important role for cPLA₂α in protecting the GI tract. However, levels of eicosanoids in the GI tract of cPLA₂α KO mice are only partially reduced suggesting a role for other PLA₂s. This may contribute to the less severe health effects of cPLA₂α deficiency in mice compared to humans. There has not been a systematic comparison of the relative expression levels of the various types of PLA₂ enzymes expressed in different tissues of mice and humans to speculate on the PLA₂s that may account for eicosanoid production in the GI tract of mice.

Although results support a role for prostaglandins in protecting the integrity of the intestine, COX-2 expression and PGE₂-mediated inflammation are associated with development of human colorectal cancer consistent with epidemiological evidence that non-steroid anti-inflammatory drug use is protective (128-130). However, cPLA₂α protects against colon cancer in mice induced by the colon
carcinogen, azoxymethane, which induces increased tumor multiplicity in heterozygous and KO mice compared to WT mice (131). The increased colon tumorigenesis in cPLA$_2$α mutant mice occurs despite a decrease in PGE$_2$ production. In contrast, genetic ablation of cPLA$_2$α protects against cancer development in mouse models of adenomatous polyposis (Apc$^{716}$ knockout mice and Apc$^{Min}$ mice) (125,132). Apc$^{716}$ knockout mice develop small intestinal polyps that express high levels of COX-2 and cPLA$_2$α mRNA compared to normal small intestine (125,133). Genetic deletion of cPLA$_2$α in Apc$^{716}$ knockout mice leads to a reduction in size of the polyps but has no effect on the number of polyps in contrast to COX-2 knockout mice (125,133). In contrast, knocking out cPLA$_2$α in Apc$^{Min}$ mice results in a reduction in size and a dramatic decrease in the number of small intestinal polyps, although there is no effect on tumor number in the large intestine (132). The results highlight differences in the role of cPLA$_2$α in promoting tumorigenesis in the small and large intestine. Several lines of evidence suggest that tumorigenesis in the small intestine involves cPLA$_2$α/COX-2/PGE$_2$ pathway rather than a role for arachidonic acid itself in promoting apoptosis (132,134). Understanding how cPLA$_2$α regulates tumorigenesis is complex since it is upstream of many lipid mediators including arachidonic acid, diverse prostaglandins, leukotrienes and lysophospholipids. In addition the large repertoire of receptors for lipid mediators are differentially expressed in tissues, and promote signaling pathways with distinct effects on cell function. This adds another level of complexity in understanding the role of cPLA$_2$α-derived bioactive lipids in organ-specific cancer development.

Liver

Studies have shown that prostaglandins promote increased triglyceride storage in adipocytes and hepatocytes, prompting investigations on the role of cPLA$_2$α in regulating lipid storage and liver damage (135-140). It is of interest that even under normal dietary conditions, cPLA$_2$α regulates lipid storage in adipose tissue and liver (138). cPLA$_2$α KO mice fed a normal diet have reduced adipose tissue mass than WT mice, and lower triglyceride deposition in liver, that correlates with lower serum levels of PGE$_2$. An
extension of this work showed that cPLA$_2$$\alpha$ KO mice are protected from fatty liver damage induced by a high-fat diet (139). Mice with cPLA$_2$$\alpha$ deficiency have lower serum PGE$_2$ levels, which may afford protection since the administration of PGE$_2$ enhances hepatic triacylglycerol content whereas inhibition of COX-2 prevents fat deposition in rodents (137,141). Obesity is associated with development of nonalcoholic fatty liver disease (NAFLD) with pathological manifestations including nonalcoholic steatohepatitis (NASH), fibrosis or cirrhosis. Recently it was shown that cPLA$_2$$\alpha$ knockout mice fed a high fat diet are protected from hepatic liver deposition and development of hepatic fibrosis (140). Similar results are observed in mice subject to repeated administration of carbon tetrachloride that induces fibrosis without lipid deposition. The mechanism is thought to involve a role for cPLA$_2$$\alpha$ in production of monocyte chemoattractant protein-1 and infiltration of macrophages in these models. Previous results have shown that leukotrienes and prostaglandins regulate induction of monocyte chemoattractant protein-1, a chemotactic factor implicated in development of hepatic fibrosis (142-145). Eicosanoids secreted by cells influence gene expression through paracrine or autocrine interaction with their specific G-protein coupled receptors (146,147). For example in an infectious disease model using macrophages from wild type and cPLA$_2$$\alpha$ knockout mice, we found that cPLA$_2$$\alpha$ activation promotes an autocrine loop involving production of prostaglandins that engage prostanoid receptors, increase cAMP and globally affect expression of genes involved in host defense and inflammation (43).

Hemostasis

Considering the importance of eicosanoids in regulating hemostasis, the role of cPLA$_2$$\alpha$ in TXA$_2$ production, platelet activation and hemostasis has been investigated. Platelets from cPLA$_2$$\alpha$ KO mice stimulated with collagen produce much less TXA$_2$, and bleeding time is increased, compared to WT mice (59). However, the low level of TXA$_2$ produced by other phospholipases in collagen-stimulated cPLA$_2$$\alpha$ KO platelets is sufficient for platelet aggregation. In vivo, cPLA$_2$$\alpha$ KO mice have increased bleeding time.
and are protected from thromboembolism that correlates with reduced TXA2 levels and vasoconstriction. cPLA2α appears to play a discrete role in the complex regulation of platelet activation in mice.

Studies of humans with inherited cPLA2α deficiency have shown an important role of cPLA2α in regulating platelet function and hemostasis. In the patient with two heterologous base pair mutations in the coding regions of PLA2G4A, the PLA2 activity in platelet lysates was reduced and levels of TXB2 and 12-hydroxyeicosatetraenoic acid in serum during blood clotting (platelet-derived) were much lower than normal levels (49). Platelet aggregation and ATP release were compromised indicating impaired function. In patients with homozygous deletion mutants of cPLA2α associated with cryptogenic multifocal ulcerating stenosing enteritis (50), similar abnormalities of platelet function were observed including defects in collagen-induced aggregation and reduced levels of TXB2 production. The results indicate that cPLA2α plays a dominant role in providing arachidonic acid for the COX-1 and 12-lipoxygenase products in platelets, although the patients described above did not exhibit generalized clotting deficiency. In contrast the siblings with inherited cPLA2α deficiency due to homozygous base pair mutations had lifelong bleeding diathesis characterized by spontaneous muco-cutaneous bleeding in addition to GI bleeding. The bleeding defect correlated with defective platelet function and extremely low levels of serum TXB2 (51).

Reproduction

Prostaglandins produced through the COX-1 and COX-2 pathways play an important role in regulating female reproduction, therefore it is not surprising that cPLA2α KO female mice exhibit decreased fertility, small litter size and delayed onset of labor (47,48,148-150). In cPLA2α KO mice, blastocyst attachment is delayed due to a loss of maternal cPLA2α but decidualization is unaffected (151). Implantation beyond the normal "window" results in defective embryo development, abnormal uterine spacing of the embryos and resorption contributing to the small litter size, and defects in parturition (151). A later study emphasized the importance of cPLA2α function early in pregnancy since the defect in
parturition in cPLA$_2$$\alpha$ KO mice could be attributed to the deferred embryo implantation (152). cPLA$_2$$\alpha$ KO mice showed less pronounced defects in ovulation and fertilization compared to COX-2 KO mice (149,151,152). A more detailed study showed that cPLA$_2$$\alpha$ modestly regulates ovulation and fertilization by promoting the induction of COX-2 for PG production (153). A role for cPLA$_2$$\alpha$ in regulating male sexual maturation has also been described (154). Male cPLA$_2$$\alpha$ KO mice are fertile but exhibit abnormal histology of the testes showing reduced number of interstitial cells. Compared to wild type mice, cPLA$_2$$\alpha$ knockout males have delayed spermatogenesis and reduced testosterone production although these defects normalized in KO mice with age.

It is difficult to extrapolate from studies in mouse models in understanding the specific role of cPLA$_2$$\alpha$ in human reproduction, which has many unique regulatory features not shared by other mammalian species (155). The female patient with homozygous deletion mutations in cPLA$_2$$\alpha$ associated with cryptogenic multifocal ulcerating stenosing enteritis is infertile although the basis for this is unknown, and may be due to the severe consequences of the disease (50). Prostaglandins regulate labor and birth in humans, and an important source of prostaglandins are amnion fibroblasts in fetal membranes (156,157). There is an increase in cortisol secretion and prostaglandin production during parturition, a paradoxical event since cortisol suppresses prostaglandin production in most tissues (158). Glucocorticoids have been shown to induce mRNA and protein expression of cPLA$_2$$\alpha$ and COX-2 in human amnion fibroblasts (72,159). The induction of cPLA$_2$$\alpha$ by glucocorticoids involves activation of G$\alpha_s$ leading to CREB-1 phosphorylation, which interacts with the glucocorticoid receptor on the glucocorticoid response element in the cPLA$_2$$\alpha$ promoter (160).

Kidney

cPLA$_2$$\alpha$ KO female mice exhibit defects in renal function possibly due to a deficiency in PGE$_2$ production, which regulates various aspects of kidney physiology (161). Mice lacking cPLA$_2$$\alpha$ have a urine-concentrating defect that develops with age. Based on abnormal aequorin staining in proximal
tubules the defect in cPLA₂α KO female mice may be due to diminished water reabsorption. There is also evidence that cPLA₂ regulate Na⁺-HCO₃⁻ transport by angiotensin II in kidney proximal tubules (162). The role of cPLA₂α in kidney function of humans is poorly understood. The female sibling with the cPLA₂α deletion developed acute renal failure requiring hemodialysis although whether this is a direct effect of cPLA₂α deletion is not clear (50). It has recently been shown that cPLA₂α plays a role in mediating the onset of non-obstructive neonatal hydronephrosis induced in mice by treatment with the dioxin 2,3,7,8-tetrachlorodibenzo-p-dioxin, which increases expression of cPLA₂α in kidneys (163). Results showed that cPLA₂α KO neonates have reduced incidence and severity of hydronephrosis that correlates with reduced expression of COX-2, mPGES-1 and lower urinary PGE₂ compared to 2,3,7,8-tetrachlorodibenzo-p-dioxin treated WT pups. The COX-2/PGE₂ pathway is also implicated in the pathogenesis of dioxin-induced hydronephrosis (164). The toxic effects of dioxins are mediated by the arylhydrocarbon receptor, which binds to the second intron of Pla2g4a and mediates its induction (165,166). The arylhydrocarbon receptor pathway also promotes signaling cascades for post-translational activation of cPLA₂α by increasing calcium and phosphorylation (167,168).

Nervous System

Many studies have implicated cPLA₂α in mediating several disease processes in the nervous system including post-ischemic brain injury, spinal cord injury and neurodegenerative diseases as will be discussed below. However, there is a role for cPLA₂α in regulating neuronal homeostasis particularly long-term depression and long-term potentiation involved in synaptic plasticity. Long-term depression is blocked in brain slices from cPLA₂α KO mice by treatment with cPLA₂α (pyrrolidine-1) and COX-2 inhibitors (169). The defect in long-term depression by cPLA₂α deficiency or COX-2 inhibition attenuates optokinetic eye movement adaptation, suggesting that cPLA₂α-regulated long-term depression is important for motor learning. Long-term potentiation is also important for motor learning and a recent
report found that long-term potentiation in cerebellar parallel fiber-Purkinje cell synapses is abolished in cPLA₂α KO mice (170,171).

The earliest work investigating the role of cPLA₂α in brain injury found that cPLA₂α KO mice are protected from post-ischemic brain injury induced by middle cerebral artery occlusion (47). Infarct volumes are reduced in KO mice after reperfusion particularly evident in posterior brain slices. The KO mice also exhibit fewer neurological deficits compare to WT mice. No differences in the vascular anatomy of the brain in KO and WT mice were noted. An extension of this work found that cPLA₂α contributes to the early events leading to injury during cerebral ischemia (172). cPLA₂α WT mice exhibit greater COX-2 expression, PGE₂ production and reactive oxygen species after ischemia, and more disruption of neuron morphology than KO mice. Other studies have reported a role for cPLA₂α in regulating the expression of COX-2 in the brain (173). After systemic LPS, there is less COX-2 expression and PGE₂ production in cPLA₂α KO compared to WT mice (174). COX-2 is induced in neuronal and non-neuronal cells of the brain during inflammation and promotes injury (175).

A role for cPLA₂α in regulating spinal cord injury (SCI) has also been investigated. One report found that cPLA₂α expression is increased in SCI particularly in neurons and oligodendrocytes (176). It was shown that inhibiting cPLA₂α (AACOCF₃) or genetic ablation (cPLA₂α KO C57Bl/6 mice) results in improved motor deficiencies and less tissue damage from SCI. Another study reported a complex role for different PLA₂ enzymes (cPLA₂α, sPLA₂ GIIA and iPLA₂ GVIA) in protecting or exacerbating SCI injury (177). All of the PLA₂s were up-regulated after SCI. In contrast to Liu et al (176), cPLA₂α was found to protect from SCI, which was determined using specific inhibitors and cPLA₂α KO mice (Balb/c), but sPLA₂ GIIA and iPLA₂ GVIA were detrimental. Results suggested that the beneficial effect of cPLA₂α in SCI involves PGE₂ signaling through the EP₁ receptor. A confounding factor in evaluating the role of cPLA₂α in these mouse models is the influence of genetic differences in mouse strains. For example, the commonly used mouse strains BALB/c and C57BL/6 generally exhibit distinct polarization of helper T cell responses with BALB/c predisposed to Th2 and C57BL/6 to Th1 responses (178).
addition, C57BL/6 mice lack sPLA<sub>2</sub> GIIA due to a natural deletion in the gene that does not occur in BALB/c mice (179).

A role for cPLA<sub>2</sub>α in mouse models of neurodegenerative disease such as Alzheimer's has been investigated (180,181). The Aβ peptides that are implicated in Alzheimer's neurodegeneration exert toxic effects in neurons, which is in part mediated by cPLA<sub>2</sub>α (182,183). Genetic ablation of cPLA<sub>2</sub>α improves cognitive function in a mouse model of familial Alzheimer's disease, and protects from the toxic effects of intra-cerebroventricular injection of Aβ oligomers (184,185). A recent study found that Aβ peptides activate cPLA<sub>2</sub>α in rat primary cortical neurons resulting in increased expression of β-amyloid precursor protein by an autocrine pathway involving PGE<sub>2</sub> production and increases in cAMP (186). Additional studies also suggest that cPLA<sub>2</sub>α activation is involved in promoting neurodegeneration that occurs in prion diseases. In primary cortical neurons, the prion-derived peptide (PrP82-146) induces cPLA<sub>2</sub>α activation and arachidonic acid release that correlates with synapse damage and cell death (187).

Treatment of neurons with PLA<sub>2</sub> inhibitors (AACOCF3, MAFP), and a PGE<sub>2</sub> receptor antagonist (AH13205), protects from synapse degeneration (188). In a model of Parkinson's disease cPLA<sub>2</sub>α contributes to neurotoxicity induced by 1-methyl-4-phenyl-1,2,3,6-tetrhydropyridine (189). The reduction in dopamine induced by 1-methyl-4-phenyl-1,2,3,6-tetrhydropyridine is significantly less in KO than WT mice. It is not known how cPLA<sub>2</sub>α contributes to neurodegeneration in these models but mechanisms involving free radical production, effects on intracellular membrane trafficking, and arachidonic acid metabolites have been proposed.

It is important to consider underlying differences that have been found in the architecture and lipid composition of the brain in cPLA<sub>2</sub>α WT and KO mice. A recent study showed by microscopy that cortical neurons in cPLA<sub>2</sub>α KO mice exhibit abnormal architecture including larger nuclei, increased number of nucleoli and aggregated intra-nuclear ribosomes (190). Abnormalities in the nuclear envelope, nuclear pore and synapses were observed. The authors speculated that this could be due to the role of cPLA<sub>2</sub>α in regulating membrane structure and curvature. In addition, a study comparing the brain lipid
content of cPLA₂α WT and KO mice found altered brain phospholipid composition in mutant mice but no
differences in the concentrations of non-esterified fatty acids in the plasma or brain (191). There were
decreased levels of esterified arachidonic acid in phosphatidylinositol and decreases of several fatty acids
including linoleic, arachidonic, and docosahexaenoic acids in phosphatidylcholine in cPLA₂α KO
compared to WT. However, an interesting finding was the increased incorporation and turnover of AA in
phosphatidylinositol and phosphatidylcholine in cPLA₂α KO perhaps due to compensation from other
PLA₂s. Consequently the differences observed in phospholipid brain composition in cPLA₂α WT and KO
mice may reflect both a lack of cPLA₂α and compensation from other enzymes with differences in lipid
specificity as noted by the authors (191). The lipid alterations in KO mice may influence membrane
properties contributing to the structural differences noted in cortical neurons, and could also influence the
response of mutant mice to brain injury. This again raises the question of whether responses of cPLA₂α
KO mice are due to a primary effect of the loss of cPLA₂α or secondary compensatory changes that
occur.

Lung

There is extensive information investigating the role of eicosanoids in regulating lung diseases
including fibrosis, acute lung injury and cancer. Comparisons of cPLA₂α KO and WT mice have
provided interesting insight into how cPLA₂α regulates these processes. When the cPLA₂α KO mouse
was initially developed, the role of cPLA₂α in regulating type I allergic response (anaphylaxis) in the lung
was investigated (48). To induce systemic anaphylaxis KO and WT mice were immunized by
intraperitoneal injection of ovalbumin (OVA) followed by intravascular OVA injection to elicit
anaphylaxis. In WT mice, there was narrowing of the airway and alveolar thickening that was not evident
in KO mice. Lung resistance was lower and recovery was faster in KO mice than WT mice after OVA
challenge. Bronchial reactivity to methacholine challenge was dramatically reduced in KO mice. Airway
hyperreactivity is also reduced in 5-LO KO mice suggesting that cPLA₂α-mediated production of
leukotrienes mediates allergic responses (192). In contrast allergic lung responses are exacerbated in COX-1 and COX-2 KO mice compared to WT mice suggesting prostaglandins play a protective role (193).

Over the past few years, Wyeth has developed indole compounds that selectively inhibit human cPLA$_2\alpha$ and have been tested in a sheep model of allergic asthma (194). Since diverse lipid mediators are implicated in mediating asthmatic responses in this model they reasoned that broad inhibition with a cPLA$_2\alpha$ inhibitor would be beneficial. The indole cPLA$_2\alpha$ inhibitors reduced late phase bronchoconstriction and airway hyperreactivity in allergen challenged sheep. The indole inhibitor also blocked allergen-dependent induction of novel genes expressed in cells from asthmatics (195). One of the most potent indole inhibitors PF-5212372 blocks eicosanoid production by stimulated human lung mast cells and lung homogenates. However, in human lung homogenates, which contain a complex cell mix, the inhibitor was less effective at blocking PGE$_2$ production than other eicosanoids suggesting that other PLA$_2$s, particularly sPLA$_2$s may contribute to arachidonic acid release for eicosanoid production (1,196). The inhibitor also blocked contraction of isolated human bronchial rings induced by AMP. The results provide convincing evidence for a role of cPLA$_2\alpha$ in mediating responses in allergic asthma. Analysis of the promoter region of PLA2G4A has shown that microsatellite fragments are shorter in patients with severe asthma than healthy controls (197). A comparison of peripheral blood monocytes from severe asthmatics with short and long microsatellite sequences in the PLA2G4A promoter found a correlation between the shorter sequences with increased expression of cPLA$_2\alpha$ mRNA and protein, and greater eicosanoid production (197,198).

Results of studies comparing cPLA$_2\alpha$ WT and KO mice have also supported a role for cPLA$_2\alpha$ in mediating acute lung injury (199). One model induced lung injury by intravenous administration of endotoxin and fungal cell walls, zymosan; the other model involved intratracheal administration of hydrochloric acid. These models simulate endotoxemia and acid aspiration, which are causes of acute respiratory distress syndrome. In both models of lung injury the cPLA$_2\alpha$ KO mouse was protected,
exhibiting less protein leak and neutrophil recruitment into the lung than WT mice that correlated with reduced levels of TXB$_2$, LTB$_4$ and cysteinyll leukotrienes. However, in a cecal ligation and puncture model of experimental sepsis there was no difference in survival of cPLA$_2$$\alpha$ WT and KO mice although there was lower production of eicosanoids (with the exception of 12-hydroxy-eicosatetraenoic acid) and cytokines (IL-6 and CCL2) in the mutant mice (200). It is possible that lipid mediators do not play a dominant role in regulating this sepsis model, or that just targeting one PLA$_2$ isoform is not sufficient (201). A study using an antisense approach to inhibit both sPLA$_2$-IIa and cPLA$_2$$\alpha$ showed improved survival of rats in a cecal ligation and puncture model (202). Another consideration is that cPLA$_2$$\alpha$ mediates the production of diverse lipid mediators that may have opposing effects, some are pro- and others anti-inflammatory (201).

The opposing role of cPLA$_2$$\alpha$-derived lipid mediators is illustrated in the regulation of pulmonary fibrosis, a chronic disease characterized by the accumulation of myofibroblasts and deposition of extracellular matrix (203). Chronic inflammation often leads to fibrosis due to aberrant wound healing resulting in permanent scarring and organ failure (204). A study of pulmonary fibrosis induced by bleomycin found that fibrosis, collagen synthesis and inflammation were reduced in cPLA$_2$$\alpha$ KO mice compared to WT mice (205). In WT mice, the effects of bleomycin were accompanied by increases in eicosanoids in bronchoalveolar lavage fluid that were markedly lower in bleomycin treated cPLA$_2$$\alpha$ KO mice. Although disease parameters were not completely attenuated in KO mice, cPLA$_2$$\alpha$ may contribute to the pathogenesis of fibrosis due to production of pro-inflammatory lipid mediators. This is supported by studies using mice with targeted disruption of 5-LO and the cysteinyll leukotriene receptors 1 and 2, which show reduced bleomycin-induced pulmonary fibrosis (206-208). In contrast, COX-2 deficiency promotes pulmonary fibrosis, and an important role for PGE$_2$ and prostaglandin I$_2$ in protecting the lung has emerged (209-212). Consequently cPLA$_2$$\alpha$ is upstream of pathways that produce mediators with positive and negative effects on lung disease. There has been considerable interest in the role of lysophosphatidic acid (LPA) in promoting fibrosis in many organ systems including the lung (213,214).
LPA levels increase during fibrosis and it exerts a profibrotic effect primarily through the LPA$_1$ receptor. Several pathways for the synthesis of LPA have been described (215,216). Although there is correlative data that cPLA$_2$$\alpha$ may play a role in the initial production of lysosphobilipids that are metabolized to LPA by autotaxin, there is as yet little definite data to support a role for cPLA$_2$$\alpha$ in LPA production (217,218).

Mouse models have been used to investigate the role of cPLA$_2$$\alpha$ in regulating lung cancer. Using the chemical carcinogen urethane to initiate lung cancer, cPLA$_2$$\alpha$ KO mice developed fewer lung tumors than WT mice, and much lower levels of tumor-associated prostaglandins (219). Another study investigated whether the presence of cPLA$_2$$\alpha$ in the tumor microenvironment influenced lung cancer progression and metastasis (220). The injection of mouse lung tumor cells into mouse lungs induced primary tumors of similar size in cPLA$_2$$\alpha$ WT and KO mice. However, KO mice had fewer secondary tumor metastases, less lymph node spread and increased survival. cPLA$_2$$\alpha$ is thought to contribute to tumorigenesis in part by enhancing the viability and proliferation of endothelial cells leading to formation of vascular networks in the tumor microenvironment (221,222). This process diminishes the effectiveness of radiotherapy. It has recently been shown that a cPLA$_2$$\alpha$ inhibitor (PLA-695) blocks pro-survival signaling in endothelial cells and renders cancer cells radiosensitive in a simulated tumor microenvironment (223). Combined therapy (PLA-695 and irradiation) also reduces lung tumor growth in mice. Results suggest that cPLA$_2$$\alpha$-mediated production of lysosphobilipid metabolites promotes vascular endothelial cell proliferation, migration and tumor vascularization (224). Therefore a number of lipid mediators including arachidonic acid, eicosanoids and lysosphobilipids may contribute to cancer development.

**Autoimmunity**

Chronic inflammation is a characteristic of certain autoimmune diseases such as rheumatoid arthritis. Collagen-induced arthritis in mice has pathological features resembling rheumatoid arthritis and
the role of eicosanoids has been studied extensively. Interestingly, there is evidence that both leukotrienes and prostaglandins contribute to disease pathogenesis based on studies using FLAP inhibitors, LTB₄ receptor antagonist and COX-2 KO mice (225-227). Therefore, it is not surprising that cPLA₂α KO mice are also protected from collagen-induced arthritis (228). Based on the collective involvement of eicosanoids in this autoimmune disease it has been suggested that a cPLA₂α inhibitor would have therapeutic value by simultaneously blocking eicosanoids from the COX and 5-LO pathways (228). In rheumatoid arthritis, bone homeostasis is disrupted due in part to chronic inflammation resulting in joint destruction and bone loss. The role of cPLA₂α in regulating inflammatory bone resorption induced in mice with endotoxin has been investigated (229). In response to endotoxin (i.p.) cPLA₂α KO mice exhibit less bone loss and reduced PGE₂ production. The results indicate that cPLA₂α mediates osteoclastic bone resorption due to endotoxin-induced PGE₂ production by osteoblasts. The role of cPLA₂α in regulating autoimmune disease in non-obese diabetic (NOD) mice has also been investigated (230). NOD mice spontaneously develop autoimmune diabetes that has similarities to human type 1 diabetes. In contrast to collagen-induced arthritis, cPLA₂α plays a protective role in the development of diabetes. NOD mice with genetic ablation of cPLA₂α have a higher incidence of diabetes than cPLA₂α WT NOD mice. Results suggest that cPLA₂α plays a protective role during the diabetic stage possibly due to the ability of PGE₂ produced by macrophages to suppress the production of the pro-inflammatory cytokine TNFα.

Experimental autoimmune encephalomyelitis (EAE) is an inflammatory disease in mice that has features of the human autoimmune disease multiple sclerosis (231). There is conflicting data on the role of specific eicosanoids in regulating EAE based on studies with 5-LO KO mice, in which EAE is exacerbated, or using an LTB₄ receptor antagonist, which protects (232). The role of prostaglandins is not clear since COX-2 KO mice are not protected but a COX-2 inhibitor is beneficial in EAE (233,234). A study of EAE induced in cPLA₂α WT and KO mice on the susceptible C57Bl/6 background found that KO mice are resistant to disease (235). Results suggested that cPLA₂α contributes in the induction and effector phase by contributing to the development of Th1 responses. Since the development of the
immune system may be altered in KO mice, a follow up study tested the effect of cPLA$_2$$\alpha$, 5-LO and COX inhibitors on the development of EAE in WT mice (236). The cPLA$_2$$\alpha$ inhibitor WAY-196025 used at the time of immunization to promote EAE blocked the development of EAE, and when used at the start of disease reduced the duration of EAE relapses. Inhibitors of COX-1/2 and 5-LO also reduced disease severity. Results using the selective cPLA$_2$$\alpha$ inhibitor are consistent with the studies using the cPLA$_2$$\alpha$ KO mice. A recent study also showed that another selective cPLA$_2$$\alpha$ inhibitor (2-oxoamide AX059) protected rats from EAE that correlated with increased production of Tregs (237). However, the conflicting studies on the role of specific eicosanoids in EAE highlight potential difficulties in interpreting results from KO mice, which can exhibit compensatory effects or changes that occur during embryogenesis. However, there are also complications in the use of inhibitors involving dosing and time of administration, as well as unknown off-target effects. Therefore multiple approaches are required to evaluate the role of these pathways in disease pathogenesis. As an example, a recent study used bone marrow transplantation to specifically block PGE$_2$ synthesis, or EP receptor expression, in peripheral immune cells and showed that PGE$_2$/EP4 signaling promotes development of EAE in mice (238). This approach demonstrates the importance of investigating the role of specific cell types and receptor pathways for understanding how lipid mediators regulate disease processes in vivo.

CONCLUDING REMARKS

Due to the key role of cPLA$_2$$\alpha$ in mediating lipid mediator production and its widespread tissue expression it has been implicated in regulating homeostatic processes and disease pathogenesis throughout all organ systems. The function of cPLA$_2$$\alpha$ in regulating certain physiological systems such as female reproduction and hemostasis is more straightforward and can be attributed to specific types of lipid mediators. However, elucidating the specific mechanism for cPLA$_2$$\alpha$ action in disease processes in many cases is not known and understandably difficult to evaluate. The cPLA$_2$$\alpha$ KO mouse has provided a
wealth of information, however, multiple approaches are needed to determine cPLA₂α function. Genetic differences in mouse strains leads to difficulties in interpretation of results, and compensatory changes in the conventional KO mouse are a complicating issue. The use of cPLA₂α conditional KO mice with ablation in specific tissues would be useful to bypass changes occurring during embryogenesis and development. The availability of specific inhibitors is also important to understand cPLA₂α function in animal models in vivo, and in human cells (2). The primary function of cPLA₂α is due to its role in mediating production of eicosanoids, however there is evidence that cPLA₂α regulates processes such as membrane trafficking that are not linked to production of eicosanoids adding an additional complexity to understanding its function (16,17,74). Many studies primarily from comparisons of cPLA₂α WT and KO mice have suggested that cPLA₂α is a useful therapeutic target for treating a plethora of diseases. There are a number of issues in considering cPLA₂α as a therapeutic target especially since most of studies are based on disease models comparing cPLA₂α WT and KO mice, which in some cases may not accurately reflect processes contributing to human disease. This is apparent from the differences observed between mice and humans as a consequence of cPLA₂α deficiency. Another complication is that cPLA₂α is the first regulatory enzyme in the pathway for production of numerous lipid mediators that often have opposing effects, although targeting cPLA₂α may be beneficial in some diseases where both 5-LO and COX metabolites contribute to disease as suggested in studies of asthma and CIA (194,228). However, the essential role of cPLA₂α in human health, particularly for maintenance of the small intestine and female reproduction, is also a consideration for targeting cPLA₂α in disease.

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REFERENCES


108. Leslie, C. C., and Channon, J. Y. (1990) Anionic phospholipids stimulate an arachidonoyl-
hydrolyzing phospholipase A₂ from macrophages and reduce the calcium requirement for

enzyme for leukotriene biosynthesis in health and disease. *Biochim. Biophys. Acta* **1851**, 331-
339.

lipoxygenase binds calcium and mediates calcium stimulation of enzyme activity. *J. Biol. Chem.*
**275**, 38787-38793.

cytosolic 5-lipoxygenase into the nucleus of neutrophils after in vivo recruitment and in vitro


about?: 5-lipoxygenase-activating protein inhibitors for inflammatory diseases. *Trends
Pharmacol. Sci.* **29**, 72-78.
117. Mandal, A. K., Jones, P. B., Bair, A. M., Christmas, P., Miller, D., Yamin, T. T., Wisniewski, D.,
Menke, J., Evans, J. F., Hyman, B. T., Bacskaï, B., Chen, M., Lee, D. M., Nikolic, B., and

118. Mandal, A. K., Skoch, J., Bacskaï, B. J., Hyman, B. T., Christmas, P., Miller, D., Yamin, T. D.,

nuclear membrane leukotriene synthetic complex is a signal integrator and transducer. Mol. Biol.
Cell 23, 4456-4464.

120. Smith, W. L., Urade, Y., and Jakobsson, P. J. (2011) Enzymes of the cyclooxygenase pathways of
prostanoid biosynthesis. Chemical reviews 111, 5821-5865.

fluorescent probe: targeting the Golgi apparatus of cancer cells. J. Amer. Chem. Soc. 135, 11663-
11669.


cPLA\textsubscript{2} deficiency. Biochemistry 50, 1731-1738.


C., Liang, B. T., Sonin, D., Hand, A. R., Zarini, S., Murphy, R. C., Belinsky, G. S., Nakanishi,


FIGURE LEGENDS

Fig. 1. Regulation of cPLA$_2$$\alpha$. cPLA$_2$$\alpha$-mediated arachidonic acid (AA) release occurs following cell stimulation that results in increases in intracellular Ca$^{2+}$ and kinase activation. In the cytosol, cPLA$_2$$\alpha$ is bound to p11/Annexin A2 and is released from this complex when phosphorylated on S727. Calcium binds to the calcium-binding loops (CBL, green) at the membrane binding face of the C2 domain. This increases the hydrophobicity of the C2 domain and promotes preferential binding of cPLA$_2$$\alpha$ to Golgi and, at higher levels of intracellular calcium, to the endoplasmic reticulum (ER) and nuclear envelope. Basic residues (orange patch) in the C2 domain are implicated in binding to ceramide-1-P in the Golgi. Association of the catalytic domain with membrane is mediated in part by a tryptophan residue in the catalytic domain (W464, red patch) that stabilizes cPLA$_2$$\alpha$ on the membrane. Phosphorylation of S505 by mitogen-activated protein kinases in the catalytic domain enhances cPLA$_2$$\alpha$ activity. Interaction of basic residues in the catalytic domain (blue patch) with anionic components in the membrane, such as polyphosphoinositides, optimizes catalytic activity. AA released by cPLA$_2$$\alpha$ is metabolized by COX-1 and -2, which are primarily localized to ER but also occur on the Golgi. Leukotriene production occurs at the nuclear envelope, the site of 5-LO translocation in response to increases in calcium. 5-LO binds to the scaffold protein FLAP, an AA binding protein, which also binds LTC$_4$ synthase (syn) for production of LTC$_4$.

Fig. 2. Ribbon diagram of cPLA$_2$$\alpha$ structure. Two ions of calcium bind to the calcium-binding loops (CBL) on the membrane binding face of the C2 domain. The catalytic domain with the active site nucleophile S228 contains residues involved in cPLA$_2$$\alpha$ regulation (pink) include phosphorylation sites (S505, S727), basic residues (K488, K544, K543) and W464 important for membrane interaction. The residues mutated in humans with cPLA$_2$$\alpha$ deficiencies are shown in green. The residue V707 is predicted to be the start of the deletion in patients with cryptogenic multifocal ulcerating stenosing enteritis.
Fig. 2

CBL1

CBL3

$\text{Ca}^{2+}$

R485H

K488

K544

K543

S111P

S505

S727

W464

Active Site

D575H

V707

C2 Domain

Catalytic Domain