

## Secretory phospholipases A<sub>2</sub> in inflammatory and allergic diseases: Not just enzymes

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Secretory phospholipases A<sub>2</sub> (sPLA<sub>2</sub>s) are molecules released in plasma and biologic fluids of patients with systemic inflammatory, autoimmune, and allergic diseases. Several sPLA<sub>2</sub> isoforms are expressed and released by such human inflammatory cells as neutrophils, eosinophils, basophils, T cells, monocytes, macrophages, and mast cells. Certain sPLA<sub>2</sub>s release arachidonic acid, thereby providing the substrate for the biosynthesis of proinflammatory eicosanoids. However, there are other mechanisms by which sPLA<sub>2</sub>s might participate in the synthesis of lipid mediators. Interestingly, sPLA<sub>2</sub>s activate inflammatory cells through mechanisms unrelated to their enzymatic activity. Several sPLA<sub>2</sub>s induce degranulation of mast cells and eosinophils and activate exocytosis in macrophages. Furthermore, sPLA<sub>2</sub>s promote cytokine and chemokine production from macrophages, neutrophils, eosinophils, monocytes, and endothelial cells. Some of these effects are mediated by the binding of sPLA<sub>2</sub>s to specific receptors expressed on effector cells. Thus sPLA<sub>2</sub>s might play important roles in the initiation and amplification of the inflammatory reaction. Selective inhibitors of sPLA<sub>2</sub>s and specific antagonists of sPLA<sub>2</sub> receptors might prove useful in the treatment of allergic and autoimmune diseases, such as bronchial asthma and rheumatoid arthritis. (*J Allergy Clin Immunol* 2005;116:1000-6.)

**Key words:** Secretory phospholipases A<sub>2</sub>, arachidonic acid, inflammation, cytokines, chemokines, asthma

Phospholipases A<sub>2</sub> (PLA<sub>2</sub>s) are enzymes that hydrolyze fatty acids at the sn-2 position of phospholipids. Several PLA<sub>2</sub> isoenzymes have been identified in mammals and are classified into 12 major groups and several subgroups (Table I). Two major types of PLA<sub>2</sub>s are currently recognized: (1) high-molecular-weight cytosolic PLA<sub>2</sub>s (cPLA<sub>2</sub>s) and (2) low-molecular-weight secretory PLA<sub>2</sub>s (sPLA<sub>2</sub>s).<sup>1</sup>

### Abbreviations used

AA:	Arachidonic acid
cPLA <sub>2</sub> :	Cytosolic phospholipase A <sub>2</sub>
DC:	Dendritic cell
ERK:	Extracellular signal-regulated kinase
HSPG:	Heparan sulfate proteoglycans
MIP:	Macrophage inflammatory protein
M-type receptor:	Muscular-type receptor
NO:	Nitric oxide
N-type receptor:	Neural-type receptor
PLA <sub>2</sub> :	Phospholipase A <sub>2</sub>
sPLA <sub>2</sub> :	Secretory phospholipase A <sub>2</sub>

cPLA<sub>2</sub>s are located in the cytosol and migrate to the perinuclear membrane and other intracellular compartments of stimulated cells. In contrast, most sPLA<sub>2</sub>s are stored in cytoplasmic granules and are released in the extracellular environment on appropriate cell activation, which explains their presence in the plasma and biologic fluids of patients with systemic inflammatory, autoimmune, or allergic diseases (eg, acute pancreatitis, rheumatoid arthritis, bronchial asthma, and allergic rhinitis).<sup>2</sup> The latter finding led to the hypothesis that sPLA<sub>2</sub>s might play a role in inflammation.

The hydrolysis of arachidonic acid (AA)-containing phospholipids by a PLA<sub>2</sub> enzyme generates free AA and lysophospholipids, which are the precursors of eicosanoids and platelet-activating factor, respectively. Therefore it was initially thought that sPLA<sub>2</sub>s served to provide substrates for the biosynthesis of proinflammatory lipid mediators. However, not all sPLA<sub>2</sub>s hydrolyze AA from intact mammalian cells (Table I),<sup>3-5</sup> suggesting that generation of lipid mediators is not a general function of sPLA<sub>2</sub>s.

A major breakthrough in sPLA<sub>2</sub> biology was the identification of other biologic effects in cells involved in inflammatory and immune responses. These effects are not always related to sPLA<sub>2</sub> enzymatic activity and are thought to depend on other mechanisms, including interactions with membrane peptidoglycans and with specific or promiscuous receptors.<sup>6</sup>

The number of sPLA<sub>2</sub>s identified in human cells or tissues has greatly increased in the last decades (Table I). Interestingly, the expression of sPLA<sub>2</sub>s is greatly different

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**TABLE I.** Classification of sPLA<sub>2</sub>s and cPLA<sub>2</sub>s

Group	Subgroups	Species	Human sources	Classes	Hydrolytic activity*
I	A	Snakes	—	Secretory	Yes
	B	Mammals	Lung, kidney, pancreas, spleen	Secretory	No
II	A	Mammals, snakes	Lung, placenta, serum, spleen, thymus, bone marrow, synovia, chondrocytes, keratinocytes, platelets, mast cells, macrophages	Secretory	No
	B	Snakes	—	Secretory	ND
	C	Rodents	—	Secretory	No
	D	Human subjects, rodents	Lymphoid organs, colon, pancreas, endothelium, eosinophils, keratinocytes, mast cells	Secretory	No
	E	Human subjects, rodents	Brain, heart, lung, placenta, smooth muscle	Secretory	No
	F	Human subjects, rodents	Kidney, liver, placenta, synovia, testis, thymus, endothelium	Secretory	No
III	A/B/C	Human subjects, reptiles	Heart, kidney, liver, skeletal muscle	Secretory	Yes
IV		Mammals	Most mammalian cells	Cytosolic	-
V		Human subjects, rodents	Lung, heart, placenta, airway epithelium, chondrocytes, fibroblasts, keratinocytes, macrophages, neutrophils	Secretory	Yes
VI	A-1/A-2/B	Mammals	Most mammalian cells	Cytosolic	-
VII		Mammals	Plasma, liver, kidney, macrophages	Secretory	ND
(acetylhydrolase)					
VIII	A/B	Human subjects	Brain	Cytosolic	-
IX		Marine snail	—	Secretory	ND
X		Human subjects, mice	Gut, lung, spleen, thymus, airway epithelium and endothelium, macrophages, keratinocytes, neutrophils	Secretory	Yes
XI	A/B	Plants	—	Secretory	ND
XII	A	Human subjects, mice	Heart, brain, gut, kidney, liver, lung, pancreas, placenta, skeletal muscle	Secretory	No
	B	Human subjects	Gut, kidney, liver	Secretory	No

ND, Not done.

\*Determined as AA release from intact mammalian cells (monocytes, macrophages, HEK293, or RBL 2H3) treated with exogenous sPLA<sub>2</sub>s.<sup>3-5</sup>

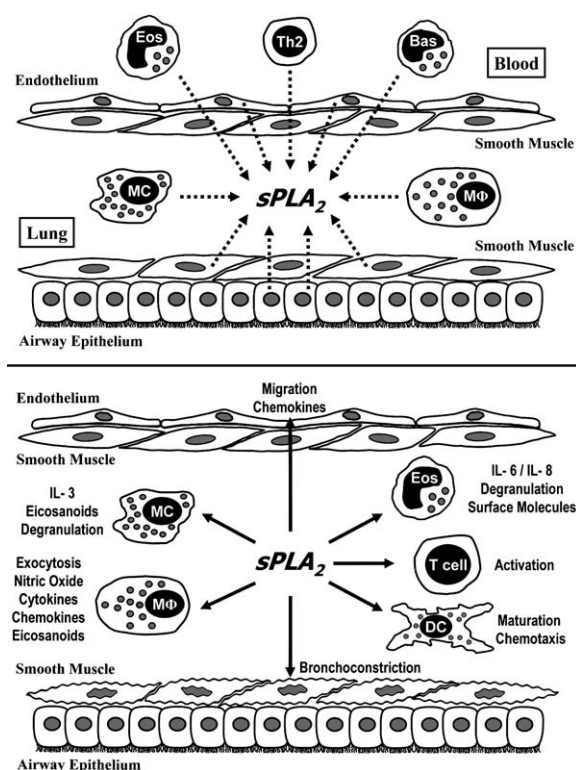
from cell to cell.<sup>1</sup> In addition, different sPLA<sub>2</sub>s are upregulated in inflamed tissues, where they might augment eicosanoid production.<sup>7,8</sup> Moreover, the sPLA<sub>2</sub>s expressed in normal and inflamed tissues can differ greatly depending on the tissue and the type of inflammation.<sup>9</sup> These observations evoke a complex scenario in which the various sPLA<sub>2</sub>s expressed and released at sites of inflammation differ in their capacity to produce lipid mediators or to modulate functions of inflammatory and immune cells.

## EXPRESSION AND RELEASE OF sPLA<sub>2</sub>s IN ALLERGIC INFLAMMATION

Stadel et al<sup>10</sup> were the first to report sPLA<sub>2</sub> activity in the nasal lavage fluid of patients with allergic rhinitis on specific antigen challenge. sPLA<sub>2</sub>s were later found in the bronchoalveolar lavage fluid of patients with bronchial asthma in which bronchial antigen challenge increased sPLA<sub>2</sub> activity 3- to 5-fold during the late-phase reaction (ie, 4-20 hours after challenge).<sup>11,12</sup> This increase was associated with the appearance of AA and lysophospholipids, which are major enzymatic products of sPLA<sub>2</sub>.<sup>11,12</sup>

The sPLA<sub>2</sub> isoforms present in the bronchoalveolar lavage or nasal fluid of allergic patients have not yet been characterized. However, several resident cells are potential sources of sPLA<sub>2</sub>s in the human airways, among which are mast cells, macrophages, epithelial cells, endothelial cells, and bronchial smooth muscle cells (Table I and Fig 1). Moreover, infiltrating leukocytes, such as basophils, eosinophils, neutrophils, lymphocytes, and monocytes, also synthesize sPLA<sub>2</sub>s.

Most studies of the expression of sPLA<sub>2</sub> isoforms have been carried out in rodents. Murine mast cells express all the group II (GII; “G” indicates group, with Roman letters indicating group number and capital letters indicating subgroup designation) subfamily sPLA<sub>2</sub>s (GIIA, GIIC, GIIE, GIIF, and GV).<sup>13</sup> By contrast, murine macrophages mainly express GV and GX.<sup>14,15</sup> Interestingly, 2 specific sPLA<sub>2</sub>s (GV and GXII) are selectively expressed in murine T<sub>H</sub>2 cells, but not in T<sub>H</sub>1 or B cells.<sup>16</sup> Human basophils<sup>17</sup> and lung mast cells<sup>18</sup> contain and release sPLA<sub>2</sub> activity when immunologically stimulated (ie, with anti-IgE or antigen), but the isoforms expressed by these cells have yet to be defined. Various sPLA<sub>2</sub>s have been detected in tissue biopsy specimens of human lung, where GV and



**FIG 1.** Schematic representation of the cellular sources of sPLA<sub>2</sub>s in human airways (upper panel) and of sPLA<sub>2</sub>-induced effects on the cells involved in the pathogenesis of asthma (lower panel). Eos, Eosinophil; Bas, basophil; MC, mast cell; MΦ, macrophage.

GX have been demonstrated in epithelial cells in both normal<sup>19</sup> and inflamed<sup>7</sup> lungs. In the latter study, interstitial and alveolar macrophages were found to produce GIID, GV, and GX, whereas GIIA expression was restricted to the vascular smooth muscles and bronchial chondrocytes.<sup>7</sup>

The expression of some sPLA<sub>2</sub> isoforms can be upregulated in activated inflammatory cells or inflamed tissues, whereas the expression of other sPLA<sub>2</sub>s appears to be constitutive.<sup>1,2,7-9</sup> Induced expression of GIIA occurs in a variety of cells exposed to such stimuli as IL-1, IL-6, TNF- $\alpha$ , IFN- $\gamma$ , LPS, phorbol esters, and cyclic AMP-elevating agents.<sup>1,9</sup> Interestingly, in mesangial cells, GIIA is an autocrine inducer of its own expression by activating peroxisome-proliferator receptor activator  $\alpha$ .<sup>20</sup> Moreover, it has recently been reported that IL-12 significantly increases the plasma levels of sPLA<sub>2</sub> *in vivo*.<sup>21</sup> Glucocorticoids<sup>22</sup> and anti-inflammatory cytokines (TGF- $\beta$  and IL-10)<sup>23,24</sup> might prevent the inducible expression of GIIA by blocking its mRNA synthesis, its posttranscriptional expression, or both. Interestingly, the expression of GV, which is also upregulated by proinflammatory stimuli, is not inhibited by glucocorticoids.<sup>25</sup>

The differential expression of the various sPLA<sub>2</sub>s is important because different isoforms might elicit distinct biologic responses. These observations underscore the

need to characterize further the sPLA<sub>2</sub> isoforms expressed by cells involved in allergic inflammation.

## ROLE OF sPLA<sub>2</sub>s IN AA GENERATION AND EICOSANOID SYNTHESIS

In most inflammatory cells, the majority of intracellular AA converted to eicosanoids is provided by the major cPLA<sub>2</sub> (GIV).<sup>1</sup> However, sPLA<sub>2</sub>s also could contribute to the generation of AA. Earlier studies performed with nonspecific inhibitors were unable to discriminate the effects of different sPLA<sub>2</sub> isoforms. Transfection technology is now being used to evaluate the role of individual sPLA<sub>2</sub>s in the mobilization of AA. For example, Satake et al<sup>26</sup> showed that zymosan-induced generation of leukotriene C<sub>4</sub> and prostaglandin E<sub>2</sub> is reduced in macrophages isolated from GV-deficient mice and that these knockout mice also have a reduced plasma exudation and leukotriene C<sub>4</sub> generation *in vivo*.<sup>26</sup>

sPLA<sub>2</sub>s contribute to the generation of lipid mediators through several mechanisms. Some of them (GIIA, GIID, GV, and GX) efficiently hydrolyze AA from membrane phospholipids by binding to the outer layer of mammalian cells.<sup>3,6</sup> Alternatively, sPLA<sub>2</sub>s possessing a heparin-binding domain (GIIA, GIID, and GV) interact with heparan sulfate proteoglycans (HSPG), which are particularly abundant in specialized membrane microdomains known as caveolae or lipid rafts.<sup>27</sup> On cell activation, these sPLA<sub>2</sub>s are internalized and concentrated into restricted compartments enriched in AA-containing phospholipids. This HSPG-shuttling pathway allows sPLA<sub>2</sub>s to come in close contact with other enzymes, such as COX and 5-lipoxygenase, thereby creating the conditions for efficient eicosanoid production.<sup>1</sup> A third mechanism by which sPLA<sub>2</sub>s contribute to AA mobilization is by upregulating the activity, expression, or both of other enzymes involved in eicosanoid synthesis. In mast cells<sup>28</sup> and neutrophils<sup>29</sup> exogenous GIB, GIIA, and GV generate intracellular signals, leading to cPLA<sub>2</sub> activation and subsequent eicosanoid production. This intracellular cross-talk between sPLA<sub>2</sub>s and cPLA<sub>2</sub> is mediated by such mitogen-activated protein kinases as extracellular signal-regulated kinase (ERK) 1/2.<sup>28,29</sup> Similarly, GIIA and GV participate in the delayed phase of prostaglandin synthesis through functional coupling with COX-2 or by enhancing COX-2 expression.<sup>30,31</sup>

Taken together, these observations indicate that certain sPLA<sub>2</sub>s play a role in the synthesis of eicosanoids. Although their contribution is usually lower than that of the cPLA<sub>2</sub>s, it might be important for initiation and amplification of eicosanoid production in activated inflammatory cells. In addition, although sPLA<sub>2</sub>s show little or no specificity for AA, they are very efficient to hydrolyze other fatty acids at the sn-2 position of phospholipids, thereby generating lysophospholipids. Conditions such as bronchial asthma, in which sPLA<sub>2</sub>s are released in a phospholipid-rich extracellular environment (ie, the airway surfactant), are associated with the presence of large quantities of lysophospholipids.<sup>11</sup> These molecules exert

multiple effects on inflammatory and immune cells by activating their G protein–coupled receptors and by providing the substrate for the synthesis of platelet-activating factor.<sup>32</sup> Therefore generation of lysophospholipids is another mechanism by which sPLA<sub>2</sub>s contribute to airway inflammation in asthma.

## BIOLOGIC EFFECTS OF sPLA<sub>2</sub>s RELEVANT TO ASTHMA AND ALLERGIC DISORDERS

Inflammation induced by sPLA<sub>2</sub>s *in vivo* is characterized by the following: (1) vasodilation and increased vascular permeability; (2) recruitment of inflammatory cells; (3) severe tissue damage; and (4) proliferation of resident cells (eg, fibroblasts in the lung or keratinocytes in the skin).<sup>2</sup> These effects are partially explained by the contribution of sPLA<sub>2</sub>s to the generation of lipid mediators. Other mechanisms by which sPLA<sub>2</sub>s might induce biologic responses are based on the ability of certain isoforms to bind specific or promiscuous membrane receptors.<sup>6,33,34</sup> Initial studies with snake venom sPLA<sub>2</sub>s showed that their major biologic effects (myotoxicity and neurotoxicity) were mediated by the interaction with 2 distinct specific receptors: the muscular-type (M-type) and neural-type (N-type) receptors, respectively.<sup>6</sup> The M-type receptor is a 180-kd protein cloned in various mammalian tissues that binds various sPLA<sub>2</sub>s, including GIB, GIIA, and GX.<sup>33</sup> The structure of this receptor is similar to that of other receptors belonging to the mannose receptor family (ie, the mannose receptor, the DEC-205, and the Endo-180) that are involved in endocytosis, antigen processing, and innate immunity.<sup>34</sup> In particular, the mannose receptor that binds mannose-containing molecules is also able to interact with some sPLA<sub>2</sub>s. By contrast, the N-type receptor, which is highly expressed in the brain, has not yet been cloned. It is constituted by 3 subunits of 36, 51, and 85 kd, and its best ligands are venom GIA and GIII.<sup>6</sup> The role of these receptors in cellular responses evoked by sPLA<sub>2</sub>s is currently under investigation (Table II).

### Effects of sPLA<sub>2</sub>s on dendritic cell and T-cell functions

Dendritic cells (DCs) are thought to play a crucial role in the pathogenesis of bronchial asthma. These cells are primarily involved in antigen presentation to T cells, and they are responsible for the polarization of immune response toward a T<sub>H</sub>2 phenotype during allergic sensitization. Human monocyte-derived DCs apparently do not release sPLA<sub>2</sub> activity,<sup>35</sup> but they might be targets for sPLA<sub>2</sub>s produced by other cells at sites of allergic inflammation. Two recent studies have shown that GIII promotes the phenotypical and functional maturation of DCs and increases their capacity to stimulate allogeneic T cells.<sup>35,36</sup> In addition, GIII increases macrophage inflammatory protein (MIP) 3β–induced DC migration<sup>35</sup> and activates several transcription factors (ie, nuclear factor κB, activator protein-1, and nuclear factor of activated T cells).<sup>36</sup>

**TABLE II.** The past, present, and future of sPLA<sub>2</sub>s in allergic inflammation

#### Established concepts

- sPLA<sub>2</sub>s are released in the airways of patients with asthma and rhinitis.
- Mast cells, basophils, eosinophils, and T<sub>H</sub>2 cells express and release sPLA<sub>2</sub>s.
- sPLA<sub>2</sub>s contribute to the generation of AA and lysophospholipids in human airways and within inflammatory cells.
- sPLA<sub>2</sub>s elicit cytokine and chemokine production by human inflammatory cells, such as eosinophils, monocytes, and macrophages.

#### Current areas of investigation

- Mechanisms by which sPLA<sub>2</sub>s activate human inflammatory cells (ie, mediated by enzymatic activity vs receptor mediated).
- Characterization of sPLA<sub>2</sub>-specific (M-type and N-type) and promiscuous receptors (mannose receptor, DEC-205, Endo-180).
- Role of sPLA<sub>2</sub> inhibitors or receptor antagonists in bronchial asthma and airway remodeling.

#### Areas of uncertainty

- What is the role of sPLA<sub>2</sub>s in allergic sensitization?
- Is there a cooperative or an opposing role for sPLA<sub>2</sub>s (deleterious) and cPLA<sub>2</sub>s (beneficial) in asthma?
- What are the effects on sPLA<sub>2</sub> expression and function of drugs currently used in asthma (inhaled corticosteroids, β<sub>2</sub>-agonists, antileukotrienes, and methylxanthines).

These effects are linked to the release of fatty acids from either resting DCs or cells primed with TNF-α or IL-1.<sup>35</sup>

Increasing evidence suggests that sPLA<sub>2</sub>s regulate T-cell activation through the generation of free fatty acids and lysophospholipids. Earlier reports showed that GIIA enhanced thymidine incorporation and IL-2Rα expression in human T cells activated by diacylglycerol and ionomycin.<sup>37</sup> This observation was extended by Tessier et al,<sup>38</sup> who showed that GIB and GV, constitutively expressed in Jurkat T cells, are crucial for cell proliferation and IL-2 production induced by phorbol 12-myristate 13-acetate and ionomycin. As previously mentioned, GV and GXII are preferentially expressed in T<sub>H</sub>2 rather than T<sub>H</sub>1 cells,<sup>16</sup> as are other enzymes involved in eicosanoid synthesis (COX-2 and hematopoietic prostaglandin D synthase).<sup>39</sup> Given that sPLA<sub>2</sub>s such as GV could be functionally coupled to COX-2 and prostaglandin D synthase,<sup>30,31</sup> the T<sub>H</sub>2 cell appears to possess a very efficient biochemical machinery for prostaglandin D<sub>2</sub> production. In addition, the low-affinity receptor for prostaglandin D<sub>2</sub> (CRTH2) is selectively expressed and is a potent chemotactic receptor for T<sub>H</sub>2 cells.<sup>40</sup> Therefore T<sub>H</sub>2-specific sPLA<sub>2</sub>s, such as GV and GXII, might be important components of an autocrine pathway that regulates differentiation, recruitment, and activation of T<sub>H</sub>2 cells.

### Effects of sPLA<sub>2</sub>s on exocytosis and nitric oxide production

It has long been known that GIIA triggers degranulation of rat mast cells.<sup>41</sup> Several sPLA<sub>2</sub>s induce exocytosis of



human macrophages,<sup>42</sup> neutrophils,<sup>43</sup> and eosinophils.<sup>44</sup> sPLA<sub>2</sub>-induced exocytosis occurs irrespective of enzymatic activity. Indeed, it is not reproduced by AA and lysophospholipids<sup>44</sup> and is still elicited by catalytically inactive sPLA<sub>2</sub>s.<sup>42-44</sup> Current evidence suggests that sPLA<sub>2</sub>-induced exocytosis is mediated by the engagement of membrane receptors, such as the M-type receptor or the mannose receptor.<sup>42,43</sup> In particular, the latter appears to be involved in sPLA<sub>2</sub>-induced activation of human macrophages because an antibody anti-mannose receptor blocks exocytosis induced by both GIIA and mannose-BSA, a ligand of this receptor without AA-releasing capacity.<sup>42</sup>

A receptor-mediated mechanism has also been proposed for sPLA<sub>2</sub>-induced production of nitric oxide (NO). Park et al<sup>45</sup> showed that GIIA induces NO production in murine macrophages by upregulating the expression of the inducible form of NO synthase. This effect of sPLA<sub>2</sub> appears to be mediated by the engagement of the M-type receptor and involves several intracellular kinases (ie, ERK1/2, phosphatidylinositol 3-kinase, and Akt) and nuclear factors, such as nuclear factor  $\kappa$ B. However, the exact pathways leading to the upregulation of inducible NO synthase in macrophages have yet to be defined.

### Effects of sPLA<sub>2</sub>s on cytokine and chemokine production and chemotaxis

GIA and GIIA were the first sPLA<sub>2</sub>s reported to induce IL-6 production from human lung macrophages.<sup>42</sup> More recently, we found that other human sPLA<sub>2</sub>s (GIB, GV, and GX) induce the production of a wide spectrum of cytokines (IL-6, TNF- $\alpha$ , and IL-10)<sup>5</sup> and chemokines of the CC (monocyte chemotactic protein 1/CCL2, MIP-1 $\alpha$ /CCL3, and MIP-1 $\beta$ /CCL4) and CXC (IL-8/CXCL8) families (Granata et al, unpublished observation). GIA, GIB, and GIIA also activate the production of IL-6, TNF- $\alpha$ , and IL-12 in human monocytes<sup>4</sup>; of IL-6 and IL-8/CXCL8 in human eosinophils and neutrophils<sup>44,46</sup>; and of Gro- $\alpha$ /CXCL1, ENA-78/CXCL5, and IL-8/CXCL8 in lung microvascular endothelial cells.<sup>47</sup> Like exocytosis, sPLA<sub>2</sub>-induced cytokine-chemokine production appears to be independent of enzymatic activity because there is no relationship between the ability of sPLA<sub>2</sub>s to release AA and their capacity to induce cytokine production.<sup>4,5</sup> In addition, sPLA<sub>2</sub>s in which hydrolytic activity has been suppressed by site-directed mutagenesis retain the ability to induce cytokine release.<sup>5</sup> Various observations link the production of cytokines and chemokines to the engagement of the M-type receptor. First, the M-type receptor has been detected at the mRNA and protein level in the cells actively producing cytokines and chemokines in response to sPLA<sub>2</sub>s.<sup>5,43,45</sup> Second, binding of sPLA<sub>2</sub>s to the M-type receptor expressed on these cells results in activation of mitogen-activated protein kinases (ie, ERK1/2 and p38) crucial for cytokine-chemokine gene transcription.<sup>5,43,44,46</sup> Finally, sPLA<sub>2</sub>-induced cytokine production from human macrophages is suppressed by indoxam,<sup>5</sup> the only pharmacologic agent known to block

the binding of sPLA<sub>2</sub>s to the M-type receptor.<sup>33</sup> These *in vitro* observations are further supported by data obtained *in vivo* showing that targeted disruption of the M-type receptor gene or pretreatment of mice with indoxam prevent the increase in IL-6 and TNF- $\alpha$  plasma levels during experimentally induced endotoxic shock.<sup>33</sup>

sPLA<sub>2</sub>s also promote the recruitment of inflammatory cells through chemokine-independent mechanisms that seem to involve both the enzymatic activity and the interaction with membrane targets. Rizzo et al<sup>48</sup> found that various sPLA<sub>2</sub>s induced endothelial cell migration and that this process was mediated, at least in part, by the catalytic activity. By contrast, it was reported that several sPLA<sub>2</sub>s activate human neutrophil chemotaxis independently of catalytic activity through mechanisms involving the interaction with HSPG.<sup>49</sup>

In conclusion, sPLA<sub>2</sub>s might be involved in allergic inflammation through exertion of multiple biologic effects (eicosanoid formation, degranulation, NO generation, cytokine-chemokine production, and inflammatory cell recruitment and activation) that are mediated by their enzymatic activity, as well as by interactions with membrane receptors.

### THERAPEUTIC IMPLICATIONS OF sPLA<sub>2</sub> INHIBITORS IN INFLAMMATORY AND ALLERGIC DISEASES

The multivalent proinflammatory activities of sPLA<sub>2</sub>s led many research groups to develop selective inhibitors to be tested in *in vitro* and *in vivo* models of inflammation. Recently, using an extracellular inhibitor of sPLA<sub>2</sub>s in a murine model of asthma, Offer et al<sup>50</sup> showed that sPLA<sub>2</sub>s induce primarily cysteinyl leukotriene generation, whereas cPLA<sub>2</sub> is mainly responsible for prostaglandin E<sub>2</sub> production. These results suggest that cPLA<sub>2</sub>s and sPLA<sub>2</sub>s might have opposing roles in asthma and that their selective inhibition could be a novel pharmacologic approach to modulate eicosanoid formation.

The new compounds S-5920/LY315920Na and the related molecule S-3013/LY333013 are potent and selective inhibitors of human GIIA with little or no effects on cPLA<sub>2</sub>.<sup>51</sup> These drugs are effective anti-inflammatory agents in experimental models of inflammatory diseases<sup>52</sup> and have been used in clinical trials in patients with rheumatoid arthritis<sup>53</sup> and bronchial asthma.<sup>54</sup> However, both of these trials failed to demonstrate a significant therapeutic effect. One possible explanation for these negative results is that sPLA<sub>2</sub>s other than GIIA are primarily involved in rheumatoid arthritis and bronchial asthma. In addition, there is no evidence that these inhibitors of GIIA enzymatic activity block the other nonenzymatic, receptor-mediated, proinflammatory activities of this sPLA<sub>2</sub>. Therefore molecules that inhibit the enzymatic activity of several sPLA<sub>2</sub> isoforms and prevent the interaction with the M-type receptor (ie, indoxam)<sup>3,5,33</sup> appear to be more promising candidates for the treatment of autoimmune and allergic diseases (Table II).

## CONCLUDING REMARKS AND FUTURE DIRECTIONS

sPLA<sub>2</sub>s are emerging as a novel class of mediators of inflammation and immune responses. These molecules are found in biologic fluids in a variety of systemic inflammatory, allergic, and autoimmune disorders. The biologic effects of sPLA<sub>2</sub>s relevant to the pathogenesis of bronchial asthma are schematically summarized in Fig 1. These effects are mediated by various mechanisms that involve the enzymatic activity of sPLA<sub>2</sub>s and their capacity to interact with membrane targets (HSPG, M-type, N-type, or mannose receptors).

Research on sPLA<sub>2</sub>s is still in its infancy, and much remains to be done to understand fully the molecular biology and pathophysiology of these molecules in human subjects (Table II). A major target of future investigations is the development of molecules that interfere with sPLA<sub>2</sub>-induced effects *in vivo*, either as inhibitors of their enzymatic activity or as antagonists of sPLA<sub>2</sub> membrane receptors. However, when considering inhibitors or antagonists for therapeutic purposes, it should be kept in mind that sPLA<sub>2</sub>s also exert protective effects in bacterial and viral infections.<sup>55</sup> Given their capacity to hydrolyze bacterial phospholipids, mammalian sPLA<sub>2</sub>s (particularly GIIA) exhibit a potent bactericidal activity against gram-negative and gram-positive bacteria both *in vitro* and *in vivo*.<sup>55,56</sup> Other sPLA<sub>2</sub>s (eg, GIII) are potent inhibitors of HIV-1 infection by blocking membrane structures (heparans, fusion receptors, or chemokine receptors) crucial for HIV-1 entry into target cells.<sup>55,57</sup>

Studies currently ongoing are expected to discriminate the deleterious and protective roles of sPLA<sub>2</sub>s in human pathophysiology and to provide pharmacologic tools potentially useful for the treatment of allergic and other inflammatory diseases.

## REFERENCES

- Kudo I, Murakami M. Phospholipase A<sub>2</sub> enzymes. Prostaglandins Other Lipid Mediat 2002;68-69:3-58.
- Uhl W, Nevalainen TJ, Buchler MW. Phospholipase A<sub>2</sub>. Basic and clinical aspects in inflammatory diseases. Basel: Karger; 1997.
- Singer AG, Ghomashchi F, Le Calvez C, Bollinger J, Bezzine S, Rouault M, et al. Interfacial kinetic and binding properties of the complete set of human and mouse groups I, II, V, X, and XII secreted phospholipases A<sub>2</sub>. J Biol Chem 2002;277:48535-49.
- Triggiani M, Granata F, Oriente A, Gentile M, Petraroli A, Balestrieri B, et al. Secretory phospholipases A<sub>2</sub> induce cytokine release from blood and synovial fluid monocytes. Eur J Immunol 2002;32:67-76.
- Granata F, Petraroli A, Boillard E, Bezzine S, Bollinger J, Del Vecchio L, et al. Activation of cytokine production by secreted phospholipase A<sub>2</sub> in human lung macrophages expressing the M-type receptor. J Immunol 2005;174:464-74.
- Valentin E, Lambeau G. Increasing molecular diversity of secreted phospholipases A<sub>2</sub> and their receptors and binding proteins. Biochim Biophys Acta 2000;1488:59-70.
- Masuda S, Murakami M, Mitsuishi M, Komiyama K, Ishikawa Y, Ishii T, et al. Expression of secretory phospholipase A<sub>2</sub> enzymes in lungs of humans with pneumonia and their potential prostaglandin-synthetic function in human lung-derived cells. Biochem J 2004;387:27-38.
- Masuda S, Murakami M, Komiyama K, Ishihara M, Ishikawa Y, Ishii T, et al. Various secretory phospholipase A<sub>2</sub> enzymes are expressed in rheumatoid arthritis and augment prostaglandin production in cultured synovial cells. FEBS J 2005;272:655-72.
- Hamaguchi K, Kuwata H, Yoshihara K, Masuda S, Shimbara S, Oh-ishi S, et al. Induction of distinct sets of secretory phospholipase A<sub>2</sub> in rodents during inflammation. Biochim Biophys Acta 2003;1635:37-47.
- Stadel JM, Hoyle K, Naclerio RM, Roshak A, Chilton FH. Characterization of phospholipase A<sub>2</sub> from human nasal lavage. Am J Respir Cell Mol Biol 1994;11:108-13.
- Chilton FH, Averill FJ, Hubbard WC, Fonteh AN, Triggiani M, Liu MC. Antigen-induced generation of lyso-phospholipids in human airways. J Exp Med 1996;183:2235-45.
- Bowton DL, Seeds MC, Fasano MB, Goldsmith B, Bass DA. Phospholipase A<sub>2</sub> and arachidonate increase in bronchoalveolar lavage fluid after inhaled antigen challenge in asthmatics. Am J Respir Crit Care Med 1997;155:421-5.
- Enomoto A, Murakami M, Valentin E, Lambeau G, Gelb MH, Kudo I. Redundant and segregated functions of granule-associated heparin-binding group II subfamily of secretory phospholipases A<sub>2</sub> in the regulation of degranulation and prostaglandin D<sub>2</sub> synthesis in mast cells. J Immunol 2000;165:4007-14.
- Balboa MA, Balsinde J, Winstead MV, Tischfield JA, Dennis EA. Novel group V phospholipase A<sub>2</sub> involved in arachidonic acid mobilization in murine P388D1 macrophages. J Biol Chem 1996;271:32381-4.
- Morioka Y, Saiga A, Yokota Y, Suzuki N, Ikeda M, Ono T, et al. Mouse group X secretory phospholipase A<sub>2</sub> induces a potent release of arachidonic acid from spleen cells and acts as a ligand for the phospholipase A<sub>2</sub> receptor. Arch Biochem Biophys 2000;381:31-42.
- Ho IC, Arm JP, Bingham CO 3rd, Choi A, Austen KF, Glimcher LH. A novel group of phospholipase A<sub>2</sub> preferentially expressed in type 2 helper T cells. J Biol Chem 2001;276:18321-6.
- Hundley TR, Marshall LA, Hubbard WC, MacGlashan DW Jr. Characteristics of arachidonic acid generation in human basophils: relationship between the effects of inhibitors of secretory phospholipase A<sub>2</sub> activity and leukotriene C<sub>4</sub> release. J Pharmacol Exp Ther 1998;284:847-57.
- De Marino V, Gentile M, Granata F, Marone G, Triggiani M. Secretory phospholipase A<sub>2</sub>: a putative mediator of airway inflammation. Int Arch Allergy Immunol 1999;118:200-1.
- Seeds MC, Jones KA, Duncan Hite R, Willingham MC, Borgerink HM, Woodruff RD, et al. Cell-specific expression of group X and group V secretory phospholipases A<sub>2</sub> in human lung airway epithelial cells. Am J Respir Cell Mol Biol 2000;23:37-44.
- Beck S, Lambeau G, Scholz-Pedretti K, Gelb MH, Janssen MJ, Edwards SH, et al. Potentiation of tumor necrosis factor-α-induced secreted phospholipase A<sub>2</sub> (sPLA<sub>2</sub>)-IIA expression in mesangial cells by an autocrine loop involving sPLA<sub>2</sub> and peroxisome proliferator-activated receptor α activation. J Biol Chem 2003;278:29799-812.
- Portielje JE, Kruit WH, Eerenberg AJ, Schuler M, Sparreboom A, Lamers CH, et al. Subcutaneous injection of interleukin 12 induces systemic inflammatory responses in humans: implications for the use of IL-12 as vaccine adjuvant. Cancer Immunol Immunother 2005;54:37-43.
- Nakano T, Ohara O, Teraoka H, Arita H. Glucocorticoids suppress group II phospholipase A<sub>2</sub> production by blocking mRNA synthesis and post-transcriptional expression. J Biol Chem 1990;265:12745-8.
- Schalkwijk C, Pfeilschifter J, Marki F, van den Bosch H. Interleukin-1 beta- and forskolin-induced synthesis and secretion of group II phospholipase A<sub>2</sub> and prostaglandin E<sub>2</sub> in rat mesangial cells is prevented by transforming growth factor-beta 2. J Biol Chem 1992;267:8846-51.
- Peilot H, Rosengren B, Bondjers G, Hurt-Camejo E. Interferon-gamma induces secretory group IIA phospholipase A<sub>2</sub> in human arterial smooth muscle cells. Involvement of cell differentiation, STAT-3 activation, and modulation by other cytokines. J Biol Chem 2000;275:22895-904.
- van der Helm HA, Aarsman AJ, Janssen MJ, Neys FW, van den Bosch H. Regulation of the expression of group IIA and group V secretory phospholipases A<sub>2</sub> in rat mesangial cells. Biochim Biophys Acta 2000;1484:215-24.
- Satake Y, Diaz BL, Balestrieri B, Lam BK, Kanaoka Y, Grusby MJ, et al. Role of group V phospholipase A<sub>2</sub> in zymosan-induced eicosanoid generation and vascular permeability revealed by targeted gene disruption. J Biol Chem 2004;279:16488-94.
- Murakami M, Koduri RS, Enomoto A, Shimbara S, Seki M, Yoshihara K, et al. Distinct arachidonate-releasing functions of mammalian secreted

- phospholipase A<sub>2</sub>s in human embryonic kidney 293 and rat mastocytoma RBL-2H3 cells through heparan sulfate shuttling and external plasma membrane mechanisms. *J Biol Chem* 2001;276:10083-96.
28. Fonteh AN, Atsumi G, LaPorte T, Chilton FH. Secretory phospholipase A<sub>2</sub> receptor-mediated activation of cytosolic phospholipase A<sub>2</sub> in murine bone marrow-derived mast cells. *J Immunol* 2000;165:2773-82.
  29. Kim YJ, Kim KP, Han SK, Munoz NM, Zhu X, Sano H, et al. Group V phospholipase A<sub>2</sub> induces leukotriene biosynthesis in human neutrophils through the activation of group IVA phospholipase A<sub>2</sub>. *J Biol Chem* 2002;277:36479-88.
  30. Bingham CO, Murakami M, Fujishima H, Hunt JE, Austen KF, Arm JP. A heparin-sensitive phospholipase A<sub>2</sub> and prostaglandin endoperoxide synthase-2 are functionally linked in the delayed phase of prostaglandin D<sub>2</sub> generation in mouse bone marrow-derived mast cells. *J Biol Chem* 1996;271:25936-44.
  31. Balsinde J, Shinohara H, Lefkowitz LJ, Johnson CA, Balboa MA, Dennis EA. Group V phospholipase A(2)-dependent induction of cyclooxygenase-2 in macrophages. *J Biol Chem* 1999;274:25967-70.
  32. Graler MH, Goetzl EJ. Lysophospholipids and their G protein-coupled receptors in inflammation and immunity. *Biochim Biophys Acta* 2002;1582:168-74.
  33. Hanasaki K, Arita H. Phospholipase A<sub>2</sub> receptor: a regulator of biological functions of secretory phospholipase A<sub>2</sub>. *Prostaglandins Other Lipid Mediat* 2002;68-69:71-82.
  34. East L, Isacke CM. The mannose receptor family. *Biochim Biophys Acta* 2002;1572:364-86.
  35. Ramoner R, Putz T, Gander H, Rahm A, Bartsch G, Schaber C, et al. Dendritic-cell activation by secretory phospholipase A<sub>2</sub>. *Blood* 2005;105:3583-7.
  36. Perrin-Cocon L, Agaue S, Coutant F, Masurel A, Bezzine S, Lambeau G, et al. Secretory phospholipase A<sub>2</sub> induces dendritic cell maturation. *Eur J Immunol* 2004;34:2293-302.
  37. Asaoka Y, Yoshida K, Sasaki Y, Nishizuka Y, Murakami M, Kudo I, et al. Possible role of mammalian secretory group II phospholipase A<sub>2</sub> in T-lymphocyte activation: implication in propagation of inflammatory reaction. *Proc Natl Acad Sci U S A* 1993;90:716-9.
  38. Tessier C, Hichami A, Khan NA. Implication of three isoforms of PLA(2) in human T-cell proliferation. *FEBS Lett* 2002;520:111-6.
  39. Tanaka K, Ogawa K, Sugamura K, Nakamura M, Takano S, Nagata K. Cutting edge: differential production of prostaglandin D<sub>2</sub> by human helper T cell subsets. *J Immunol* 2000;164:2277-80.
  40. Hirai H, Tanaka K, Yoshie O, Ogawa K, Kenmotsu K, Takamori Y, et al. Prostaglandin D<sub>2</sub> selectively induces chemotaxis in T helper type 2 cells, eosinophils, and basophils via seven-transmembrane receptor CRTH2. *J Exp Med* 2001;193:255-61.
  41. Murakami M, Hara N, Kudo I, Inoue K. Triggering of degranulation in mast cells by exogenous type II phospholipase A<sub>2</sub>. *J Immunol* 1993;151:5675-84.
  42. Triggiani M, Granata F, Oriente A, De Marino V, Gentile M, Calabrese C, et al. Secretory phospholipases A<sub>2</sub> induce  $\beta$ -glucuronidase release and IL-6 production from human lung macrophages. *J Immunol* 2000;164:4908-15.
  43. Silliman CC, Moore EE, Zallen G, Gonzalez R, Johnson JL, Elzi DJ, et al. Presence of the M-type sPLA<sub>2</sub> receptor on neutrophils and its role in elastase release and adhesion. *Am J Physiol Cell Physiol* 2002;283:C1102-13.
  44. Triggiani M, Granata F, Balestrieri B, Petraroli A, Scalia G, Del Vecchio L, et al. Secretory phospholipases A<sub>2</sub> activate selective functions in human eosinophils. *J Immunol* 2003;170:3279-88.
  45. Park DW, Kim JR, Kim SY, Sonn JK, Bang OS, Kang SS, et al. Akt as a mediator of secretory phospholipase A<sub>2</sub> receptor-involved inducible nitric oxide synthase expression. *J Immunol* 2003;170:2093-9.
  46. Jo EJ, Lee HY, Lee YN, Kim JI, Kang HK, Park DW, et al. Group IB secretory phospholipase A<sub>2</sub> stimulates CXC chemokine ligand 8 production via ERK and NF-kappaB in human neutrophils. *J Immunol* 2004;173:6433-9.
  47. Beck G, Yard BA, Schulte J, Haak M, van Ackern K, van der Woude FJ, et al. Secreted phospholipases A<sub>2</sub> induce the expression of chemokines in microvascular endothelium. *Biochem Biophys Res Commun* 2003;300:731-7.
  48. Rizzo MT, Nguyen E, Aldo-Benson M, Lambeau G. Secreted phospholipase A(2) induces vascular endothelial cell migration. *Blood* 2000;96:3809-15.
  49. Gambero A, Landucci EC, Toyama MH, Marangoni S, Giglio JR, Nader HB, et al. Human neutrophil migration in vitro induced by secretory phospholipases A<sub>2</sub>: a role for cell surface glycosaminoglycans. *Biochem Pharmacol* 2002;63:65-72.
  50. Offer S, Yedgar S, Schwob O, Krinsky M, Bibi H, Eliraz A, et al. Negative feedback between secretory and cytosolic phospholipase A<sub>2</sub> and their opposing roles in ovalbumin-induced bronchoconstriction in rats. *Am J Physiol Lung Cell Mol Physiol* 2005;288:L523-9.
  51. Snyder DW, Bach NJ, Dillard RD, Draheim SE, Carlson DG, Fox N, et al. Pharmacology of LY315920/S-5920, [[3-(aminooxoacetyl)-2-ethyl-1-(phenylmethyl)-1H-indol-4-yl]oxy] acetate, a potent and selective secretory phospholipase A<sub>2</sub> inhibitor: a new class of anti-inflammatory drugs, SPI. *J Pharmacol Exp Ther* 1999;288:1117-24.
  52. Tomita Y, Kuwabara K, Furue S, Tanaka K, Yamada K, Ueno M, et al. Effect of a selective inhibitor of secretory phospholipase A<sub>2</sub>, S-5920/LY315920Na, on experimental acute pancreatitis in rats. *J Pharmacol Sci* 2004;96:144-54.
  53. Bradley JD, Dmitrienko AA, Kivitz AJ, Gluck OS, Weaver AL, Wiesenhutter C, et al. A randomized, double-blinded, placebo-controlled clinical trial of LY333013, a selective inhibitor of group II secretory phospholipase A<sub>2</sub>, in the treatment of rheumatoid arthritis. *J Rheumatol* 2005;32:417-23.
  54. Bowton DL, Dmitrienko AA, Israel E, Zeiher BG, Sides GD. Impact of a soluble phospholipase A<sub>2</sub> inhibitor on inhaled allergen challenge in subjects with asthma. *J Asthma* 2005;42:65-71.
  55. Villarrubia VG, Costa LA, Diez RA. Secreted phospholipases A<sub>2</sub> (sPLA<sub>2</sub>): friends or foes? Are they actors in antibacterial and anti-HIV resistance? *Med Clin (Barc)* 2004;123:749-57.
  56. Koduri RS, Gronroos JO, Laine VJ, Le Calvez C, Lambeau G, Nevalainen TJ, et al. Bactericidal properties of human and murine groups I, II, V, X, and XII secreted phospholipases A(2). *J Biol Chem* 2002;277:5849-57.
  57. Fenard D, Lambeau G, Maurin T, Lefebvre JC, Doglio A. A peptide derived from bee venom-secreted phospholipase A<sub>2</sub> inhibits replication of T-cell tropic HIV-1 strains via interaction with the CXCR4 chemokine receptor. *Mol Pharmacol* 2001;60:341-7.