Biochemistry and Physiology of Mammalian Secreted Phospholipases A_2

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Key Words

atherosclerosis, host defense, inflammation, interfacial enzymology, lipid mediators

Abstract

Phospholipases A_2 (PLA2s) are esterases that hydrolyze the *sn*-2 ester of glycerophospholipids and constitute one of the largest families of lipid hydrolyzing enzymes. The mammalian genome contains 10 enzymatically active secreted PLA2s (sPLA2s) and two sPLA2-related proteins devoid of lipolytic enzymatic activity. In addition to the well-established functions of one of these enzymes in digestion of dietary phospholipids and another in host defense against bacterial infections, accumulating evidence shows that some of these sPLA2s are involved in arachidonic acid release from cellular phospholipids for the biosynthesis of eicosanoids, especially during inflammation. More speculative results suggest the involvement of one or more sPLA2s in promoting atherosclerosis and cancer. In addition, the mammalian genome encodes several types of sPLA2-binding proteins, and mounting evidence shows that sPLA2s may have functions related to binding to cellular target proteins in a manner independent of their lipolytic enzymatic activity.

Contents

INTRODUCTION

Phospholipases A_2 (PLA2s) are a class of enzymes that hydrolyze the *sn*-2 ester of glycerophospholipids to release a free fatty acid and a lysophospholipid (**Figure 1**). The PLA2 family is larger than the phospholipase C and D families and is composed of secreted PLA2s (sPLA2s) and intracellular PLA2s (1, 2).

At the cellular level, sPLA2s may function during secretion (in the secretory compartment or in the extracellular space in an autocrine or paracrine manner) and after internalization. The control of sPLA2 function occurs at the transcriptional level, but also by regulatory mechanisms during secretion, by proteolytic maturation, by biophysical properties of the membrane surface in which these enzymes operate, and by inhibition and degradation owing to the binding of the sPLA2 to specific binding proteins.

Many biological functions have been attributed to sPLA2s, but definitive in vivo evidence is lacking in many cases. Tools including sPLA2-deficient mice and small molecular weight sPLA2 inhibitors are being developed to help understand the physiological roles of sPLA2s. There is the possibility that some sPLA2 biological functions are not conserved from mouse to humans. This is based on differences in the tissue distribution of some sPLA2s in mice and humans (3).

This review summarizes our current knowledge of sPLA2 structure, catalytic mechanisms, membrane-binding properties, and physiological roles of mouse and human sPLA2s. Also emphasized are the most recent aspects of sPLA2s reported over the past decade. In addition, the mammalian genome encodes a secreted PLA2 that hydrolyzes the platelet-activating factor and oxidized phospholipids (PAF-acetylhydrolyase) (2). The PAF-acetylhydrolyase enzyme is not discussed as it does not belong to the same structural family as the large group of sPLA2s covered in this chapter.

PLA2: phospholipase A_2

sPLA2: secreted PLA2

STRUCTURAL FEATURES OF sPLA2s

As far as we can tell by sequence alignment searching, the human genome contains 9 sPLA2 genes, and the mouse genome contains 10, all of which have experimentally observed *sn*-2 phospholipolysis enzymatic activity. The mouse genome contains group IIC sPLA2, which exists in humans as a pseudogene. In addition, the mammalian genome also contains two proteins that are structurally related to sPLA2s but lack enzymatic activity (4, 5).

Table 1 lists some of the structural properties of enzymatically active mouse and human sPLA2s. These 10 sPLA2s were given group names on the basis of their disulfide bond pattern and on the order of their discovery in relationship to other PLA2s (2).

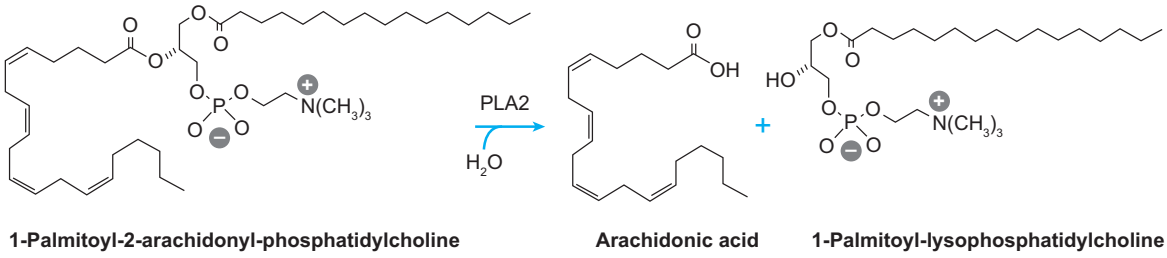

Figure 1

Reaction catalyzed by PLA2. Shown is the hydrolysis of the *sn*-2 ester of 1-palmitoyl-2-arachidonyl-phosphatidylcholine to give free arachidonic acid plus 1-palmitoyl-lysophosphatidylcholine. Many PLA2s can hydrolyze the *sn*-2 ester of phospholipids with other polar head groups and fatty acyl chains. Some substrates have an ether linkage at the *sn*-1 position (6).

There is little value in explaining the origin of the group naming here; we simply list them in **Table 1**. These proteins are disulfide rich and have molecular weights of ~16 kDa except for the group III sPLA2, whose cDNA predicts a protein with a molecular weight of ~55 kDa. Group III sPLA2 has a 130-amino acid N-terminal extension followed by an sPLA2 domain, which is similar to the bee venom sPLA2 (a classical group III sPLA2), followed by a 219-amino acid C-terminal extension. The functions of these extensions are not known, and they are not homologous to other known proteins. The group IIF sPLA2 has a 23-amino acid, proline-rich C-terminal extension with a single cysteine residue. Because this cysteine is not involved in a classical sPLA2-type disulfide bond, it is possible that the group IIF sPLA2, similar to some snake venom sPLA2s, exists as a disulfide-linked homodimer or heterodimer in a physiological setting (55), but this requires investigation.

As mammalian sPLA2s are disulfide rich, reduction by 2-mercaptoethanol or by dithiothreitol is often used to determine if the PLA2 enzymatic activity in a biological sample is caused by an sPLA2. However, caution is advised because the sensitivity of the mammalian sPLA2s to inactivation by treatment with dithiothreitol varies greatly among the different groups (6). For example, human group IIA sPLA2 is completely inactivated by 10 mM dithiothreitol after 30 min at room temperature, whereas human group IIE sPLA2 retains 55% of its activity even after 30 min at 50°C.

All mouse and human sPLA2s appear to have an N-terminal signal peptide, and these enzymes are secreted either from their native tissues or from transfected mammalian cells. The group IB sPLA2, also known as the pancreatic sPLA2, is secreted into the gastrointestinal tract as a component of pancreatic juice, as a zymogen of low enzymatic activity containing a 7-amino acid propeptide. The propeptide is removed by trypsin to generate the active enzyme (7). The group X sPLA2 also contains a propeptide, and studies with transfected cells show that a portion of the sPLA2 is found in the culture medium as a mature protein lacking the propeptide (8, 9). The protease responsible for propeptide removal from group X sPLA2 is not known.

Among mammalian sPLA2s, X-ray structures are available for porcine and bovine pancreatic sPLA2 (group IB) (10), human group IIA sPLA2 (10), and human group X sPLA2 (11). All of these enzymes share a common protein fold (**Figure 2**). Using this structural data and sequence alignment, group IB, IIA, IIC, IID, IIE, IIF, V, and X mammalian sPLA2s share a common three-dimensional structure. The sPLA2 domain of human group III sPLA2 has a distinct fold and is expected to be similar to that of the group III sPLA2 from bee venom (10).

Table 1 Structural and catalytic properties of mouse and human sPLA2s

sPLA2 group name	Propeptide[a]	Number of disulfides	N-glycosylation[b]	Extra structural features	Specific activity on POPG μmol/(min x mg)[b]	K_{Ca}^{app} (μM)[c]
IB	Y(expt)	7	N(expt)	Pancreatic loop	1030 (hu)	8 (hu)
					720 (mo)	8 (mo)
IIA	N (expt)	7	N(expt)	Short C-terminal extension	220 (hu)	13 (hu)
					140 (mo)	4 (mo)
IIC[d]	N(pred)	8	Y(expt)	Short C-terminal extension	— (hu)	— (hu)
					12 (mo)	14 (mo)
IID	N(expt)	7	Y(expt) (hu)	Short C-terminal extension	0.9 (hu)	11 (hu)
			N(expt) (mo)		0.9 (mo)	46 (mo)
IIE	N(pred)	7	N(pred)	Short C-terminal extension	0.5 (hu)	112 (hu)
					0.1 (mo)	100 (m)
IIF	N(pred)	7	Y(expt)	23-amino acid, long Pro-rich C-terminal extension with odd Cys	18 (hu)	27 (hu)
					4 (mo)	35 (mo)
III	Y(pred)	5 (sPLA2 domain)	Y(expt)	130-amino acid N-terminal extension, 219-amino acid C-terminal extension	— (hu)[e]	6 (hu)
					— (mo)[e]	— (mo)
V	N(expt)	6	N(expt)		24 (hu)	1 (hu)
					40 (mo)	1 (m)
X	Y(expt)	8	Y(expt) (hu)	Short C-terminal extension	14 (hu)	1.5 (hu)
			N(expt) (mo)		30 (mo)	48 (mo)
XIIA	N(pred)	7	N(pred)		0.3 (hu)	4 (hu)
					— (mo)	— (mo)

[a]Y(expt) means the existence of a propeptide was experimentally verified; Y(pred) means the existence of a propeptide was predicted by amino acid sequence analysis (using **http://www.cbs.dtu.dk/services/ProP/**), but there is no experimental data; same for N(expt) and N(pred). It is unclear for group III sPLA2 if the long N-terminal and C-terminal extensions serve as a propeptide or as a functional domain, possibly regulating catalytic activity (162).

[b]Abbreviations: hu, human; mo, mouse; POPG, 1-palmitoyl-2-oleoyl-sn-glycero-3-glycerol.

[c]All values are for hydrolysis of phosphatidylglycerol vesicles except phosphatidylmethanol vesicles were used for hGIII (6, 163).

[d]Group IIC is a pseudogene in humans.

[e]The kinetic properties of highly purified hGIII and mGIII have not yet been reported. Studies with partially purified hGIII expressed in HEK293 cells suggest that this enzyme displays a high level of sPLA2 activity on phosphatidylglycerol and phosphatidylcholine vesicles typical of other highly active sPLA2s (i.e., group IB, IIA, IIC, IIF, V and X sPLA2s) (163).

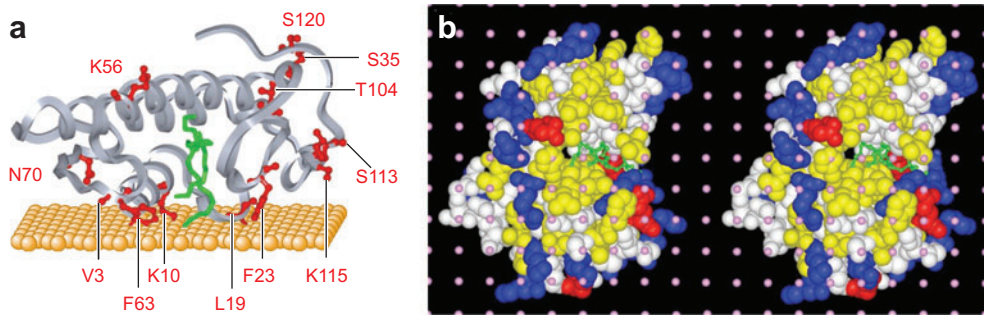

Figure 2

(*a*) Ribbon diagram of hGIIA at the membrane interface using the X-ray structure of the protein (164) and studies using site-specific spin labels (21). A short chain phospholipid analog in the active site is shown in the center. (*b*) Stereoview of membrane-docked hGIIA as viewed perpendicular to the membrane. Reprinted from Reference 21 with permission.

Finally, group XIIA sPLA2 represents the third structural class among mammalian sPLA2s. All sPLA2s share the common structural feature of a ~15-Å-deep active site slot where a single phospholipid molecule binds to position the enzyme-susceptible ester next to the catalytic residues (**Figure 2*a***). The region of the protein's surface that surrounds the opening to the active site slot constitutes the interfacial binding surface that is in direct contact with the membrane when the enzyme transfers from the aqueous phase to the membrane surface (**Figure 2*b***). The occurrence of these structurally distinct surfaces of sPLA2s has important consequences for understanding the interfacial kinetic behavior of these enzymes as is briefly described below.

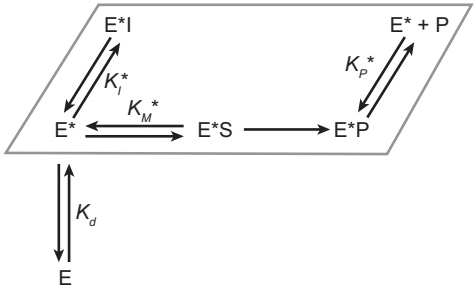

Figure 3

Interfacial binding and catalysis by PLA2. The steps occurring on the membrane are enclosed in the parallelogram. K_d is the interfacial dissociation constant for the enzyme at the interface, E^*, to dissociate into the aqueous phase. $K_M{}^*$, $K_P{}^*$, and $K_I{}^*$ (for a competitive inhibitor) are the interfacial dissociation constants for substrate, product, and inhibitor, respectively, but are for steps confined to the membrane. Abbreviations: E*P, containing bound product; E*S, containing bound substrate.

sPLA2 INTERFACIAL KINETICS AND BINDING

Figure 3 shows the minimal kinetic scheme for the catalytic cycle of PLA2s, including sPLA2s. Because naturally occurring phospholipids have virtually no solubility in water and because sPLA2s can exist in a water-soluble state, the enzyme must adsorb to the substrate membrane interface for phospholipid hydrolysis to occur. The interfacial binding surface of the sPLA2 is distinct from the active site (**Figure 2**), so the enzyme bound to the membrane can exist with its active site devoid of bound substrate (E* state), containing bound substrate (E*S), which is a classical Michaelis complex, or containing bound product (E*P) (**Figure 3**). Kinetic proof for these distinct enzyme states comes from the observation that when binding of the enzyme to the interface is sufficiently tight, multiple catalytic cycles occur without the desorption of the enzyme into the aqueous phase (scooting mode catalysis) (12). Interfacial catalysis by sPLA2s has been reviewed in detail (13, 14), and only some critical features are mentioned below.

The substrate specificity of a PLA2 is controlled by a composite of two factors (15). First is the ability of the enzyme to bind to the membrane surface (E* to E equilibrium dissociation constant, K_d in **Figure 3**). Second, once bound, the relative velocity for the hydrolysis of different substrates species by E* is controlled by the relative interfacial specificity constant (k_{cat}^*/K_M^* in **Figure 3**). Many previously reported PLA2 substrate specificity studies do not disentangle these two factors, and thus the results cannot be interpreted. The interfacial and kinetic properties of all of the mouse and human sPLA2s (except group III) acting on phospholipid liposomes have been reported (6). The group V and X sPLA2s are unique among mammalian sPLA2s in that they display relatively high affinity for both anionic and zwitterionic phospholipid vesicles, whereas the other sPLA2s display stronger affinity for anionic phospholipid vesicles. For example, mouse and human IIA sPLA2 binds extremely weakly to phosphatidylcholine vesicles compared to anionic vesicles, including phosphatidylglycerol and phosphatidylserine (16, 17). Because group IIA sPLA2 bound to anionic vesicles is able to hydrolyze phosphatidylcholine with a catalytic efficiency similar to other phospholipid species, the extremely low activity of this enzyme on vesicles of high phosphatidylcholine content is caused by the presence of most of the enzyme in the aqueous layer rather than the inability of the active site to accept this zwitterionic phospholipid as a substrate (6). Group V and X sPLA2s hydrolyze phosphatidylcholine-rich vesicles at rates comparable to anionic phospholipid vesicles (6, 16). These properties of mammalian sPLA2s have important physiological consequences as described below.

It can be seen from **Table 1** that the range of specific activities for the hydrolysis of phosphatidylglycerol vesicles by the different mouse and human sPLA2s spans about four orders of magnitude. Poorly active sPLA2s are still able to bind to these vesicles and to bind calcium (6). The biological significance of this remains unclear because the functions of many of these enzymes are not known. The recent cloning of the group XIIB sPLA2-like protein from mice and humans, which is highly homologous to the group XIIA sPLA2 but totally lacks lipolytic enzymatic activity because of natural mutation to a key catalytic residue (4), most strikingly underscores the fact that the functions of some sPLA2s and sPLA2-like proteins are unrelated to their ability to hydrolyze the *sn*-2 position of glycerophospholipids. Some of these proteins may act as ligands for specific cellular targets as described below.

The systematic study of the ability of exogenously added sPLA2 to liberate arachidonic acid from mammalian cells in vitro was carried out (6). Only those sPLA2s that have a high specific activity of phospholipid hydrolysis and that can bind well to phosphatidylcholine-rich membranes, the group V and X sPLA2s, display the ability to release fatty acids when added to the culture medium of non-apoptotic mammalian cells (6). This is further discussed in the section on arachidonic acid release and eicosanoid generation, see below.

Because phospholipid binding to the catalytic site of the sPLA2 is calcium dependent, owing to direct coordination of substrate oxygen atoms with calcium, and because this catalytic step (E* to E.S* in **Figure 3**) cannot occur unless the enzyme is bound to the interface (E to E* step in **Figure 3**), the concentration of calcium that activates the sPLA2 depends on the structure of the phospholipid that forms the membrane to which the enzyme binds and that serves as the substrate (18). Thus, values of the dissociation constant for calcium, K_{Ca}, must be taken as apparent values. Values of this apparent K_{Ca} for the action of the full set of mouse and human sPLA2s acting on phosphatidylglycerol vesicles are listed in **Table 1**. The values are all less than 112 μM, and most are in the low micromolar range. Thus, there is no basis for the often used statement that sPLA2s are activated by millimolar concentrations of calcium.

It is now clear that the interfacial binding surface of sPLA2 contacts on the order of 20–40 phospholipids in the membrane interface. When vesicles are titrated with enzyme until no more enzyme can fit on the vesicles, each bound sPLA2 makes ~40 phospholipids unavailable to other bound sPLA2s (19). Electron paramagnetic resonance (EPR) studies of site-selectively spin-labeled bee venom group III and human group IIA sPLA2s define the approximate boundary of the interfacial binding site, revealing that it is not due to van der Waals contact of protein residues with just a few phospholipids (20, 21) (**Figure 2**). The EPR studies also show that these enzymes sit on the membrane surface with amino acid side chains penetrating into the glycerol ester backbone region or perhaps a bit deeper. In detergent micelles containing solubilized phospholipids (mixed micelles), the interfacial catalytic cycle was modeled according to the dual-phospholipid model (22). In this model, the enzyme in the aqueous phase binds a single phospholipid in the mixed micelle, and then a second phospholipid binds into the active site. However, on basis of the phospholipid bilayer studies mentioned above, it seems more likely that sPLA2s may cause segregation of several phospholipids in the mixed micelle so that their interfacial binding surface can interact with multiple phospholipids. The fact that each mixed micelle may contain only a few phospholipid molecules does not clarify the situation because phospholipids can exchange between mixed micelles.

Dissecting the role of specific sPLA2 amino acids in supporting interfacial binding to membranes is a complex subject that has been partially resolved. A detailed discussion is outside the scope of this review, but a few points are mentioned here. On a per amino acid basis, tryptophan stands out as the most potent promoter of interfacial binding to zwitterionic and anionic phospholipid vesicles (16, 23–25). The indole side chain is thought to penetrate into the glycerol backbone region of the bilayer. Similarly, tryptophan is often found near the membrane-aqueous boundary of transmembrane proteins (i.e., ion channels), and a systematic study of binding of amphipathic helices to membranes has shown that tryptophan is the most powerful contributor of aqueous-to-membrane partitioning (26). Not surprisingly, there are hydrophobic and electrostatic contributions to interfacial binding. The earlier report that basic residues (Lys and Arg) on the interfacial binding surface of a snake venom sPLA2 contribute about 50% of the total binding energy (27) has been challenged on the grounds that the authors changed basic residues to acidic ones (charge reversal), which overestimates the contribution of the basic residues because of a repulsion effect, and on thermodynamic arguments (28). Recent detailed studies with bee venom sPLA2 show that mutation of all five basic residues on the interfacial binding surface to neutral glutamines has a very modest effect on binding to anionic vesicles, and the effect is much more pronounced if the basic residues are mutated to glutamate (charge reversal) owing to a repulsive effect of anionic residues with the anionic interface (28). In the case of group IIA sPLA2s, the human enzyme contains a large excess of positive charge over its entire solvent-exposed surface (Lys and Arg residues) (24). It does not bind to phosphatidylcholine vesicles even in the presence of millimolar phospholipid concentration, but it binds very tightly to vesicles that contain a critical amount of anionic phospholipid (16, 17, 29). Detailed structural studies using EPR spectroscopy to map the interfacial binding surface of human group IIA sPLA2 show that it forms a protein-vesicle microaggregate with anionic vesicles (21). Thus, aggregate formation makes it difficult to study interfacial binding of this enzyme to anionic vesicles. Recent calculations show that electrostatic interaction of basic residues of group IIA sPLA2 with anionic vesicles is an important contributor to interfacial binding (30). In short, the highly cationic nature of group IIA sPLA2 probably accounts for its selective binding to anionic vesicles. Basic residues work together with hydrophobic

residues, mainly aromatic amino acids (especially tryptophan), to support interfacial adsorption of sPLA2 to membranes. Further understanding of the precise nature by which amino acid side chains engage in molecular contacts with membrane phospholipids will have to await high-resolution structural information; current structural information, including EPR (20, 21) and IR spectroscopy (31), only provides protein-membrane orientation and depth of membrane penetration information.

The important topic of inhibition of both sPLA2s and interfacial enzymes has been reviewed (13, 32–34), and only a few critical points are given here. Many previously reported inhibitors work not by binding directly to the enzyme's active site but by partitioning into the phospholipid interface and changing the physiochemical properties of the interface, which causes less enzyme to bind to membranes (resulting in a decrease in reaction velocity owing to a drop in the amount of E^*) (**Figure 3**). Such inhibition is nonspecific, and such compounds should not be used to study the role of sPLA2s in cellular processes or be used as therapeutic agents. Such nonspecific inhibitors can often be spotted by observing a lack of tight inhibitor structure-activity relationships and by the use of inhibitor concentrations approaching that of the substrate when a substantial fraction of the inhibitor is partitioned into the membranes. Studies of reliable sPLA2 inhibitors are given in more detail below.

sPLA2 CATALYTIC MECHANISM

The originally proposed active site catalytic mechanism for hydrolysis of the *sn*-2 ester of phospholipids by sPLA2s is shown in **Figure 4*a*,*b*** (35). It is based on high-resolution X-ray structures of several sPLA2s and the conservation of structural features of the active site, including an Asp-His catalytic diad and a Ca^{2+} ion bound by a peptide loop. Additional evidence for this mechanism came from the X-ray structures of different sPLA2s containing a bound phosphonate-phospholipid analog (10), a putative transition state analog inhibitor (36). The relevance of this bound inhibitor to the suggested catalytic mechanism is shown in **Figure 4*a*-*c***. However, a second mechanism for sPLA2 catalysis has been proposed by more recent kinetic and crystallographic studies by Jain & Berg (37). This mechanism involves two active site waters, one bound to calcium (**Figure 4*d*,*e***). Both mechanisms have two common features: Histidine functions as a general base to deprotonate a water molecule, and the calcium ion stabilizes the oxyanion derived from the substrate's carbonyl oxygen. At this point, there is insufficient data to determine which of the two proposed mechanisms is operative. The task of distinguishing the two mechanisms appears difficult.

A remarkable feature of sPLA2s is their ability to couple binding of enzyme to the membrane interface to catalytic efficiency such that interfacial activation occurs. Detailed studies by Jain & Berg (37) have shown that interfacial binding promotes binding of substrate to the active site (interfacial K_M^* allostery) and catalytic efficiency (interfacial k_{cat}^* allostery). Structural components of this allostery have been proposed (37).

sPLA2 INHIBITORS

More than 40 structural classes of sPLA2 inhibitors have been reported (for a review, see Reference 38). We do not review all of them here. All but a few structural classes of these inhibitors lack potency in that they block sPLA2 action with 50% inhibitory concentration (IC_{50}) values measured with in vitro PLA2 enzymatic assays in the low-micromolar range or at higher concentrations. It is our opinion that such compounds are not sufficiently potent to be used as reliable sPLA2 inhibitors in studies with mammalian cells or animals because addition of micromolar and higher concentrations of agents to cells often leads to off-target effects. In addition, the use of inhibitor concentrations comparable to the substrate

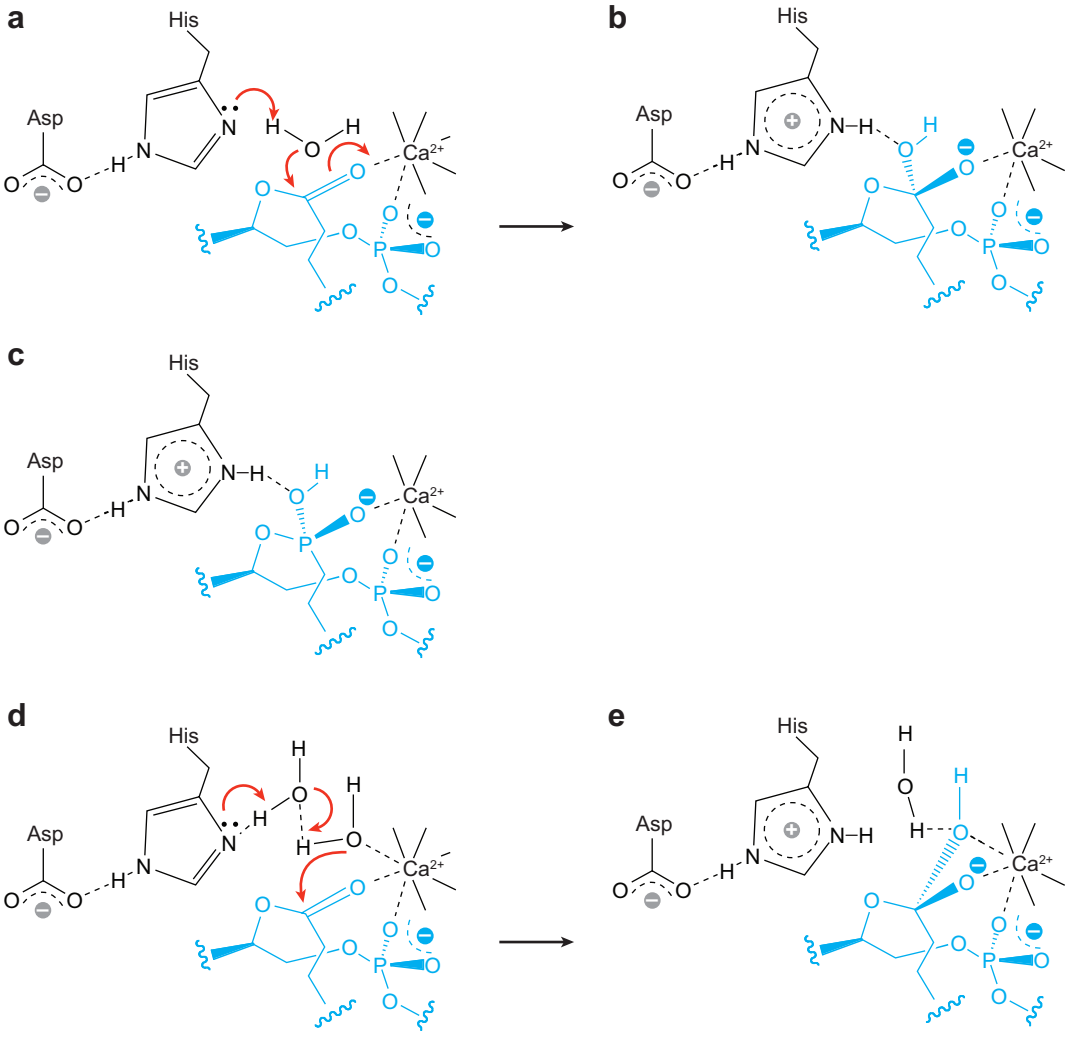

Figure 4

(***a***) The first proposed mechanism for sPLA2-catalyzed ester hydrolysis. A water molecule H bonded to the imidazole N of histidine attacks the substrate carbonyl carbon with a concomitant proton transfer from water to imidazole N. The carbonyl carbon of the ester substrate is bound to the catalytic calcium. (*b*) A proposed tetrahedral intermediate derived from the mechanism shown in panel *a*. The oxyanion derived from the substrate carbonyl oxygen is proposed to be stabilized by coordination to calcium. (*c*) The phosphonate-phospholipid analog bound in the active site as revealed by X-ray crystallography. The indicated structure mimics that proposed in panel *b*. (*d*) A two-water mechanism proposed by Jain and coworkers. A calcium-bound water was proposed as the attacking nucleophile, and a proton from this water was transferred to a second water concomitant with proton transfer from this second water to the histidine imidazole ring. (*e*) Proposed tetrahedral intermediate derived from the mechanism shown in panel *d*. Substrate, substrate-derived intermediates, and inhibitors are shown in blue.

concentration in the sPLA2 assay is problematic for interfacial enzymes (as discussed in detail in References 13 and 34). Also problematic are the uses of the marine natural products manoalide and scalaradial, which have been reported to irreversibly inactivate sPLA2s in vitro (39, 40). Mechanistic studies show that these compounds covalently modify multiple lysine residues found on the surface of many different sPLA2s. In the case of one detailed study, it was shown that manoalogue, a structural analog of manoalide, forms more than 20 distinct molecular adducts with bee venom sPLA2 (41). Thus, it is highly likely that these compounds inactivate several different classes of enzymes in cells. Despite these concerns, such low potency inhibitors and highly reactive inactivators continue to be reported in many publications as evidence for the involvement of sPLA2 in cellular processes; these studies should be re-examined using specific and potent inhibitors of sPLA2.

As far as we are aware, only those structural classes of sPLA2 inhibitors shown in **Figure 5** have low nanomolar potency against one or more sPLA2s in vitro. It is the surface concentration of inhibitor in the membrane that determines the degree of PLA2 inhibition. Because the fraction of total inhibitor in the assay that is portioned to the vesicles is not known, we expressed the potency in terms of molarity of inhibitor. The indole series, exemplified by varespladib (**Figure 5**), was first reported by workers at Eli Lilly and was discovered by a high-throughput screen to obtain lead compounds that were improved by a structure-based approach (42). The focus of the inhibition analysis was on human group IIA sPLA2 because this was the first non-pancreatic sPLA2 to be discovered, and there were several studies showing that high levels of this enzyme are associated with inflammatory diseases in humans. Several analogs in this indole series have been reported [for example, (43–47)], and recent studies have reported an analysis of these compounds on the full set of human and mouse sPLA2s (6, 46, 47). Crystal structures are available for analogs in this series bound to human group IIA and X sPLA2s and show that the amide carbonyl and carboxylate are direct ligands to the active site calcium (46, 48).

Another class of highly potent sPLA2 inhibitors is exemplified by compound 2b (**Figure 5**) with a reported IC_{50} against the human group IIA enzyme in vitro of 29 nM (49). This compound is an analog of a series of potent amide-based sPLA2 inhibitors first reported by workers at Fisons (50). Compounds in this series have been tested so far only on human group IIA and porcine pancreatic sPLA2s. The X-ray structure of compound 2b bound to human group IIA sPLA2 has been reported and shows that the amide carbonyl and carboxylate are direct ligands to the active site calcium (49). Oral administration of compound 2b to rats at 5 mg kg^{-1} day^{-1} results in inhibition of edema in an adjuvant-induced arthritis model (49). This inhibitor is also active in several other models of inflammatory diseases (38). These results are encouraging, but whether they are due to inhibition of rat group IIA sPLA2, another sPLA2, or a non-sPLA2 target remains to be established.

Pyrazole-1 (**Figure 5**) was obtained in a structure-based design by workers at Eli Lilly (51). This compound was tested on the full set of mouse and human sPLA2s in vitro and found to inhibit human group IIA and IID sPLA2s with an IC_{50} of 80 and 40 nM, respectively, and to be much less active on all other enzymes ($IC_{50} > 1$ μM) (6). Experimental data for the structure of enzyme-bound pyrazole-1 have not been reported.

YM-26734 (**Figure 5**) from Yamanouchi Pharma was tested on a subset of mammalian sPLA2s and found to inhibit the group IIA, IID, IIE, V, and X sPLA2s with IC_{50} values in the 0.2–1 μM range (it is not clear if these are mouse or human enzymes) (52). Administration of YM-26734 reduced the accumulation of exudate fluids and leukocytes in a carageenin-induced rat pleurisy model (52).

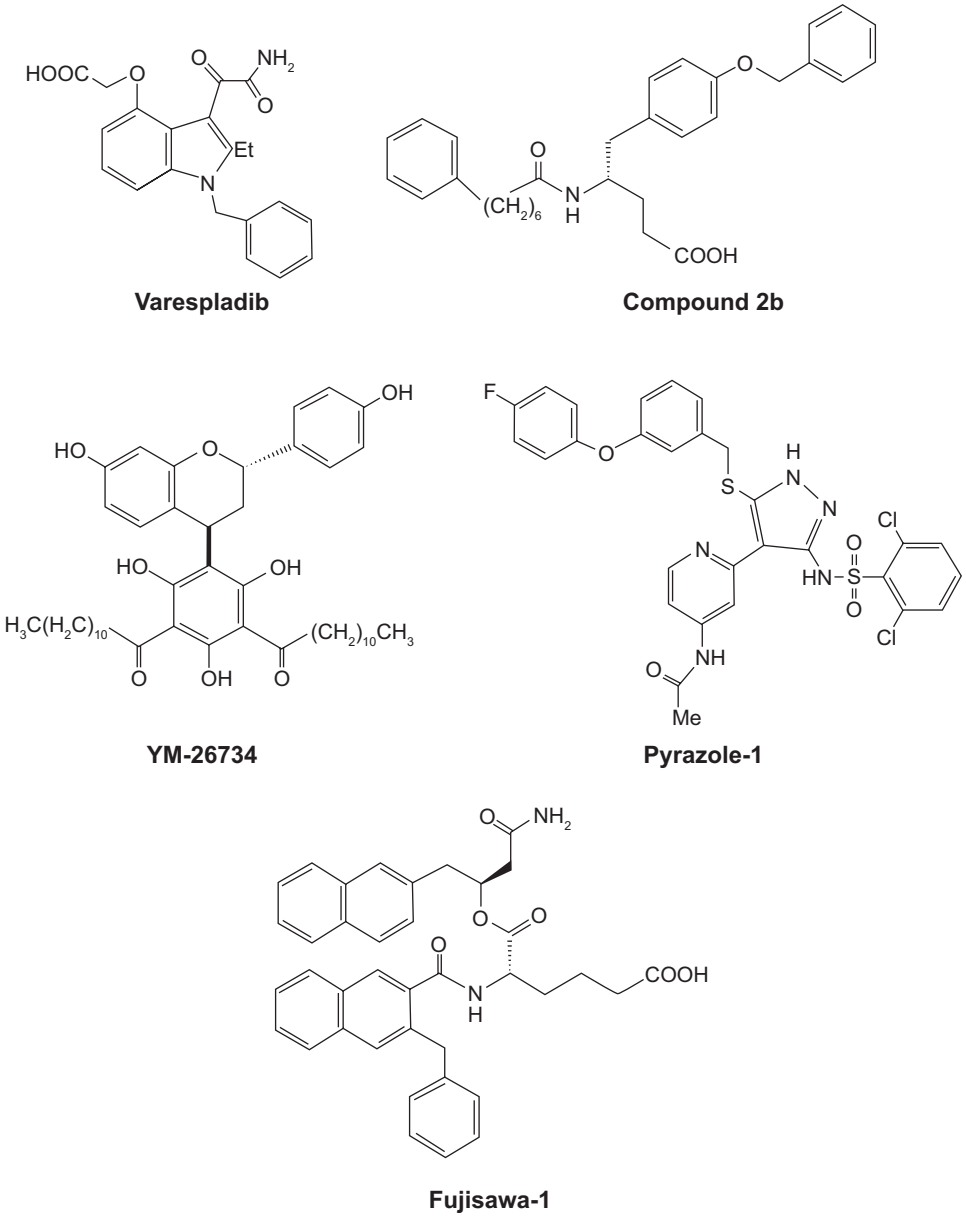

Figure 5

Structure of sPLA2 inhibitors with submicromolar potency (51–53).

The compound Fujisawa-1 (**Figure 5**) was reported to inhibit human group IIA sPLA2 by 100% at a concentration of 1 μM using an in vitro liposomal assay (53). No additional data about this compound is available.

GROUP IB sPLA2 AND DIETARY PHOSPHOLIPID DIGESTION

Group IB (pancreatic) sPLA2 is present at high levels in pancreatic juice that enters the gastrointestinal tract. In mice with the group

IB sPLA2 gene disrupted, no PLA2 activity was detected in a pancreatic lysate when assayed with phosphatidylcholine/cholate-mixed micelles (54). It may be noted that the ability of all mouse sPLA2s to hydrolyze these mixed micelles has not been reported, so it is possible that the pancreas lysate contains other sPLA2s besides the group IB enzyme (3, 55). Nevertheless, this data as well as the high expression of group IB sPLA2 in pancreas and stomach (56) suggest that group IB sPLA2 is the major enzyme responsible for hydrolysis of mixed micelles of cholate with the major dietary phospholipid, phosphatidylcholine.

Mice deficient in group IB sPLA2 appear normal and develop normal body weight when fed a standard chow diet (54). When these mice were fed phosphatidylcholine including an *sn*-2 [^{14}C]oleoyl group in the form of phosphatidylcholine liposomes containing cholesterol, all of the radiolabel was found in the lymph as [^{14}C]oleate (54). Cholesterol uptake under these conditions was the same in group IB sPLA2-deficient and wild-type mice (54). This is despite the fact that other studies show that dietary phospholipid reduces cholesterol uptake. All of these results are consistent with a compensation in the loss of group IB sPLA2 by other enzymes capable of hydrolyzing phospholipids. Candidate-compensating enzymes are pancreatic carboxyl ester lipase (54) and intestinal brush border phospholipase B (57).

In contrast to the studies described above, group IB sPLA2-deficient mice fed a high fat, western diet are resistant to obesity and obesity-related insulin resistance (58). Insulin resistance was measured by injecting insulin into mice fed a high-fat diet and measuring the drop in blood glucose. All together, the studies show that the absence of group IB sPLA2 can be compensated in mice only if a low-fat diet is fed. Cholesterol uptake in these studies with mice fed a high-fat diet was not reported, and it is still an open question whether inhibitors of pancreatic PLA2 would modulate cholesterol uptake in mice and humans fed a high-fat diet.

Additional studies show that lysophosphatidylcholine levels in the blood and liver rise after feeding wild-type mice a high-fat diet and that this increase is significantly attenuated in group IB sPLA2-deficient mice (59). Furthermore, the lower levels of lysophosphatidylcholine in the sPLA2-deficient mice are thought to be directly responsible for the resistance to obesity-related insulin resistance observed in group IB sPLA2-deficient mice as infusion of lysophosphatidylcholine into these mice restores their insulin resistance to that of high-fat-fed wild-type mice (59). Addition of lysophosphatidylcholine to hepatoma cells suppressed insulin-stimulated glycogen synthesis (59) by an unknown mechanism. These studies with group IB sPLA2-deficient mice suggest that pancreatic PLA2 inhibitors may find use in humans for the treatment of high-fat diet-induced obesity and type II diabetes.

sPLA2s, ARACHIDONIC ACID RELEASE, AND EICOSANOID GENERATION

There is a large body of evidence showing that cytosolic phospholipase A_2-α (group IVA PLA2) liberates arachidonic acid from phospholipids of mammalian cells as the first step for the biosynthesis of eicosanoids (prostaglandins, leukotrienes, and others) (60). Evidence for the role of sPLA2s in eicosanoid generation is beginning to appear, but much more work is needed to fully understand if and how these enzymes modulate eicosanoid production. There is a large amount of data showing that human group IIA sPLA2 accumulates to high levels during inflammation, for example, in the synovial fluid of patients suffering from arthritis, especially rheumatoid arthritis, and in serum of septic patients (61), as well as in experimental

animals treated with lipopolysaccharide; however, we are not aware of any data clearly showing that human group IIA sPLA2 contributes significantly to this inflammation. A clinical trial of a compound related to varespladib (**Figure 5**) in rheumatoid arthritis showed a lack of efficacy (62); however, see Reference 63 for a critical discussion of this trial, including the fact that the concentration of the sPLA2 inhibitor in synovial fluid was not measured.

Hard evidence for the involvement of group IIA sPLA2 in arachidonic acid release is very limited. Murakami and coworkers (64) showed that stable overexpression of mouse group IIA sPLA2 in HEK293 cells leads to enhanced arachidonic acid release and prostaglandin E_2 production. This has been confirmed by Mounier et al. (65), but in this study, the reported ability of LY311727 (an analog of varespladib, **Figure 5**) to block arachidonic release (64) could not be confirmed. Additional studies show that human group IIA sPLA2-induced arachidonic acid release in HEK293 cells involves cytosolic phospholipase A_2-α (65). A different study showed that stable overexpression of human group IIA sPLA2 in mouse mesangial cells leads to enhanced arachidonic acid release but not from cells isolated from cytosolic phospholipase A_2-α-deficient mice (66). There are numerous studies from workers at Eli Lilly showing that addition of recombinant human group IIA sPLA2 to mammalian cells in vitro, such as those isolated from bronchoalveolar lavage, leads to eicosanoid production (67). Cho and coworkers (68) have shown that exogenous addition of human group V sPLA2 to human neutrophils leads to leukotriene production that is dependent on cytosolic phospholipase A_2-α. Addition of this sPLA2 to eosinophils also induces leukotriene production but in a cytosolic phospholipase A_2-α-independent manner (69).

All of the above studies were carried out with the exogenous addition or forcible overexpression of the sPLA2. Only a few studies show that endogenous group IIA sPLA2 can enhance arachidonic acid release. Extensive studies by Kudo and colleagues (70) with rat 3Y1 fibroblastic cells suggest a cooperation between rat group IIA sPLA2 and calcium-independent, intracellular group VIB PLA2 in arachidonic acid release. In cytokine-stimulated rat gastric mucosa (RGM1) cells, rat group IIA sPLA2 and cytosolic phospholipase A_2-α cooperate to release arachidonic acid, leading to prostaglandin E_2 production (71, 72). In P388D1 mouse macrophage-like cells, antisense oligonucleotide reduction of mouse group V sPLA2 leads to reduced eicosanoid production (73). Disruption of the gene for this sPLA2 leads to a ~50% reduction in zymosan-stimulated eicosanoid production from mouse peritoneal macrophages (74). Mouse group V sPLA2-deficient macrophages also show reduced ability to phagocytize zymosan particles, and this may be the basis for the reduced eicosanoid response to these particles. Studies with cytosolic phospholipase A_2-α inhibitors and deficient cells show that this enzyme is also involved with arachidonic acid release and eicosanoid production in response to multiple agonists (60), again showing that the sPLA2 acts together with cytosolic phospholipase A_2-α to maximize eicosanoid production.

In these cellular systems where an endogenous or overexpressed sPLA2 seems to be involved in arachidonic acid release and eicosanoid production, the highly potent indole-type sPLA2 inhibitors, including varespladib (**Figure 5**) and its analogs, fail to inhibit arachidonic acid release (65) or block release only at very high concentrations compared to those needed to block sPLA2 in vitro (72). This result, as well as a large body of additional evidence (65, 72), strongly shows that the group IIA sPLA2 acts prior to externalization from cells, presumably in the secretory compartment. Whether or not this is the case for all cellular/sPLA2

systems remains to be determined. The inability of the indole-type sPLA2 inhibitors to block sPLA2 in these cell systems constitutes a serious limitation to the use of these compounds in probing the role of sPLA2s in cellular processes. Efforts are underway in both authors' laboratories to explore the cellular action of additional analogs of these compounds as well as others shown in **Figure 5**.

Perhaps the strongest evidence for the involvement of an sPLA2 in eicosanoid-mediated inflammation comes from recent studies with mouse group X-deficient mice (76). These mice were studied in the context of an asthma model in which the allergen ovalbumin was given systemically followed by challenge to the airways. In this model, cysteinyl leukotrienes are known to play a major role in promoting airway inflammation. Immunohistochemical studies show that mouse group X sPLA2 is expressed in the airway epithelium and also in alveolar macrophages (76). Compared to wild-type mice, mouse group X sPLA2-deficient mice showed a dramatic reduction in a number of airway inflammation parameters, including eicosanoid production, inflammatory cell migration into the airways, mucus hypersecretion, Th2 cytokine production, and airway resistance to methacholine challenge (76). Knockout of cytosolic phospholipase A_2-α also causes a dramatic reduction of airway inflammation in this model (77), again showing both the involvement of an sPLA2 and a cytosolic PLA2 in mediating eicosanoid production.

In systems where an sPLA2 and a cytosolic PLA2 deficiency lead to a reduction in arachidonic acid release and eicosanoid production, it will be extremely difficult to determine the fraction of total released arachidonic acid that is the direct result of each enzyme's catalytic action. This is because both types of PLA2s have the enzymatic capacity to hydrolyze the *sn*-2 ester of membrane phospholipids, and the liberated arachidonic acid does not remain in the membrane from which it is generated. The molecular basis for the cooperation between sPLA2 and cytosolic PLA2 action remains to be clarified.

sPLA2s AND HOST DEFENSE AGAINST INFECTION BY BACTERIA, VIRUSES AND PARASITES

The most well-established biological function of group IIA sPLA2 is probably its role in innate immunity as a potent antimicrobial agent (78). The antibacterial activity of this sPLA2 was first demonstrated in the early 1980s by Elsbach, Weiss and colleagues (79, 80) who showed that the group IIA enzyme is present in rabbit serum, inflammatory exudates, and polymorphonuclear leukocytes, where it exerts a strong activity against Gram-negative *Escherichia coli* in synergy with the bactericidal/permeability-increasing protein. In this case, the bactericidal/permeability-increasing protein binds to and disrupts the lypopolysaccharide coat of Gram-negative bacteria and will allow the enzyme access to the underlying bacterial cell wall (78). The enzyme was then found to have a direct and potent antibacterial activity against many Gram-positive bacteria including *Staphyloccus aureus*, *Streptococcus pyogenes*, *Listeria monocytogenes*, and *Bacillus anthracis* (80–83). High concentrations of group IIA sPLA2 were found in tears, seminal plasma, intestinal lumen, inflammatory exudates, bronchoalveolar lavage, and serum of patients with bacterial infections, indicating that the enzyme is present at strategic locations to contribute to antibacterial defense in vivo (84–86).

The bactericidal activity of group IIA sPLA2 first requires electrostatic interactions between the highly basic enzyme and the acidic lipoteichonic acids of Gram-positive bacteria and then its enzymatic activity to hydrolyze the phospholipid membrane (87, 88). In vitro the purified enzyme or extracellular fluids containing the enzyme can readily

kill several types of Gram-positive bacteria (80–82, 88). Furthermore, addition of group IIA sPLA2 to human neutrophils, which do not express this enzyme, leads to a synergistic bactericidal activity toward *S. aureus* (89). In vivo transgenic mice overexpressing human group IIA sPLA2 are resistant to infection by *S. aureus*, *E. coli*, and *B. anthracis* (82, 90), and injection of the recombinant sPLA2 protects mice from infection by *B. anthracis* (82). The antibacterial function of group IIA sPLA2 is conserved among mammalian species because rabbit, guinea-pig, rat, mouse, and human enzymes all have strong antibacterial activity.

Several other sPLA2s exhibit antibacterial activity in vitro against various bacterial strains (81, 83, 88), but their in vivo role remains to be studied. The rank order potency among human sPLA2s to kill Gram-positive bacteria in vitro is: group IIA > X > V > XII > IIE > IB, IIF (88). Interestingly, group V sPLA2 was present in the phagosome of macrophages and regulated phagocytosis (75), suggesting that it may participate in killing of ingested bacteria. Several sPLA2s are also present in mouse and human eye (55, 56), and many participate in antibacterial activity. Many snake venom sPLA2s, including some catalytically inactive Lys-49 sPLA2-like proteins, also present antibacterial activity against Gram-negative and Gram-positive bacteria (91, 92). In the case of Lys-49 proteins, the catalytic activity is not necessary for the antibacterial activity.

Mammalian sPLA2s may also participate in host defense against viruses and parasites. Indeed, some venom sPLA2s and human group X sPLA2, but not human group IB and IIA sPLA2s, can inhibit infection by human immunodeficiency virus (93, 94). Similarly, group V and X sPLA2s prevent adenoviral infection in mammalian cells (95). Finally, venom and mammalian sPLA2s, such as group IB pancreatic sPLA2, can block infection of erythrocytes by *Plasmodium falciparum*, the parasite of malaria (96). Whether the other sPLA2s are effective against these pathogens and exert such antiviral and antiparasitic activities in vivo remains to be analyzed.

Together, the above information suggests that certain mammalian sPLA2s have distinct roles in host defense against various types of pathogens. By contrast, it is remarkable that certain bacterial pathogens, such as Gram-negative *Pseudomonas aeruginosa* and group A *Streptococcus*, as well as parvoviruses have acquired genes encoding specific sPLA2s that participate in infection as virulence factors and increase the severity of the associated diseases (97). Finally, it is interesting to note that, although endotoxins from *E. coli* are well-known inducers of the expression of group IIA sPLA2, some other factors, such as the lethal toxin of *B. anthracis*, can downregulate expression of this sPLA2, thus helping the bacteria to escape from the bactericidal action of group IIA sPLA2 (98).

sPLA2s AND ATHEROSCLEROSIS

Accumulating evidence indicates that at least three members of the sPLA2 family, group IIA, V, and X sPLA2s, are likely to exert multiple proatherogenic properties in the vessel wall (99–104). These sPLA2s may act by (*a*) generating proinflammatory lipid mediators, such as prostaglandins, thromboxanes, leukotrienes, and lysophospholipids; (*b*) hydrolyzing low-density lipoprotein (LDL) particles and converting them into more proatherogenic particles; and (*c*) promoting multiple inflammatory processes on various cells from the arterial wall. Over the past few years, particular attention was first paid to human group IIA sPLA2 for its potential role in atherosclerosis (105). Specifically, this sPLA2 is expressed in both normal and atherosclerotic human arteries, and increased sPLA2 plasma concentration has been associated with the risk of coronary events in stable patients (106, 107).

Although group IIA sPLA2 acts poorly on unmodified LDL, presumably because of its poor binding to phosphatidylcholine-rich membranes as discussed above, this enzyme shows enhanced ability to hydrolyze oxidized LDL. Also, this sPLA2 can bind to components of the extracellular matrix produced by smooth muscle cells of the vessel wall. Transgenic mice constitutively overexpressing human group IIA sPLA2 in various tissues (108) developed atherosclerotic lesions on both standard chow and atherogenic diets and have altered lipoprotein profiles. Transplanting bone marrow cells from these transgenic mice into LDL receptor-deficient mice increased atherosclerotic lesion formation (109). Furthermore, human group IIA sPLA2 transgenic mice with a macrophage-specific overexpression show increased lesion area and enhanced collagen deposition (110). Together, these studies suggest that human group IIA sPLA2 may be a factor in the initiation, progression, and/or rupture of lipid-rich atherosclerotic plaques by affecting plasma lipoprotein metabolism or by acting locally in the atherosclerotic plaque.

However, the recent discovery of group V and X sPLA2s in atherosclerotic foci plus the fact that these two enzymes more readily hydrolyze lipoproteins have raised the question of which sPLA2 types may contribute to atherosclerosis (100–102, 111). Among the 10 mammalian, enzymatically active sPLA2s, group V and X sPLA2s hydrolyze LDL and high-density lipoprotein (HDL) at least 20-fold more efficiently than group IIA sPLA2 (111–113). Like group IIA sPLA2, group V and group X enzymes are also present in human and mouse atherosclerotic lesions, but their respective distributions are partially different, suggesting nonredundant functions (100–102, 104, 111). Furthermore, a four-week high-fat diet upregulates the expression of mouse group V sPLA2 in aorta by fivefold, but not that of group IIA sPLA2 (102). The presence of these sPLA2s in atherosclerotic lesions and their capacity to hydrolyze LDL particles and to promote macrophage foam cell formation make them interesting proatherogenic factors for further study.

The recent work of Bostrom et al. (114) provides the first set of in vivo data demonstrating that group V sPLA2 is a potential factor in promoting atherosclerosis, at least in the mouse species. Overexpression of mouse group V sPLA2 by retrovirus-mediated gene transfer increased lesion area, whereas mice deficient in bone marrow-derived group V sPLA2 had reduced lesion area. The absence of an effect on plasma lipoproteins observed in this study indicates that the contribution of group V sPLA2 to atherosclerosis is mediated within the vascular intima, probably by altering the interaction of lipoproteins with proteoglycans at the surface of macrophages and by increasing collagen deposition. Although direct evidence for an in vivo role of group X sPLA2 is currently lacking, this enzyme is the most efficient among sPLA2s at hydrolyzing LDL and HDL, it promotes macrophage foam cell formation (111), and it renders LDL particles more proatherogenic to trigger mitogen-activated protein kinase activation, arachidonic acid release, expression of adhesion molecules on endothelial cells, and subsequent adhesion of monocytes (101).

The above studies raise the question of the respective contribution and possible redundancy of group IIA, V, and X sPLA2s in atherosclerosis, acting either on plasma lipoproteins at the systemic level and/or locally at the level of the vessel wall or inside atherosclerotic lesions. Despite the above studies with transgenic mice overexpressing group IIA sPLA2, the in vivo role of this enzyme is questionable because the endogenous mouse sPLA2 appears to be dispensable for atherosclerosis and because group IIA sPLA2 has much lower capacity to hydrolyze LDL than group V and X sPLA2s (112), especially in the presence of serum (102). Rather, group IIA sPLA2 may be an inflammatory marker of atherosclerosis. At first sight, all three enzymes are expected to be

released in the extracellular space within the lesion and thus may act in concert to trigger various atherosclerotic processes, including modification of LDL, local production of eicosanoids and lysophospholipids, activation of endothelial cells, and proliferation of vascular smooth muscle cells (100–102, 115). However, as the cellular sources of the three sPLA2s are only partially overlapping in lesions and may be temporally controlled (104), it remains to be seen if they really exert similar overlapping functions or distinct roles within the plaque. Last but not least, it was recently demonstrated that the potent sPLA2 inhibitor A-002 from Anthera Pharmaceuticals (structurally related to varespladib, **Figure 5**) is effective in treating atherosclerosis in C57BL/6 ApoE-deficient mice, with up to 50% reduction in the formation of atherosclerotic lesions (**http://www.anthera.com/**). The fact that this sPLA2 inhibitor, which was originally developed by Eli Lilly, inhibits group IIA, V, and X sPLA2s with high affinities (47) while C57BL/6 ApoE-deficient mice do not express mouse group IIA sPLA2 is evidence that either group V or group X sPLA2 or both contribute to atherosclerosis in the mouse.

MISCELLANEOUS ROLES OF sPLA2s

A large variety of biological functions have been proposed for sPLA2s, but definitive evidence for an in vivo role is lacking in many cases. Most of these functions have been attributed to the first identified sPLA2s, the group IB and IIA enzymes, and these functions appear related to disease states, including cancer (see below) and inflammatory diseases, e.g., rheumatoid arthritis, inflammatory bowel disease, colitis, Crohn's disease, pancreatitis, psoriasis, and multiple sclerosis, among others. Because of the discovery of novel sPLA2s, the redundant or specific roles of the full set of sPLA2s in these settings need to be evaluated.

In vitro group IB and IIA sPLA2s have been proposed to play a role in proliferation, migration, apoptosis, and removal of damaged cells, suggesting that sPLA2s play a role in cell fate and cancer (116–119). In vivo MacPhee et al. (120) first reported that in the $Apc^{Min/+}$ mouse, a model of familial adenomatous polyposis and colorectal cancer, strains carrying a mutated mouse group IIA sPLA2 gene developed more tumors than wild-type strains. These data suggested a tumor modifier role of this sPLA2 in the mouse and led to numerous studies exploring the association of the human group IIA sPLA2 gene with colorectal cancer, which at present have remained inconclusive (121). Additionally, group IIA sPLA2 may also play a role in prostate (122) and gastric (123) cancers. Group IIA and some other sPLA2s are expressed in various types of cancers (124), and group X and III sPLA2s may also play a role in tumorigenesis (125, 126). Despite numerous studies, the mechanistic roles of sPLA2s in promoting cell proliferation and cancer are either unknown, diverse, or controversial (121, 124, 127).

It was proposed that Group IIA sPLA2 plays an important role in acute respiratory distress syndrome, notably by degrading phospholipid lung surfactant (128). Pharmacological inhibition of the enzyme protects animals against acute lung injury (129). However, recent studies using group V sPLA2 transgenic mice suggest that this sPLA2 (130) and possibly other sPLA2s expressed in the lung (131) contribute to lung dysfunction because of surfactant hydrolysis. In vitro activation by sPLA2s IB, IIA, V, and X of human inflammatory cells present in the lung has also been widely documented and was recently reviewed by Triggiani and colleagues (132). Remarkably, the ability of some sPLA2s to induce degranulation, exocytosis, and production of cytokines and chemokines from alveolar macrophages and other inflammatory cells does not require lipolytic enzymatic activity but may be related to primary binding of sPLA2s to specific membrane targets.

sPLA2s are also likely to be involved in physiological and pathophysiological conditions in skin. Many sPLA2s are expressed in the different layers of mouse and human skin (133). A role of sPLA2 in lipid barrier integrity and photoprotection was proposed (134, 135). Transgenic mice overexpressing mouse and human group IIA sPLA2 develop hyperkeratosis, hyperplasia, and increased sensitivity to chemical carcinogenesis (136, 137).

Several mammalian and venom sPLA2s possess potent in vitro anticoagulant activity (138). sPLA2s that are highly cationic at physiological pH, as is the human group IIA enzyme, inhibit the formation of the prothrombinase complex through direct binding to factor Xa. This effect is independent of sPLA2 lipolytic enzymatic activity. By contrast, acidic sPLA2s, similar to the human group X sPLA2, inhibit coagulation in a phospholipid-dependent manner. It is unclear if this anticoagulant activity has a significant impact in vivo during injury and in cardiovascular diseases.

Although the role of sPLA2s in the central nervous system is not well documented, some sPLA2s, including group IIA and X, may contribute to neurotransmitter release, neuritogenesis, neuronal apoptosis, and pain and may participate in ischemia, multiple sclerosis, and Alzheimer's disease (139, 140).

Some sPLA2s may play a role during embryogenesis. Group IIF, V, and XIIA sPLA2s as well as the group XIIB sPLA2-like protein are highly and selectively expressed during mouse embryogenesis (5, 55). The first in vivo indication supporting a role of an sPLA2 during embryogenesis was demonstrated in a gain-of-function study in *Xenopus laevis*, showing that group XIIA sPLA2 acts as a neural inducer of ectopic olfactory structures by blocking bone morphogenetic protein signaling (141). In vitro studies in P19 mouse embryonic carcinoma cells indicate that mouse group XIIA sPLA2, and also *Drosophila* and *Xenopus* group XIIA sPLA2s, exert the same inhibitory effects on the signaling of bone morphogenetic proteins, suggesting a conserved mechanism of action from invertebrates to mammals. Interestingly, the mechanism of action appears to be independent of the enzymatic activity of group XIIA sPLA2.

Many sPLA2s and the group XIIB sPLA2-like protein are also expressed in reproductive organs, gestational tissues, skeletal muscle, heart, liver, kidney, thymus, spleen, and retina (4, 55, 142–144). The sPLA2-like protein otoconin-95, which is devoid of PLA2 activity, is highly and specifically expressed in the inner ear (5). The function of the different sPLA2s and sPLA2-like proteins expressed in these organs is essentially unknown.

sPLA2-BINDING PROTEINS

Mammalian sPLA2s display a huge range of specific activities for the hydrolysis of glycerophospholipids in vitro (6). In addition to mammals, sPLA2s are also present in the venom of snakes, bees, cnidaria, and scorpions. These venom sPLA2s are known to exert multiple toxic and pharmacological effects that cannot be fully explained by their enzymatic properties. Furthermore, some snake venom sPLA2s are catalytically inactive because they have a mutation in a critical active site residue, but they can be myotoxic or cytotoxic. This is reminiscent of the group XIIB sPLA2-like protein, which is found in the mouse and human genomes and lacks PLA2 activity owing to an active site mutation (4). Because these mutations occur in catalytic residues that have a well-understood function in promoting lipolysis, it seems unlikely that these enzymes show no enzymatic activity because their in vivo substrates have not yet been discovered.

Almost two decades ago, a model explaining the pharmacological effects of snake venom PLA2s was proposed by Kini & Evans (145). The model is that venom sPLA2s bind to cell targets distinct from

phospholipids to exert many of their toxic effects. In addition, enzymatic activity may contribute to the toxicity of venom sPLA2s that have functional catalytic sites. Endogenous mammalian sPLA2s with PLA2 activity and sPLA2-like proteins that lack this activity may be toxic as well if left unregulated. Therefore, it is likely that both venomous animals and mammals produce components that help regulate their endogenous sPLA2s and sPLA2-like proteins. All together, these concepts led to the search for and identification of several types of sPLA2-binding proteins that are likely to function as sPLA2 inhibitors or to promote some of the endogenous functions of mammalian sPLA2s.

The N-type and M-type receptors were the two first groups of sPLA2-binding proteins identified using, as ligand, the neurotoxic snake venom sPLA2 called OS_2 (146). OS_2 binds to the two receptors with exceptionally high affinities (picomolar range). The N-type receptors bind with high affinity several neurotoxic sPLA2s, are diverse in nature, and are highly expressed in mammalian brain membranes. They have not been purified or cloned, but a collection of distinct neuronal proteins has been identified using various other neurotoxic sPLA2s known to compete with iodinated OS_2 binding (146–148). For example, calmodulin, 14-3-3 proteins, R25, PDI, and two novel proteins of 45 and 46 kDa were identified using the neurotoxic ammodytoxins (148). Interestingly, the neuronal proteins identified using taipoxin, another neurotoxic sPLA2 homologous to OS_2, are members of the pentraxin superfamily (146). This family includes the short pentraxins, acute phase proteins, C-reactive protein, and serum amyloid P component, as well as the long pentraxins that play key roles in innate immunity (149, 150).

The M-type sPLA2 receptor is a 180-kDa protein that was cloned in various mammalian species as a membrane-bound and soluble secreted receptor (117, 146). This receptor is expressed in various tissues and is upregulated by various inflammatory stimuli (117, 151). It is most similar to the mannose receptor and belongs to the superfamily of C-type lectins, containing one or more carbohydrate recognition domains (117, 146). Importantly, several mammalian sPLA2s were identified as endogenous ligands of this receptor with low-nanomolar affinity, but a subset of mammalian sPLA2s do not bind to this receptor (152). For those sPLA2s that bind to the M-type receptor, addition of a soluble form of this receptor to sPLA2 lipolysis mixtures in vitro inhibits the reaction. Studies with mammalian cells that express the M-type receptor show that the membrane-bound receptor can internalize and lead to the degradation of the sPLA2, suggesting a role of the receptor in controlling sPLA2 activity, possibly in clearance of sPLA2s after secretion into the extracellular fluid (146). However, this receptor was also proposed to mediate several biological effects of group IB sPLA2 and to participate in inflammation during endotoxic shock (117).

The sPLA2 inhibitory role of the M-type receptor is further supported because other proteins, which also belong to the C-type lectin superfamily, bind and inhibit sPLA2s. Indeed, the lung surfactant protein SP-A can bind venom and mammalian sPLA2s in the nanomolar range and block their enzymatic activity (154). Similarly, snakes contain in their serum the PLI-α inhibitors, which are homologous to C-type lectins and block the enzymatic and toxic activities of their catalytically active and inactive (yet toxic) venom sPLA2s in the nanomolar range (155). Interestingly, snake serum also contains two other types of sPLA2 inhibitors with different molecular structures that bind and inactivate the enzymatic and toxic properties of endogenous snake venom sPLA2s (155). One inhibitor belongs to the superfamily of three-finger proteins and shares similarity with the urokinase-type plasminogen activator receptor and cell surface antigens of the Ly-6 superfamily. The second inhibitor shares identity with human α-2-glycoprotein, a leucine-rich

serum protein of unknown function. Serum from oppossum also contains a 64-kDa protein, which belongs to the immunoglobulin gene superfamily and is homologous to human α-1B-glycoprotein (155). Recently, a catalytically inactive group IIA venom sPLA2 was found to bind to the vascular endothelial growth factor receptor-1 and -2 with nanomolar affinities (156). Finally, several mammalian sPLA2s, including group IIA and V sPLA2s, are known to bind to heparan sulfate proteoglycans, including decorin, biglycan, and glypican-1 (105, 119, 157).

This diversity of sPLA2-binding proteins supports the view that sPLA2s are not only enzymes but are also ligands for a set of distinct proteins. The latter seems to apply to both enzymatically active and inactive sPLA2s. Most of the biological effects of mammalian sPLA2s have been proposed to occur by mechanisms dependent on their enzymatic activity. In some cases, specific sPLA2s have been proposed to act by mechanisms that are independent of their PLA2 activity (132, 152, 153). However, the nature of the sPLA2 membrane targets at the cell surface remains elusive in many if not all of these latter cases. A clear understanding of the binding properties of each mammalian sPLA2 in endogenous physiological or pathophysiological situations is required. There is much more to be learned about the best-characterized sPLA2-binding protein, the M-type receptor, because its role in several biological effects is either unknown or remains controversial. Finally, it was recently shown that many highly potent, competitive sPLA2 inhibitors (including the Lilly indole types, such as varespladib in **Figure 5**) are also blockers of binding to the M-type receptor (158). This is not surprising as the M-type receptor is thought to contact the face of the sPLA2 that includes the opening of the catalytic site, and many sPLA2 inhibitors protrude from the active site. These results underscore the possibility that many sPLA2 inhibitors may not block the biological functions of sPLA2s simply by blocking their PLA2 activity but also by blocking binding to specific sPLA2-binding proteins. At present, there are no active site inhibitors of sPLA2 that block receptor binding independent of blocking enzymatic activity and vice versa.

CONCLUDING REMARKS

Elucidating the in vivo biological functions of each sPLA2 is probably the most challenging area in this field. As indicated above, sPLA2s have been implicated or proposed to play a role in very divergent biological functions over the last two decades. The reasons for this stem from the discoveries that (*a*) the lipid products of sPLA2 activity and their derived metabolites are important in a large variety of physiological and pathological conditions; (*b*) certain sPLA2s, and most significantly group IIA, are upregulated in various disease states; (*c*) as secreted proteins, sPLA2s are able to attack both cellular and noncellular phospholipids of different origins, such as dietary phospholipids, bacterial membranes, lipoproteins, microvesicles (159), lung surfactant (128), and epidermal lamellar bodies (134); and (*d*) sPLA2s may act independently of their enzymatic activity, involving specific sPLA2-binding proteins. The occurrence of enzymatically active and inactive sPLA2s is reminiscent of triglyceride lipases, another class of secreted, lipid hydrolases, which also include enzymatically active and inactive variants, some with toxic properties (i.e., scorpion lipases) (160, 161). Many biological functions of sPLA2s have been proposed on the basis of in vitro data, but definitive evidence for a role in vivo is lacking in most cases. This is in part because many functions were originally attributed to group IIA sPLA2 (the first nonpancreatic sPLA2 to be discovered), these functions may not be conserved from mouse to humans, and in some cases, venom sPLA2s have been used in place of mammalian enzymes.

Now that the identification of all of the sPLA2s in the mammalian genome appears complete and tools for studying these

enzymes are becoming available, one can anticipate much progress in the coming decade in delineating the physiological functions of these enzymes in mammals.

FUTURE ISSUES

Some of the most important future areas of research include: (*a*) further analysis of the role of sPLA2s in eicosanoid biosynthesis, including the mechanisms of cross talk between sPLA2s and intracellular PLA2s; (*b*) the role of sPLA2s in inflammation leading to atherosclerosis; (*c*) the role of the M-type receptor in sPLA2 physiology and the molecular characterization of other sPLA2-binding proteins; and (*d*) the role of sPLA2s with low or no enzymatic activity.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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LITERATURE CITED

1. Valentin E, Lambeau G. 2000. *Biochim. Biophys. Acta* 1488:59–70
2. Schaloske RH, Dennis EA. 2006. *Biochim. Biophys. Acta* 1761:1246–59
3. Valentin E, Singer AG, Ghomashchi F, Lazdunski M, Gelb MH, Lambeau G. 2000. *Biochem. Biophys. Res. Commun.* 279:223–28
4. Rouault M, Bollinger JG, Lazdunski M, Gelb MH, Lambeau G. 2003. *Biochemistry* 42:11494–503
5. Verpy E, Leibovici M, Petit C. 1999. *Proc. Natl. Acad. Sci. USA* 96:529–34
6. Singer AG, Ghomashchi F, Le Calvez C, Bollinger J, Bezzine S, et al. 2002. *J. Biol. Chem.* 277:48535–49
7. Verheij HM, Slotboom AJ, De Haas GH. 1981. *Rev. Physiol. Biochem. Pharmacol.* 91:91–203
8. Cupillard L, Koumanov K, Mattéi MG, Lazdunski M, Lambeau G. 1997. *J. Biol. Chem.* 272:15745–52
9. Hanasaki K, Ono T, Saiga A, Morioka Y, Ikeda M, et al. 1999. *J. Biol. Chem.* 274:34203–11
10. Scott DL, Sigler PB. 1994. *Adv. Protein Chem.* 45:53–88
11. Pan YH, Yu BZ, Singer AG, Ghomashchi F, Lambeau G, et al. 2002. *J. Biol. Chem.* 277:29086–93
12. Jain MK, Rogers J, Jahagirdar DV, Marecek JF, Ramirez F. 1986. *Biochim. Biophys. Acta* 860:435–47
13. Gelb MH, Jain MK, Hanel AM, Berg O. 1995. *Ann. Rev. Biochem.* 64:653–88
14. Berg OG, Jain MK. 2002. *Interfacial Enzyme Kinetics*. West Sussex, Engl.: Wiley
15. Ghomashchi F, Yu B-Z, Berg O, Jain MK, Gelb MH. 1991. *Biochemistry* 30:7318–29

16. Bezzine S, Bollinger JG, Veatch SL, Keller SL, Gelb MH. 2002. *J. Biol. Chem.* 277:48523–34
17. Kinkaid AR, Wilton DC. 1995. *Biochem. J.* 308:507–12
18. Jain MK, Yu B-Z, Rogers J, Ranadive GN, Berg O. 1991. *Biochemistry* 30:7306–17
19. Ramirez F, Jain MK. 1991. *Proteins* 9:229–39
20. Lin Y, Nielsen R, Murray D, Mailer C, Hubbell WL, et al. 1998. *Science* 279:1925–29
21. Canaan S, Nielsen R, Ghomashchi F, Robinson BH, Gelb MH. 2002. *J. Biol. Chem.* 277:30984–90
22. Hendrickson HS, Dennis EA. 1984. *J. Biol. Chem.* 259:5740–44
23. Han SK, Kim KP, Koduri R, Bittova L, Munoz NM, et al. 1999. *J. Biol. Chem.* 274:11881–88
24. Beers SA, Buckland AG, Giles N, Gelb MH, Wilton DC. 2003. *Biochemistry* 42:7326–28
25. Gelb MH, Cho W, Wilton DC. 1999. *Curr. Opin. Struct. Biol.* 9:428–32
26. Wimley WC, White SH. 1996. *Nat. Struct. Biol.* 3:842–48
27. Han SK, Yoon ET, Scott DL, Sigler PB, Cho W. 1997. *J. Biol. Chem.* 272:3573–82
28. Bollinger JG, Diraviyam K, Ghomashchi F, Murray D, Gelb MH. 2004. *Biochemistry* 43:13293–304
29. Snitko Y, Koduri RS, Han SK, Othman R, Baker SF, et al. 1997. *Biochemistry* 36:14325–33
30. Diraviyam K, Murray D. 2006. *Biochemistry* 45:2584–98
31. Tatulian SA, Qin S, Pande AH, He X. 2005. *J. Mol. Biol.* 351:939–47
32. Gelb MH, Berg O, Jain MK. 1991. *Curr. Opin. Struct. Biol.* 1:836–43
33. Jain MK, Yu B-Z, Gelb MH, Berg OG. 1992. *Mediat. Inflamm.* 1:85–100
34. Gelb MH, Jain MK, Berg OG. 1994. *FASEB J.* 8:916–24
35. Kuipers OP, van den Bergh CJ, Verheij HM, de Haas GH. 1990. *Adv. Exp. Med. Biol.* 279:65–84
36. Lin H-K, Gelb MH. 1993. *J. Am. Chem. Soc.* 115:3932–42
37. Jain MK, Berg OG. 2006. *Curr. Opin. Chem. Biol.* 10:473–79
38. Reid RC. 2005. *Curr. Med. Chem.* 12:3011–26
39. Glaser KB, de Carvalho MS, Jacobs RS, Kernan MR, Faulkner DJ. 1989. *Mol. Pharmacol.* 36:782–88
40. de Carvalho MS, Jacobs RS. 1991. *Biochem. Pharmacol.* 42:1621–26
41. Ghomashchi F, Yu BZ, Jain MK, Gelb MH. 1991. *Biochemistry* 30:9559–69
42. Schevitz RW, Bach NJ, Carlson DG, Chirgadze NY, Clawson DK, et al. 1995. *Nat. Struct. Biol.* 2:458–65
43. Dillard RD, Bach NJ, Draheim SE, Berry DR, Carlson DG, et al. 1996. *J. Med. Chem.* 39:5137–58
44. Dillard RD, Bach NJ, Draheim SE, Berry DR, Carlson DG, et al. 1996. *J. Med. Chem.* 39:5119–36
45. Draheim SE, Bach NJ, Dillard RD, Berry DR, Carlson DG, et al. 1996. *J. Med. Chem.* 39:5159–75
46. Smart BP, Pan YH, Weeks AK, Bollinger JG, Bahnson BJ, Gelb MH. 2004. *Bioorg. Med. Chem.* 12:1737–49
47. Smart BP, Oslund RC, Walsh LA, Gelb MH. 2006. *J. Med. Chem.* 49:2858–60
48. Mihelich ED, Schevitz RW. 1999. *Biochim. Biophys. Acta* 1441:223–28
49. Hansford KA, Reid RC, Clark CI, Tyndall JDA, Whitehouse MW, et al. 2003. *ChemBioChem.* 4:181–85
50. Beaton HG, Bennion C, Connolly S, Cook AR, Gensmantel NP, et al. 1994. *J. Med. Chem.* 37:557–59

51. Hite GA, Mihelich ED, Tulio S, Edmund WS. 1998. *Eur. Patent No. 0 846 687 A1*
52. Hamaguchi K, Kuwata H, Yoshihara K, Masuda S, Shimbara S, et al. 2003. *Biochim. Biophys. Acta* 1635:37–47
53. Yoshimura S, Namiki TI, Imai KI, Namiki TI. 1997. *Jpn. Patent WO 97/03951*
54. Richmond BL, Boileau AC, Zheng S, Huggins KW, Granholm NA, et al. 2001. *Gastroenterology* 120:1193–202
55. Valentin E, Ghomashchi F, Gelb MH, Lazdunski M, Lambeau G. 1999. *J. Biol. Chem.* 274:31195–202
56. Eerola LI, Surrel F, Nevalainen TJ, Gelb MH, Lambeau G, Laine VJ. 2006. *Biochim. Biophys. Acta* 1761:745–56
57. Takemori H, Zolotaryov FN, Ting L, Urbain T, Komatsubara T, et al. 1998. *J. Biol. Chem.* 272:2222–31
58. Huggins KW, Boileau AC, Hui DY. 2002. *Am. J. Physiol. Endocrinol. Metab.* 283:E994–1001
59. Labonte ED, Kirby RJ, Schildmeyer NM, Cannon AM, Huggins KW, Hui DY. 2006. *Diabetes* 55:935–41
60. Ghosh M, Tucker DE, Burchett SA, Leslie CC. 2006. *Prog. Lipid Res.* 45:487–510
61. Pruzanski W, Vadas P, Browning J. 1993. *J. Lipid Mediat.* 8:161–67
62. Bradley JD, Dmintrienko AA, Kivitz AJ, Gluck OS, Weaver AL, et al. 2005. *J. Rheumatol.* 32:417–23
63. Pruzanski W. 2005. *J. Rheumatol.* 32:400–2
64. Murakami M, Shimbara S, Kambe T, Kuwata H, Winstead MV, et al. 1998. *J. Biol. Chem.* 273:14411–23
65. Mounier C, Ghomashchi F, Lindsay MR, James S, Singer AG, et al. 2004. *J. Biol. Chem.* 279:25024–38
66. Han WK, Sapirstein A, Hung CC, Alessandrini A, Bonventre JV. 2003. *J. Biol. Chem.* 278:24153–63
67. Fleisch JH, Armstrong CT, Roman CR, Mihelich ED, Spaethe SM, et al. 1996. *J. Pharmacol. Exp. Ther.* 278:252–57
68. Kim YJ, Kim KP, Han SK, Munoz NM, Zhu X, et al. 2002. *J. Biol. Chem.* 277:36479–88
69. Muñoz NM, Kim YJ, Meliton AY, Kim KP, Han S-K, et al. 2003. *J. Biol. Chem.* 278:38813–20
70. Kuwata H, Fujimoto C, Yoda E, Shimbara S, Nakatani Y, et al. 2007. *J. Biol. Chem.* 282:20124–32
71. Akiba S, Hatazawa R, Ono K, Kitatani K, Hayama M, Sato T. 2001. *J. Biol. Chem.* 276:21854–62
72. Ni Z, Okeley NM, Smart BP, Gelb MH. 2006. *J. Biol. Chem.* 281:16245–55
73. Shinohara H, Balboa MA, Johnson CA, Balsinde J, Dennis EA. 1999. *J. Biol. Chem.* 274:12263–68
74. Satake Y, Diaz BL, Balestrieri B, Lam BK, Kanaoka Y, et al. 2004. *J. Biol. Chem.* 279:16488–94
75. Balestrieri B, Hsu VW, Gilbert H, Leslie CC, Han WK, et al. 2006. *J. Biol. Chem.* 281:6691–98
76. Henderson WR Jr, Chi EY, Bollinger JG, Tien YT, Ye X, et al. 2007. *J. Exp. Med.* 204:865–77
77. Uozumi N, Kume K, Nagase T, Nakatani N, Ishii S, et al. 1997. *Nature* 390:618–22
78. Buckland AG, Wilton DC. 2000. *Biochim. Biophys. Acta* 1488:71–82
79. Elsbach P, Weiss J, Franson RC, Beckerdite-Quagliata S, Schneider A, Harris L. 1979. *J. Biol. Chem.* 254:11000–9

80. Weinrauch Y, Abad C, Liang NS, Lowry SF, Weiss J. 1998. *J. Clin. Investig.* 102:633–38
81. Beers SA, Buckland AG, Koduri RS, Cho W, Gelb MH, Wilton DC. 2002. *J. Biol. Chem.* 277:1788–93
82. Piris-Gimenez A, Paya M, Lambeau G, Chignard M, Mock M, et al. 2005. *J. Immunol.* 175:6786–91
83. Huhtinen HT, Gronroos JO, Gronroos JM, Uksila J, Gelb MH, et al. 2006. *APMIS* 114:127–30
84. Harwig SS, Tan L, Qu XD, Cho Y, Eisenhauer PB, Lehrer RI. 1995. *J. Clin. Investig.* 95:603–10
85. Saari KM, Aho V, Paavilainen V, Nevalainen TJ. 2001. *Investig. Ophthalmol. Vis. Sci.* 42:318–20
86. Nevalainen TJ, Eerola LI, Rintala E, Laine VJ, Lambeau G, Gelb MH. 2005. *Biochim. Biophys. Acta* 1733:210–23
87. Koprivnjak T, Peschel A, Gelb MH, Liang NS, Weiss JP. 2002. *J. Biol. Chem.* 277:47636–44
88. Koduri RS, Gronroos JO, Laine VJ, Le Calvez C, Lambeau G, et al. 2002. *J. Biol. Chem.* 277:5849–57
89. Femling JK, Nauseef WM, Weiss JP. 2005. *J. Immunol.* 175:4653–61
90. Laine VJ, Grass DS, Nevalainen TJ. 2000. *Infect. Immun.* 68:87–92
91. Paramo L, Lomonte B, Pizarro-Cerda J, Bengoechea JA, Gorvel JP, Moreno E. 1998. *Eur. J. Biochem.* 253:452–61
92. Chioato L, Aragao EA, Lopes Ferreira T, Ivo de Medeiros A, Faccioli LH, Ward RJ. 2007. *Biochim. Biophys. Acta* 1768:1247–57
93. Fenard D, Lambeau G, Maurin T, Lefebvre JC, Doglio A. 2001. *Mol. Pharmacol.* 60:341–47
94. Kim JO, Chakrabarti BK, Guha-Niyogi A, Louder MK, Mascola JR, et al. 2007. *J. Virol.* 81:1444–50
95. Mitsuishi M, Masuda S, Kudo I, Murakami M. 2006. *Biochem. J.* 393:97–106
96. Deregnaucourt C, Schrevel J. 2000. *J. Biol. Chem.* 275:39973–80
97. Sitkiewicz I, Stockbauer KE, Musser JM. 2007. *Trends Microbiol.* 15:63–69
98. Gimenez AP, Wu Y-Z, Paya M, Delclaux C, Touqui L, Goossens PL. 2004. *J. Immunol.* 173:521–30
99. Murakami M, Kudo I. 2003. *Curr. Opin. Lipidol.* 14:431–36
100. Webb NR. 2005. *Curr. Opin. Lipidol.* 16:341–44
101. Karabina SA, Brocheriou I, Le Naour G, Agrapart M, Durand H, et al. 2006. *FASEB J.* 20:2547–49
102. Rosengren B, Jonsson-Rylander AC, Peilot H, Camejo G, Hurt-Camejo E. 2006. *Biochim. Biophys. Acta* 1761:1301–8
103. Menschikowski M, Hagelgans A, Siegert G. 2006. *Prostaglandins Other Lipid Mediat.* 79:1–33
104. Kimura-Matsumoto M, Ishikawa Y, Komiyama K, Tsuruta T, Murakami M, et al. 2008. *Atherosclerosis.* 196:81–91
105. Hurt-Camejo E, Camejo G, Peilot H, Oorni K, Kovanen P. 2001. *Circ. Res.* 89:298–304
106. Kugiyama K, Ota Y, Takazoe K, Moriyama Y, Kawano H, et al. 1999. *Circulation* 100:1280–84
107. Mallat Z, Benessiano J, Simon T, Ederhy S, Sebella-Arguelles C, et al. 2007. *Arterioscler. Thromb. Vasc. Biol.* 27:1177–83
108. Ivandic B, Castellani LW, Wang XP, Qiao JH, Mehrabian M, et al. 1999. *Arterioscler. Thromb. Vasc. Biol.* 19:1284–90

109. Webb NR, Bostrom MA, Szilvassy SJ, van der Westhuyzen DR, Daugherty A, de Beer FC. 2003. *Arterioscler. Thromb. Vasc. Biol.* 23:263–68
110. Ghesquiere SA, Gijbels MJ, Anthonsen M, van Gorp PJ, van der Made I, et al. 2005. *J. Lipid Res.* 46:201–10
111. Hanasaki K, Yamada K, Yamamoto S, Ishimoto Y, Saiga A, et al. 2002. *J. Biol. Chem.* 277:29116–24
112. Pruzanski W, Lambeau G, Lazdunski M, Cho W, Kopilov J, Kuksis A. 2005. *Biochim. Biophys. Acta* 1736:38–50
113. Gesquiere L, Cho W, Subbaiah PV. 2002. *Biochemistry* 41:4911–20
114. Bostrom MA, Boyanovsky BB, Jordan CT, Wadsworth MP, Taatjes DJ, et al. 2007. *Arterioscler. Thromb. Vasc. Biol.* 27:600–6
115. Pruzanski W, Stefanski E, Kopilov J, Kuksis A. 2001. *Lab. Investig.* 81:757–65
116. Lee C, Park DW, Lee J, Lee TI, Kim YJ, et al. 2006. *Eur. J. Pharmacol.* 536:47–53
117. Hanasaki K, Arita H. 2002. *Prostaglandins Other Lipid Mediat.* 68–69:71–82
118. Brueseke TJ, Bell JD. 2006. *Biochim. Biophys. Acta* 1761:1270–79
119. Boilard E, Bourgoin SG, Bernatchez C, Poubelle PE, Surette ME. 2003. *FASEB J.* 17:1068–80
120. MacPhee M, Chepenik PK, Liddel AR, Nelson KK, Siracusa DL, Buchberg MA. 1995. *Cell* 81:957–66
121. Nakanishi M, Rosenberg DW. 2006. *Biochim. Biophys. Acta* 1761:1335–43
122. Dong Q, Patel M, Scott KF, Graham GG, Russell PJ, Sved P. 2006. *Cancer Lett.* 240:9–16
123. Leung SY, Chen X, Chu KM, Yuen ST, Mathy J, et al. 2002. *Proc. Natl. Acad. Sci. USA* 99:16203–8
124. Cummings BS. 2007. *Biochem. Pharmacol.* 74:949–59
125. Morioka Y, Ikeda M, Saiga A, Fujii N, Ishimoto Y, et al. 2000. *FEBS Lett.* 487:262–66
126. Murakami M, Masuda S, Shimbara S, Ishikawa Y, Ishii T, Kudo I. 2005. *J. Biol. Chem.* 280:24987–98
127. Scott KF, Graham GG, Bryant KJ. 2003. *Expert Opin. Ther. Targets* 7:427–40
128. Touqui L, Arbibe L. 1999. *Mol. Med. Today* 5:244–49
129. Furue S, Mikawa K, Nishina K, Shiga M, Ueno M, et al. 2001. *Crit. Care Med.* 29:719–27
130. Ohtsuki M, Taketomi Y, Arata S, Masuda S, Ishikawa Y, et al. 2006. *J. Biol. Chem.* 281:36420–33
131. Hite RD, Seeds MC, Safta AM, Jacinto RB, Gyves JI, et al. 2005. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 288:L618–24
132. Triggiani M, Granata F, Frattini A, Marone G. 2006. *Biochim. Biophys. Acta* 1761:1289–300
133. Haas U, Podda M, Behne M, Gurrieri S, Alonso A, et al. 2005. *J. Investig. Dermatol.* 124:204–11
134. Mao-Qiang M, Jain M, Feingold KR, Elias PM. 1996. *J. Investig. Dermatol.* 106:57–63
135. Scott GA, Jacobs SE, Pentland AP. 2006. *J. Investig. Dermatol.* 126:855–61
136. Grass DS, Felkner RH, Chiang MY, Wallace RE, Nevalainen TJ, et al. 1996. *J. Clin. Investig.* 97:2233–41
137. Mulherkar R, Kirtane BM, Ramchandani A, Mansukhani NP, Kannan S, Naresh KN. 2003. *Oncogene* 22:1936–44
138. Mounier CM, Bon C, Kini RM. 2001. *Haemostasis* 31:279–87
139. Masuda S, Murakami M, Takanezawa Y, Aoki J, Arai H, et al. 2005. *J. Biol. Chem.* 280:23203–14
140. Farooqui AA, Ong WY, Horrocks LA. 2006. *Pharmacol. Rev.* 58:591–620

141. Munoz-Sanjuan I, Brivanlou AH. 2005. *Mol. Cell. Biol.* 25:3608–19
142. Lappas M, Rice GE. 2004. *Prostaglandins Leukot. Essent. Fat. Acids* 70:87–100
143. Murakami M, Kudo I. 2004. *Biol. Pharm. Bull.* 27:1158–64
144. Kolko M, Christoffersen NR, Barreiro SG, Bazan NG. 2004. *J. Neurosci. Res.* 77:517–24
145. Kini RM, Evans HJ. 1989. *Toxicon* 27:613–35
146. Lambeau G, Lazdunski M. 1999. *Trends Pharmacol. Sci.* 20:162–70
147. Rouault M, Rash LD, Escoubas P, Boilard E, Bollinger J, et al. 2006. *Biochemistry* 45:5800–16
148. Kovacic L, Sribar J, Krizaj I. 2007. *Bioorg. Chem.* 35:295–305
149. Bottazzi B, Bastone A, Doni A, Garlanda C, Valentino S, et al. 2006. *J. Leukocyte Biol.* 79:909–12
150. Kirkpatrick LL, Matzuk MM, Dodds DC, Perin MS. 2000. *J. Biol. Chem.* 275:17786–92
151. Beck S, Beck G, Ostendorf T, Floege J, Lambeau G, et al. 2006. *Kidney Int.* 70:1251–60
152. Rouault M, Le Calvez C, Boilard E, Surrel F, Singer A, et al. 2007. *Biochemistry* 46:1647–62
153. Beck S, Beck G, Ostendorf T, Floege J, Lambeau G, et al. 2006. *Kidney Int.* 70:1251–60
154. Chabot S, Koumanov K, Lambeau G, Gelb MH, Chignard M, et al. 2003. *J. Immunol.* 171:995–1000
155. Marcussi S, Sant'Ana CD, Oliveira CZ, Rueda AQ, Menaldo DL, et al. 2007. *Curr. Top. Med. Chem.* 7:743–56
156. Yamazaki Y, Matsunaga Y, Nakano Y, Morita T. 2005. *J. Biol. Chem.* 280:29989–92
157. Murakami M. 2004. *Biol. Pharm. Bull.* 27:1179–82
158. Boilard E, Rouault M, Surrel F, Le Calvez C, Bezzine S, et al. 2006. *Biochemistry* 45:13203–18
159. Fourcade O, Simon MF, Viode C, Rugani N, Leballe F, et al. 1995. *Cell* 80:919–27
160. Aloulou A, Rodriguez JA, Fernandez S, van Oosterhout D, Puccinelli D, Carriere F. 2006. *Biochim. Biophys. Acta* 1761:995–1013
161. Wong H, Schotz MC. 2002. *J. Lipid Res.* 43:993–99
162. Murakami M, Masuda S, Shimbara S, Bezzine S, Lazdunski M, et al. 2003. *J. Biol. Chem.* 278:10657–67
163. Valentin E, Ghomashchi F, Gelb MH, Lazdunski M, Lambeau G. 2000. *J. Biol. Chem.* 275:7492–96
164. Scott DL, White SP, Browning JL, Rosa JJ, Gelb MH, Sigler PB. 1991. *Science* 254:1007–10

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