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REVIEW

Biology of Secretory Phospholipase A_2

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Abstract
Introduction The secretory phospholipase A_2 ($sPLA_2$) family provides a seemingly endless array of potential biological functions that is only beginning to be appreciated. In humans, this family comprises 9 different members that vary in their tissue distribution, hydrolytic activity, and phospholipid substrate specificity. Through their lipase activity, these enzymes trigger various cell-signaling events to regulate cellular functions, directly kill bacteria, or modulate inflammatory responses. In addition, some $sPLA_2$'s are high affinity ligands for cellular receptors.
Objective This review merely scratches the surface of some of the actions of $sPLA_2$s in innate immunity, inflammation, and atherosclerosis. The goal is to provide an overview of recent findings involving $sPLA_2$s and to point to potential pathophysiologic mechanisms that may become targets for therapy.

Key words Atherosclerosis · Inflammation · Innate immunity · Lipoprotein · Eicosanoid

Introduction

The phospholipase A_2 (PLA_2, EC 3.1.1.4) family of enzymes catalyze the hydrolysis of the *sn-2* ester of glycerophospholipids to produce free fatty acids and lysophospholipids. Based on their primary actions, PLA_2s are divided into cytosolic and secretory forms [1]. In addition, PLA_2s are also subdivided into Ca^{2+}-dependent and independent based on their requirement for Ca^{2+} for catalytic activity [2]. This review will specifically focus on the secretory members of the PLA_2 ($sPLA_2$) family with an emphasis placed on their respective biologic functions, in particular, their role in host immunity, inflammation and atherosclerosis. Other proposed roles for $sPLA_2$s, including cell migration [3], apoptosis [4–6], reactive oxygen species generation and cytotoxicity [5, 7], proliferation and differentiation [8, 9], coagulation [10] and cancer [11, 12] are beyond the scope of this review.

Ten members of the $sPLA_2$ family have been identified in mammals, which are numbered and grouped in order of their discovery: group IB (GIB), IIA, IIC, IID, IIE, IIF, III, V, X and XII (Table 1) [13]. The exact molecular structure, classification, genome localization and mechanisms of catalytic activity are reviewed in detail elsewhere [14–17]. The human genome encodes nine $sPLA_2$'s, with group IIC existing as a pseudogene [18]. All $sPLA_2$s contain a histidine/arginine catalytic dyad forming the active center and a conserved Ca^{2+}-binding loop that is essential for the enzymes' proper function. Although not closely related at the amino acid level (20–50% identity), the $sPLA_2$'s share a common molecular weight (14–16 kDa) and are rich in disulfide bonds. The mammalian $sPLA_2$'s have been subdivided into three structural classes, based on the position of disulfide bonds and sequence alignment [19]. Groups I, II, V and X $sPLA_2$ comprise one subclass, and as such have similar primary structures and partially overlapping sets of disulfides. The ~55 kDa mammalian GIII $sPLA_2$ is an atypical member of the $sPLA_2$ family, containing a central domain that is similar to the classical

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Table 1 Mammalian $sPLA_2$s

$sPLA_2$ group	Tissue distribution[a]	Features and functions	Phospholipase activity	Binding
I B	Pancreatic secretions [23], lung [25], liver, spleen, kidney, and ovary [30], brain [31]	Digestion of dietary PLs [23], antibacterial [65], eicosanoid formation, cell contraction, proliferation [30], migration [11]	PG > PS >> PC [44]	M-type [40]
II A	Acute phase serum, intestinal mucosa, lacrimal gland cells, prostatic epithelial cells [36]	Acute phase protein [1,35], antibacterial [60–62, 64–66], cell proliferation [3, 4], migration [5], apoptosis [6], atherogenic [124]	PG > PS >> PC [44]	M-, N-type [40] HSPG [41]
C	Pseudogene (human) [18] Testis, pancreas (mouse) [44]	N/D	PG >> PC [44]	Low affinity to M-type [40]
D	Pancreas, spleen, thymus, skin, lung, ovary, eosinophils [44]	N/D	low phospholipase activity PG~PC [44]	Low affinity to M-type [40]
E	Thyroid gland, uterus, embryo [44]	Antibacterial [65]	PG > PC [44]	M-type [40]
F	Placenta, testis, thymus, liver, kidney [44]	Antibacterial [65]	PG >> PC [44]	M-type [40]
III	Kidney, heart, liver, skeletal muscle, placenta, leukocytes [20]	High molecular weight [20]. Antiviral [78]	PG > PC [44]	Low affinity to M-type [40]
V	Heart, eye, pancreas [44], macrophages [99], neutrophils [69], mastocytes [70]	Antibacterial [17,65], antiviral [77], atherogenic [137,142], AA release, eicosanoid generation [51], phagocytosis [76]	PE > PC > PS [46]	HSPG [41] M-type [40]
X	Intestine, lung, testis, stomach [44], neutrophils [69], macrophages [73]	Secreted as pro-enzyme [86]. Antibacterial [17, 65], antiviral [77], atherogenic [135], AA release, eicosanoid generation [52, 99]	PC > PS [45]	M-type [40]
XII A	Heart, skeletal muscle, kidney, pancreas [14]	Antibacterial [65]	Low phospholipase activity [14] PG > PS >> PC	Low affinity to M-type [40]
B	Liver, kidney, skeletal muscle, heart [22]	N/D	Inactive [22]	No binding to M-type [40]

[a] GIB and GIIA data are obtained from humans. GV and GX data are obtained from humans and mice. Data for the other $sPLA_2$s are obtained from mice.

N/D not determined, *PG* phosphatidylglycerol, *PS* phosphatidylserine, *PC* phosphatidylcholine, *HSPG* heparan sulphate proteoglycans

GIII bee venom $sPLA_2$ flanked by a 130-amino acid N-terminal domain and a 219-amino acid C-terminal domain [20]. Finally, GXIIA $sPLA_2$ comprises the third subclass that is characterized by an unusual Ca^{2+} binding loop and position and spacing of cysteine residues [14, 21]. Group XIIB, a molecule homologous to GXIIA, has also been identified [22]. Group XIIB has a mutation in the catalytic site and has been shown to lack enzymatic activity.

The diversity in structure, enzymatic properties, and tissue distribution of $sPLA_2$'s argue for a wide variety of physiological functions. The observation that one cell type can express more than one $sPLA_2$ also implies that their biologic functions are not redundant. Historically, the first $sPLA_2$, GIB $sPLA_2$ (also known as pancreatic $sPLA_2$) was identified in pancreatic secretions and recognized for its role in the digestion of dietary phospholipids [23–25]. Interestingly, mice deficient in GIB $sPLA_2$ exhibit no defects in intestinal phospholipid digestion when fed a normal rodent diet, suggesting that other enzymes, such as pancreatic carboxyl ester lipase [26] and intestinal brush border phospholipase B [27] may compensate for the absence of GIB $sPLA_2$. However, subsequent studies revealed that GIB $sPLA_2$-deficient mice are resistant to high fat diet-induced obesity and obesity-related insulin resistance, suggesting this enzyme may play a critical role in dietary lipid absorption in the setting of a high fat diet [28]. The protection against insulin resistance was later attributed to reduced absorption of lysophospholipids, in particular, lyso-phosphatidylcholine (lyso-PC), in the GIB $sPLA_2$-deficient mice [29]. These studies leave open the question whether inhibitors of pancreatic PLA_2 would be of benefit in the context of high fat consumption in humans. GIB $sPLA_2$ has also been detected in other tissues such as lung, liver, spleen, kidney, ovary and brain where additional functions have been proposed [11, 30, 31].

Subsequent to the identification of GIB $sPLA_2$, GIIA $sPLA_2$ (also known as non-pancreatic $sPLA_2$) was isolated from the synovial fluid of patients with rheumatoid arthritis [32]. GIIA $sPLA_2$, an acute phase reactant that is highly upregulated during inflammation, has been strongly associated with inflammatory conditions and its role in host defense has been established [32–36].

Defining the physiological functions of each of the members of the $sPLA_2$ family poses a significant challenge.

A common approach for studying their biologic functions has been to exogenously add purified recombinant $sPLA_2$ to cells in vitro, or to over-express these enzymes in transfected cells. Gene-directed mRNA suppression and pharmacological suppression of $sPLA_2$ activity in vitro has also been employed. However, with few exceptions, direct in vivo data to support in vitro findings have been lacking. To date, only a few animal models with altered expression of $sPLA_2$ have been created, and findings from these in vivo models have not always been predicted by in vitro studies.

Recently, new functions have been attributed to $sPLA_2$s that do not require enzymatic activity. Among these is the binding to and possible cell signaling through cell surface molecules [37–39]. For example, the relative binding affinity of the full set of mouse $sPLA_2$'s to the M-type receptor has been documented [40]. Other members of the $sPLA_2$ family, such as GIIA and GV $sPLA_2$, bind proteoglycans with high affinity due to their overall positive charge [41, 42]. These properties suggest that the biologic functions of $sPLA_2$s may extend beyond their enzymatic activity and may at least in part explain the existence of $sPLA_2$s with poor phospholipase activity.

Biochemical properties

As their name indicates, $sPLA_2$s catalyze the hydrolysis of phospholipids at the *sn-2* position, a reaction that generates both free fatty acid and lysophospholipid. Due to the amphipathic structure of phospholipids, under physiologic conditions they are either incorporated in membranes or are part of vesicles with various complexity, such as micelles, lipoproteins or cellular membranes, with their hydrophilic head-groups turned to the aqueous phase and hydrophobic tails embedded in the inner parts of the membranes or the vesicles. Therefore, $sPLA_2$s must penetrate the interphase comprised of phospholipid head groups to exert their action. As such, an important prerequisite for the action of $sPLA_2$s is the successful binding to the phospholipid surface, and this property determines some specificities of their enzymatic activity. Indeed, the "interfacial specificity" for each of the different $sPLA_2$'s can vary by several orders of magnitude [43].

In vitro studies utilizing recombinant enzymes and artificial phospholipid substrates have provided substantial information about the biochemical properties of the different members of the $sPLA_2$ family. For example, GIIA as well as GIB $sPLA_2$ have been shown to act on anionic phospholipids such as phosphatidylglycerol (PG), phosphatidylserine (PS) and phosphatidylethanolamine (PE) but are virtually inactive to phosphatidylcholine (PC) due to the lack of high affinity binding [44]. In contrast, GV and GX $sPLA_2$ hydrolyze PC with much higher efficiency compared to other members of the $sPLA_2$ family due to a high binding affinity [43, 45]. Perhaps not surprisingly, there is a strong correlation between the ability of $sPLA_2$s to hydrolyze PC-containing artificial substrates and their ability to hydrolyze mammalian cells and lipoprotein particles, which contain primarily PC on the outer surface. Thus, GV and GX $sPLA_2$ have been shown to be the most potent $sPLA_2$s in mediating phospholipid hydrolysis when added exogenously to mammalian cells, HDL and LDL [43, 45, 46], and accordingly, these two members of the $sPLA_2$ family have received much attention.

Tryptophan residues located on the interfacial binding surfaces near the N terminus appear to be critical for the penetration of $sPLA_2$ into zwitterionic interfaces. Thus, the molecular basis for the extremely low activity of GIIA $sPLA_2$ to hydrolyze PC-rich vesicles, mammalian cell membranes, and serum lipoproteins is the absence of tryptophan residues that are present in both GV and GX $sPLA_2$ [47, 48]. The capacity of GIIA $sPLA_2$ to discriminate between PC-rich mammalian membranes and PG-rich membranes may be critical for its role in innate immunity. During acute infection, circulating levels of GIIA $sPLA_2$ can increase up to three orders of magnitude [33]. High affinity binding to bacterial membranes that are rich in PG allows for selective phospholipid hydrolysis by GIIA $sPLA_2$, providing effective bacterial killing while protecting mammalian membranes during an acute phase response.

In contrast to cytosolic PLA_2 ($cPLA_2$)-α (also designated GIVA PLA_2), which has a marked specificity for arachidonic acid at the *sn-2* position of its substrate phospholipids [49, 50], members of the $sPLA_2$ family are generally thought not to be arachidonyl-selective. Nevertheless, GV and GX $sPLA_2$ have been shown to be quite potent in mobilizing arachidonic acid and stimulating eicosanoid synthesis in various cell types [51, 52], and thus may play an important role in inflammation and inflammatory diseases, as discussed in more detail in a subsequent section of this review. Interestingly, membrane sphingomyelin/ceramide content has been shown to modulate both the activity and arachidonic acid selectivity of GV and GX $sPLA_2$ [46, 53–55], suggesting a novel mechanism whereby sphingolipids may regulate $sPLA_2$-induced inflammatory responses. The exact mechanism by which sphingomyelin/ceramide modulates $sPLA_2$ activity is not clearly understood, although the change in fatty acid specificity has been attributed to segregation of PC and $sPLA_2$ between disordered and ordered sphingomyelin/free cholesterol/PC lipid phases [53].

Conditions associated with cellular injury including apoptosis have been shown to increase the susceptibility of cellular membranes to hydrolysis by GIIA $sPLA_2$ (reviewed in [56]). This may relate to the increased

exposure of PS on the outer leaflet of the membrane [57]. Oxidative modification of phospholipids also alters the physiochemical state of the membrane, which in turn affects the susceptibility of oxygenated and non-oxygenated fatty acid residues toward $sPLA_2$ [58]. Perhaps relevant to atherosclerotic processes, the ability of GIIA $sPLA_2$ to hydrolyze low density lipoproteins in vitro has been shown to be enhanced by mild oxidation [59].

Host defense

The innate immunity is a highly conserved function of mammalian organisms that provides a rapid yet non-specific response to various invading agents. This targeted and complex reaction serves to contain an infection prior to the induction of the adaptive immune response and involves various cell types, such as neutrophils, macrophages and natural killer cells and non-cellular host defense molecules, which range from simple inorganic molecules, such as hypochloric acid and nitric oxide, to more complex proteins or lipids. Abundant evidence indicates that certain members of the mammalian $sPLA_2$ family play important roles in host defense against microbial pathogens (recently reviewed in [36]).

GIIA $sPLA_2$ is predominantly recognized as part of the innate immune system and appears to be a major antibacterial factor against Gram-positive bacteria in human acute phase serum [60–62]. A study of patients with sepsis showed that of the nine human $sPLA_2$'s, only GIIA $sPLA_2$ could be detected in serum by time-resolved fluoroimmunoassay [63]. GIB $sPLA_2$ could also be detected in sera of patients suffering from acute pancreatitis, presumably derived from injured pancreatic acinar cells that normally store this enzyme in an inactive form in zymogen granules. During acute infection, activation of the nuclear factor (NF)-κB signaling pathway leads to the induction of pro-inflammatory mediators, including TNF-α, IL-1 and IL-6, which in turn induce the expression of GIIA $sPLA_2$ in multiple tissues [35].

GIIA $sPLA_2$ efficiently kills various microorganisms, such as *S. aureus*, *E. coli*, *S. typhimurium* and *L. monocytogenes* [64–66]. Mice with transgenic expression of human GIIA $sPLA_2$ are resistant to infection by *S. aureus*, *E. coli*, and *B. anthracis* [60, 67]. To achieve bacterial killing, the enzyme needs to gain access to bacterial cell membrane phospholipids. GIIA $sPLA_2$ is able to penetrate the peptidoglycan envelope of Gram-positive bacteria due to its highly positive charge [65]. In contrast, the cell membrane of Gram-negative bacteria, which is coated by lipopolysaccharide is resistant to $sPLA_2$ hydrolysis. Therefore, GIIA $sPLA_2$ is capable of hydrolyzing the phospholipid of the bacterial cell membrane only after the lipopolysaccharide layer is disrupted by the action of the bactericidal/permeability-increasing protein or the membrane attack complex of complement [62, 68].

Several other $sPLA_2$s in addition to GIIA have been shown to have antibacterial activity. In rank order, the potency of $sPLA_2$s in killing Gram-positive bacteria in vitro is: GIIA > GX > GV > GXIIA > GIIE > GIB > GIIF [65]. Identifying the precise antimicrobial roles of the various $sPLA_2$s in different inflammatory cell types await further study. Current evidence suggests that GV and GX $sPLA_2$ are expressed by human neutrophils, whereas GIIA $sPLA_2$ is not detected [69]. In mouse bone marrow-derived mast cells, GIIA $sPLA_2$ is found associated with secretory granules, while GV $sPLA_2$ is present on Golgi membranes, the nuclear envelope, and the plasma membrane [70]. Increased phospholipase activity was reported in bronchoalveolar lavage fluid of patients with respiratory distress syndrome [71] and bronchial asthma [72], which was tentatively ascribed to GIIA $sPLA_2$. However, a subsequent immunohistochemistry study showed that GIID, GV, and GX $sPLA_2$ but not GIIA $sPLA_2$ is present in human lung macrophages [73].

In addition to direct killing, $sPLA_2$s may have indirect antibacterial effects through the activation of inflammatory cells [39]. For example, GIIA $sPLA_2$ stimulates neutrophils to produce superoxide and release bactericidal enzymes [74]. It also has been proposed that GIIA plays a role in the removal of extracellular cell debris through a non-enzymatic process that involves bridging of the GIIA $sPLA_2$ protein between anionic phospholipid vesicles and heparan sulfate proteoglycans on macrophages [75]. In response to zymosan stimulation, GV $sPLA_2$ is recruited to phagosomes of macrophages where it activates $cPLA_2$-α and leukotriene synthase, suggesting that it may participate in the killing of ingested bacteria through the regulation of eicosanoid production and as a component of the phagocytic machinery [76].

Antiviral functions have also been attributed to GIII, GV and GX $sPLA_2$ [77, 78]. These $sPLA_2$s apparently block adenoviral infection of cells through different mechanisms. In the case of GV and GX $sPLA_2$, the anti-viral action appears to be due to the conversion of PC to lyso-PC in the host cell membrane, which interferes with virus fusion [77]. Interestingly, the anti-adenovirus effect of GIII $sPLA_2$ is reportedly dependent on the catalytically active central domain as well as its unique N-terminal domain [79]. Studies to investigate the antiviral efficacy of these and other $sPLA_2$s in vivo are warranted.

Inflammation and eicosanoid generation

In addition to its antimicrobial activity, $sPLA_2$s contribute to innate immunity through the generation of various

biologically active molecules that modulate immune responses [39]. As noted above, GIIA $sPLA_2$ activity in serum can increase by several orders of magnitude during an acute phase response. The induction of other $sPLA_2$'s during inflammation is not well established, and it is possible that there are species-specific differences in their regulation. In humans, the genes for GIIC, IID, IIE, IIF, and V $sPLA_2$ are clustered at the same chromosomal locus of chromosome 1 as GIIA (1p34–36) [80, 81], and it is been suggested that the transcription of these genes may be co-regulated. However, studies investigating the induction of GV $sPLA_2$ during inflammation have been conflicting [42, 82–85]. GX $sPLA_2$ transcription does not appear to be induced by inflammatory stimuli [85]. Interestingly, findings from transgenic GX $sPLA_2$ mice are consisted with post-translational regulation of GX $sPLA_2$, such that during inflammation this molecule may be converted from an inactive pro-peptide form to the mature, catalytically active enzyme [86].

The generation of arachidonic acid via $sPLA_2$ hydrolysis has the potential to give rise to a wide variety of bioactive lipid mediators, including prostaglandins, thromboxanes, leukotrienes, and lipoxins. These molecules can have potent and pleiotropic effects that modulate inflammatory responses [87]. $sPLA_2$s may provide arachidonic acid for eicosanoid synthesis via multiple proposed mechanisms. Through the "external plasma membrane pathway", $sPLA_2$ acts directly on the outer leaflet of the plasma membrane to release free fatty acids. As summarized in a previous section, this pathway is thought to be operative in the case of GV and GX $sPLA_2$, which are known to potently hydrolyze PC on the surface of cells. Another possibility is the "heparan sulphate proteoglycan (HSPG)-shuttling pathway", whereby heparin-binding $sPLA_2$s may be internalized and trafficked to intracellular compartments to release arachidonic acid for eicosanoid production [88]. GIIA and GV $sPLA_2$ bind negatively charged HSPGs by virtue of cationic domains in their C-terminal region. Alternatively, $sPLA_2$s may function intracellularly prior to secretion [89].

Numerous in vitro studies document the ability of GV and GX $sPLA_2$, and to a lesser extent GIIA, to generate arachidonic acid for eicosanoid production in inflammatory cells, including macrophages, neutrophils, eosinophils and mast cells (for recent review, see [17]). In some cases, this activity has been shown to involve cross-talk with cytosolic GIV or GVIB $sPLA_2$s [89–97]. With few exceptions, such studies have involved transfection-mediated overexpression or exogenous addition of $sPLA_2$. Suppression of endogenous GV $sPLA_2$ using anti-sense oligonucleotides has been shown to significantly reduce LPS-stimulated prostaglandin-E2 production in 388D1 mouse macrophage-like cells [51]. Furthermore, peritoneal macrophages isolated from mice deficient in GV $sPLA_2$ exhibit impaired zymosan-stimulated eicosanoid production [98]. However, only modest changes in eicosanoid levels were detected in bronchoalveolar lavage fluid of mice with transgenic overexpression of GV $sPLA_2$, despite approximately sevenfold increased $sPLA_2$ activity in their lungs compared to wild-type mice [86]. In the case of GX $sPLA_2$, mice with gene-targeted deletion of this enzyme exhibited dramatically reduced allergen-induced eicosanoid production and airway inflammation in an in vivo model of asthma [99]. A more recent study showed that GX $sPLA_2^{-/-}$ mice had attenuated myocardial ischemia/reperfusion injury which was at least partly due to the suppression of neutrophil cytotoxic activities [100].

An important area of research concerns the involvement of $sPLA_2$s in the pathology of adult respiratory distress syndrome (ARDS), which is characterized by alterations in pulmonary surfactant composition that lead to increased alveolar surface tension, alveolar collapse, and severe disturbance of pulmonary gas exchange. Several studies have reported increased $sPLA_2$ activity in bronchoalveolar lavage fluid of patients with ARDS [101–103] or severe pneumonia [73], and that pharmacological inhibition of $sPLA_2$ protects animals against experimental ARDS [104]. A role for GV $sPLA_2$ in lung pathology is also suggested by recent studies in GV $sPLA_2$ transgenic mice [86]. Unexpectedly, these mice died in the neonatal period because of respiratory failure that was attributed to marked reduction of the lung surfactant phospholipids, PC and PG. In contrast, GX $sPLA_2$ transgenic neonates displayed minimal abnormality of the respiratory tract with normal alveolar architecture and surfactant composition. Although this finding appears to be inconsistent with in vitro data that GX $sPLA_2$ is more potent in hydrolyzing surfactant phospholipids compared to GV $sPLA_2$, the lack of a phenotype in GX $sPLA_2$ transgenic mice may be due to the fact that GX $sPLA_2$, unlike GV $sPLA_2$, is originally expressed in an inactive form that requires removal of 11 amino acid residues at the N terminus for catalytic activity [52]. The authors showed that the bulk of GX $sPLA_2$ in lungs of the transgenic mice was present in the precursor form. This conclusion was borne out by a more recent study, in which the mature form of GX $sPLA_2$ was expressed in transgenic mice using the macrophage-specific CD68 promoter [105]. These mice died neonatally due to severe lung pathology that was characterized by severe interstitial pneumonia, increased eicosanoid levels, and enhanced hydrolysis of lung surfactant. Future studies of experimental ARDS in GV and GX $sPLA_2$-deficient mice will provide definitive evidence whether or not these $sPLA_2$s contribute to lung dysfunction either by promoting inflammation-induced surfactant damage and/or pathological eicosanoid generation.

In addition to ARDs, numerous studies provide ample evidence for $sPLA_2$ involvement in other diverse inflammatory

conditions, including rheumatoid arthritis [106], central nervous system inflammation and neurodegenerative diseases [107, 108], inflammatory bowel diseases including Crohn's disease and ulcerative colitis [109, 110], and endotoxin-induced septic shock [111]. While GIIA $sPLA_2$ is the major enzyme found elevated in the systemic circulation of patients with various acute and chronic inflammatory diseases, other $sPLA_2$s have also been reported to be elevated locally at sights of inflammation and cell injury.

Interestingly, many of the biological effects exerted by $sPLA_2$s on inflammatory and other cells appear to be independent of their catalytic activity [39]. The recognition that $sPLA_2$'s may act through binding cellular targets distinct from membrane phospholipids was first appreciated through studies of snake and insect venoms [112, 113]. The N-type receptors, which bind with high affinity several neurotoxic $sPLA_2$s, are highly expressed in mammalian brain membranes but have not yet been isolated or cloned. The M-type receptor mediates myotoxic effects of $sPLA_2$s and is probably the best characterized binding protein for $sPLA_2$s [113, 114]. This receptor is a 180-kDa member of the C-type lectin family that is structurally similar to the macrophage mannose receptor. Based on studies in vitro, the M-type receptor has been proposed to mediate several biological effects of GIB, GIIA, and GX $sPLA_2$, including eicosanoid release, cell proliferation, cell migration, and cytokine induction [40, 115, 116]. On the other hand, the M-type receptor is an endocytic receptor, and it has been suggested that it may function to internalize and inactivate $sPLA_2$'s [117, 118]. Mice deficient in the M-type receptor are resistant to LPS-induced lethality, suggesting a role in inflammation [111]. The full complement of mouse $sPLA_2$'s have been assessed for their ability to bind the mouse M-type receptor [40]: $sPLA_2$ IB, IIA, IIE, IIF, and X bind with highest affinity ($K_{0.5}$=0.3–3 nM); $sPLA_2$ IIC and V bind with lower affinity ($K_{0.5}$=30–75 nM) and the remaining $sPLA_2$'s bind only weakly or not at all ($K_{0.5}$> 100 nM).

Intriguingly, human GIIA $sPLA_2$ has very low binding affinity to the M-type receptor [119], suggesting that different receptors may mediate the actions that are independent of the enzyme's catalytic activity in humans. Indeed, very recently Saegusa et al., reported that human GIIA $sPLA_2$ binds with very high affinity to integrins $\alpha V\beta 3$ and $\alpha 4\beta 1$ and induces proliferation of a monocytic cell line directly connecting the pro-inflammatory functions of GIIA $sPLA_2$ and integrins [120].

Atherosclerosis

According to the "response to retention" hypothesis, a key event in atherosclerosis is the retention of atherogenic lipoproteins in the vessel wall, which initiates the recruitment of monocyte/macrophages [121, 122]. In an effort to remove excess lipid accumulated in the subendothelium, these cells may convert into "foam cells". Simultaneously, inflammatory cytokines produced by various cell types present in the lesion trigger and sustain the inflammatory milieu [123, 124]. Lipid-laden cells and chronic inflammation eventually lead to core necrosis and plaque instability [125]. Current data suggest that $sPLA_2$s may contribute to lipoprotein retention, foam cell formation, and inflammation in a developing lesion (Fig. 1).

A potential involvement of GIIA $sPLA_2$ in atherosclerotic processes has been recognized for well over a decade

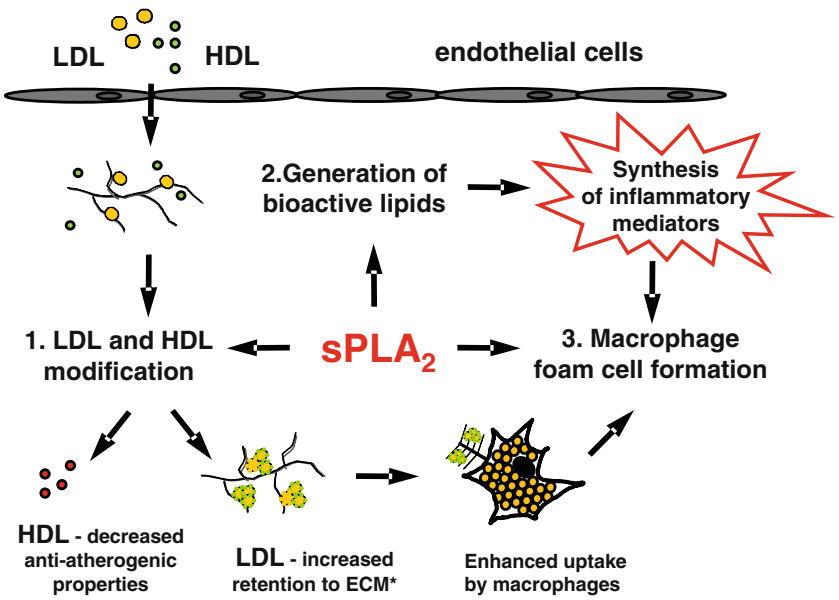

Fig. 1 Model for atherogenic role of sPLA2. *1* Upon influx in the subendothelial space LDL and HDL are subject to hydrolysis by sPLA2 generating atherogenic LDL and HDL with decreased anti-atherogenic properties. *2* sPLA2 generates various bioactive lipids that promote inflammatory processes. *3* sPLA2 modified LDL is readily taken up by macrophages. *ECM** extracellular matrix

[126]. This interest was further amplified with the 1999 finding that circulating levels of GIIA $sPLA_2$ are an independent risk factor for cardiovascular events in humans [127]. The concurrent finding in 1999 that transgenic mice with constitutive expression of human GIIA $sPLA_2$ spontaneously develop atherosclerosis even in the absence of hyperlipidemia provided compelling evidence that this enzyme contributes to atherogenic processes, and is not merely a marker for the disease [128]. GIIA $sPLA_2$ transgenic animals exhibit systemic changes in lipoproteins including elevated VLDL/LDL cholesterol levels, lower HDL and decreased paraoxonase activity [128] that could contribute to increased atherosclerosis susceptibility. The decreased HDL was later shown to be due to an increase in the rate of hepatic selective uptake of HDL-cholesterol ester and plasma clearance of HDL [129, 130]. Subsequent studies in LDL receptor-deficient mice demonstrated that expression of human GIIA $sPLA_2$ only in bone-marrow derived cells or specifically in macrophages increased lesion area without any detectable changes in systemic lipoproteins, indicating that $sPLA_2$ in the local environment of a developing lesion is pro-atherogenic [131–133]. The recent discovery of additional members of the $sPLA_2$ family that are present in lesions has raised the question of which $sPLA_2$ subtypes contribute to atherosclerotic processes [134–136].

There are several potential mechanisms by which $sPLA_2$s can affect atherosclerotic lesion development. As briefly reviewed in the previous section, $sPLA_2$s may contribute to pro-inflammatory processes through the generation of bioactive lipid mediators, including free fatty acids and lysophospholipids. By liberating arachidonic acid, $sPLA_2$ may promote the localized production of prostaglandins, leukotrienes and thromboxanes, all of which have potent pro-inflammatory and thrombogenic potential. A second mechanism by which $sPLA_2$ may enhance lesion formation is through the generation of atherogenic lipoproteins [137–142]. Both GV and GX $sPLA_2$ are capable of hydrolyzing LDL and HDL in vitro with higher potency compared to GIIA $sPLA_2$, producing free fatty acids and lysophospholipids and structurally modified lipoprotein particles [45, 46, 137, 139]. LDL hydrolyzed by either GV or GX $sPLA_2$ promotes macrophage foam cell formation in vitro [137, 139]. Our laboratory has investigated the molecular mechanism by which macrophages take up GV $sPLA_2$-modified LDL, and have shown that this process is independent of scavenger receptors SR-A and CD36 and dependent on cell-surface proteoglycans [140]. This finding is in agreement with studies that $sPLA_2$ modification of LDL leads to conformational changes in apoB that enhance apoB binding to proteoglycans of the extracellular matrix [143]. Interestingly, upon binding to proteoglycans, GV $sPLA_2$ activity on LDL also increases [42]. Hydrolysis by $sPLA_2$ may also impact lipid accumulation in the vessel wall by reducing the anti-atherogenic functions of HDL [141].

Consistent with the large body of in vitro data, a recent gain-of-function and loss-of-function study in LDL receptor-deficient mice confirmed that GV $sPLA_2$ promotes atherosclerotic lipid deposition in vivo [144]. In the near future, valuable information about the role of GX $sPLA_2$, and perhaps other members of the $sPLA_2$ family known to be present in lesions [134], will be provided as appropriate mouse models become available. An important area of research that requires further investigation is the impact of $sPLA_2$ on plaque stability. Several studies have reported that macrophage-specific overexpression of GIIA or GV $sPLA_2$ leads to increased collagen content of atherosclerotic lesions in mice fed a high fat diet, compared to their wild type littermates [132, 133, 144]. Although not directly demonstrated, increased collagen deposition might be expected to provide a more stable plaque phenotype in lesions with enhanced expression of $sPLA_2$. Clearly, more studies are needed to determine whether $sPLA_2$ alters collagen deposition by enhancing its production or reducing its degradation in plaques, and whether such changes have an effect on plaque stability.

Summary and future directions

Elucidating the biologic functions of specific $sPLA_2$s remains a significant challenge, given the relatively large number of family members, their overlapping tissue distribution, and their distinct biochemical properties. The development of specific reagents and animal models to probe their respective functions will undoubtedly lead to novel and important insights.

The inhibition of $sPLA_2$ activity remains an attractive target in the treatment of acute and chronic inflammatory diseases. However, the appropriate strategy should take into account their diversity, their potentially redundant activities and beneficial properties. Although $sPLA_2$ inhibition has been achieved through the development of compounds that partition into the phospholipid membrane and decrease enzyme binding, such inhibitors exhibit low specificity and only modest efficacy [145]. In addition to the potentially adverse effects of the inhibitors per se, non-selective blockade of PLA_2s may prove to be detrimental since they are involved in vital cell processes and have defensive functions that should not be overlooked. Since it appears that more than one $sPLA_2$ may be involved in a particular process, selective targeting of only one $sPLA_2$ may not be sufficient. An additional caveat is the recent recognition that in addition to acting as lipolytic enzymes, $sPLA_2$s can serve as high affinity ligands for cell surface receptors. The

fact that there are currently no available reagents that block enzymatic activity independent of receptor binding and vice versa is one complication in carrying out studies to elucidate the divergent functions of $sPLA_2$s.

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