Enzymatic and receptor mediated effects of secretory phospholipase A_2 on the pathophysiology of inflammatory diseases

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Overview of phospholipases

Phospholipases A_2 are enzymes that share the common attribute to hydrolyze fatty acids from the *sn*-2 position of glycerol phospholipids [1–3]. Groups I, II, V and X PLA_2 are four sets of enzymes in a highly conserved family of secreted PLA_2 found in mammals [4–14]. Other non-secreted PLA_2 enzymes include group IV, cytosolic PLA_2 ($cPLA_2$) [15–17], group VI, calcium-independent PLA_2 ($iPLA_2$) [18–22] and groups VII and VIII, selective acetyl hydrolases [23–28]. The secretory family of enzymes has a number of features that distinguish them from other PLA_2 families including a relatively low molecular weight (~ 14 kDa), high disulfide bond content and a requirement for relatively high concentrations of calcium for maximal activation [29, 30]. In contrast, cytosolic enzymes are generally higher molecular weight proteins and require no calcium or very low calcium concentrations for optimal activation [18, 22]. Many $sPLA_2$ isotypes are synthesized as proenzymes that contain a signal peptide sequence that facilitates its release from cells. $sPLA_2$ isotypes have been studied extensively in mammals and in snake venoms, yet there is no clear understanding of their physiological and pathophysiological roles. Inspection of numerous publications dealing with $sPLA_2$s reveals that they have potential to mediate a wide range of biological activities including:

1) Producers of AA that contributes to eicosanoid formation [31–36];
2) Generation of lysophospholipids that contribute to electrophysiologic alteration that lead to arrythmogenesis in the heart or altered airway permeability and surfactant properties in the lung [37–48];
3) Potent antibacterial effects and implications in viral infections [49–54];
4) Key components in glycerophospholipid digestion [55];
5) Serum markers and potential regulators of severe illnesses such as sepsis, shock, organ injury and pancreatitis, all of which are linked to the development of adult respiratory distress syndrome or multiple organ failure [56–75];

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6) Regulators of platelet aggregation in hemorrhagic diseases [76–78];
7) Prevention of apoptosis of inflammatory cells and initiators of cell proliferation in several cancer cell lines [79–82];
8) A potent modifying locus in intestinal tumorigenesis in mice that is absent in human [83];
9) Pro-inflammatory components in diseases such as rheumatoid arthritis and asthma [84-93].

This overwhelming list of biological activities and diseases raises deep-seated questions as to whether $sPLA_2$ cause or is merely associated with the aforesaid effects. It also raises questions about molecular mechanisms that this family of enzymes could influence in order to control such a wide range of biological activities.

Role of $sPLA_2$ on cell function and animal physiology

With the milieu of so many potential biological activities, inhibitors, antibodies, antisense oligonucleotides and genetic models have been used to better define the essential processes induced by $sPLA_2$ secretion into sites of inflammation [94–105]. While some inhibitors, antibodies and antisense oligonucleotides have been developed that block $sPLA_2$ activity and inflammatory processes, the lack of selectivity among these reagents against different $sPLA_2$ isotypes make data interpretation ambiguous. Various genetic models have been discovered or developed in order to address the complex issue of the role $sPLA_2$ isotypes may play in diseases [106–111]. For example, peritoneal macrophages from mice with targeted gene disruption of group IV $cPLA_2$ show a marked reduction in their capacity to synthesize leukotriene B_4 (LTB_4), leukotriene C_4 (LTC_4), prostaglandin E_2 (PGE_2) and platelet-activating factor (PAF) [111]. These animals have attenuation in pulmonary responses and hyper-responsiveness after allergen challenge. In terms of $sPLA_2$, Nevalainen and colleagues designed experiments where transgenic mice expressed more than eighty fold more group II PLA_2 in most tissues including liver, lung, kidney and skin than non-transgenic littermates. Histopathological analysis of these animals revealed a disorder in skin consisting of hyperkeratosis, epidermal and adrenectral hypoplasia [112]. Chronic hypoplasia and hyperkeratosis observed in these animals is similar to that seen in a variety of skin disorders including human psoriasis. Certain mouse strains (C57BL/6, 129, A/J, C58 and P/J) have been shown to have a natural disruption of group IIA PLA_2 gene [106]. Thus, these strains are deficient in functional group IIA PLA_2 and have been used to determine the need for this enzyme in cell function. Interestingly, mast cells obtained from $PLA_2g2a^{+/+}$ and $PLA_2g2a^{-/-}$ mice both contained $sPLA_2$ activity and release similar quantities of AA upon antigen stimulation. Studies using these animals and antisense oligonucleotides reveal that group V $sPLA_2$, and not IIA, is likely an important $sPLA_2$ iso-

type in mast cell immune activation [113]. Similar studies using antisense oligonucleotide specific for group V PLA_2 in macrophages also demonstrated that group V PLA_2 has an important role in extracellular AA release after endotoxin and PAF stimulation [114]. Fonteh and colleagues have also shown that cells over-expressing group IIA PLA_2 and group V PLA_2 release more AA that mock-transfected cells [115]. Together, these studies show that various $sPLA_2$ isotypes can induce AA release from a variety of inflammatory cells.

Cytokine-like effects of $sPLA_2$ in inflammatory diseases

$sPLA_2$ may contribute to the pathogenesis of an inflammatory disease such as asthma in one of the following ways. First, $sPLA_2$ may induce lipid mediator formation. Second, $sPLA_2$ may induce degranulation of inflammatory cells leading to the release of preformed mediators such as histamine. Third, $sPLA_2$ may induce the synthesis of inflammatory cytokines. Our data show that $sPLA_2$ can also induce the formation of cytokines that prevent mast cells from undergoing apoptosis, thus preventing the resolution of inflammation. These potential effects of $sPLA_2$ summarized in Figure 1 and discussed in detail below can have significant ramifications in the management of inflammatory diseases.

Eicosanoid biosynthesis

Although the existence of $sPLA_2$ receptors has been recognized for several years, few studies have focused on the significance of receptor occupancy or the signaling mechanisms associated with receptor occupancy and how these events manifests themselves in inflammatory disease processes. Early work on $sPLA_2$ receptors focused on the neurotoxic effects of snake venom $sPLA_2$ acting through high affinity for $sPLA_2$ receptors. It has been speculated that somewhere along the evolution of $sPLA_2$, the mannose receptor may have been duplicated to accommodate other forms of and other functions of $sPLA_2$. These $sPLA_2$ receptors and their agonists ($sPLA_2$) regulate events in inflammatory cells that are critical to the pathogenesis of diseases such as asthma. Thus, $sPLA_2$ isotypes can act through their receptors in both autocrine and cytokine-like fashion. For example, mast cells contain and release group V PLA_2 during antigen activation and our data reveal that mast cells contain plasma membrane receptors and respond to receptor occupancy by $sPLA_2$ [116, 117]. A $sPLA_2$ receptor pathway could clarify several studies in the literature. For example, Arm and colleagues have shown that there are two phases of prostaglandin production in cultured mast cells that are primed with *c-kit* ligand and stimulated with antigen [118, 119]. AA is supplied for the first phase of prostanoids synthesis by $cPLA_2$ activation and AA for the second phase is provided by $sPLA_2$. It has

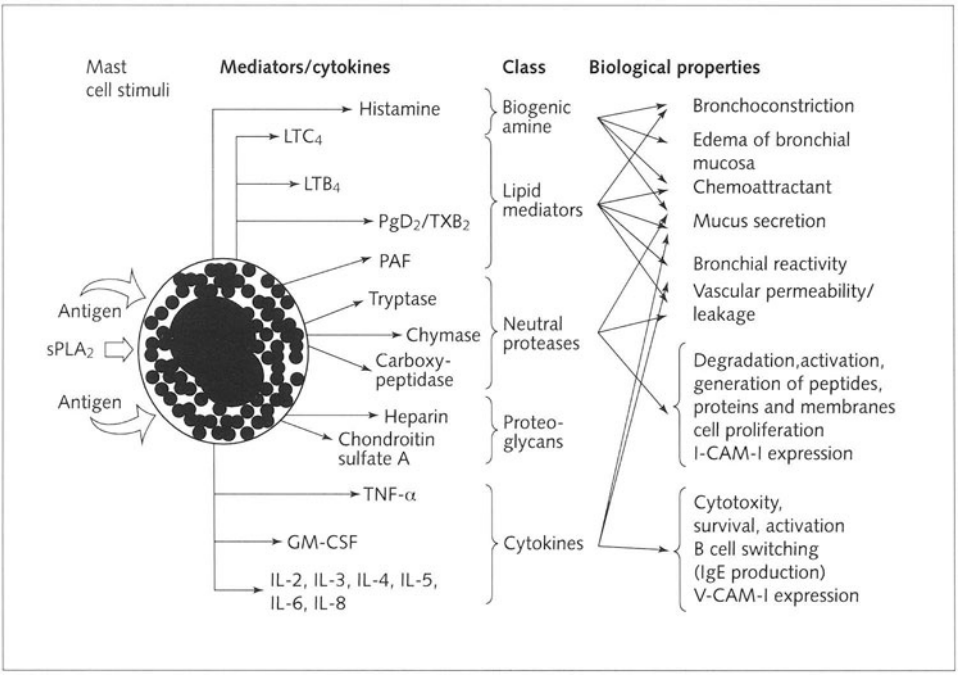

Figure 1
Stimulation of mast cells by $sPLA_2$, formation of mediators and their biological properties
$sPLA_2$ receptor occupancy results in the activation of mast cells. Mediators released include preformed biogenic amines (histamine), newly formed lipid mediators of inflammation (leukotrienes (LTC_4, LTB_4), prostaglandins (PGD_2), thromboxane (TXB_2) and platelet activating factor (PAF)), neutral proteases, proteoglycans and cytokines. These mediators induce various biological functions that are linked to disease processes.

been proposed that $sPLA_2$ hydrolyzes AA from membrane phospholipids to supply the substrate for PGD_2 biosynthesis. Fonteh and colleagues have provided evidence that $sPLA_2$ binds to cell surface receptors on mast cells that may initiate subsequent activation of $cPLA_2$, cyclo-oxygenase and lipoxygenase enzymes needed for AA release, prostaglandin formation and leukotriene biosynthesis, respectively [115, 116]. $sPLA_2$ also could have cytokine-like roles in that it could be released from one cell type and subsequently act on a variety of other cells types expressing $sPLA_2$ receptors. For example, it is well documented that high levels of $sPLA_2$ are found in serum of patients with sepsis, shock, organ injury or pancreatitis. Tools for studying these diseases suggest that group IIA $sPLA_2$ is involved. However, many of these studies used antibodies that are non-specific recognizing group IIA, V and X PLA_2. Thus, it is not clear which of the various $sPLA_2$ isotypes play a role in these diseases.

We have postulated that $sPLA_2$ isotypes released from mast cells after antigen challenge are found in sites of inflammation and these $sPLA_2$ isotypes induce lipid mediator formation or influence the recruitment and function of cells that participate in airway diseases. A study by Reddy and colleagues showing that mast cells can provide $sPLA_2$ to fibroblasts for prostaglandin production supports this postulation [120]. Cells other than mast cells can also provide $sPLA_2$ in airways. For example, using an enzyme-linked immunoassay specific for groups IIA and V PLA_2, has shown that human eosinophils have approximately 14-fold more of the enzymes than human neutrophils and this activity is released very rapidly upon cell activation [121]. In a related study, Hundley and colleagues have shown that $sPLA_2$ is released from human basophils and likely participates in leukotriene generation [122]. Thus, there is also potential for $sPLA_2$ from eosinophils or basophils to have both autocrine and cytokine effects in airway disease by inducing the formation of eicosanoids.

Degranulation of inflammatory cells

In addition to eicosanoid production, $sPLA_2$ has been shown to induce degranulation of several cells. Fonteh and colleagues showed that incubation of mast cells with different $sPLA_2$ isotypes resulted in the release of histamine. Likewise Triggiani and colleagues have duplicated these mast cell studies using macrophages, monocytes and eosinophils [123–125]. Their studies show that $sPLA_2$ induce the release of β-glucuronidase and the production of IL-6, IL-8, IL-12 and TNF-α by these cells. They conclude that this process is mediated *via* the mannose receptor and another receptor based on experiments showing that $sPLA_2$ isotypes are not cytotoxic to macrophages that were used in their studies. Together, these studies suggest that $sPLA_2$ may induce immune and inflammatory responses in cells by inducing exocytosis resulting in the release of mediators such as histamine and cytokines. Mast cells and macrophages also contain other proteases, which are released by activated inflammatory cells [126–129]. Induction of the release of these can result in bronchoconstriction, edema of the bronchial mucosa, chemoattraction, mucus secretion, vascular permeability and leakage, cell proliferation and expression of adhesion molecules (Fig. 1). Together, these biological properties constitute events related to inflammatory diseases of the airway.

Induction of cytokine formation

An important observation in mast cells, macrophages, monocytes and eosinophils incubated with $sPLA_2$ is that these cells while releasing AA still remain viable, indicating that these enzymes are not cytotoxic to these cells. This paradoxical effect can

be explained by the fact that $sPLA_2$ acts on receptors to induce processes that prevent apoptosis. For example, $sPLA_2$ acting on $sPLA_2$ receptors may activate mitogen-activated protein kinase (MAP kinase) and this process may result in the biosynthesis of cytokines, which prevent apoptosis. We confirmed this process by showing that incubation of bone marrow-derived mast cells with low concentrations of $sPLA_2$ resulted in the induction of IL-3 release into supernatant fluids. Only $sPLA_2$ isotypes that induced IL-3 production prevent apoptosis of mast cells [130]. A similar study showed that some $sPLA_2$ isotypes induce IL-6, IL-8, IL-12 and TNF-α formation by other inflammatory cells [123–125]. In addition to preventing apoptosis, cytokines induced by $sPLA_2$ also induce several biological effects including bronchial reactivity, chemoattraction, and activation of other inflammatory cells and the induction of adhesion molecules. Prevention of apoptosis of inflammatory cells such as mast cells and macrophages keep these cells longer in the site of inflammation and thus prevent quick resolution of the inflammatory process.

Mechanisms that account for the biologic functions of $sPLA_2$

$sPLA_2$ receptors

To date, most of the biological activities of $sPLA_2$ have been attributed to its capacity to hydrolyze membrane phospholipids. However, several of the biological functions described above cannot be easily reconciled with enzymatic activity alone. For example, intradermal injection of inactivated $sPLA_2$ causes similar phenotypic changes in skin to those observed when the fully active enzyme was injected [131]. Similarly, others have shown that the physiologic action of $sPLA_2$ is not due to hydrolytic activity [125, 132]. We have demonstrated that very low concentrations of $sPLA_2$ (low nanomolar levels) of certain $sPLA_2$ isotypes induce AA release, histamine release, and proliferation of some cells and enhance the survival of other cells in a receptor-mediated fashion [82]. Our studies also show that $sPLA_2$'s cause the selective release of AA and not other more abundant fatty acids from cells that express $sPLA_2$ receptors [115]. In contrast, cells that do not express $sPLA_2$ receptors do not selectively release AA when incubated with low amounts of $sPLA_2$.

Recently, different subtypes of membrane receptors for $sPLA_2$ have been identified in a variety of cells by determining their affinities for various types of $sPLA_2$. Arita and colleagues described the existence of a specific receptor family termed PLA_2-I receptor that is abundant in brain and several other tissues and has high affinity for the binding of pancreatic-type PLA_2 [133–136]. More recently, receptors have been divided into two classes termed N-type receptors (neuronal) or M-type receptor (muscle). Lambeau and colleagues report that a major difference between N-type and M-type receptors is their capacity to bind group III PLA_2 from bee venom [137, 138]. N-type receptor associates very tightly with both pancreatic

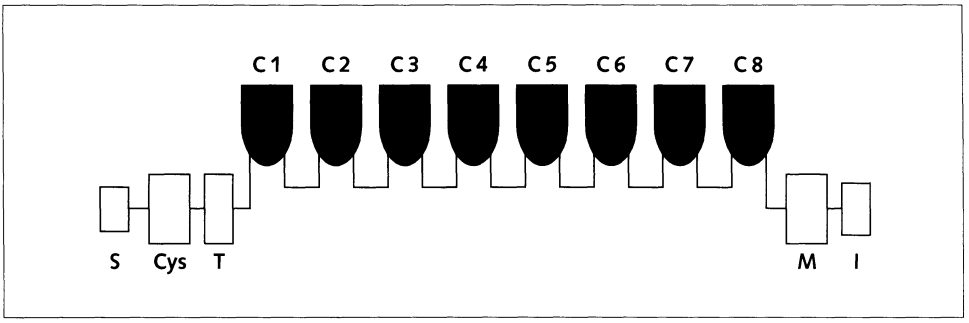

Figure 2
Domain organization of the $sPLA_2$ receptor
S, signal sequence; Cys, cysteine-rich domain; T, fibronectin type repeat; C1–C8, carbohydrate-like domain; M, membrane spanning domain; I, intracellular region

$sPLA_2$ and bee venom group III PLA_2 while rabbit muscle M-type receptor tightly binds human synovial fluid group II PLA_2 but does not bind bee venom $sPLA_2$. Our studies show that bee venom $sPLA_2$ at extremely low concentrations induced the selective release of AA from mast cells suggesting that this mast cell line expresses a protein that is similar if not identical to N-type receptors. Both membrane and plasma bound $sPLA_2$ receptors have been described [139]. The plasma bound $sPLA_2$ receptors seem to prevent the LPS-induced inflammatory process by binding $sPLA_2$ and thus preventing septic shock during bacterial infections [140].

Despite the fact that these receptor subtypes show somewhat different $sPLA_2$ binding profiles, their amino acid sequences are strikingly similar with as much as 82% homology. Additionally, the sequences of the cloned $sPLA_2$ receptor is homologous to that of the macrophage mannose receptor and DEC-205, suggesting that these proteins constitute a new family of membrane proteins [137]. The $sPLA_2$ receptor is composed of an N-terminal cysteine rich domain (Cys) a fibronectin-like type II domain (T), eight carbohydrate recognition domains (C1–C8), a membrane spanning domain (M) and an intracellular tail (I) (Fig. 2). $sPLA_2$ is thought to bind to the M-type receptor *via* the carbohydrate domains (particular C5) [137]. Although occupancy of the $sPLA_2$ receptor has been suggested to enhance cell survival, proliferation, cell migration, much remains to be learned about the molecular events and physiological ramifications of $sPLA_2$ receptor activation.

Hydrolytic activity of $sPLA_2$

In addition to receptor binding, the hydrolytic activity of PLA_2 may also play an important role in releasing fatty acids from cells. There are several distinct features

that distinguish receptor-mediated effects of $sPLA_2$ from enzymatic activity. First, whereas $sPLA_2$ receptor-mediated release is specific for AA [35, 115, 116], hydrolytic activity releases other more abundant fatty acids and degrades phospholipids [141]. Various reports have shown more release of oleic acid than AA in cells where hydrolytic activity is the major mechanism of action. Secondly, very low levels of $sPLA_2$ (nanomolar amounts) are required for receptor-mediated release of AA. In contrast, 1,000 fold more $sPLA_2$ is needed to release fatty acids by hydrolytic activity. Thirdly, disruption of cell membrane is not required for receptor-mediated AA release, while perturbation of cellular membranes is needed for hydrolytic activity [79, 130, 142]. Disruption of cell membranes or loss of membranes phospholipid asymmetry is usually accomplished using cell-activating agents such as ionophore and thrombin in the case of platelets or antigen in the case of mast cells [143, 144]. Additionally, there is alteration in membrane asymmetry when cells are undergoing apoptosis. A combination of disruptive agents and $sPLA_2$ treatment, or treatment of apoptotic cells, usually results in enhanced fatty acid mobilization. Fourthly, $sPLA_2$ receptor-mediated AA release is predominantly from the phosphatidylethanolamine pool whereas hydrolytic release favors phosphatidylcholine [35, 145, 146]. As shown in Figure 3, very low amounts of $sPLA_2$ (0.1 nM) induce the selective formation of lysophosphatidylethanolamine from [^{3}H]-ethanolamine-labeled mast cells while higher concentrations (100 nM) are required to significantly form lysophosphatidylcholine from [^{3}H]-choline labeled mast cells. It is important to note that the receptor-mediated release from a pool of phospholipid that is usually found within the inner bilayer of cell membranes will only be possible if there is recruitment of another lipase activity within cells. Importantly, it is worth noting that the profile of AA release in mast cells incubated with $sPLA_2$ (receptor-mediated) is similar to that of IgE-receptor mediated release of AA. Finally, $sPLA_2$-receptor mediated processes may lead to enhanced cell survival or cell proliferation while hydrolysis inevitably results in cell death as a result of lysis of cell membranes. Similarities between receptor-mediated and hydrolytic activity revolve around the fact that both processes release AA from phospholipid pools and also form lysophospholipids. These lysophospholipids can be acetylated to form PAF or can act as mediators of several processes in cells [147–149]. Thus, AA and lysophospholipids released by hydrolytic action of $sPLA_2$ can be converted to eicosanoid or PAF, respectively and these lipid mediators can then induce several biological effects at sites of inflammation.

$sPLA_2$ receptor-mediated signaling pathways

Although several $sPLA_2$ binding proteins have been described in various mammalian cells, little is currently known about signaling events that are initiated once $sPLA_2$ isotypes or mannose receptors are occupied by ligands. Although little is known about binding of group I PLA_2 to N-type receptors, it is clear that calcium is not

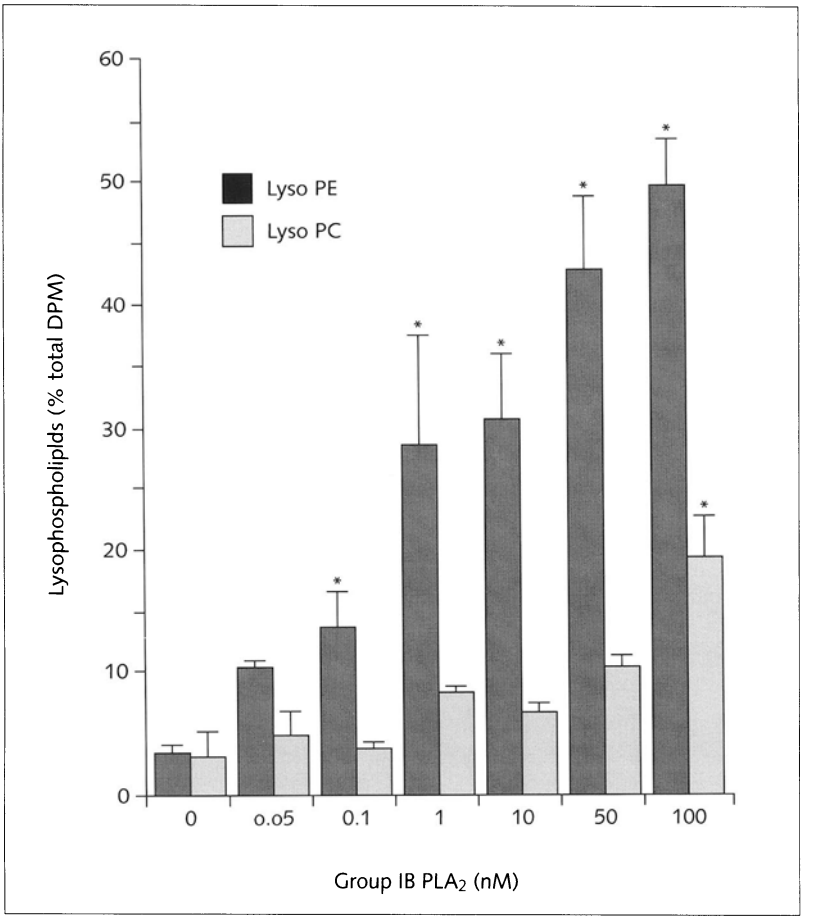

Figure 3
Mobilization of lysophospholipids by group IB PLA_2
Mast cells labeled with [^{3}H]-ethanolamine or [^{3}H]-choline are incubated with increasing concentrations of group IB $sPLA_2$. Lysophosphatidylethanolamine (Lyso PE) and lysophosphatidylcholine (Lyso PC) were isolated using thin layer chromatography and radioactivity in these lipid species determined using lipid scintillation counting ($p < 0.05$).*

required, while calcium is required for the mannose receptor. Mannose receptor occupancy is linked with tyrosine phosphorylation while $sPLA_2$ receptors have been recently shown to mediate cell proliferation and AA release by MAP kinase activation [116, 150]. Demonstration of $sPLA_2$ receptor function as opposed to catalytic action is based on studies using catalytically inactive $sPLA_2$ or ligands of $sPLA_2$ receptors that are devoid of hydrolytic activity. In these studies, catalytically inactive $sPLA_2$ induced the selective release of AA from mast cells and prevented apop-

tosis of these same cells when they were cultured in cytokine-depleted cell culture medium. Likewise, a ligand of the mannose receptor, *p*-amino-phenyl-D-mannopyranoside BSA (APDM-BSA) is shown to induce AA release from mast cells and to compete with $sPLA_2$ in this process. Binding studies also show that only $sPLA_2$ isotypes that selectively induce AA release can compete with each other for specific binding. Interestingly, APDM-BSA does not prevent mast cells from undergoing apoptosis. This suggests that there are at least two $sPLA_2$ receptor subtypes in mast cells, one that is linked to AA release (binds APDM-BSA) and another that prevents apoptosis. Alternatively, there are multiple signaling pathways in mast cells activated differentially likely due to difference in receptor affinity of the different ligands. These signaling events are reviewed below.

In receptor-mediated AA·release, another lipase activity must be recruited if $sPLA_2$ activity is not required. As described above, AA is mobilized from phospholipid pools (mainly PE) that are normally found within cells. Therefore, an ideal PLA_2 that can release this AA pool is the hormonally regulated cytosolic PLA_2 ($cPLA_2$). $cPLA_2$ is translocated to a membrane location in response to an increase in cytosolic calcium and activated by phosphorylation of serine 505 or other phosphorylation sites by MAP kinases [151–154]. The extracellular signal-regulated kinase (ERKs, p42/p44) initially was thought to be the major kinases responsible for $cPLA_2$ phosphorylation. However, recent studies suggest that p38 kinase pathway, which can be activated by environmental stresses and inflammatory cytokines, may also phosphorylate/activate $cPLA_2$. In stimulated platelets, inhibitors of p38 kinase have been shown to prevent $cPLA_2$ activation while these same inhibitors indicate that ERKs and not p38 kinase may activate $cPLA_2$ in other cell types [155, 156]. Wykle and colleagues have shown in human neutrophils that both ERKs and p38 kinases are important in $cPLA_2$ activation depending on the stimuli used. Fonteh and colleagues have also shown that tyrosine kinase inhibitors attenuate $sPLA_2$-induced $cPLA_2$ and Ras activation [116]. Since Ras activation is upstream of MAP kinase activation, we have proposed the signaling pathway depicted in Figure 4A for $cPLA_2$ recruitment and AA release after $sPLA_2$ receptor occupancy. We have proposed that the $sPLA_2$ receptor is similar to other protein tyrosine kinase (PTK) receptors that may have an intrinsic kinase activity or may be able to recruit kinases from cytosol upon ligand binding. Once tyrosine kinases are activated, a sequence of events including Ras, ERKs or p38 activation lead to the phosphorylation and translocation of $cPLA_2$ from cytosol to membranes [116]. This results in the mobilization of AA that is utilized for eicosanoid formation (Fig. 4A). It is likely that the c-Jun pathway may also be linked to $cPLA_2$ activation. As the tools become available for studying and discriminating between the various signaling pathways, it will become clearer whether c-Jun kinases phosphorylate $cPLA_2$ and induce AA release from cells.

The high affinity IgE receptor is a well-characterized membrane bound protein that belongs to the multi-chain system of receptors involved in hypersensitivity reac-

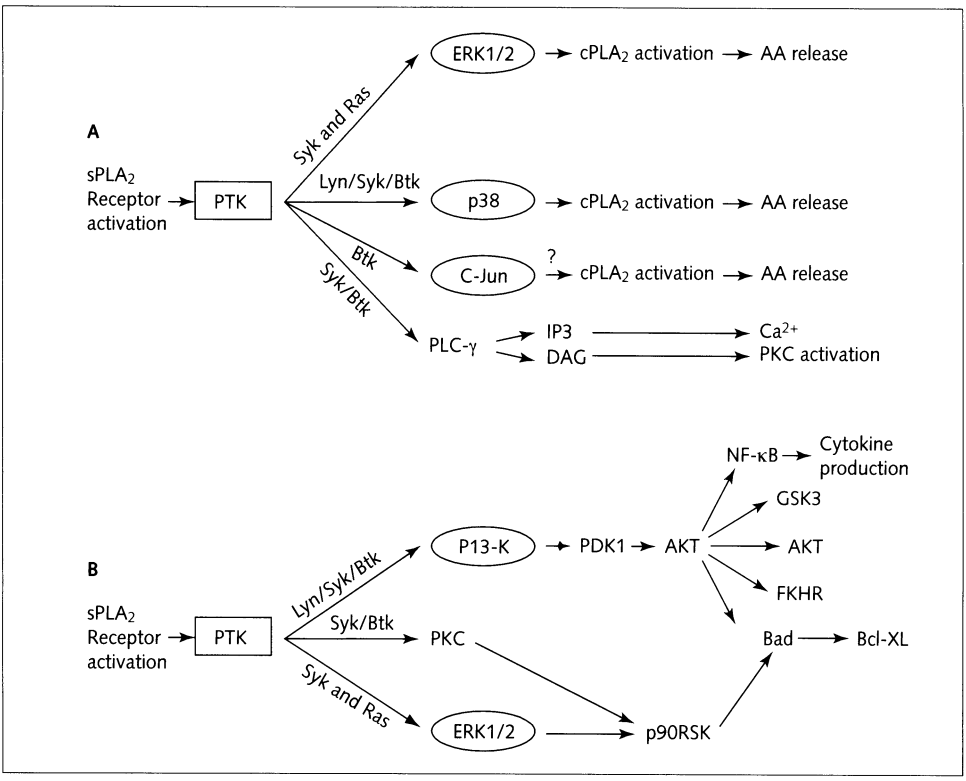

Figure 4
$sPLA_2$-receptor mediated signaling pathways
A) Cytosolic PLA_2 activation. Binding of $sPLA_2$ to its receptors results in the activation of kinase pathways (protein tyrosine kinase (PTK), tyrosine kinases (Syk, Syk and Btk), MAP kinases (ERK1/2, p38, c-Jun)). These kinases activate cytosolic phospholipase A_2 ($cPLA_2$) via *phosphorylation of various amino acid residues. Activated $cPLA_2$ translocates to membrane fraction of cells and releases AA that is used for eicosanoid biosynthesis. Other second messengers (DAG and Ca^{2+}) are also initiated* via *PLCγ.*
B) Anti-apoptotic signaling pathways. $sPLA_2$ binds to its receptors and activates kinase pathways (phosphoinositide 3-kinase (PI3-K), phosphatidylinositol dependent kinase (PDK, glycogen synthase kinase (GSK)), transcription factors (nuclear factor kappa B (NF-κB) and anti-apoptotic proteins such as Bad. Activation of these pathways prevents apoptosis of cells and/or induces cell growth and differentiation.

tions [157]. These receptors lack intrinsic tyrosine kinase activity and so have to recruit cytoplasmic tyrosine kinase that phosphorylates the receptor at sites known as immunoreceptor tyrosine-based activation motifs (ITAMs). Phosphorylation of ITAMs results in protein tyrosine kinase activation. Three major PTKs have been

described in mast cells including Lyn, Syk and Burton's tyrosine kinase (Btk) that are upstream of the three subfamilies of MAK kinases (ERKs, p38 and c-Jun NH_2 terminal kinases) [158–160]. Activation of PTKs and their respective downstream MAP kinases result in $cPLA_2$ activation and the formation of pro-inflammatory mediators described above. Another group of mast cell receptors characterized by *kit*, have intrinsic tyrosine kinase activity and are involved in mast cell survival and proliferation [160, 161]. Similar to *kit*, the high affinity nerve growth factor (NGF, 165 kDa) receptor autophosphorylates tyrosine residues to activate multiple downstream effectors including PLCγ, MAP kinases and phosphoinositide-3-kinase (PI3-K/Akt). Of the many signaling pathways influenced by *kit* and NGF, the PI3-K/Akt pathway has been implicated in mast cell survival and growth. PI3-K is a dual kinase consisting of an 85 kDa regulatory unit and a 110 kDa catalytic unit. PI3-K adds a phosphate molecule specifically to the 3 position of the inositol ring of phosphatidylinositols resulting in the formation of products that have been implicated in survival, proliferation or cell migration. There are striking similarities between *kit*, NGF and the $sPLA_2$ receptor when one examines mast cell survival. First, the cloned $sPLA_2$ receptor (180 kDa) has one membrane-spanning domain, as does the NGF receptor. Secondly, NGF prevents apoptosis of mast cells, as does $sPLA_2$ isotypes that bind specifically to $sPLA_2$ receptors. Thirdly, NGF activates PI3-K/Akt pathway. We have shown that PI3-K specific inhibitors reverse the anti-apoptotic effects observed in mast cells incubated with very low levels of $sPLA_2$. Moreover, $sPLA_2$ also induce Akt phosphorylation in mast cells while inhibitors of nuclear factor kappa B (NF-κB) are shown to prevent $sPLA_2$ effects on mast cells. Both active and catalytically inactive $sPLA_2$ induce IL-3 production from mast cells and NF-κB inhibitors reverse this property of $sPLA_2$ [130]. Taken together, these data show that $sPLA_2$ produce IL-3 by activating the PTK/PI3-K/Akt/NF-κB pathway. Similarly antigen-stimulated mast cells have been shown to produce cytokines *via* PTK/PI3-K/Akt/NF-κB activation.

Conclusions

The first step in designing new pharmaceutical agents is the identification of a candidate target. Elucidation of all biological properties of the identified target plays a crucial role in conceptualizing new strategies for selectively blocking disease-related events.

The above review examines $sPLA_2$ from mast cells as a candidate ligand and $sPLA_2$ receptor as a potential target responsible for important biological functions linking $sPLA_2$ to inflammatory diseases. To our knowledge, the concept that $sPLA_2$ receptors play an important role in inflammatory diseases is novel and potentially interesting to pursue in the development of agents to avert allergic and inflammatory reactions associated with these diseases.

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References

1 Dennis EA (1997) The growing phospholipase A_2 superfamily of signal transduction enzymes. *Trends Biochem Sci* 22: 1–2
2 Murakami M, Nakatani Y, Atsumi G, Inoue K, Kudo I (1997) Regulatory functions of phospholipase A_2. *Crit Rev Immunol* 17: 225–283
3 Kramer RM (1993) Structure, function and regulation of mammalian phospholipases A_2. *Adv Second Messenger Phosphoprotein Res* 28: 81–89
4 Kramer RM, Hession C, Johansen B, Hayes G, McGray P, Chow EP, Tizard R, Pepinsky RB (1989) Structure and properties of a human non-pancreatic phospholipase A_2. *J Biol Chem* 264: 5768–5775
5 Cupillard L, Koumanov K, Mattei MG, Lazdunski M, Lambeau G (1997) Cloning, chromosomal mapping, and expression of a novel human secretory phospholipase A_2. *J Biol Chem* 272: 15745–15752
6 Balboa MA, Balsinde J, Winstead MV, Tischfield JA, Dennis EA (1996) Novel group V phospholipase A_2 involved in arachidonic acid mobilization in murine P388D1 macrophages. *J Biol Chem* 271: 32381–32384
7 Cho W, Han SK, Lee BI, Snitko Y, Dua R (1999) Purification and assay of mammalian group I and group IIa secretory phospholipase A_2. *Methods Mol Biol* 109: 31–38
8 Han SK, Lee BI, Cho W (1997) Bacterial expression and characterization of human pancreatic phospholipase A_2. *Biochim Biophys Acta* 1346: 185–192
9 Chen J, Shao C, Lazar V, Srivastava CH, Lee WH, Tischfield JA (1997) Localization of group IIc low molecular weight phospholipase A_2 mRNA to meiotic cells in the mouse. *J Cell Biochem* 64: 369–375
10 Tischfield JA (1997) A reassessment of the low molecular weight phospholipase A_2 gene family in mammals. *J Biol Chem* 272: 17247–17250
11 Seeds MC, Jones DF, Chilton FH, Bass DA (1998) Secretory and cytosolic phospholipases A_2 are activated during TNF priming of human neutrophils. *Biochim Biophys Acta* 1389: 273–284
12 Shimbara S, Murakami M, Kambe T, Kudo I (1999) Comparison of recombinant types IIA, V and IIC phospholipase A_2S, the three related mammalian secretory phospholipase A_2 isozymes [In Process Citation]. *Adv Exp Med Biol* 469: 209–214
13 Gelb MH, Valentin E, Ghomashchi F, Lazdunski M, Lambeau G (2000) Cloning and recombinant expression of a structurally novel human secreted phospholipase A_2. *J Biol Chem* 275: 39823–39826
14 Tischfield JA (1997) A reassessment of the low molecular weight phospholipase A_2 gene family in mammals. *J Biol Chem* 272: 17247–17250

15 Kramer RM, Roberts EF, Hyslop PA, Utterback BG, Hui KY, Jakubowski JA (1995) Differential activation of cytosolic phospholipase A_2 ($cPLA_2$) by thrombin and thrombin receptor agonist peptide in human platelets. Evidence for activation of $cPLA_2$ independent of the mitogen-activated protein kinases ERK1/2. *J Biol Chem* 270: 14816–14823

16 Sharp JD, White DL, Chiou XG, Goodson T, Gamboa GC, McClure D, Burgett S, Hoskins J, Skatrud PL, Sportsman JR (1991) Molecular cloning and expression of human Ca(2+)-sensitive cytosolic phospholipase A_2. *J Biol Chem* 266: 14850–14853

17 Pickard RT, Strifler BA, Kramer RM, Sharp JD (1999) Molecular cloning of two new human paralogs of 85-kDa cytosolic phospholipase A_2. *J Biol Chem* 274: 8823–8831

18 Ackermann EJ, Dennis EA (1995) Mammalian calcium-independent phospholipase A_2. *Biochim Biophys Acta* 1259: 125–136

19 Gross RW (1998) Activation of calcium-independent phospholipase A_2 by depletion of internal calcium stores. *Biochem Soc Trans* 26: 345–349

20 Hazen SL, Stuppy RJ, Gross RW (1990) Purification and characterization of canine myocardial cytosolic phospholipase A_2. A calcium-independent phospholipase with absolute f1-2 regiospecificity for diradyl glycerophospholipids. *J Biol Chem* 265: 10622–10630

21 Portilla D, Crew MD, Grant D, Serrero G, Bates LM, Dai G, Sasner M, Cheng J, Buonanno A (1998) cDNA cloning and expression of a novel family of enzymes with calcium-independent phospholipase A_2 and lysophospholipase activities. *J Am Soc Nephrol* 9: 1178–1186

22 Underwood KW, Song C, Kriz RW, Chang XJ, Knopf JL, Lin LL (1998) A novel calcium-independent phospholipase A_2, $cPLA_2$-gamma, that is prenylated and contains homology to $cPLA_2$. *J Biol Chem* 273: 21926–21932

23 Endo S, Inada K, Yamashita H, Takakuwa T, Nakae H, Kasai T, Kikuchi M, Ogawa M, Uchida K, Yoshida M (1994) Platelet-activating factor (PAF) acetylhydrolase activity, type II phospholipase A_2, and cytokine levels in patients with sepsis. *Res Commun Chem Pathol Pharmacol* 83: 289–295

24 MacPhee CH, Moores KE, Boyd HF, Dhanak D, Ife RJ, Leach CA, Leake DS, Milliner KJ, Patterson RA, Suckling KE et al (1999) Lipoprotein-associated phospholipase A_2, platelet-activating factor acetylhydrolase, generates two bioactive products during the oxidation of low-density lipoprotein: Use of a novel inhibitor. *Biochem J* 338 (Pt 2): 479–487

25 Nijssen JG, Roosenboom CF, van den? BH (1986) Identification of a calcium-independent phospholipase A_2 in rat lung cytosol and differentiation from acetylhydrolase for 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (PAF-acether). *Biochim Biophys Acta* 876: 611–618

26 Soubeyrand S, Lazure C, Manjunath P (1998) Phospholipase A_2 from bovine seminal plasma is a platelet-activating factor acetylhydrolase. *Biochem J* 329 (Pt 1): 41–47

27 Tjoelker LW, Eberhardt C, Unger J, Trong HL, Zimmerman GA, McIntyre TM, Stafforini DM, Prescott SM, Gray PW (1995) Plasma platelet-activating factor acetylhydrolase is a secreted phospholipase A_2 with a catalytic triad. *J Biol Chem* 270: 25481–25487

28 Touqui L, Herpin-Richard N, Gene RM, Jullian E, Aljabi D, Hamberger C, Vargaftig BB, Dessange JF (1994) Excretion of platelet activating factor-acetylhydrolase and phospholipase A_2 into nasal fluids after allergenic challenge: Possible role in the regulation of platelet activating factor release. *J Allergy Clin Immunol* 94: 109–119

29 Fonteh AN, Bass DA, Marshall LA, Seeds M, Samet JM, Chilton FH (1994) Evidence that secretory phospholipase A_2 plays a role in arachidonic acid release and eicosanoid biosynthesis by mast cells. *J Immunol* 152: 5438–5446

30 Scott DL, Sigler PB (1994) Structure and catalytic mechanism of secretory phospholipases A_2. *Adv Protein Chem* 45: 53–88

31 Bomalaski JS, Baker DG, Brophy L, Resurreccion NV, Spilberg I, Muniain M, Clark MA (1989) A phospholipase A_2-activating protein (PLAP) stimulates human neutrophil aggregation and release of lysosomal enzymes, superoxide, and eicosanoids. *J Immunol* 142: 3957–3962

32 Hsueh W, Tan XD, Qu XW, Sun XM, Gonzalez-Crussi F (1997) Injurious and protective mechanisms in the gut. Interaction of PAF, phospholipase A_2, eicosanoids, and nitric oxide synthase. *Adv Exp Med Biol* 407: 365–369

33 Kramer RM, Jakubowski JA, Deykin D (1988) Hydrolysis of 1-alkyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine, a common precursor of platelet-activating factor and eicosanoids, by human platelet phospholipase A_2. *Biochim Biophys Acta* 959: 269–279

34 Nakae H, Endo S, Inada K, Yamashita H, Yamada Y, Takakuwa T, Kasai T, Ogawa M, Uchida K (1995) Plasma concentrations of type II phospholipase A_2, cytokines and eicosanoids in patients with burns. *Burns* 21: 422–426

35 Fonteh AN, Bass DA, Marshall LA, Seeds M, Samet JM, Chilton FH (1994) Evidence that secretory phospholipase A_2 plays a role in arachidonic acid release and eicosanoid biosynthesis by mast cells. *J Immunol* 152: 5438–5446

36 Fonteh AN, Samet JM, Chilton FH (1995) Regulation of arachidonic acid, eicosanoid, and phospholipase A_2 levels in murine mast cells by recombinant stem cell factor. *J Clin Invest* 96: 1432–1439

37 Balsinde J (2002) Roles of various phospholipases A_2 in providing lysophospholipid acceptors for fatty acid phospholipid incorporation and remodelling. *Biochem J* 364: 695–702

38 Metz SA (1986) Lysophosphatidylinositol, but not lysophosphatidic acid, stimulates insulin release. A possible role for phospholipase A_2 but not *de novo* synthesis of lysophospholipid in pancreatic islet function. *Biochem Biophys Res Commun* 138: 720–727

39 Arbibe L, Koumanov K, Vial D, Rougeot C, Faure G, Havet N, Longacre S, Vargaftig BB, Bereziat G, Voelker DR et al (1998) Generation of lyso-phospholipids from surfactant in acute lung injury is mediated by type-II phospholipase A_2 and inhibited by a direct surfactant protein A-phospholipase A_2 protein interaction. *J Clin Invest* 102: 1152–1160

40 Edelson JD, Vadas P, Villar J, Mullen JB, Pruzanski W (1991) Acute lung injury induced

by phospholipase A_2. Structural and functional changes. *Am Rev Respir Dis* 143: 1102–1109

41 Fisher AB, Dodia C, Chander A, Jain M (1992) A competitive inhibitor of phospholipase A_2 decreases surfactant phosphatidylcholine degradation by the rat lung. *Biochem J* 288 (Pt 2): 407–411

42 Furue S, Mikawa K, Nishina K, Shiga M, Ueno M, Tomita Y, Kuwabara K, Teshirogi I, Ono T, Hori Y et al (2001) Therapeutic time-window of a group IIA phospholipase A_2 inhibitor in rabbit acute lung injury: Correlation with lung surfactant protection. *Crit Care Med* 29: 719–727

43 Koike K, Yamamoto Y, Hori Y, Ono T (2000) Group IIA phospholipase A_2 mediates lung injury in intestinal ischemia-reperfusion. *Ann Surg* 232: 90–97

44 Liu L (1999) Regulation of lung surfactant secretion by phospholipase A_2. *J Cell Biochem* 72: 103–110

45 Chilton FH, Averill FJ, Hubbard WC, Fonteh AN, Triggiani M, Liu MC (1996) Antigen-induced generation of lyso-phospholipids in human airways. *J Exp Med* 183: 2235–2245

46 De Windt LJ, Willems J, Roemen TH, Coumans WA, Reneman RS, Van Der Vusse GJ, Van Bilsen M (2001) Ischemic-reperfused isolated working mouse hearts: Membrane damage and type IIA phospholipase A_2. *Am J Physiol Heart Circ Physiol* 280: H2572–H2580

47 Fletcher JE, Yang CC, Rosenberg P (1982) Basic phospholipase A_2 from Naja nigricollis snake venom: Phospholipid hydrolysis and effects on electrical and contractile activity of the rat heart. *Toxicol Appl Pharmacol* 66: 39–54

48 Van Bilsen M, Van Der Vusse GJ (1995) Phospholipase-A_2-dependent signalling in the heart. *Cardiovasc Res* 30: 518–529

49 Gronroos JO, Laine VJ, Nevalainen TJ (2002) Bactericidal group IIA phospholipase A_2 in serum of patients with bacterial infections. *J Infect Dis* 185: 1767–1772

50 Juffrie M, Meer GM, Hack CE, Haasnoot K, Sutaryo Veerman AJ, Thijs LG (2001) Inflammatory mediators in dengue virus infection in children: Interleukin-6 and its relation to C-reactive protein and secretory phospholipase A_2. *Am J Trop Med Hyg* 65: 70–75

51 Laine VJ, Grass DS, Nevalainen TJ (2000) Resistance of transgenic mice expressing human group II phospholipase A_2 to *Escherichia coli* infection. *Infect Immun* 68: 87–92

52 Rintala EM, Nevalainen TJ (1993) Group II phospholipase A_2 in sera of febrile patients with microbiologically or clinically documented infections. *Clin Infect Dis* 17: 864–870

53 Tunaz H, Park Y, Buyukguzel K, Bedick JC, Nor Aliza AR, Stanley DW (2003) Eicosanoids in insect immunity: Bacterial infection stimulates hemocytic phospholipase A_2 activity in tobacco hornworms. *Arch Insect Biochem Physiol* 52: 1–6

54 Degousee N, Ghomashchi F, Stefanski E, Singer A, Smart BP, Borregaard N, Reithmeier R, Lindsay TF, Lichtenberger C, Reinisch W et al (2002) Groups IV, V, and X phospholipases A_2s in human neutrophils: Role in eicosanoid production and gram-negative bacterial phospholipid hydrolysis. *J Biol Chem* 277: 5061–5073

55 Borgstrom B (1980) Importance of phospholipids, pancreatic phospholipase A_2, and fatty acid for the digestion of dietary fat: *In vitro* experiments with the porcine enzymes. *Gastroenterology* 78: 954–962

56 Gijon MA, Perez C, Mendez E, Sanchez CM (1995) Phospholipase A_2 from plasma of patients with septic shock is associated with high-density lipoproteins and C3 anaphylatoxin: Some implications for its functional role. *Biochem J* 306 (Pt 1): 167–175

57 Gonzalez RJ, Moore EE, Ciesla DJ, Meng X, Biffl WL, Silliman CC (2001) Post-hemorrhagic shock mesenteric lymph lipids prime neutrophils for enhanced cytotoxicity via phospholipase A_2. *Shock* 16: 218–222

58 Green JA, Smith GM, Buchta R, Lee R, Ho KY, Rajkovic IA, Scott KF (1991) Circulating phospholipase A_2 activity associated with sepsis and septic shock is indistinguishable from that associated with rheumatoid arthritis. *Inflammation* 15: 355–367

59 Marshall LA, Hall RH, Winkler JD, Badger A, Bolognese B, Roshak A, Flamberg PL, Sung CM, Chabot-Fletcher M, Adams JL et al (1995) SB 203347, an inhibitor of 14 kDa phospholipase A_2, alters human neutrophil arachidonic acid release and metabolism and prolongs survival in murine endotoxin shock. *J Pharmacol Exp Ther* 274: 1254–1262

60 Sorensen J, Kald B, Tagesson C, Lindahl M (1994) Platelet-activating factor and phospholipase A_2 in patients with septic shock and trauma. *Intensive Care Med* 20: 555–561

61 Vadas P, Pruzanski W, Stefanski E (1988) Extracellular phospholipase A_2: Causative agent in circulatory collapse of septic shock? *Agents Actions* 24: 320–325

62 Vadas P, Pruzanski W (1991) Serum phospholipase A_2 values and septic shock. *Crit Care Med* 19: 988–990

63 Xu D, Lu Q, Deitch EA (1995) Calcium and phospholipase A_2 appear to be involved in the pathogenesis of hemorrhagic shock-induced mucosal injury and bacterial translocation. *Crit Care Med* 23: 125–131

64 Aufenanger J, Samman M, Quintel M, Fassbender K, Zimmer W, Bertsch T (2002) Pancreatic phospholipase A_2 activity in acute pancreatitis: A prognostic marker for early identification of patients at risk. *Clin Chem Lab Med* 40: 293–297

65 Bird NC, Goodman AJ, Johnson AG (1989) Serum phospholipase A_2 activity in acute pancreatitis: An early guide to severity. *Br J Surg* 76: 731–732

66 Buchler M, Malfertheiner P, Schadlich H, Nevalainen TJ, Friess H, Beger HG (1989) role of phospholipase A_2 in human acute pancreatitis. *Gastroenterology* 97: 1521–1526

67 Eskola JU, Nevalainen TJ (1986) Pancreatic phospholipase A_2 in human acute pancreatitis. *Mater Med Pol* 18: 132–135

68 Friess H, Shrikhande S, Riesle E, Kashiwagi M, Baczako K, Zimmermann A, Uhl W, Buchler MW (2001) Phospholipase A_2 isoforms in acute pancreatitis. *Ann Surg* 233: 204–212

69 Gronroos JM, Nevalainen TJ (1992) Increased concentrations of synovial-type phospholipase A_2 in serum and pulmonary and renal complications in acute pancreatitis. *Digestion* 52: 232–236

70 Kemppainen E, Hietaranta A, Puolakkainen P, Sainio V, Halttunen J, Haapiainen R, Kivilaakso E, Nevalainen T (1999) Bactericidal/permeability-increasing protein and

group I and II phospholipase A_2 during the induction phase of human acute pancreatitis. *Pancreas* 18: 21–27

71 Matsuda Y, Ogawa M, Nishijima J, Miyauchi K, Mori T (1986) Usefulness of determination of serum immunoreactive pancreatic phospholipase A_2 content for early identification of severe acute pancreatitis. *Hepatogastroenterology* 33: 214–216

72 Miura M, Endo S, Kaku LL, Inoue Y, Sato N, Wakabayshi G, Baba E, Katsuya H, Inada K, Sato S (2001) Plasma type II phospholipase A_2 levels in patients with acute pancreatitis. *Res Commun Mol Pathol Pharmacol* 109: 159–164

73 Nevalainen TJ (1989) The role of phospholipase A_2 in human acute pancreatitis. *Klin Wochenschr* 67: 180–182

74 Schuppisser JP, Grotzinger U, Reichlin B, Tondelli P (1985) The role of phospholipase A2 in respiratory failure of acute pancreatitis. *Helv Chir Acta* 51: 665–667

75 Touqui L, Arbibe L (1999) A role for phospholipase A_2 in ARDS pathogenesis. *Mol Med Today* 5: 244–249

76 Blackwell GJ (1978) Phospholipase A_2 and platelet aggregation. *Adv Prostaglandin Thromboxane Res* 3: 137–142

77 Hazlett TL, Deems RA, Dennis EA (1990) Activation, aggregation, inhibition and the mechanism of phospholipase A_2. *Adv Exp Med Biol* 279: 49–64

78 Nakano T, Hanasaki K, Matsumoto S, Arita H (1988) Retinol induces platelet aggregation *via* activation of phospholipase A_2. *Biochem Biophys Res Commun* 154: 1075–1080

79 Atsumi G, Murakami M, Tajima M, Shimbara S, Hara N, Kudo I (1997) The perturbed membrane of cells undergoing apoptosis is susceptible to type II secretory phospholipase A_2 to liberate arachidonic acid. *Biochim Biophys Acta* 1349: 43–54

80 Yagami T, Ueda K, Asakura K, Hayasaki-Kajiwara Y, Nakazato H, Sakaeda T, Hata S, Kuroda T, Takasu N, Hori Y (2002) Group IB secretory phospholipase A_2 induces neuronal cell death via apoptosis. *J Neurochem* 81: 449–461

81 Yagami T, Ueda K, Asakura K, Hata S, Kuroda T, Sakaeda T, Takasu N, Tanaka K, Gemba T, Hori Y (2002) Human group IIA secretory phospholipase A_2 induces neuronal cell death *via* apoptosis. *Mol Pharmacol* 61: 114–126

82 Fonteh AN, Marion CR, Barham BJ, Edens MB, Atsumi G, Samet JM, High KP, Chilton FH (2001) Enhancement of mast cell survival: A novel function of some secretory phospholipase A_2 isotypes. *J Immunol* 167: 4161–4171

83 Riggins GJ, Markowitz S, Wilson JK, Vogelstein B, Kinzler KW (1995) Absence of secretory phospholipase A2 gene alterations in human colorectal cancer. *Cancer Res* 55: 5184–5186

84 Bomalaski JS, Clark MA (1990) Activation of phospholipase A_2 in rheumatoid arthritis. *Adv Exp Med Biol* 279: 231–238

85 Bowton DL, Seeds MC, Fasano MB, Goldsmith B, Bass DA (1997) Phospholipase A_2 and arachidonate increase in bronchoalveolar lavage fluid after inhaled antigen challenge in asthmatics. *Am J Respir Crit Care Med* 155: 421–425

86 Busse W (1998) The role and contribution of leukotrienes in asthma. *Ann Allergy Asthma Immunol* 81: 17–26

87 Calabrese C, Triggiani M, Marone G, Mazzarella G (2000) Arachidonic acid metabolism in inflammatory cells of patients with bronchial asthma. *Allergy* 55 (Suppl 61): 27–30

88 Hurt-Camejo E, Paredes S, Masana L, Camejo G, Sartipy P, Rosengren B, Pedreno J, Vallve JC, Benito P, Wiklund O (2001) Elevated levels of small, low-density lipoprotein with high affinity for arterial matrix components in patients with rheumatoid arthritis: Possible contribution of phospholipase A_2 to this atherogenic profile. *Arthritis Rheum* 44: 2761–2767

89 Komatsubara T, Tojo H, Ying Z, Tomita T, Ochi T, Okamoto M (1995) Serum phospholipase A_2 activity and immunoreactive group II phospholipase A_2 in rheumatoid arthritis. *Clin Chim Acta* 236: 109–112

90 Kortekangas P, Aro HT, Nevalainen TJ (1994) Group II phospholipase A_2 in synovial fluid and serum in acute arthritis. *Scand J Rheumatol* 23: 68–72

91 Kramer RM, Pepinsky RB (1991) Assay and purification of phospholipase A_2 from human synovial fluid in rheumatoid arthritis. *Methods Enzymol* 197: 373–381

92 Lin MK, Farewell V, Vadas P, Bookman AA, Keystone EC, Pruzanski W (1996) Secretory phospholipase A_2 as an index of disease activity in rheumatoid arthritis. Prospective double blind study of 212 patients. J *Rheumatol* 23: 1162–1166

93 Pruzanski W, Vadas P (1988) Secretory synovial fluid phospholipase A_2 and its role in the pathogenesis of inflammation in arthritis. *J Rheumatol* 15: 1601–1603

94 Barbour SE, Dennis EA (1993) Antisense inhibition of group II phospholipase A_2 expression blocks the production of prostaglandin E_2 by P388D1 cells. *J Biol Chem* 268: 21875–21882

95 Bayburt T, Yu BZ, Lin HK, Browning J, Jain MK, Gelb MH (1993) Human nonpancreatic secreted phospholipase A_2: Interfacial parameters, substrate specificities, and competitive inhibitors. *Biochemistry* 32: 573–582

96 Bernard P, Pintore M, Berthon JY, Chretien JR (2001) A molecular modeling and 3D QSAR study of a large series of indole inhibitors of human non-pancreatic secretory phospholipase A_2. *Eur J Med Chem* 36: 1–19

97 Blanchard SG, Andrews RC, Brown PJ, Gan LS, Lee FW, Sinhababu AK, Wheeler TN (1998) Discovery of bioavailable inhibitors of secretory phospholipase A_2. *Pharm Biotechnol* 11: 445–463

98 Flower R (1978) Steroidal anti-inflammatory drugs as inhibitors of phospholipase A_2. *Adv Prostaglandin Thromboxane Res* 3: 105–112

99 Glaser KB (1995) Regulation of phospholipase A_2 enzymes: Selective inhibitors and their pharmacological potential. *Adv Pharmacol* 32: 31–66

100 Hansford KA, Reid RC, Clark CI, Tyndall JD, Whitehouse MW, Guthrie T, McGeary RP, Schafer K, Martin JL, Fairlie DP (2003) D-tyrosine as a chiral precusor to potent inhibitors of human non-pancreatic secretory phospholipase A_2 (IIa) with anti-inflammatory activity. *Chembiochem* 4: 181–185

101 Jain MK, Streb M, Rogers J, DeHaas GH (1984) Action of phospholipase A_2 on bilayers containing lysophosphatidylcholine analogs and the effect of inhibitors. *Biochem Pharmacol* 33: 2541–2551

102 Kokotos G, Kotsovolou S, Six DA, Constantinou-Kokotou V, Beltzner CC, Dennis EA (2002) Novel 2-oxoamide inhibitors of human group IVA phospholipase A_2. *J Med Chem* 45: 2891–2893

103 Kokotos G, Constantinou-Kokotou V, Noula C, Nicolaou A, Gibbons WA (1996) Synthesis of lipidic amino acid and dipeptide inhibitors of human platelet phospholipase A_2. *Int J Pept Protein Res* 48: 160–166

104 Lappas M, Munns MJ, King RG, Rice GE (2001) Antisense oligonucleotide inhibition of type II phospholipase A_2 expression, release and activity *in vitro*. *Placenta* 22: 418–424

105 Tanaka K, Arita H (1995) Secretory phospholipase A_2 inhibitors. Possible new anti-inflammatory agents. *Agents Actions* (Suppl) 46: 51–64

106 Kennedy BP, Payette P, Mudgett J, Vadas P, Pruzanski W, Kwan M, Tang C, Rancourt DE, Cromlish WA (1995) A natural disruption of the secretory group II phospholipase A_2 gene in inbred mouse strains. *J Biol Chem* 270: 22378–22385

107 Lilja I, Smedh K, Olaison G, Sjodahl R, Tagesson C, Gustafson-Svard C (1995)Phospholipase A_2 gene expression and activity in histologically normal ileal mucosa and in Crohn's ileitis. *Gut* 37: 380–385

108 MacPhee M, Chepenik KP, Liddell RA, Nelson KK, Siracusa LD, Buchberg AM (1995) The secretory phospholipase A_2 gene is a candidate for the Mom1 locus, a major modifier of ApcMin-induced intestinal neoplasia. *Cell* 81: 957–966

109 Fox N, Song M, Schrementi J, Sharp JD, White DL, Snyder DW, Hartley LW, Carlson DG, Bach NJ, Dillard RD et al (1996) Transgenic model for the discovery of novel human secretory non-pancreatic phospholipase A_2 inhibitors. *Eur J Pharmacol* 308: 195–203

110 Nevalainen TJ, Laine VJ, Grass DS (1997) Expression of human group II phospholipase A_2 in transgenic mice. *J Histochem Cytochem* 45: 1109–1119

111 Uozumi N, Kume K, Nagase T, Nakatani N, Ishii S, Tashiro F, Komagata Y, Maki K, Ikuta K, Ouchi Y et al (1997) Role of cytosolic phospholipase A_2 in allergic response and parturition. *Nature* 390: 618–622

112 Grass DS, Felkner RH, Chiang MY, Wallace RE, Nevalainen TJ, Bennett CF, Swanson ME (1996) Expression of human group II PLA_2 in transgenic mice results in epidermal hyperplasia in the absence of inflammatory infiltrate. *J Clin Invest* 97: 2233–2241

113 Bingham CO III, Murakami M, Fujishima H, Hunt JE, Austen KF, Arm JP (1996) A heparin-sensitive phospholipase A_2 and prostaglandin endoperoxide synthase-2 are functionally linked in the delayed phase of prostaglandin D_2 generation in mouse bone marrow-derived mast cells. *J Biol Chem* 271: 25936–25944

114 Balsinde J, Barbour SE, Bianco ID, Dennis EA (1994) Arachidonic acid mobilization in P388D1 macrophages is controlled by two distinct Ca^{2+}-dependent phospholipase A_2 enzymes. *Proc Natl Acad Sci USA* 91: 11060–11064

115 Fonteh AN, Samet JM, Surette M, Reed W, Chilton FH (1998) Mechanisms that account for the selective release of arachidonic acid from intact cells by secretory phospholipase A_2. *Biochim Biophys Acta* 1393: 253–266

116 Fonteh AN, Atsumi G, LaPorte T, Chilton FH (2000) Secretory phospholipase A_2 receptor-mediated activation of cytosolic phospholipase A_2 in murine bone marrow-derived mast cells. *J Immunol* 165: 2773–2782

117 Bingham CO III, Fijneman RJ, Friend DS, Goddeau RP, Rogers RA, Austen KF, Arm JP (1999) Low molecular weight group IIA and group V phospholipase A_2 enzymes have different intracellular locations in mouse bone marrow-derived mast cells. *J Biol Chem* 274: 31476–31484

118 Fujishima H, Sanchez Mejia RO, Bingham CO III, Lam BK, Sapirstein A, Bonventre JV, Austen KF, Arm JP (1999) Cytosolic phospholipase A_2 is essential for both the immediate and the delayed phases of eicosanoid generation in mouse bone marrow-derived mast cells. *Proc Natl Acad Sci USA* 96: 4803–4807

119 Murakami M, Austen KF, Arm JP (1995) The immediate phase of c-*sn*-ligand stimulation of mouse bone marrow-derived mast cells elicits rapid leukotriene C_4 generation through post-translational activation of cytosolic phospholipase A_2 and 5-lipoxygenase. *J Exp Med* 182: 197–206

120 Reddy ST, Winstead MV, Tischfield JA, Herschman HR (1997) Analysis of the secretory phospholipase A_2 that mediates prostaglandin production in mast cells. *J Biol Chem* 272: 13591–13596

121 Blom M, Tool AT, Wever PC, Wolbink GJ, Brouwer MC, Calafat J, Egesten A, Knol EF, Hack CE, Roos D et al (1998) Human eosinophils express, relative to other circulating leukocytes, large amounts of secretory 14-kD phospholipase A_2. *Blood* 91: 3037–3043

122 Hundley TR, Marshall LA, Hubbard WC, MacGlashan DW Jr (1998) Characteristics of arachidonic acid generation in human basophils: Relationship between the effects of inhibitors of secretory phospholipase A_2 activity and leukotriene C4 release. *J Pharmacol Exp Ther* 284: 847–857

123 Triggiani M, Granata F, Oriente A, De MV, Gentile M, Calabrese C, Palumbo C, Marone G (2000) Secretory phospholipases A_2 induce beta-glucuronidase release and IL-6 production from human lung macrophages. *J Immunol* 164: 4908–4915

124 Triggiani M, Granata F, Oriente A, Gentile M, Petraroli A, Balestrieri B, Marone G (2002) Secretory phospholipases A_2 induce cytokine release from blood and synovial fluid monocytes. *Eur J Immunol* 32: 67–76

125 Triggiani M, Granata F, Balestrieri B, Petraroli A, Scalia G, Del Vecchio L, Marone G (2003) Secretory phospholipases A_2 activate selective functions in human eosinophils. *J Immunol* 170: 3279–3288

126 Galli SJ (2000) Mast cells and basophils. *Curr Opin Hematol* 7: 32–39

127 Galli SJ (1993) New concepts about the mast cell. *N Engl J Med* 328: 257–265

128 Gordon JR, Galli SJ (1991) Release of both preformed and newly synthesized tumor necrosis factor alpha (TNF-α)/cachectin by mouse mast cells stimulated *via* the Fc

epsilon RI. A mechanism for the sustained action of mast cell-derived TNF-α during IgE-dependent biological responses. *J Exp Med* 174: 103–107

129 Wedemeyer J, Tsai M, Galli SJ (2000) Roles of mast cells and basophils in innate and acquired immunity. *Curr Opin Immunol* 12: 624–631

130 Fonteh AN, Marion CR, Barham BJ, Edens MB, Atsumi G, Samet JM, High KP, Chilton FH (2001) Enhancement of mast cell survival: A novel function of some secretory phospholipase A_2 isotypes. *J Immunol* 167: 4161–4171

131 Nair X, Nettleton D, Clever D, Tramposch KM, Ghosh S, Franson RC (1993) Swine as a model of skin inflammation. Phospholipase A_2-induced inflammation. *Inflammation* 17: 205–215

132 Babu AS, Gowda TV (1994) Dissociation of enzymatic activity from toxic properties of the most basic phospholipase A_2 from *Vipera russelli* snake venom by guanidination of lysine residues. *Toxicon* 32: 749–752

133 Arita H, Hanasaki K (1993) Physiological aspects of a high affinity binding site for pancreatic-type phospholipase A_2. *J Lipid Mediat* 6: 217–222

134 Hanasaki K, Arita H (2002) Phospholipase A_2 receptor: A regulator of biological functions of secretory phospholipase A_2. *Prostaglandins Other Lipid Mediat* 68–69: 71–82

135 Hanasaki K, Arita H (1996) Structure and function of phospholipase A_2 receptor. *Adv Exp Med Biol* 416: 315–319

136 Ishizaki J, Kishino J, Teraoka H, Ohara O, Arita H (1993) Receptor-binding capability of pancreatic phospholipase A_2 is separable from its enzymatic activity. *FEBS Lett* 324: 349–352

137 Lambeau G, Lazdunski M (1999) Receptors for a growing family of secreted phospholipases A_2. *Trends Pharmacol Sci* 20: 162–170

138 Lambeau G, Ancian P, Nicolas JP, Beiboer SH, Moinier D, Verheij H, Lazdunski M (1995) Structural elements of secretory phospholipases A_2 involved in the binding to M-type receptors. *J Biol Chem* 270: 5534–5540

139 Ancian P, Lambeau G, Mattei MG, Lazdunski M (1995) The human 180-kDa receptor for secretory phospholipases A_2. Molecular cloning, identification of a secreted soluble form, expression, and chromosomal localization. *J Biol Chem* 270: 8963–8970

140 Hanasaki K, Yokota Y, Ishizaki J, Itoh T, Arita H (1997) Resistance to endotoxic shock in phospholipase A_2 receptor-deficient mice. *J Biol Chem* 272: 32792–32797

141 Koduri RS, Gronroos JO, Laine VJ, Le Calvez C, Lambeau G, Nevalainen TJ, Gelb MH (2002) Bactericidal properties of human and murine groups I, II, V, X, and XII secreted phospholipases A_2. *J Biol Chem* 277: 5849–5857

142 Fourcade O, Simon MF, Viode C, Rugani N, Leballe F, Ragab A, Fournie B, Sarda L, Chap H (1995) Secretory phospholipase A_2 generates the novel lipid mediator lysophosphatidic acid in membrane microvesicles shed from activated cells. *Cell* 80: 919–927

143 Bevers EM, Comfurius P, Zwaal RF (1983) Changes in membrane phospholipid distribution during platelet activation. *Biochim Biophys Acta* 736: 57–66

144 Dekkers DW, Comfurius P, Bevers EM, Zwaal RF (2002) Comparison between Ca^{2+}-

induced scrambling of various fluorescently labelled lipid analogues in red blood cells. *Biochem J* 362: 741–747

145 Fonteh AN, Chilton FH (1993) Mobilization of different arachidonate pools and their roles in the generation of leukotrienes and free arachidonic acid during immunologic activation of mast cells. *J Immunol* 150: 563–570

146 Fonteh AN, Chilton FH (1992) Rapid remodeling of arachidonate from phosphatidylcholine to phosphatidylethanolamine pools during mast cell activation. *J Immunol* 148: 1784–1791

147 Benveniste J, Chignard M, Le Couedic JP, Vargaftig BB (1982) Biosynthesis of platelet-activating factor (PAF-ACETHER) II. Involvement of phospholipase A_2 in the formation of PAF-ACETHER and lyso-PAF-ACETHER from rabbit platelets. *Thromb Res* 25: 375–385

148 Blank ML, Fitzgerald V, Smith ZL, Snyder F (1995) Generation of the precursor (lyso-PAF) of platelet-activating factor via a CoA-dependent transacylase. *Biochem Biophys Res Commun* 210: 1052–1058

149 Snyder F, Lee TC, Blank M, Malone B, Woodard D, Robinson M (1985) Platelet-activating factor: Alternate pathways of biosynthesis, mechanism of inactivation, and reacylation of lyso-PAF with arachidonate. *Adv Prostaglandin Thromboxane Leukot Res* 15: 693–696

150 East L, Isacke CM (2002) The mannose receptor family. *Biochim Biophys Acta* 1572: 364–386

151 Durstin M, Durstin S, Molski TF, Becker EL, Sha'afi RI (1994) Cytoplasmic phospholipase A_2 translocates to membrane fraction in human neutrophils activated by stimuli that phosphorylate mitogen-activated protein kinase. *Proc Natl Acad Sci USA* 91: 3142–3146

152 Gordon RD, Leighton IA, Campbell DG, Cohen P, Creaney A, Wilton DC, Masters DJ, Ritchie GA, Mott R, Taylor IW et al (1996) Cloning and expression of cystolic phospholipase A_2 (cPLA$_2$) and a naturally occurring variant. Phosphorylation of Ser505 of recombinant cPLA$_2$ by p42 mitogen-activated protein kinase results in an increase in specific activity. *Eur J Biochem* 238: 690–697

153 Hazan-Halevy I, Levy R (2000) Activation of cytosolic phospholipase A_2 by opsonized zymosan in human neutrophils requires both ERK and p38 MAP-kinase. *Adv Exp Med Biol* 479: 115–123

154 Hefner Y, Borsch-Haubold AG, Murakami M, Wilde JI, Pasquet S, Schieltz D, Ghomashchi F, Yates JR III, Armstrong CG, Paterson A et al (2000) Serine 727 phosphorylation and activation of cytosolic phospholipase A_2 by MNK1-related protein kinases. *J Biol Chem* 275: 37542–37551

155 Kramer RM, Roberts EF, Hyslop PA, Utterback BG, Hui KY, Jakubowski JA (1995) Differential activation of cytosolic phospholipase A_2 (cPLA$_2$) by thrombin and thrombin receptor agonist peptide in human platelets. Evidence for activation of cPLA$_2$ independent of the mitogen-activated protein kinases ERK1/2. *J Biol Chem* 270: 14816–14823

156 Kramer RM, Roberts EF, Um SL, Borsch-Haubold AG, Watson SP, Fisher MJ,

Jakubowski JA (1996) p38 mitogen-activated protein kinase phosphorylates cytosolic phospholipase A_2 (cPLA2) in thrombin-stimulated platelets. Evidence that proline-directed phosphorylation is not required for mobilization of arachidonic acid by $cPLA_2$. *J Biol Chem* 271: 27723–27729

157 Kawakami T, Galli SJ (2002) Regulation of mast-cell and basophil function and survival by IgE. *Nat Rev Immunol* 2: 773–786

158 Hata D, Kawakami Y, Inagaki N, Lantz CS, Kitamura T, Khan WN, Maeda-Yamamoto M, Miura T, Han W, Hartman SE et al (1998) Involvement of Bruton's tyrosine kinase in Fc epsilon RI-dependent mast cell degranulation and cytokine production. *J Exp Med* 187: 1235–1247

159 Petro JB, Khan WN (2001) Phospholipase C-gamma 2 couples Bruton's tyrosine kinase to the NF-kappa B signaling pathway in B lymphocytes. *J Biol Chem* 276: 1715–1719

160 Tsai M, Chen RH, Tam SY, Blenis J, Galli SJ (1993) Activation of MAP kinases, pp90rsk and pp70-S6 kinases in mouse mast cells by signaling through the c-kit receptor tyrosine kinase or Fc epsilon RI: Rapamycin inhibits activation of pp70-S6 kinase and proliferation in mouse mast cells. *Eur J Immunol* 23: 3286–3291

161 Galli SJ, Tsai M, Wershil BK (1993) The c-kit receptor, stem cell factor, and mast cells. What each is teaching us about the others. *Am J Pathol* 142: 965–974