Chapter 2
Phospholipase A_2

Makoto Murakami and Yoshitaka Taketomi

Abstract Phospholipase A_2s (PLA_2s) are a group of enzymes that hydrolyze the *sn*-2 position of phospholipids to generate fatty acids and lysophospholipids, which serve as lipid mediators or their precursors. Mammalian genomes encode genes for more than 30 PLA_2s or related enzymes, which are subdivided into several groups on the basis of their structures, enzymatic properties, and evolutional relationships. Among them, the Ca^{2+}-dependent cytosolic PLA_2 ($cPLA_2$), Ca^{2+}-independent PLA_2 ($iPLA_2$), and secreted PLA_2 ($sPLA_2$) families are regarded as the "big three." From a general point of view, $cPLA_2\alpha$ (the prototypic $cPLA_2$) plays a major role in the initiation of arachidonic acid (AA) metabolism, the $iPLA_2$ family contributes to membrane homeostasis or energy metabolism, and the $sPLA_2$ family affects various biological events by modulating extracellular phospholipid milieus in response to given microenvironmental cues. In this chapter, we overview current understanding of the biological functions of PLA_2s as revealed by gene-manipulated mice and human diseases.

Keywords Arachidonic acid • Eicosanoid • Fatty acid • Glycerophospholipid • Immunity • Inflammation • Lipoprotein • Lysophospholipid • Metabolic disease • Phospholipase A_2

2.1 Introduction

Phospholipase A_2 (PLA_2) catalyzes the hydrolysis of the *sn*-2 position of membrane glycerophospholipids to liberate free fatty acids and lysophospholipids. To date, more than 30 enzymes that possess PLA_2 or related activities have been identified in

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mammals, and these have been subdivided into several groups based on their structures, catalytic mechanisms, localizations. and evolutionary relationships. The $cPLA_2$ family contains 6 enzymes ($cPLA_2\alpha$–ζ), which (except for $cPLA_2\gamma$) contain an N-terminal C2 domain for Ca^{2+}-dependent association with the membrane. The $iPLA_2$ or patatin-like phospholipase domain-containing lipase (PNPLA) family includes 9 enzymes, some of which act principally on phospholipids and others on neutral lipids such as triglyceride (TG). The $sPLA_2$ family, in which 10 catalytically active enzymes have been identified, are low molecular weight, extracellular enzymes that require Ca^{2+} of the mM order for optimal enzymatic activity. Because of this diversity, PLA_2 enzymes have been implicated in various biological processes such as lipid mediator production, membrane remodeling, and energy metabolism.

During the past few decades, studies of various PLA_2 transgenic and/or knockout mice as well as human diseases with PLA_2 gene mutations have provided new insights into the emerging biological roles of individual PLA_2s. The functions of individual PLA_2s may not simply reflect changes in lipid mediator signaling, or more particularly eicosanoid signaling, but may also be attributable to hydrolysis of one or a combination of various target membrane lipids. Herein, we focus on the pathophysiology of various PLA_2s as revealed by information from transgenic or knockout mice, as well as human diseases.

2.2 The $cPLA_2$ Family

2.2.1 General Aspects of $cPLA_2$s

Enzymes belonging to the $cPLA_2$ family are characterized by the presence of a C2 domain in their N-terminal region, with the exception of $cPLA_2\gamma$, which lacks this domain. Evolutionarily, the $cPLA_2$ family emerged from the ancestral $iPLA_2$ family at the branching point of vertebrates, correlating with the development of eicosanoid signaling cascades. $cPLA_2\alpha$ is no doubt the best-known PLA_2, with a major role in releasing arachidonic acid (AA), a precursor of eicosanoids (prostaglandins, PGs, and leukotrienes, LTs), from cellular membrane phospholipids.

2.2.2 $cPLA_2\alpha$

$cPLA_2\alpha$, also known as group IVA PLA_2, is localized in the cytosol of resting cells, and in response to an increase in cytosolic Ca^{2+} levels after cell activation, it translocates to the perinuclear or, more specifically, the Golgi membranes to encounter its preferred substrate, AA-containing phosphatidylcholine (PC). Ceramide-1-phosphate or phosphoinositide-4,5-bisphosphate (PIP_2) enhances the membrane

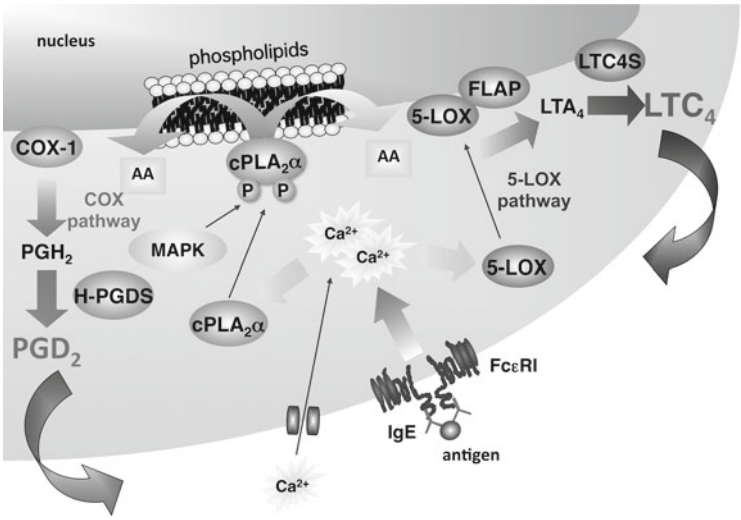

Fig. 2.1 Activation of cytosolic PLA_2 ($cPLA_2$)α in mast cells (MCs). In response to Ca^{2+} influx following FcεRI activation with IgE and cognate antigen, $cPLA_2\alpha$ translocates from the cytosol to the perinuclear membrane and is phosphorylated by mitogen-activated protein kinase (MAPK) for optimal activation. The arachidonic acid (AA) released from membrane phospholipids by $cPLA_2\alpha$ is then converted to PGD_2 by the sequential action of cyclooxygenase (COX)-1 (or COX-2 when the cells are primed by particular stimuli) and hematopoietic PGD_2 synthase (H-PGDS) to PGD_2 or by the sequential action of 5-lipoxygenase (5-LOX) incorporation with 5-LOX-activating protein (FLAP) and LTC_4 synthase (LTC4S) to LTC_4

interaction of $cPLA_2\alpha$. Mitogen-activated protein kinases phosphorylate Ser^{505} on $cPLA_2\alpha$, leading to its activation. The AA released by $cPLA_2\alpha$ is then converted to PGs and LTs by cyclooxygenases and 5-lipoxygenase, respectively. As an example, the $cPLA_2\alpha$ activation mechanism in mast cells (MCs) is shown in Fig. 2.1.

Mice lacking $cPLA_2\alpha$ (*Pla2g4a*$^{-/-}$) exhibit a number of striking phenotypes that can be explained by defects in pathways involving PGs, LTs, or platelet-activating factor (PAF). For instance, *Pla2g4a*$^{-/-}$ mice are protected from asthma, acute respiratory distress syndrome, and pulmonary fibrosis, which can be explained by marked reductions of detrimental lipid mediators such as LTs and PAF [1–3]. *Pla2g4a*$^{-/-}$ mice or wild-type mice treated with a $cPLA_2\alpha$ inhibitor are less susceptible to experimental autoimmune encephalomyelitis or collagen-induced arthritis [4, 5], are protected from brain injuries caused by ischemia or Aβ amyloid [6, 7], and have reduced incidences of intestinal and lung cancer [8, 9], all of which can be attributed to reduced PGE_2 signaling. Consistent with the protective role of PGE_2 in the gastrointestinal mucosa, the intestinal epithelium of *Pla2g4a*$^{-/-}$ mice has numerous small ulcerative lesions [9]. The impairment of female fertility observed in *Pla2g4a*$^{-/-}$ mice suggests that $cPLA_2\alpha$ has an important role in parturition and implantation by providing $PGF_{2\alpha}$ and PGE_2 [10, 7]. Because of reduced production

of thromboxane A_2 (TXA_2) by platelets, *Pla2g4a*$^{-/-}$ mice are protected from thromboembolism and have prolonged bleeding times [11]. Furthermore, ablation or knockdown of cPLA$_2$α ameliorates metabolic disorders including atherosclerosis, hepatic fibrosis, insulin resistance, and adipose tissue inflammation [12–14]. In all cases, the levels of lipid mediators responsible for the corresponding pathophysiological events are markedly reduced in *Pla2g4a*$^{-/-}$ mice relative to wild-type mice. In humans, an inherited *PLA2G4A* mutation is linked to impaired eicosanoid biosynthesis, ulceration of the small intestine, and platelet dysfunction [15].

2.2.3 Other cPLA$_2$s

cPLA$_2$β, δ, ε, and ζ (group IVB, IVD, IVE, and IVF PLA$_2$s) map to the same chromosomal locus and are therefore evolutionally more related [16]. cPLA$_2$β is a dual PLA$_1$/PLA$_2$ enzyme, although cPLA$_2$δ has a robust PLA$_1$ activity in preference to PLA$_2$ activity. cPLA$_2$γ (group IVC PLA$_2$) is unique in that it lacks the C2 domain and displays lysophospholipase and transacylase activities in addition to PLA$_2$ activity [17]. The *in vivo* functions of these cPLA$_2$ isoforms are entirely unknown because knockout studies have yet to be performed. A recent study has shown that cPLA$_2$ε may drive recycling through clathrin-independent endocytosis [18].

2.3 The iPLA$_2$/PNPLA Family

2.3.1 General Aspects of iPLA$_2$s

The human genome encodes nine iPLA$_2$/PNPLA enzymes, which share a protein motif known as the "patatin domain" with an unusual folding topology that differs from classical lipases (Fig. 2.2). The cPLA$_2$ and iPLA$_2$ families seem to have evolved from a common ancestral gene, as their catalytic domains are commonly characterized by a three-layer α/β/α architecture employing a conserved Ser/Asp catalytic dyad instead of the classical catalytic triad [19]. iPLA$_2$/PNPLA enzymes are found in virtually all eukaryotes including yeast, plants, invertebrates, and vertebrates, suggesting that they possess fundamental roles in cellular lipid metabolism conserved in the eukaryote kingdom. The designation "PNPLA" appears to be more appropriate than "iPLA$_2$," as some of the isoforms have enzymatic activities apparently distinct from *bona fide* PLA$_2$ activity. For instance, iPLA$_2$ζ/PNPLA2 functions as a major TG lipase in adipose and many other tissues, whereas iPLA$_2$ε/PNPLA3 may act mainly as an acyltransferase or transacylase for accumulation of TG, particularly in the liver [20]. Here, we focus on two particular iPLA$_2$s, iPLA$_2$β/PNPLA9 and iPLA$_2$γ/PNPLA8, which have robust PLA$_2$ activity.

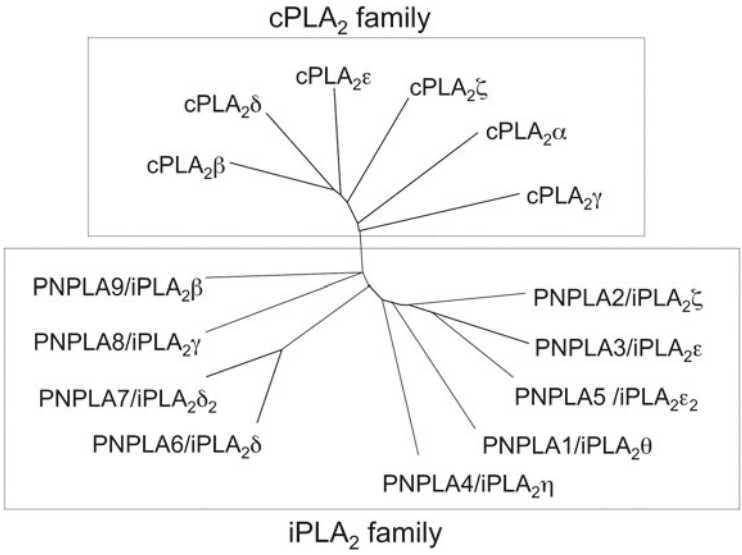

Fig. 2.2 Evolutional relationship between the $cPLA_2$ and $iPLA_2$ families. The $iPLA_2$ family is present in all eukaryotes, whereas the $cPLA_2$ family emerged from the $iPLA_2$ family at the stage of divergence of vertebrates

2.3.2 iPLA$_2$β

$iPLA_2\beta$ (PNPLA9 or group VIA PLA_2), the best characterized $iPLA_2$, has long been thought to be involved in homeostatic phospholipid remodeling through deacylation of phospholipids in the Lands' cycle. Indeed, the composition of phospholipids, particularly those containing docosahexaenoic acid (DHA), is noticeably altered in the brain (but not other tissues) of mice lacking $iPLA_2\beta$ (*Pla2g6*$^{-/-}$) [21]. Notably, human *PLA2G6* mutations are associated with neurodegenerative diseases such as infantile neuroaxonal dystrophy (INAD), neurodegeneration with brain iron accumulation (NBIA), and Schindler's disease, which share the distinctive pathological feature of axonal degeneration with spheroid bodies in the nervous system [22]. Similar neurodegenerative phenotypes are also evident in *Pla2g6*$^{-/-}$ mice or *Pla2g6* mutant mice (*Pla2g6-inad*, in which the *Pla2g6* gene harbors a point mutation), which show motor dysfunction caused by widespread degeneration of axons and synapses, accompanied by the appearance of spheroids and vacuoles [23, 24]. $iPLA_2\beta$ has also been proposed to have more diverse signaling roles. These *Pla2g6*$^{-/-}$ phenotypes include male infertility [25], defective opening of store-operated Ca^{2+} entry, probably caused by reduced production of lysophosphatidylcholine (LPC) [26], impaired insulin secretion by pancreatic β-cells [27], reduced apoptosis [28], decreased eicosanoid generation in vascular cells [29], and protection from ovarian cancer, possibly through reduction of lysophosphatidic acid (LPA) [30]. In most cases, however, the $iPLA_2\beta$-driven lipid metabolic processes underlying these events are poorly characterized.

2.3.3 iPLA$_2$γ

iPLA$_2$γ, also known as PNPLA8 or group VIB PLA$_2$, is localized to mitochondria or peroxisomes and displays PLA$_2$ or PLA$_1$ activity depending on the substrates involved [31]. Mice null for iPLA$_2$γ (*Pnpla8*$^{-/-}$) exhibit bioenergetic dysfunctional phenotypes, including growth retardation, cold intolerance, reduced exercise endurance, increased mortality from cardiac stress, and abnormal mitochondrial function with an altered cardiolipin composition [32]. Furthermore, *Pnpla8*$^{-/-}$ mice are resistant to diet-induced obesity, fatty liver, and hyperlipidemia [33, 34]. These mice also display lipodystrophy, impaired glucose-stimulated insulin secretion, and decreased mitochondrial β-oxidation. Myocardium-specific *Pnpla8*-transgenic mice show a dramatic reduction of myocardial phospholipid mass, marked accumulation of TG, impaired mitochondrial function, and hemodynamic dysfunction [35]. Thus, iPLA$_2$γ appears to be crucial for maintaining efficient bioenergetic mitochondrial function by tailoring mitochondrial lipid metabolism. However, considering that defective β-oxidation usually leads to increased fat accumulation in peripheral tissues, the protective effect of iPLA$_2$γ ablation against diet-induced metabolic disorders might involve an as yet unknown mechanism. *Pnpla8*$^{-/-}$ mice also display a profound alteration in hippocampal mitochondrial homeostasis, leading to cognitive dysfunction [36]. The *Pnpla8*$^{-/-}$ hippocampus has an increased level of cardiolipin and a decrease of plasmalogen, implying a function of iPLA$_2$γ in remodeling of these phospholipids. Overall, the neurological abnormalities in *Pnpla8*$^{-/-}$ mice are reminiscent of features in patients with Barth syndrome, a disease caused by disturbed cardiolipin metabolism [37].

2.4 The sPLA$_2$ Family

2.4.1 General Aspects of sPLA$_2$s

More than one third of the PLA$_2$ enzymes belong to the sPLA$_2$ family, which contains ten catalytically active isoforms (IB, IIA, IIC, IID, IIE, IIF, III, V, X, XIIA). Individual sPLA$_2$s exhibit unique tissue and cellular localizations and enzymatic properties, suggesting their distinct pathophysiological roles. Classical group I/II/V/X sPLA$_2$s are closely related, 14- to 19-kDa secreted enzymes with a highly conserved Ca^{2+}-binding loop and a His/Asp catalytic dyad as well as conserved disulfide bonds, whereas group III and XII sPLA$_2$s are atypical and classified into distinct classes. As sPLA$_2$s are secreted, their target membranes should reside in the extracellular spaces. Individual sPLA$_2$s contribute to various biological events through production of lipid mediators, promotion of membrane remodeling, modification of extracellular noncellular lipid components such as surfactant, microparticles, and lipoproteins, or degradation of foreign phospholipids such as those originating from microbes and dietary components. Here we overview the

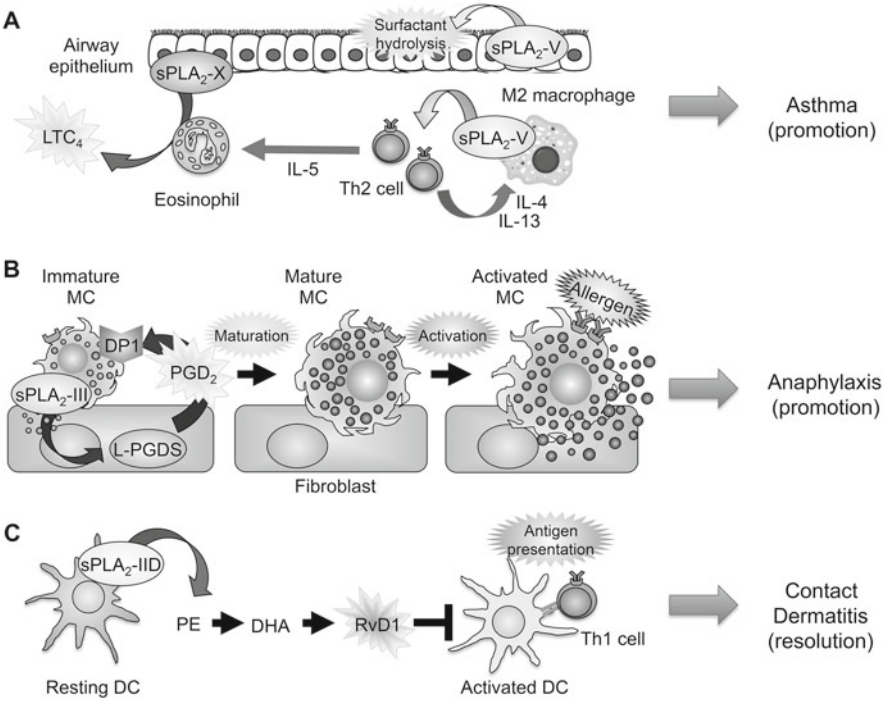

Fig. 2.3 Several examples of $sPLA_2$-driven lipid networks. (**a**) $sPLA_2$-V in M2 macrophages facilitates the Th2 response and that in airway epithelial cells degrades lung surfactant. $sPLA_2$-X from the airway epithelium acts on eosinophils to augment LTC_4 generation. Accordingly, the two $sPLA_2$s independently promote asthma. (**b**) $sPLA_2$-III released from immature MCs acts on fibroblasts to promote L-PGDS-dependent generation of PGD_2, which in turn acts on the PGD_2 receptor DP1 on MCs to promote MC maturation. Accordingly, PLA2G3 facilitates MC-dependent anaphylaxis. Activation of $cPLA_2\alpha$ in mature MCs is highlighted in Fig. 2.1. (**c**) In lymph nodes, $sPLA_2$-IID in DCs hydrolyzes PE to release DHA, which is then converted to the pro-resolving lipid mediator resolving D1 (RvD1) to sequester Th1 immunity. Accordingly, $sPLA_2$-IID ameliorates Th1-dependent contact dermatitis

pathophysiological functions of several classical $sPLA_2$s (IB, IIA, IID, IIE, V, X) and an atypical $sPLA_2$ (group III), as revealed by their transgenic overexpression or gene targeting in mice. Several examples of these $sPLA_2$-mediated lipid networks are illustrated in Fig. 2.3.

2.4.2 sPLA$_2$-IB

$sPLA_2$-IB, often called "pancreatic $sPLA_2$," is abundantly expressed in the pancreas. After secretion into the duodenal lumen, an N-terminal heptapeptide of the inactive zymogen is cleaved by trypsin to yield an active enzyme. The main role of this

enzyme is digestion of dietary and biliary phospholipids. Gene disruption of $sPLA_2$-IB (*Pla2g1b*$^{-/-}$) results in decreased phospholipid digestion and absorption in the gut [38]. The reduced intestinal production of LPC, which is a causal factor for hepatic insulin resistance, results in protection from diet-induced obesity, glucose intolerance, hyperlipidemia, and atherosclerosis in *Pla2g1b*$^{-/-}$ mice [39–41].

2.4.3 *sPLA$_2$-IIA*

$sPLA_2$-IIA is the only isozyme detectable in the circulation, particularly under pathological conditions. It is often referred to as "inflammatory $sPLA_2$" as its levels in sera or inflammatory exudates correlate with the severity of inflammatory diseases, and it is robustly induced by pro-inflammatory stimuli in various cells [42]. However, the precise role of $sPLA_2$-IIA in inflammation remains debatable, as a natural mutation in its gene (*Pla2g2a*) in C57BL/6 and 129Sv mice [43] prevents adequate evaluation of its functions by gene targeting. So far, therefore, most of the *in vivo* functions of $sPLA_2$-IIA have currently been addressed mainly using *Pla2g2a*-transgenic mice.

Pla2g2a-transgenic mice have skin abnormalities manifested by hair loss and epidermal hyperplasia [44] and by increased carcinogen-induced skin cancer [45]. In line with clinical evidence that the serum level of $sPLA_2$-IIA correlates with cardiovascular diseases [46], *Pla2g2a*-transgenic mice develop advanced atherosclerotic lesions [47]. Given that atherosclerosis represents chronic inflammation in the aorta, $sPLA_2$-IIA can be regarded as a pro-inflammatory enzyme in atherosclerosis. The most probable physiological role of $sPLA_2$-IIA is degradation of bacterial membranes, thereby providing a first line of antimicrobial defense [48]. $sPLA_2$-IIA is capable of hydrolyzing phosphatidylethanolamine (PE) and phosphatidylglycerol in marked preference to PC, which can account for the preferential action of this enzyme on bacteria rather than on mammalian cells. Accordingly, *Pla2g2a*-transgenic mice or wild-type mice treated with $sPLA_2$-IIA are resistant to pneumonia and sepsis following bacterial infection [49]. For this reason, $sPLA_2$-IIA is often referred to as a "bactericidal $sPLA_2$."

Mouse strains with natural disruption of the *Pla2g2a* gene (see foregoing) are more sensitive to intestinal tumorigenesis [50]. Transgenic transfer of the *Pla2g2a* gene into these strains reduces the incidence of intestinal polyposis [51]. Thus, $sPLA_2$-IIA appears to have an antitumorigenic role in the gastrointestinal tract. Presumably, bactericidal $sPLA_2$-IIA may affect the gastrointestinal microflora, thereby influencing tumor development. On the other hand, $sPLA_2$-IIA expression is positively correlated with the malignancy of prostate cancer [52], revealing distinct impacts of $sPLA_2$-IIA on different types of cancer. Recently, the mutated *Pla2g2a* allele in the C57BL/6 mouse strain was delivered into the BALB/c mouse strain to produce *Pla2g2a*$^{-/-}$ BALB/c mice. Autoantibody-induced arthritis is attenuated in these *Pla2g2a*$^{-/-}$ mice relative to *Pla2g2a*-sufficent mice, whereas it is conversely aggravated in *Pla2g2a*-transgenic mice [53]. This study has provided the

first compelling evidence for the pro-inflammatory role of $sPLA_2$-IIA. Recently, it has been shown that $sPLA_2$-IIA targets phospholipids in extracellular mitochondria, and thereby amplifies inflammation by producing eicosanoids as well as mitochondrial DNA, a kind of danger-associated molecular pattern (DAMP) [54].

2.4.4 $sPLA_2$-IID

$sPLA_2$-IID is structurally most related to $sPLA_2$-IIA and is expressed preferentially in dendritic cells (DCs) in secondary lymphoid organs [55], suggesting its immunoregulatory role. In a model of Th1-dependent contact dermatitis, resolution of inflammation is compromised in skin and lymph nodes of mice lacking $sPLA_2$-IID (*Pla2g2d*$^{-/-}$) [55]. $sPLA_2$-IID in regional lymph nodes mobilizes a pool of polyunsaturated fatty acids that can be metabolized to pro-resolving lipid mediators such as DHA-derived resolvin D1, which reduces Th1 cytokine production and DC activation. $sPLA_2$-IID preferentially hydrolyzes DHA-containing PE in lymph node membranes. In accordance with its antiinflammatory role, $sPLA_2$-IID expression in DCs is downregulated after cell activation. Furthermore, administration of $sPLA_2$-IID-Fc protein attenuates autoimmune diseases in mice [56]. Together, the existing data suggest that $sPLA_2$-IID is a "resolving $sPLA_2$" that ameliorates inflammation by mobilizing DHA-derived pro-resolving lipid mediators.

2.4.5 $sPLA_2$-IIE

Similar to $sPLA_2$-IID, $sPLA_2$-IIE is structurally most homologous to $sPLA_2$-IIA. Expression of $sPLA_2$-IIE is markedly induced in adipocytes during adipogenesis *in vitro* and after high-fat feeding *in vivo*. Mice deficient in $sPLA_2$-IIE (*Pla2g2e*$^{-/-}$) are modestly protected from diet-induced obesity, fatty liver, and hyperlipidemia [57]. $sPLA_2$-IIE preferentially hydrolyzes minor lipoprotein phospholipids, phosphatidylserine (PS), and PE, with no apparent fatty acid selectivity. As such, $sPLA_2$-IIE alters lipid composition in lipoproteins, thereby affecting fat deposition in adipose tissue and liver. Thus, $sPLA_2$-IIE is a "metabolic $sPLA_2$" that controls systemic metabolic states by modulating lipoprotein phospholipids. These findings shed light on the importance of the minor lipoprotein phospholipids (PS and PE) in metabolic regulation.

2.4.6 $sPLA_2$-V

Because $sPLA_2$-V is able to hydrolyze PC more efficiently than $sPLA_2$-IIA, most investigators in this research field have been interested in the potential roles of this enzyme in inflammation in the context of AA metabolism. Indeed, zymosan-induced

peritonitis or lipopolysaccharide (LPS)-induced air pouch inflammation is partially attenuated in mice lacking sPLA$_2$-V (*Pla2g5*$^{-/-}$) [58, 59]. sPLA$_2$-V is highly expressed in the myocardium, and *Pla2g5*$^{-/-}$ mice exhibit a markedly decreased infarct size in a myocardial ischemia and reperfusion model [60]. sPLA$_2$-V is expressed in bronchial epithelial cells and alveolar macrophages, and *Pla2g5*$^{-/-}$ mice are protected from airway disorders such as antigen-induced asthma and LPS-induced respiratory distress syndrome [61, 62]. Moreover, in keeping with the view that hydrolysis of phospholipids in low density lipoprotein (LDL) by sPLA$_2$-V can promote foam cell formation by macrophages *in vitro* [63], *Ldlr*$^{-/-}$ mice transplanted with *Pla2g5*$^{-/-}$ bone marrow cells are partially protected from atherosclerosis [64]. Although most of these studies support the offensive roles of sPLA$_2$-V, the underlying mechanisms by which sPLA$_2$-V regulates each of these pathologies have remained controversial. Of note, sPLA$_2$-V prefers phospholipids bearing fatty acids with a lower degree of unsaturation (e.g., oleate and linoleate) to those containing highly polyunsaturated fatty acids (e.g., AA and DHA), making it unclear whether sPLA$_2$-V indeed mobilizes AA-derived eicosanoids *in vivo*. Because increased inflammation is generally accompanied by cPLA$_2$α activation, the observed changes in eicosanoid levels in *Pla2g5*$^{-/-}$ mice might simply reflect disease-associated changes in cPLA$_2$α activation, rather than hydrolytic liberation of AA by sPLA$_2$-V. Indeed, transgenic overexpression of sPLA$_2$-V leads to respiratory distress and neonatal death without alterations in pulmonary eicosanoid levels [65]. This phenotype has been ascribed to aberrant hydrolysis of surfactant phospholipids (dipalmitoyl-PC) and is apparently eicosanoid independent.

The roles of sPLA$_2$-V in inflammation have been proven to be more complex. Although sPLA$_2$-V was thought to be induced by pro-inflammatory stimuli (as in the case of sPLA$_2$-IIA), it has recently become obvious that its expression is induced by the Th2 cytokines IL-4 and IL-13, rather than proinflammatory stimuli including LPS, zymosan, and Th1 cytokines, which decrease sPLA$_2$-V expression [57, 66]. sPLA$_2$-V is expressed in IL-4-driven M2 macrophages and Th2 cells, which facilitate Th2-type inflammation while attenuating Th1 or Th17 immunity. Importantly, Th2 responses, as monitored by IL-4 expression and IgE production, are greatly reduced in *Pla2g5*$^{-/-}$ mice, thus underscoring the reduced asthmatic phenotype from the lack of sPLA$_2$-V. Thus, sPLA$_2$-V appears to function in at least two regulatory steps in asthma: (1) in antigen-presenting cells to regulate antigen processing and thereby the Th2 response, and (2) in airway-resident cells to promote airway inflammation that may involve surfactant degradation. *Pla2g5*$^{-/-}$ mice are more susceptible to *Candida* infection (Th1 immunity) and arthritis (Th17 immunity) [53, 67], which could also be partly explained by the ability of sPLA$_2$-V to promote Th2 immunity (and therefore to suppress Th1/Th17 immunity).

The function of sPLA$_2$-V as a "Th2-prone sPLA$_2$" also influences obesity, as Th2 or M2 response dampens adipose tissue inflammation. In obesity, sPLA$_2$-V is induced in hypertrophic adipocytes [57]. When fed a high-fat diet, *Pla2g5*$^{-/-}$ mice display hyperlipidemia with higher plasma levels of lipid-rich LDL and increased obesity, fatty liver, and insulin resistance. sPLA$_2$-V has a protective function against metabolic disorders by hydrolyzing and thereby normalizing PC in LDL and by

tipping the immune balance toward an Th2/M2 state that counteracts adipose tissue inflammation. Mechanistically, $sPLA_2$-V-driven oleate and linoleate from PC in LDL dampen M1 macrophage polarization by saturated fatty acids (e.g., palmitate), probably through attenuation of endoplasmic reticulum stress. Clinically also, $sPLA_2$-V expression in human visceral adipose tissue inversely correlates with plasma LDL levels. These studies underscore the physiological relevance of lipoprotein hydrolysis by $sPLA_2$s, highlight two adipocyte-driven "metabolic $sPLA_2$s" ($sPLA_2$-IIE and $sPLA_2$-V) as integrated regulators of immune and metabolic responses, and bring about a paradigm shift toward a better understanding of the roles of the $sPLA_2$ family as a metabolic coordinator.

2.4.7 $sPLA_2$-X

As in the case of $sPLA_2$-IB, $sPLA_2$-X is synthesized as a zymogen, and removal of an N-terminal pro-peptide produces an active mature enzyme [68]. Among the $sPLA_2$s, $sPLA_2$-X has the highest binding affinity for PC and thus exhibits the most potent ability to hydrolyze plasma membrane phospholipids in intact cells [69]. Because of this property, many investigators have speculated that $sPLA_2$-X has a major role in inflammation. In line with this scenario, mice lacking $sPLA_2$-X (*Pla2g10*$^{-/-}$) are refractory to antigen-induced asthma, with markedly reduced infiltration of eosinophils and lymphocytes, attenuated goblet cell hyperplasia and smooth muscle layer thickening, and decreased levels of Th2 cytokines and pro-asthmatic eicosanoids [70]. The attenuated asthmatic responses in *Pla2g10*$^{-/-}$ mice are fully restored by knock-in of human $sPLA_2$-X, and treatment of the knock-in mice with an inhibitor specific for human $sPLA_2$-X suppresses airway inflammation [71]. Mechanistically, $sPLA_2$-X secreted from the airway epithelium may act on infiltrating eosinophils to augment LT production in a process involving LPC-dependent activation of $cPLA_2\alpha$ [72]. *Pla2g10*$^{-/-}$ mice are also protected from the early phase of influenza infection [73], further highlighting the role of this enzyme in the airway. Moreover, $sPLA_2$-X is one of the major $sPLA_2$ isoforms detected in the airway of patients with asthma [74], thus directing attention to $sPLA_2$-X as a novel therapeutic target for asthma. In contrast to $sPLA_2$-V, however, $sPLA_2$-X does not influence the Th2 response itself, as antigen-sensitized *Pla2g10*$^{-/-}$ mice have normal IgE and IL-4 levels.

Several phenotypes have been reported for *Pla2g10*$^{-/-}$ mice, but the data are controversial. These phenotypes include protection from myocardial infarction or aneurysm [75, 76], exacerbation or attenuation of atherosclerosis [77, 78], increased or decreased adiposity [79, 80], altered macrophage responses [81], and lower response to peripheral pain [79]. In some of these studies, experiments were performed under the assumption that $sPLA_2$-X is expressed in immune cells such as neutrophils and macrophages. However, the expression of $sPLA_2$-X in such immune cells is very low or almost undetectable [75, 79], raising a question as to the physiological relevance of studies involving adoptive transfer of *Pla2g10*$^{-/-}$ bone marrow-derived

cells. Rather, the possibility that paracrine sPLA$_2$-X may alter the properties of inflammatory cells should be taken into account. Because sPLA$_2$-X is abundantly expressed in the gut epithelium, it is likely that the decreased digestion and absorption of dietary and biliary phospholipids are eventually linked to reduced fat accumulation in adipose tissue of *Pla2g10*$^{-/-}$ mice [79], a situation similar to *Pla2g1b*$^{-/-}$ mice (see foregoing).

sPLA$_2$-X is most abundantly expressed in the testis, where it is stored in acrosomes (secretory granules) in the head of sperm cells [82]. *Pla2g10*$^{-/-}$ spermatozoa display an impaired acrosome reaction and low fertility despite showing a normal number and motility [83, 82]. Thus, sPLA$_2$-X plays a specific role in sperm activation, boosting the acrosome reaction by producing LPC from sperm membranes in a paracrine or autocrine manner. Last, a striking skin phenotype characterized by alopecia in *Pla2g10*-transgenic mice points to a unique role of sPLA$_2$-X in hair homeostasis [84]. Although grossly the coat hairs of *Pla2g10*$^{-/-}$ mice appear normal, they have ultrastructural abnormalities including a hypoplasic outer root sheath and reduced melanin granules in their hair follicles.

2.4.8 sPLA$_2$-III

sPLA$_2$-III, an atypical sPLA$_2$, more closely resembles bee venom sPLA$_2$ rather than other mammalian sPLA$_2$s [85]. Transgenic overexpression of sPLA$_2$-III in mice with an *ApoE*$^{-/-}$ background results in increased atherosclerosis from accelerated LDL hydrolysis and increased TXA$_2$ synthesis [86]. These mice also develop systemic inflammation as they age because of elevated eicosanoid formation [87]. Thus, beyond the overexpression strategy, sPLA$_2$-III has a pro-inflammatory potential.

sPLA$_2$-III is highly expressed in the epididymal epithelium. Studies using mice lacking sPLA$_2$-III (*Pla2g3*$^{-/-}$) have revealed that epididymal sPLA$_2$-III acts on immature sperm cells passing through the duct in a paracrine manner to regulate phospholipid remodeling. During epididymal transit of spermatozoa, PC in the sperm membrane undergoes a dramatic shift in its acyl groups from oleate, linoleate, and AA to docosapentaenoic acid (DPA) and DHA, and the increased proportion of DPA/DHA consequently contributes to increased sperm membrane fluidity and thereby sperm motility. In *Pla2g3*$^{-/-}$ mice, this sperm membrane remodeling is severely compromised. Accordingly, spermatozoa from *Pla2g3*$^{-/-}$ mice have a low DPA/DHA content, aberrant acrosomes and flagella with an abnormal axoneme configuration, and display hypomotility and reduced fertility [88]. Thus, the two "reproductive sPLA$_2$s" (sPLA$_2$-III and sPLA$_2$-X), which are expressed in different locations within the male genital organs, exert nonredundant but interrelated functions in two major steps of male fertility, the former during sperm maturation in the epididymis and the latter during capacitation and the acrosome reaction, likely after ejaculation in the uterus and oviduct.

Microenvironmental alterations in MC phenotypes affect susceptibility to allergy, yet the mechanisms underlying the proper maturation of MCs toward an allergy-sensitive phenotype were poorly understood. $sPLA_2$-III is stored in and released from MC granules, and MC-associated passive and active anaphylactic responses are markedly attenuated in *Pla2g3*$^{-/-}$ mice, whereas they are augmented in *Pla2g3*-transgenic mice [89]. Tissue MCs in *Pla2g3*$^{-/-}$ mice are immature and are therefore resistant to IgE-dependent and -independent activation. Similar MC abnormalities are also seen in mice lacking lipocalin-type prostaglandin D_2 (PGD_2) synthase (L-PGDS) or those lacking the PGD_2 receptor DP1, suggesting their functional relationship. Indeed, genetic or pharmacological ablation of DP1 in MCs or L-PGDS in fibroblasts phenocopies that of $sPLA_2$-III in MCs in terms of defective MC maturation and anaphylaxis. Taken together, the data suggest that $sPLA_2$-III secreted from immature MCs is coupled with fibroblastic L-PGDS to provide microenvironmental PGD_2, which in turn promotes MC maturation via DP1. The $sPLA_2$-III/L-PGDS/DP1 paracrine loop is a novel lipid-orchestrated mechanism, providing a missing microenvironmental cue that underlies the proper maturation of MCs.

2.5 Concluding Remarks

With the growing list of knockout and transgenic mouse strains for PLA_2s, much progress has been made in delineating the physiological functions of each PLA_2. It is now becoming obvious that $cPLA_2\alpha$ is a central regulator of AA metabolism, supported by the view that the molecular evolution of $cPLA_2\alpha$ coincided with that of eicosanoid receptors when vertebrates evolved, that the $iPLA_2$ family is a fundamental regulator of membrane homeostasis and energy metabolism, and that individual $sPLA_2$s exert unique and tissue-specific biological functions by acting on extracellular phospholipids, which include adjacent cell membranes, noncellular lipid components, and foreign phospholipids such as those in microbes and the diet. The diversity of target phospholipids and products may explain why each PLA_2 family contains many isoforms. Further advances in this research field and their integration for therapeutic applications are likely to benefit from improved, time- and space-resolved lipidomics technology that will allow monitoring of individual PLA_2s and their associated forms of lipid metabolism within specific tissue niches. Hopefully, the next decade will yield a comprehensive map of the PLA_2-driven lipid networks, which will allow the therapeutic application of inhibitors for some PLA_2s central to human diseases.

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