

Secretory Phospholipases A₂ as Multivalent Mediators of Inflammatory and Allergic Disorders

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Key Words

Secretory phospholipase A₂ · Cytokines · Eicosanoids · Inflammatory cells

Abstract

Phospholipases A₂ (PLA₂s) are enzymes responsible for mobilization of fatty acids, including arachidonic acid (AA), from phospholipids. These enzymes are classified as high-molecular-weight cytosolic PLA₂s (cPLA₂s) and low-molecular-weight secretory PLA₂s (sPLA₂s). There is increasing evidence that large quantities of sPLA₂s are released in the plasma of patients with systemic inflammatory and autoimmune diseases. In addition, high levels of sPLA₂s can be detected at sites of allergic inflammation including the upper airways of patients with rhinitis and the lower airways of patients with asthma. These extracellular enzymes play an important role in inflammation by releasing AA, which can be subsequently converted to proinflammatory prostaglandins and leukotrienes. Generation of AA mediated by sPLA₂s occurs through different mechanisms, including (1) the direct hydrolysis of outer cell membrane phospholipids, (2) internalization and transfer of sPLA₂s to intracellular pools of phospholipids enriched in AA, and (3) activation of cPLA₂s. In addition, sPLA₂s induce degranulation and

production of cytokines and chemokines from a variety of cells involved in inflammatory and immune responses. These effects are exerted by mechanisms that are independent of the enzymatic activity and are mediated by the interaction of sPLA₂s with specific or promiscuous membrane receptors. Therefore, sPLA₂s may have an important role in inflammatory and allergic reactions by activating multiple mechanisms within inflammatory and immune cells, leading to the production of eicosanoids, cytokines and chemokines.

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Introduction

Phospholipases A₂ (PLA₂s) are widely distributed enzymes whose primary action is to hydrolyze fatty acids at the *sn*-2 position of phospholipids [1]. In mammalian cells, arachidonic acid (AA), the precursor of the bioactive eicosanoids (prostaglandins, thromboxane and leukotrienes), is almost exclusively esterified at the *sn*-2 position of membrane phospholipids [2]. The main function of PLA₂s is therefore to generate AA available for eicosanoid synthesis [3]. For a long time, only one intracellular form and one extracellular form of PLA₂ (released in the pancreatic fluid) were recognized. However, in the last 20 years, more

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Table 1. Classification of PLA₂s

Group	Subgroup	Source	Localization	Size kD	Ca ²⁺ dependence
I	A	cobra/krait venom	secretory	13–15	mM
	B	mammalian pancreas/lung/kidney/spleen	secretory	13–15	mM
II	A	human synovial fluid/platelets/BAL	secretory	13–15	mM
	B	Gaboon viper venom	secretory	13–15	mM
	C	rat/mouse testis	secretory	15	mM
	D	human/mouse pancreas/spleen/mast cells	secretory	14–15	mM
	E	human/mouse brain/heart/uterus	secretory	14–15	mM
	F	mouse testis/embryo	secretory	16–17	mM
III		bee/lizard/scorpion venom	secretory	15–18	mM
		human kidney/heart/liver/ skeletal muscle			
IV	A/B/C	most mammalian cells	cytosolic	85–114	<μM
V		human heart/lung/macrophages	secretory	14	mM
VI	A-1/A-2/B	most mammalian cells	cytosolic	84–90	none
VII (acetylhydrolase)		human/mouse/porcine/bovine plasma/liver/ kidney/macrophages	secretory	40–45	none
VIII	A/B	human brain	cytosolic	26	none
X		human spleen/thymus/lung/pancreas/colon/ leukocytes	secretory	14	mM
XII		human heart/skeletal muscle/kidney/liver/lung	secretory	19	mM?

than twelve other isoforms of PLA₂s have been reported in mammalian and nonmammalian cells (table 1), opening new perspectives with regard to the biochemistry and biological functions of these molecules [4]. PLA₂s are divided into two major classes: low-molecular-weight secretory PLA₂s (sPLA₂s) and high-molecular-weight cytosolic PLA₂s (cPLA₂s) [5]. These two classes have different cellular sources, subcellular locations and Ca²⁺ requirements for their enzymatic activity [6]. Most of the secretory isoforms are stored within cytoplasmic granules and are released in the extracellular environment upon appropriate stimulation of the cell. In contrast, cPLA₂s are cytosolic enzymes that migrate to the perinuclear membrane or other intracellular compartments within activated cells.

The first recognized forms of sPLA₂s were those contained in reptile (group I) and bee (group III) venoms [7]. In humans, the first isoform reported, after pancreatic sPLA₂ (group IB), was group IIA sPLA₂, an enzyme stored in and released from platelets [8]. In 1989, two different groups demonstrated that group IIA sPLA₂ accumulates in large quantities in the synovial fluid of patients with rheumatoid arthritis [9, 10]. This was the first evidence

that an extracellular form of sPLA₂ could be released in an inflammatory area. Since then, other isoforms of sPLA₂ (groups IIC, IID, IIE, IIF, V, X and XII) have been identified in mammalian cells [11–16]. The expression of most of these enzymes has now been demonstrated in a variety of human cells and tissues [5, 6]. The ability of several immune and inflammatory cells to synthesize and release various sPLA₂s strongly supports the hypothesis that these molecules may have an important role in inflammation. This review will focus on the potential effects of sPLA₂s relevant to inflammatory and allergic disorders.

The biochemistry and biological functions of the major cPLA₂s (groups IV and VI) and acetyl hydrolases (groups VII and VIII) have been reviewed elsewhere [17–20].

Release of sPLA₂s in Inflammatory and Allergic Diseases

Since the early observation of high levels of group IIA sPLA₂ in the synovial fluid of patients with rheumatoid arthritis, increased concentrations of sPLA₂ have been

Table 2. Diseases associated with high levels of sPLA₂

Disease	sPLA ₂ localization	References
<i>Inflammatory diseases</i>		
Acute pancreatitis	serum	21
Septic shock	serum	22, 27
Extensive burns	serum	31
Multiple trauma (crush syndrome)	serum	27, 34, 42
Adult respiratory distress syndrome	BAL fluid, serum	30, 24, 39
Acute arthritis	synovial fluid	25, 42
<i>Autoimmune diseases</i>		
Crohn's disease	intestinal mucosa, serum	36, 40
Ulcerative colitis	intestinal mucosa, serum	37
Rheumatoid arthritis	synovial fluid, serum	9, 10, 33
Juvenile rheumatoid arthritis	serum	26
<i>Allergic diseases</i>		
Bronchial asthma	BAL fluid, serum	23, 32, 35
Allergic rhinitis	nasal fluid, serum	23, 28
<i>Other diseases</i>		
Typhoid fever	serum	29
Cancer		41, 44, 46
Prostate	serum	
Colon	serum	
Myocardial ischemia	serum	38
Atherosclerosis	atherosclerotic lesions, serum	43, 45

reported in a variety of inflammatory, autoimmune and allergic diseases (listed in table 2) [9, 10, 21–46]. Accumulation of sPLA₂ occurs in the serum or in biological fluids retrieved from an inflamed area, e.g. synovial or bronchoalveolar lavage (BAL) fluid. In some cases, such as the intestinal mucosa of patients with inflammatory bowel diseases, sPLA₂ is significantly overexpressed [36, 37, 40]. In general, serum levels of sPLA₂ are higher in systemic inflammatory diseases, e.g. septic shock or extensive burns, than in tissue-specific inflammation such as rheumatoid arthritis or inflammatory bowel diseases. For example, the plasma concentrations of sPLA₂ in patients with septic shock are as high as 5 µg/ml [22]. Interestingly, the circulating level of sPLA₂ is a useful prognostic marker since it correlates significantly with morbidity and mortality rates in acute syndromes and it is a reliable index of disease activity in chronic disorders such as rheumatoid arthritis [26].

High levels of sPLA₂ are released at sites of allergic reactions, including the airways of patients with bronchial asthma [32, 35] and the nasal lavage of patients with allergic rhinitis [28]. Baseline concentrations of sPLA₂ in the BAL of patients with mild to moderate asthma are significantly increased as compared to those of healthy individ-

uals [32, 35]. In addition, bronchial antigen challenge increases sPLA₂ activity in the BAL fluid of sensitized patients 3- to 5-fold, but not in nonallergic controls [32]. BAL sPLA₂ activity increases during the late-phase reaction, i.e. 20 h after challenge, simultaneously with a significant accumulation of the two major enzymatic products of sPLA₂, AA and lysophospholipids [32].

At least three different isoforms of sPLA₂ (groups IIA, V and X) are expressed in the human airways [47]. However, the possibility that other more recently described sPLA₂s, such as groups IID and XII, may accumulate in the airways of patients with bronchial asthma cannot be excluded.

Potential cellular sources of sPLA₂ in the human airways are mast cells [48], macrophages [49], epithelial cells [47] and fibroblasts [50]. Inflammatory cells infiltrating the airways that also contain sPLA₂s are basophils [51], eosinophils [52] and neutrophils [53, 54].

Stadel et al. [28] have shown that sPLA₂ activity increases in the nasal lavage of patients with allergic rhinitis but not in that of healthy controls following antigen challenge. The rise in sPLA₂ activity was studied only during the early phase. Together, these observations indicate that large quantities of sPLA₂ may be released extracellu-

larly at sites of allergic inflammation, presumably by different cells involved in either the early or the late phase.

Role of sPLA₂s in Inflammatory Reactions: In vitro Studies

The detection of increased levels of sPLA₂ in plasma or biological fluids from patients with systemic or tissue-specific inflammatory disorders led to a large number of studies aimed at understanding the pathophysiological role of these molecules. A variety of experimental models in vitro have been used to address whether sPLA₂ is truly a mediator of inflammatory reactions. It is conceivable that sPLA₂ may participate in inflammation by hydrolyzing AA from phospholipids of the outer leaflet of cell membranes or even present in the extracellular environment [55, 56]. The latter possibility is not trivial since phospholipids are contained in large quantities in plasma and biological fluids, such as synovial exudate or BAL fluid. A large proportion of these phospholipids contains AA at the *sn*-2 position [32, 35]. Therefore, sPLA₂s once released in the extracellular environment containing the millimolar concentrations of Ca²⁺ required for activation are capable of generating large quantities of AA from different sources. Free AA can be subsequently taken up by resident or infiltrating inflammatory cells and converted to eicosanoids. The ability to hydrolyze AA efficiently from cell membranes or free phospholipids has been clearly demonstrated for all major groups of sPLA₂. Thus, at least in vitro, sPLA₂s may function as an extracellular provider of AA to be used for the synthesis of eicosanoids. This phenomenon is likely to occur also in vivo since the accumulation of free AA in the BAL of asthmatics undergoing antigen challenge correlates significantly with the levels of extracellular sPLA₂ [32].

It should be mentioned, however, that most sPLA₂s have no preference for fatty acids at the *sn*-2 position of phospholipids [3]. In contrast, cPLA₂ (group IV) selectively hydrolyzes AA-containing phospholipids [57]. This raises the question of which class of PLA₂s (secretory or cytosolic) is mainly responsible for supplying intracellular AA within inflammatory cells. This issue has been investigated in cells from both human and animal sources by using selective inhibitors of sPLA₂ and cPLA₂. These studies clearly indicate that the majority of intracellular AA converted to eicosanoids is provided by group IV cPLA₂ [6, 58–60]. However, there are at least two other mechanisms by which sPLA₂s may concur in intracellular generation of AA. The first is by upregulating the activity

of cPLA₂ and other enzymes involved in eicosanoid synthesis. Fonteh et al. [61] showed that group IB, IIA and V sPLA₂s generate intracellular signals leading to cPLA₂ phosphorylation and activation with a subsequent increase in AA mobilization and eicosanoid production. In addition, group IIA sPLA₂ appears to be crucial for the delayed phase of cytokine-driven prostaglandin synthesis in murine mast cells by enhancing the expression of the inducible form of cyclooxygenase (COX-2) [48, 50, 62, 63]. A second mechanism by which sPLA₂ may release intracellular AA has been demonstrated recently in mast cells [64] and neutrophils [54]. This mechanism is based upon the ability of certain sPLA₂s (groups IIA, IID and V) to bind tightly to anionic heparanoids such as heparin and heparan sulfate [54, 65, 66]. Heparan sulfate-containing proteoglycans (HSPGs) are highly expressed on cell surfaces and constitute preferential sites for sPLA₂ binding [67]. Binding of sPLA₂s to HSPGs, particularly to glypican, has been shown to promote internalization of the complex and its migration to the perinuclear membrane within the caveolin-rich compartment of the cell [68]. This vesicular/intracellular sorting route, called the HSPG-shuttling pathway, allows sPLA₂s to be concentrated into restricted intracellular compartments that are enriched in AA-containing phospholipids [68]. Taken together, these observations indicate that sPLA₂s may contribute to AA release and eicosanoid generation in inflammatory cells by at least three mechanisms: (1) direct hydrolysis of outer membranes or extracellular phospholipids; (2) activation of cPLA₂ and induction of COX-2 expression, and (3) internalization and compartmentalization into AA-enriched intracellular domains.

Even though sPLA₂s have no specificity for AA, they are very efficient at hydrolyzing fatty acids at the *sn*-2 position of phospholipids [57]. Therefore, under circumstances in which sPLA₂s are released in a phospholipid-rich environment, e.g. airway surfactant, these molecules generate large quantities of lysophospholipids [32, 69]. Lysophospholipids are membrane-perturbing agents that can directly cause tissue injury [70]. In addition, these molecules increase endothelial and epithelial permeability and promote recruitment and activation of inflammatory cells [71, 72]. Therefore, generation of lysophospholipids may be an additional mechanism by which sPLA₂s may contribute to inflammation and tissue damage.

In the last few years, increasing evidence has suggested that the proinflammatory effects of sPLA₂ are not limited to the initiation of AA metabolism or the generation of lysophospholipids. Several diseases associated with high levels of extracellular sPLA₂ are characterized by a signifi-

cant increase in plasma or tissue concentrations of proinflammatory cytokines and chemokines such as TNF- α , IL-1, IL-6 and IL-8 [73, 74]. This observation gave rise to the hypothesis of potential interactions between sPLA₂s and proinflammatory cytokines. Initial observations demonstrated that TNF- α and IL-1 β are potent inducers of sPLA₂ expression in macrophages [75] and mesangial cells [76, 77]. The reverse relationship, i.e. the effect of sPLA₂s on cytokine and chemokine production, has been investigated in various human inflammatory cells by our and other groups. Group IA and IIA sPLA₂s induce IL-6 and TNF- α production from human macrophages purified from the lung parenchyma (fig. 1a) [78]. This effect is associated with increased expression of the mRNA for these cytokines. Group IA, IB and IIA but not group III sPLA₂s also induce TNF- α (fig. 1b), IL-6 and IL-12 production from blood monocytes of normal donors and from mononuclear cells isolated from synovial fluid of patients with rheumatoid arthritis [79]. In addition, sPLA₂s activate exocytosis and IL-8 production from human neutrophils, eosinophils and lung macrophages [80, 81], and induce MCP-1 production in monocytes [82] and chemokine expression in lung microvascular endothelial cells [83]. Finally, group IIA sPLA₂ enhances nitric oxide production from macrophages by upregulating the expression of inducible nitric oxide synthase via NF- κ B [84]. Together, these observations indicate that circulating sPLA₂s or those released at sites of inflammation are potent stimuli for the production of cytokines, chemokines and enzymes responsible for tissue damage and inflammatory cell recruitment. Cytokines and sPLA₂s potentiate each other's synthesis, thereby creating an amplification loop for propagation of inflammatory responses.

Role of sPLA₂s in Inflammatory Reactions: In vivo Studies

Several models have been used to define the role of sPLA₂s as mediators of inflammation in vivo. Initial studies performed with sPLA₂s from animal sources (mouse, snake or pig) have shown that the biological effects of sPLA₂s in vivo are clearly species specific [85].

A widely used in vivo model to investigate the proinflammatory activities of sPLA₂s has been that of experimentally induced arthritis. Intraarticular injection of purified sPLA₂ (groups IA or IIA) in rabbits or rats induces a severe arthritis characterized by extensive tissue damage, proliferative synovitis, neutrophil infiltration and accumulation of prostaglandins (mainly prostaglandin E₂) and

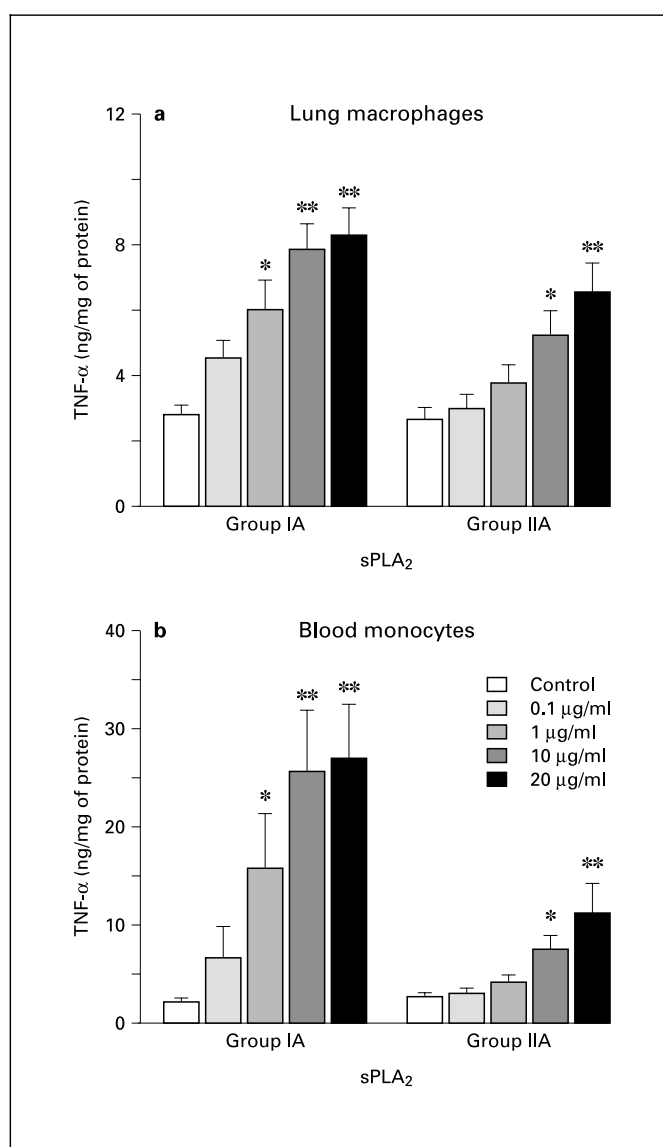


Fig. 1. Effect of increasing concentrations of group IA and IIA sPLA₂s on TNF- α release from human lung macrophages (a) and blood monocytes (b). The cells were incubated (37°C, 6 h) with the indicated concentrations of group IA and IIA sPLA₂s. At the end of the incubation, the supernatant was collected and centrifuged (1,000 g, 4°C, 5 min). TNF- α release in culture supernatants was determined by ELISA. The data are the mean $\pm$ SE of 5 experiments. * p < 0.05, ** p < 0.01 versus control.

cytokines (IL-1, IL-6 and TNF- α) [86, 87]. Injection of enzymatically inactive sPLA₂ produced similar findings, although much more attenuated [87]. Administration of an aerosolized form of sPLA₂ to guinea pigs and intratracheal administration of PLA₂ to rats evokes a neutrophilic pneumonitis and reproduces local and systemic features

of the acute respiratory distress syndrome (ARDS) [88, 89]. Intradermal injection of group IA and IB sPLA₂s causes a concentration-dependent hyperemia, neutrophil infiltration and extensive necrosis at the site of injection, followed after 14–21 days by keratinocyte proliferation and dermal hyperplasia [90]. Finally, intravenous infusion of group IA or IIA sPLA₂s in dogs, cats and rats causes a rapid and profound drop in the mean arterial blood pressure, associated with severe respiratory failure and development of an ARDS-like syndrome [39, 85]. The fall in arterial pressure correlates directly with the rise in the serum concentration of 6-keto-prostaglandin F₁, the stable metabolite of prostacyclin.

Together, these observations, albeit made in different models, suggest that the major effects induced by sPLA₂s *in vivo* are: (1) vasodilation and increased vascular permeability, (2) severe tissue damage, (3) recruitment of inflammatory cells, predominantly neutrophils, and (4) proliferation of resident cells (synoviocytes, fibroblasts or keratinocytes).

Receptor-Mediated Proinflammatory Effects of sPLA₂s

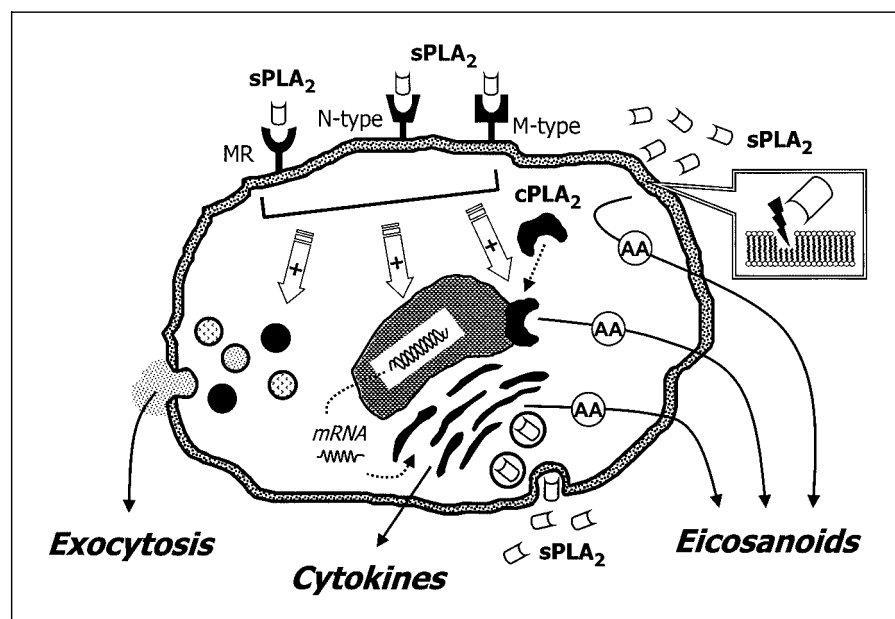
Several *in vitro* and *in vivo* activities of sPLA₂s are clearly related to the mobilization of AA and to the generation of proinflammatory and vasoactive eicosanoids. However, some of the effects of sPLA₂s cannot be readily explained by the enzymatic activity of these molecules. For example, cytokine production in monocytes and macrophages is induced by group IA, IB and IIA but not by group III sPLA₂s, even though the latter isoform has a comparable capacity to mobilize AA [78, 79]. Other *in vitro* effects, such as the induction of exocytosis in eosinophils [81], the inhibition of apoptosis and induction of IL-3 synthesis in mast cells [91] and the contraction of smooth muscle cells, are elicited by sPLA₂s in which the enzymatic activity has been irreversibly inactivated by pharmacologic treatment or by mutations at the catalytic site [92, 93]. In addition, intraarticular injection of catalytically inactive sPLA₂ also induces inflammation, although less efficiently than the active molecules [87]. Finally, sPLA₂-induced activation of inflammatory cells like monocytes and macrophages is not blocked by inhibitors of the enzymatic activity such as LY311727 [78, 79]. Together, these data suggest that at least some of the biological activities of sPLA₂ are not primarily due to the enzymatic activity of these molecules.

Studies on snake venoms containing group IA and III sPLA₂s showed that two major biological effects of venoms (myotoxicity and neurotoxicity) were mediated by the interaction of sPLA₂s with two types of receptor: the M-type (muscular type) and the N-type (neural type), respectively [94]. The M-type receptor is a 180-kD protein that was first identified in rabbit skeletal muscle [95]. This receptor has been cloned in various species, including humans, and binds various sPLA₂s, including groups I, II, V and X [96]. Structural analysis of the M-type receptor reveals a single transmembrane domain, a short cytoplasmic tail and a large extracellular region made of an N-terminal cysteine-rich domain, a fibronectin-like type II domain and a tandem repeat of 8 carbohydrate recognition domains [94–96]. This structure is very similar to that of other receptors involved in endocytosis and antigen processing, such as the mannose receptor on macrophages, the DEC-205 on dendritic cells and the endothelial cell lectin lambda receptor [97]. These receptors constitute the ‘mannose receptor’ family and they are all capable of binding sPLA₂s [97]. More recently, a soluble form of the M-type receptor was identified in normal mouse serum. The soluble receptor is produced by proteolytic cleavage of the membrane form and it inhibits both the enzymatic activity and the binding of sPLA₂ to the membrane-bound M-type receptor [98].

The N-type receptor is highly expressed in the brain and, to a lesser extent, in the heart, skeletal muscle, kidney, lung, liver and pancreas [94, 96, 99]. This receptor is composed of different subunits of 36, 51 and 85 kD and binds group IA and III but not group IB and IIA sPLA₂s.

The identification of specific (M-type and N-type) and shared (mannose, DEC-205 and lectin lambda) receptors raised the question whether some of the biological activities of sPLA₂s may be mediated by receptor activation. The lack of specific M-type or N-type receptor antagonists has so far precluded the distinction between receptor- and catalytically mediated activities of the sPLA₂s. However, several study groups have shown that some effects of sPLA₂s, such as the contraction of bronchial and vascular smooth muscle [92, 93], the activation of exocytosis in macrophages, neutrophils and eosinophils [78, 79, 81], the induction of cytokine production in macrophages, monocytes and mast cells [78, 79, 91] and the phosphorylation of cPLA₂ in murine mast cells [61], are primarily mediated by the engagement of the M-type receptor. These responses are induced by enzymatically inactive forms of sPLA₂ as well as by ligands of the M-type receptor void of enzymatic activity (mannose-containing albumin) and can be repro-

Fig. 2. Schematic representation of the mechanisms of sPLA₂-induced activation of human inflammatory cells. Extracellular sPLA₂s activate several functions in human inflammatory cells including exocytosis, cytokine expression and mobilization of AA that can be subsequently converted to eicosanoids. Activation of exocytosis and induction of cytokine gene expression are events independent of the enzymatic activity of sPLA₂s and occur through the activation of specific (M-type and N-type) and/or promiscuous receptors [mannose receptor (MR)]. AA mobilization may occur through three different mechanisms: (1) direct hydrolysis of phospholipids of the outer leaflet of cell membrane; (2) binding to membrane HSPGs, internalization and shuttling to intracellular compartments enriched in AA-containing phospholipids, and (3) activation of cPLA₂ mediated by interaction with the receptors.



duced by sPLA₂s in transfected cells expressing the M-type receptor [61, 78].

The role of the sPLA₂ receptors, particularly the M-type, in mediating some of the biological effects of these molecules *in vivo* is further emphasized by studies from the group of Hanasaki and Arita [100]. These investigators showed that mice deficient for the M-type receptor were protected during experimentally induced endotoxic shock [101]. The reduced mortality of M-type receptor-deficient mice was associated with lower plasma levels of cytokines such as IL-1, IL-6 and TNF- α [101]. In addition, indoxam, an inhibitor of both sPLA₂ enzymatic activity and binding to the M-type receptor, protects mice from endotoxic shock and inhibits plasma cytokine elevation [102]. These *in vivo* data are in line with our *in vitro* observations that sPLA₂s induce cytokine production from monocytes and macrophages by a nonenzymatic mechanism. Thus, it is reasonable to hypothesize that at least some effects, such as the production of cytokines from circulating monocytes and tissue macrophages, are mediated both *in vivo* and *in vitro* by binding of sPLA₂s to M-type receptors. These findings provide an additional mechanism by which sPLA₂s may have a role in systemic and local inflammation. Cytokine production and exocytosis induced by the engagement of specific receptors may concur with the AA mobilization induced by either direct or indirect mechanisms. Figure 2 summarizes the multiple effects of sPLA₂s on inflammatory cells.

Potential Role of sPLA₂s in Allergic Diseases

It is generally accepted now that sPLA₂s are important mediators in inflammatory and autoimmune diseases. However, the role of these molecules in allergic inflammation is still uncertain. sPLA₂s are released at sites of allergic inflammation such as the nose and the lung in patients with rhinitis and asthma, respectively [28, 32, 35]. *In vitro* experiments indicate that human basophils, eosinophils and mast cells constitutively express sPLA₂s, although a precise characterization of the isoforms produced and released is still lacking [51, 52, 61]. We provided evidence that mast cells purified from the human lung exhibit sPLA₂ activity that is partially induced upon cell activation by anti-IgE [80]. Recently, Murakami et al. [103] showed that human cord blood-derived mast cells cultured in the presence of stem cell factor and IL-6 express group IID sPLA₂ [103].

There is also substantial evidence that sPLA₂s may influence the function of primary and secondary effector cells in asthma, by mechanisms that are either enzymatic or receptor mediated (fig. 3). It should be emphasized, however, that the role of sPLA₂s is suggested only by *in vitro* data and that any conclusion regarding sPLA₂s as mediators of allergic diseases *in vivo* requires further investigation.

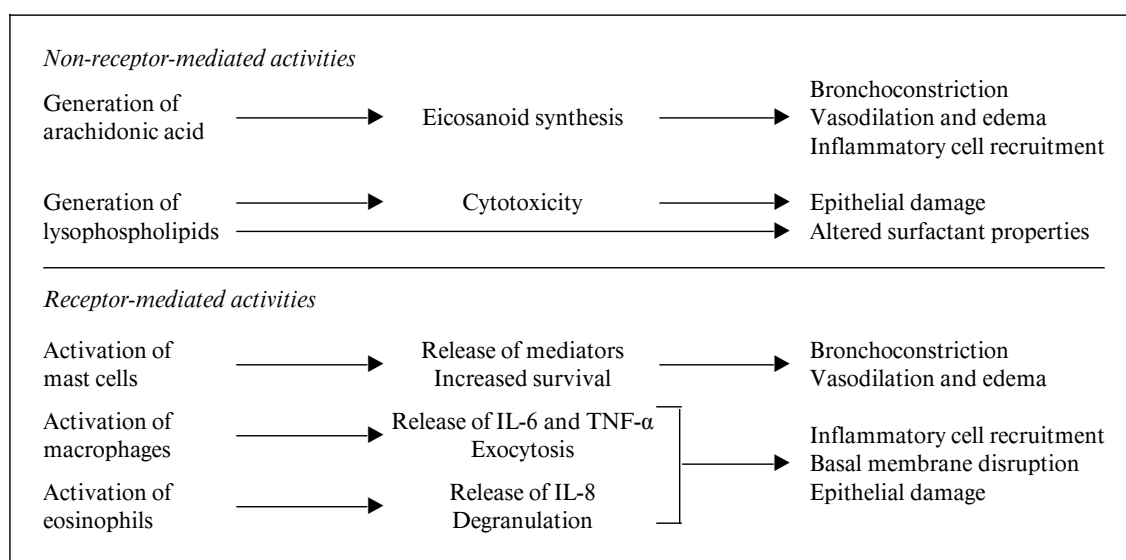


Fig. 3. Schematic representation of the biological activities of sPLA₂ potentially involved in bronchial asthma.

Conclusions and Future Directions

Our knowledge of the biochemistry, molecular biology and functions of extracellular sPLA₂s has greatly expanded over the last 20 years. It has been clearly demonstrated that several isoforms of sPLA₂ are released in both systemic and local inflammation. In certain diseases, such as septic shock, ARDS, pancreatitis and rheumatoid arthritis, serum levels of sPLA₂ correlate significantly with disease activity and prognosis. There is also substantial evidence that sPLA₂s are not only diagnostic and prognostic markers of acute and chronic inflammation, but they also play an active role in the pathophysiology of inflammatory disorders. These molecules may activate several types of inflammatory cells through multiple mechanisms schematically represented in figure 2. These mechanisms are related either to (1) the enzymatic activity leading to AA mobilization and eicosanoid production, (2) the interaction with membrane peptidoglycans or (3) the activation of specific or shared receptors. These mechanisms of cell activation often coexist and may synergistically cooperate.

Novel and exciting findings strongly support the role of sPLA₂s in other pathophysiological conditions such as bacterial or viral infections [104–107], cardiovascular diseases [38, 82, 108] and cancer [41, 44, 46, 109, 110]. These observations open new perspectives on the functions of sPLA₂s in human diseases. The search for specific

inhibitors of the enzymatic activity of sPLA₂s and for selective blockers of their receptors is currently at the forefront of research in this area. Once these pharmacological tools are available, we will hopefully be able to establish more conclusive evidence of the role of this novel class of multivalent mediators of inflammation in human diseases.

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