



Mini-review

The role of mast cell-derived secreted phospholipases A₂ in respiratory allergy[☆]

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ABSTRACT

Secreted phospholipases A₂ (sPLA₂s) are molecules released in plasma and biological fluids of patients with systemic inflammatory, autoimmune and allergic diseases. These molecules exert proinflammatory effects by either enzymatic-mechanisms or through binding to surface molecules expressed on inflammatory cells. sPLA₂s are released at low levels in the normal airways and tend to increase during respiratory allergies (e.g., rhinitis and bronchial asthma) as the result of local secretion. Several sPLA₂ isoforms are expressed in the human lung and some of them (e.g., group IIA and group X) are released in the airways of patients with rhinitis or asthma. Mast cells play a major role in the pathogenesis of respiratory allergies and other chronic inflammatory lung diseases. Recent evidence indicates that mast cells purified from human lung express most of the sPLA₂ isoforms so far described. IgE-mediated activation of these cells induce the release of sPLA₂s suggesting that mast cells are a main source of extracellular sPLA₂s during allergic reactions. Once released, sPLA₂s may contribute to the generation of eicosanoids (e.g., PGD₂ and LTC₄) and to the release of preformed mediators (e.g., histamine) by an autocrine loop involving the interaction of sPLA₂s with surface molecules such as heparan sulphate proteoglycans or the M-type receptor. Thus, mast cell-derived sPLA₂s may play an important role in the initiation and amplification of the inflammatory reactions in patients with allergic rhinitis and bronchial asthma.

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1. Introduction

Phospholipases A₂ (PLA₂)¹ are enzymes that hydrolyze the ester bond at the *sn*-2 position of membrane phospholipids generating free fatty acids and lysophospholipids [1]. PLA₂s are currently classified as high molecular weight cytosolic PLA₂s (cPLA₂s), and low molecular weight secreted PLA₂s (sPLA₂s). sPLA₂s are usually preformed within cell granules and are released in the extracellular environment upon cell activation [2]. This feature of sPLA₂s explains their presence in plasma and biological fluids of patients with acute and chronic inflammatory diseases (e.g., infections,

acute pancreatitis, septic shock, autoimmune diseases, allergic diseases) and led to the hypothesis that they may play a role in these disorders [2–4].

Several *in vitro* and *in vivo* studies support the role of sPLA₂s as proinflammatory mediators (reviewed in [4]). This is because sPLA₂s induce a variety of cellular responses due to their enzymatic activity as well as to their capacity to activate proinflammatory cells by non-enzymatic mechanisms [2,5]. As enzymes, sPLA₂s are known to act within the cell as well as following secretion in the extracellular environment thereby contributing to the mobilization of arachidonic acid (AA) that is, in turn, converted to eicosanoids (e.g., prostaglandins, thromboxanes, and leukotrienes). In addition, sPLA₂s activate other cellular functions (e.g., exocytosis, production of cytokines, chemokines and matrix metalloproteinases, expression of surface molecules, generation of nitric oxide, induction of cell adhesion and proliferation) by non-enzymatic mechanisms involving the interaction with surface molecules such as heparan sulphate proteoglycans (HSPGs) or specific (M-type) and promiscuous receptors (mannose receptor, integrins) [5].

sPLA₂s are secreted at low levels in the airways of healthy individuals and tend to increase during inflammatory reactions as the result of both plasma extravasation and local production. Some sPLA₂ activity is detectable in the bronchoalveolar lavage fluid

Abbreviations: AA, arachidonic acid; BALF, bronchoalveolar lavage fluid; BBMCs, bone marrow-derived mast cells; ERK1/2, extracellular-regulated kinase 1/2; HLMC, human lung mast cells; HSPGs, heparan sulphate proteoglycans; LTC₄, leukotriene C₄; PGD₂, prostaglandin D₂; PLA₂, phospholipases A₂; cPLA₂, cytosolic PLA₂; sPLA₂, secreted PLA₂.

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¹ The Roman number after the letter G indicates the group, the letter in caps after the number indicates the subgroup (e.g. G1B indicates group IB PLA₂).

(BALF) and in the nasal lavage fluid of healthy individuals [6,7]. The levels of sPLA₂ dramatically increase in the airways of patients with acute inflammatory diseases such as pneumonia and adult respiratory distress syndrome as well as in chronic disorders such as sarcoidosis, interstitial lung fibrosis (ILF), and chronic obstructive pulmonary disease (COPD) (reviewed in [8]). Interestingly, sPLA₂ activity is greatly increased in the nasal fluid of patients with allergic rhinitis [7] and in the BALF of patients with bronchial asthma following specific antigen challenge [9,10], which suggests that sPLA₂ enzymes are released locally in both upper and lower airways during allergic reactions.

Several isoforms of sPLA₂ have been identified in mammalian tissues [2,11]. These sPLA₂s are referred to as group IB (GIB), GII (subgroups A–F), GIII, GV, GX and GXII (subgroups A–B), according to a numbering system that assigns a group number to each sPLA₂ isoform on the basis of the disulfide bond pattern and the order of discovery [1,2]. The expression of these sPLA₂ isoforms is quite different between normal and inflamed tissues depending on the species, the type of tissue, and the degree of inflammation. For example, intraperitoneal administration of lipopolysaccharides in mice induces a widespread expression of multiple sPLA₂s (i.e., GIID, GIIE, GIIF, and GV) whereas carageenin-induced pleurisy in the rat selectively increased the expression of GIIA in the lung [12]. In humans, gastritis with intestinal metaplasia induces several sPLA₂s (i.e., GIIA, GIIE, GIIF, GIID, GV, and GX) whereas hepatic inflammation is associated with overexpression of only GIIA and GV [13]. Furthermore, respiratory allergies, namely rhinitis and bronchial asthma, are associated with the selective expression of certain sPLA₂s (i.e., GIIA, and GX) in the upper and lower airways, respectively [14,15]. Potential sources of sPLA₂s in human airways are both resident (e.g., macrophages, mast cells, and epithelial cells) and blood-borne cells (e.g., neutrophils, basophils, eosinophils, T cells) recruited at sites of allergic inflammation (reviewed in [5,11]). Among resident cells, mast cells are particularly abundant at body's interface with the external environment such as the mucosa of the respiratory and gastrointestinal tracts and the skin [16]. This unique location justifies the important role of mast cells in allergic inflammation as well as in innate immunity and host defence against bacterial and viral infections [17,18]. Recent studies indicate that mast cells express several isoforms of sPLA₂s which, in turn, may act in a paracrine or autocrine fashion to modulate the functions of these cells. This review will focus on the role of mast cell-derived sPLA₂s in respiratory allergies.

2. sPLA₂s in allergic rhinitis and bronchial asthma

It was initially reported that sPLA₂ activity was increased by specific antigen challenge in the nasal lavage of patients with allergic rhinitis but not in that of healthy controls [7]. The rise in sPLA₂ activity was observed within 30 min after challenge presumably as the result of local secretion from the cells involved in

the early phase of allergic reaction. sPLA₂s were later found in the BALF of patients with mild and moderate persistent asthma [8–10,19]. Baseline activity of sPLA₂ in the BALF of these patients was significantly increased as compared to those of healthy individuals [8,19]. In addition, bronchial antigen challenge increased three- to five fold sPLA₂ activity in the BALF of sensitized patients but not in that of non-allergic controls [9,10]. In lower airways sPLA₂ activity increased during the late-phase reactions (i.e., 4–20 h after challenge), simultaneously with a significant accumulation of AA and lysophospholipids, the two major enzymatic products of sPLA₂ [10]. These observations indicate that sPLA₂s can be released at sites of allergic inflammation during either the early or the late phase. Table 1 summarizes the main data on sPLA₂ expression in both upper and lower airways in respiratory allergy.

While at least seven sPLA₂s (GIB, GIIA, GIIE, GIIF, GV, GX and GXIIA) are expressed in the human lung tissue (reviewed in [8]), the isoforms secreted in the airways of patients with asthma are only partially characterized. Some information comes from studies in rodents showing that isoforms such as GIIA, GIID, GIIE, GV, and GX are overexpressed in lung biopsies by experimentally-induced airway and lung inflammation [12,20,21]. Two sPLA₂s, namely GV and GX, are of particular interest. GV is expressed at low levels in the lung tissue of normal mice but its expression markedly augments when lung inflammation is induced by either intraperitoneal injection of lipopolysaccharides [12] or allergic sensitization with ovalbumin [21]. Interestingly, a blocking antibody against GV, or the deletion of *GVPLA2* gene reduce lung inflammation and completely block airway hyperresponsiveness in the murine model of allergic inflammation [21]. On the other hand, GX is constitutively expressed in the normal lung of mice and rats [12,20]. The expression of this sPLA₂ does not change upon lipopolysaccharides- or carrageenin-induced lung inflammation [12] whereas it dramatically increases in the lung biopsies of mice with ovalbumin-induced asthma [20]. Furthermore, in this asthma model, knocking-out GX reduced the main histological and biochemical features of airway inflammation and remodeling. These studies suggest that certain sPLA₂s, such as GV and GX, play an important role in the pathogenesis of inflammatory and allergic diseases of the lung, at least in rodents (Table 1).

In humans, the expression of sPLA₂s in the compartments of upper and lower airways has been examined in patients with chronic rhinosinusitis [15], pneumoniae [22], or asthma [14]. Lindbom et al. first reported the relative abundance of mRNAs for several sPLA₂s (i.e., GIIA, GIIE, GIID, and GX) [23] but subsequent immunohistochemistry studies failed to demonstrate the presence of these sPLA₂ proteins in the nasal mucosa of healthy donors [15]. By contrast, in patients with chronic rhinosinusitis, the epithelium and submucosal glands of nasal mucosa stained strongly positive for GIIA and other group II enzymes [15]. Concerning the lower airways, only GX was consistently detected in bronchial epithelial cells and subepithelial interstitium from normal donors or patients with pneumoniae [22]. However, lung inflammation induced other sPLA₂s such as GIIA (in

Table 1
Expression of sPLA₂s in respiratory allergies.

Species	Condition	Samples ^a	Methods ^b	PLA ₂ level ^c	sPLA ₂ isoform	Reference
Mouse	Experimental asthma	Airway microsections	IH, WB	Increased upon Ag challenge	GV	[21]
Mouse	Experimental asthma	Lung biopsies, BALF	IH, IC	Increased upon Ag challenge	GX	[20]
Human	Allergic Rhinitis	Nasal lavage fluid	EA	Increased upon Ag challenge	Undefined	[7]
Human	Allergic Rhinitis	Nasal biopsies	PCR, IH	Increased as compared to healthy controls	GIIA	15
Human	Bronchial asthma	BALF	EA	Increased upon Ag challenge	Undefined	[9,10]
Human	Bronchial asthma	BALF	EA	Increased as compared to non-asthmatic controls	Undefined	8, 19
Human	Bronchial asthma	Cells from induced sputum	PCR, IC, WB	Increased as compared to non-asthmatic controls	GX	[14]

^a BALF = Bronchoalveolar lavage fluid.

^b EA = enzymatic activity, IC = immunocytochemistry, IH = immunohistochemistry, PCR = polymerase chain reaction, WB = western blot.

^c Ag = antigen.

vascular smooth muscle cells and bronchial chondrocytes), GIID and GV (in the epithelial cells and macrophages) [22]. More recently, immunostaining of cells from induced sputum confirmed the presence of GX in the airways of healthy donors and patients with asthma [14]. Again, the expression of GX was significantly higher in asthmatics than in normal subjects and it was further increased upon exercise-induced bronchoconstriction. By contrast, other sPLA₂s such as GIIA, GIID, GIIE, GIIF, GV, and GXIIA were poorly expressed in asthmatics and they were not induced by exercise [14]. Together, these observations indicate that specific sPLA₂s accumulate in the human airways during different types of inflammatory lung diseases (i.e., infections vs. allergic diseases), and suggest that cells resident in the lung differentially contribute to sPLA₂ production by releasing distinct isoforms (Table 1).

3. Mast cells as a source and target of sPLA₂s

Mast cells play a primary role in the pathogenesis of asthma and other chronic inflammatory lung diseases [16]. These cells are activated by IgE- and non IgE-mediated stimuli to release a variety of preformed and *de novo* synthesized proinflammatory mediators [18]. These cells play also a major role in innate immunity and host defence against bacterial and viral infections [17].

Fonteh et al. first reported that immunologically-activated murine bone marrow-derived mast cells (BMMCs) release a sPLA₂ that was involved in AA release and eicosanoid biosynthesis [24,25]. Later, two independent groups reported that BMMCs express and release GIIA and GV upon immunological activation [26,27]. Enomoto et al. extended these observations by showing that BMMCs from two different strains of mice (BALB/cj and C57BL/6j) express several isoforms of the group II subfamily (i.e., GIIC, GIID, GIIE, GIIF and GV) [28]. Group IIA was expressed only in BALB/cj because C57BL/6j BMMCs carry a natural disruption of the gene encoding for GIIA [28]. Interestingly, incubation of BMMCs with various IgE- and non IgE-mediated stimuli selectively induced the expression of GV and this isoform was shown to contribute to the production of PGD₂, the main eicosanoid produced by these cells upon immunological challenge [28].

Initial data on sPLA₂ expression in human mast cells came from immunohistochemistry studies showing that both synovial [29] and gut mast cells [30] stained positive for GIIA. We recently examined the expression of sPLA₂s in primary mast cells (HLMC) purified from the human lung [19] and in LAD-2 cells, a mast cell line isolated from the bone marrow of a patient with systemic mastocytosis [31]. Table 2 shows that HLMC and LAD-2 constitutively express similar levels of several sPLA₂s (i.e., GIIA, GIID, GIIE, GIIF, GIIL, GV, GX, GXIIA, and GXIIB). A remarkable exception is GIB that is expressed in HLMC but not in LAD-2 cells. These data demonstrate that human mast cells express a wide profile of sPLA₂s, at least at the mRNA level. This is at variance with other inflammatory cells such as neutrophils [32], eosinophils [33], and macrophages [34] that express a limited number of sPLA₂s.

While IgE-mediated activation of murine BMMCs induces the release of sPLA₂ activity and enhances the expression of certain sPLA₂ isoforms [26–28], activation of HLMC induces the secretion of sPLA₂ activity without modifying the mRNA expression of any sPLA₂s, at least in short term incubation [19]. The release of sPLA₂s from HLMC is rapid and coincident with that of histamine suggesting that sPLA₂s are synthesized as preformed mediators and stored within secretory granules [19]. This hypothesis is supported by our recent observation that GIIA colocalizes with tryptase in skin mast cells from patients with cutaneous mastocytosis (Granata et al., unpublished observation). Therefore, differently from other inflammatory cells involved in asthma, mast cells not only express

Table 2

Expression of sPLA₂s in human lung mast cells (HLMC) and LAD-2 cells.

	mRNA expression ^a			
	HLMC		LAD-2	
	sPCR	qPCR	sPCR	qPCR
sPLA₂s				
GIB	+	Medium	–	N.d.
GIIA	+	Low	+	Medium
GIID	+	Medium	+	Medium
GIIE	+	Low	+	Low
GIIF	+	Medium	+	Medium
GIIL	+	High	+	High
GV	+	High	+	Medium
GX	+	High	+	Medium
GXIIA	+	Medium	+	High
GXIIB	+	Low	+	Low

^a Data are based on semiquantitative PCR (sPCR) and real-time quantitative PCR (qPCR) [19]. For sPCR the symbols + and – mean detection or no detection of a band of the expected size at subsaturating cycle number (35 cycles), respectively. qPCR levels have been classified on the basis of ΔC_t calculated as the number of PCR cycles for lift-off for the target mRNA of interest minus the number of PCR cycles for lift-off for GAPDH mRNA ($\Delta C_t = C_t \text{ sPLA}_2 \text{ of interest} - C_t \text{ GAPDH}$). High means $\Delta C_t < 9$, Medium means $\Delta C_t = 9–12$, Low means $\Delta C_t > 12$. N.d. means not detected.

sPLA₂s but they also release one or more of these enzymes upon cell activation. This is a relevant issue because other cells (basophils, eosinophils, and macrophages) that may contribute to increase sPLA₂ levels in allergic inflammation synthesize one or more sPLA₂s but fail to release them, at least *in vitro* [33–35]. For example, human basophils and eosinophils contain detectable amounts of sPLA₂ (as assessed by ELISA) but they apparently do not release any sPLA₂ activity upon cell activation [33,35]. Primary human lung macrophages express a profile of sPLA₂s which include GIIA, GIID, GIIE, GIIF, GV, GX and GXIIA but little if any sPLA₂ protein is secreted by LPS-stimulated cells [34]. It is worth mentioning that, among human inflammatory cells, only neutrophils appear to release sPLA₂s. Degousee and collaborators first demonstrated that neutrophils synthesize GV and GX, as judged from the detection of both mRNA and protein, but they secrete only GV upon cell activation [32].

While mast cells appear to be a source of sPLA₂s in the human airways, increasing evidence supports the hypothesis that these cells can also be a target of these molecules. It was initially thought that sPLA₂ enzymes could contribute to the production of lipid mediators in mast cells by mobilizing AA from the extracellular side of cell membrane [24,25]. However, it was later shown that only few sPLA₂s (i.e., GV and GX) can efficiently hydrolyze phospholipids by interacting with the outer leaflet of the cell membrane (reviewed in [2]). Interestingly, some sPLA₂s (i.e., GIIA, GIID, GIIE) unable to release AA from membrane phospholipids contain a heparin-binding domain [28] that allows the interaction with HSPGs, such as glypican, which is particularly abundant in specialized membrane microdomains known as caveolae or lipid rafts [36]. Once released, these sPLA₂s are taken up through HSPGs and concentrated into restricted compartments enriched in AA-containing phospholipids [37]. This HSPG-shuttling pathway allows the interaction of sPLA₂s with phospholipids and drives the sPLA₂s in close contact with other AA metabolizing enzymes (e.g., cyclooxygenases and 5-lipoxygenase) thereby creating favorable conditions for efficient eicosanoid production (reviewed in [11]). In this context, it is noteworthy that selective inhibitors of the GIV-cPLA₂ completely block AA release and/or eicosanoid generation by mast cells in response to IgE- and non IgE-mediated stimuli [19,38,39]. This observation indicates that GIV-cPLA₂ is required for eicosanoid production in stimulated mast cells and suggests that

sPLA₂s do not directly provide AA as a substrate for cyclooxygenases and lipoxygenases under these conditions.

An alternative mechanism by which sPLA₂s can contribute to prostaglandin and leukotriene biosynthesis in mast cells would be a cross-talk with the GIV-cPLA₂. We have recently shown that leukotriene C₄ (LTC₄) production by immunologically activated HLMC is primarily dependent on the activity of the GIV-cPLA₂ since inhibition of this enzyme completely suppresses leukotriene formation [19]. However, the cell-impermeable sPLA₂ inhibitor Me-Indoxam reduces LTC₄ production by 40%, suggesting that the sPLA₂s released by mast cells contribute to leukotriene production by augmenting the activity of GIV-cPLA₂. These data are reminiscent of those previously obtained in murine mast cells showing that sPLA₂s alone do not initiate eicosanoid production but rather potentiate the eicosanoid generating activity of GIV-cPLA₂ [40–42]. The mechanisms of the cross-talk between cPLA₂ and sPLA₂s in mast cells are still unclear but it is currently believed that sPLA₂-induced intracellular signals increase the phosphorylation and activation of the GIV-cPLA₂ via an ERK1/2-mediated pathway [40,41]. Several sPLA₂s (e.g., GIB, GIIA, GIIE, GIIF, GV, GX) bind to the M-type receptor whose activation is coupled to phosphorylation of ERK1/2 [5,43]. Therefore, the cross-talk with cPLA₂ might be due to an autocrine effect of the sPLA₂s released by mast cells mediated by the M-type receptor expressed on these cells [40]. Alternatively, it is also worth noting that those sPLA₂s able to hydrolyze phospholipids by interacting with the outer leaflet of the cell membrane (e.g., GV and GX) generate lysophospholipids that are known to cause intracellular calcium fluxes and lead to phosphorylation and activation of GIV-cPLA₂ [44].

The role of HSPGs and of the M-type receptor in mast cell activation is probably not limited to the biosynthesis of eicosanoids but it extends to the degranulation process and the release of preformed mediators. Previous studies have shown that GIIA triggers histamine release from rat mast cells [45]. Subsequent studies expanded this observation by demonstrating that other sPLA₂s (e.g., GIID, GIIE, and GV) also induce mast cell degranulation through a cooperation between the enzymatic activity and the HSPG-binding property [28,42]. Furthermore, exogenously added sPLA₂s can support the survival of murine mast cells and elicit IL-3 production presumably by interacting with the M-type receptor [40,46]. We reported that the cell-impermeable sPLA₂ inhibitor Me-Indoxam does not influence β -hexosaminidase release from IgE-activated HLMC [19]. This observation would imply that endogenous sPLA₂s released by mast cells do not participate to the degranulation process. We have extended these observations by investigating the effect of exogenous sPLA₂s on β -hexosaminidase release from LAD-2 cells. In these experiments, LAD-2 cells were incubated (90 min, 37 °C) with increasing concentration (0.3–10 μ g/ml) of two human recombinant sPLA₂s, namely GIIE and GIIF. These sPLA₂s were used because, in the mouse system, GIIE can efficiently interact with both HSPGs [42] and the M-type receptor [43] whereas GIIF does not bind HSPGs [47] but it is an high affinity ligand of the M-type receptor [43]. Fig. 1 shows that both hGIIE and hGIIF induce a concentration-dependent release of β -hexosaminidase when added to LAD-2 cells. The effect of both sPLA₂s is significant at a concentration of 1 μ g/ml which is considerably lower than that required to induce degranulation of rodent mast cells [28,42,45]. In addition, Me-Indoxam, a compound that blocks both the enzymatic activity and the receptor-mediated effects of sPLA₂s, inhibited the release of β -hexosaminidase induced by hGIIE (data not shown). Thus, it is conceivable that sPLA₂s, while not involved in IgE-mediated degranulation of mast cells, can function as secretagogues *per se* when the cells are exposed to relatively high concentrations of these molecules such as those found at sites of allergic inflammation [8].

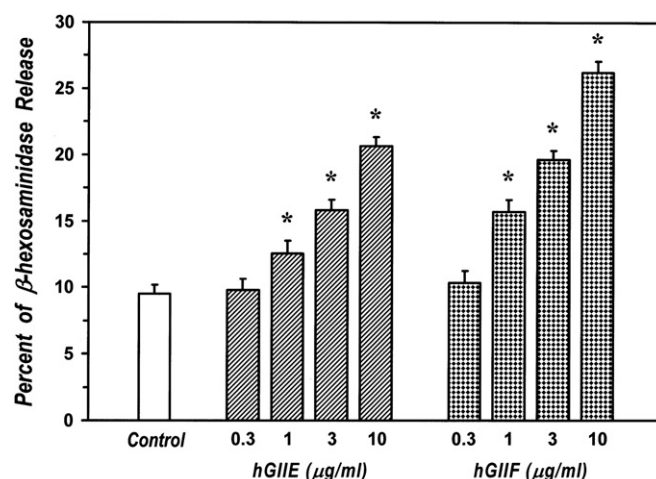


Fig. 1. Effects of sPLA₂s on β -hexosaminidase release from LAD-2 cells. LAD-2 cells [31] (a generous gift of Dr. D.D. Metcalfe, NIH, Bethesda, MD) were incubated (37 °C, 90 min) with increasing concentrations (0.3–10 μ g/ml) of human recombinant group IIE (hGIIE) and group IIF (hGIIF) sPLA₂s. The reactions were stopped by centrifugation (800 $\times$ g, 4 °C) and β -hexosaminidase release was measured by a colorimetric assay [19]. Data are the mean $\pm$ SEM of three experiments. * p < 0.05 vs. control as assessed by one-way analysis of variance (ANOVA) followed by Dunnett's test performed with Analyse-it 1.71 software (Analyse-it Ltd.).

4. Proposed role of mast cell-derived sPLA₂s in bronchial asthma and allergic rhinitis

It is currently unclear what is the real contribution of sPLA₂s to the inflammatory setting of respiratory allergies. However, one may speculate that sPLA₂-induced inflammatory responses in human airways could be similar to those evoked by exogenous administration of these molecules in experimental animals. sPLA₂s *in vivo* induce a robust inflammatory response characterized by: 1) vasodilation and increased vascular permeability; 2) recruitment of inflammatory cells; 3) severe tissue damage; and 4) proliferation of resident structural cells (e.g. fibroblasts in the lung or keratinocytes in the skin) (Reviewed in [4]). Furthermore, sPLA₂s induce airway smooth muscle contraction and bronchoconstriction [48,49]. These experimental observations suggest that sPLA₂s may reproduce many clinical and pathological features of allergic rhinitis and bronchial asthma.

The pathogenesis of respiratory allergies relies on a crucial event which is allergen-induced activation of resident mast cells [50]. This process is mediated by IgE cross-linking of Fc ϵ RI which, in turn, triggers intracellular signals that lead to the release of preformed mediators (e.g., histamine), and the generation of *de novo* synthesized eicosanoids (e.g., PGD₂ and LTC₄). Immunological activation of mast cells also results in the release of sPLA₂s (Fig. 2). Once released by mast cells and, presumably, by other inflammatory and structural cells [3], sPLA₂s may influence mast cell activity in an autocrine or paracrine fashion (Fig. 2). By interacting with HSPGs and/or the M-type receptor, sPLA₂s can contribute to the production of PGD₂ and LTC₄ and to further enhance degranulation of mast cell.

Overall, the simultaneous presence of sPLA₂s together with other mediators released by mast cells effectively modulate the activity of several immune cells involved in allergic sensitization, persistent airway inflammation and tissue remodeling. The role of histamine, prostaglandins, and leukotrienes in allergic inflammation is well established [51–53]. Regarding sPLA₂s, there is substantial evidence that these molecules may influence the function of several effector cells in asthma by mechanisms that are either enzymatic or receptor-mediated [5]. For example, it has been reported that sPLA₂s promote phenotypical and functional maturation of dendritic cells and increase their capacity to stimulate allogeneic T cells through their

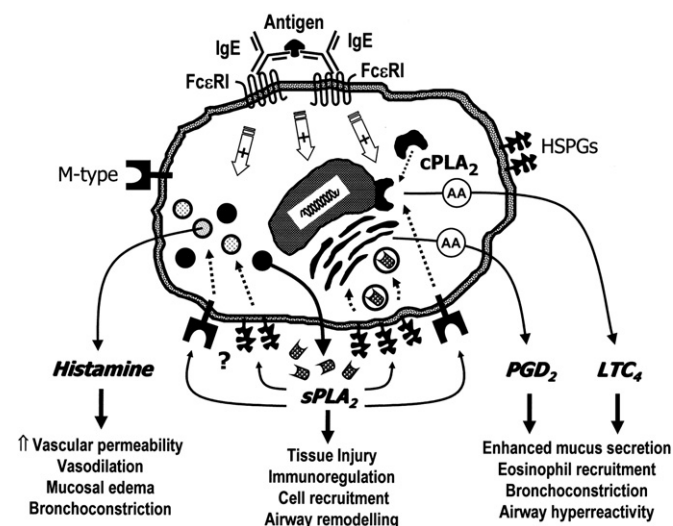


Fig. 2. Schematic representation of the autocrine loops of mast-cell derived sPLA₂s. IgE-mediated activation of human mast cells induces intracellular signals that lead to the release of preformed mediators (histamine and sPLA₂s) and to the generation of de novo synthesized eicosanoids (PGD₂ and LTC₄). Once released, sPLA₂s participate to the generation of PGD₂ by an autocrine loop that involves the interaction with HSPGs. In addition, sPLA₂s can cooperate with cPLA₂ to produce LTC₄ by a mechanism that involves the interaction with the M-type receptor. Furthermore, extracellular sPLA₂s can contribute to the degranulation of human mast cells either through the engagement of HSPGs or the M-type receptor. The simultaneous presence of these mediators in human airways may be responsible for several signs and symptoms of allergic diseases.

enzymatic activity [54,55]. Similarly, sPLA₂s regulate differentiation, recruitment and activation of Th2 cells through the generation of free fatty acids and lysophospholipids [56,57]. On the other hand, by receptor-mediated mechanisms, sPLA₂s activate exocytosis and production of cytokines (IL-6, TNF- α , and IL-10), chemokines (MCP-1/CCL2, MIP-1 α /CCL3, MIP-1 β /CCL4, and IL-8/CXCL8), and matrix metalloproteinases (MMP-2, MMP-3, MMP-7, MMP-12) in lung macrophages [5,58,59] and eosinophils [5,60]. Finally, sPLA₂s promote chemokine-independent migration of endothelial cells and recruitment of neutrophils by mechanisms that involve both the enzymatic activity and the interaction with membrane targets [61,62]. Taken together these observations explain why knocking out even just one sPLA₂ isoform (GV or GX) dramatically reduces all the histological and functional features associated with the inflammatory responses and airway remodeling in murine models of asthma [20,21].

These observations were the rationale for studying sPLA₂s inhibitors in experimental models of lung inflammation and in clinical trials in patients with asthma [63–65]. In all these studies, a potent and selective inhibitor of GIIA (i.e., S-3013/LY333013) was used. This inhibitor was effective in experimental models [64,65] but failed to demonstrate a beneficial effect in patients with asthma [63]. The negative results in the clinical setting may be explained by either the possibility that sPLA₂s other than GIIA (e.g., GV or GX) may be involved in allergic diseases or the lack of inhibition of LY333013 on receptor-mediated effects of sPLA₂s. Thus, further studies are required to define the major isoforms of sPLA₂ released in asthma and rhinitis and to clarify the complex mechanisms by which these molecules sustain allergic inflammation.

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