The Involvement of Phospholipases A<sub>2</sub> in Asthma and Chronic Obstructive Pulmonary Disease

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The increased morbidity, mortality, and ineffective treatment associated with the pathogenesis of chronic inflammatory diseases such as asthma and chronic obstructive pulmonary disease (COPD) have generated much research interest. The key role is played by phospholipases from the A<sub>2</sub> superfamily: enzymes which are involved in inflammation through participation in pro- and anti-inflammatory mediators production and have an impact on many immunocompetent cells. The 30 members of the A<sub>2</sub> superfamily are divided into 7 groups. Their role in asthma and COPD has been studied in vitro and in vivo (animal models, cell cultures, and patients). This paper contains complete and updated information about the involvement of particular enzymes in the etiology and course of asthma and COPD.

1. Introduction

Both asthma and COPD are airway diseases characterized by impaired airflow in the respiratory tract, chronic airway inflammation, as well as symptoms such as coughing, dyspnea, and wheezing. Intensive studies focused on the pathogenesis of these conditions implicate, among others, the group of phospholipases A<sub>2</sub>, which possess enzymatic and nonenzymatic properties. This paper presents general information about phospholipases and details the current knowledge about particular phospholipases A<sub>2</sub> involved in asthma and COPD in human and animal models. The data regarding interactions between members of this superfamily is summarized, as well as the role of these enzymes in exacerbations of inflammatory diseases.

2. Phospholipases

Phospholipases are enzymes that hydrolyze phospholipids. The main substrates for these enzymes are glycerophospholipids which contain glycerol with a saturated fatty acid in the sn-1 position and an unsaturated fatty acid in the sn-2 position. The phospholipases responsible for hydrolysis of glycerophospholipids are divided into two groups: acyl-hydrolases and phosphodiesterases. The first group comprises phospholipase A<sub>1</sub> (PLA<sub>1</sub>) and A<sub>2</sub> (PLA<sub>2</sub>), which hydrolyze the ester bond at the sn-1 and sn-2 positions, respectively. The second group comprises phospholipase C (PLC) which cleaves the glycerol-phosphate bond, and phospholipase D (PLD), which liberates phosphatidic acid and alcohol (Figure 1). Phospholipase B shares both the properties of PLA<sub>1</sub> and PLA<sub>2</sub>.

The structure, function, and catalytic mechanism of the enzyme determine its place within the phospholipase A<sub>2</sub> superfamily, be it secretory PLA<sub>2</sub> (sPLA<sub>2</sub>), cytosolic PLA<sub>2</sub> (cPLA<sub>2</sub>), Ca<sup>2+</sup>-independent phospholipase A<sub>2</sub> (iPLA<sub>2</sub>), PAF acetylhydrolases (PAF-AH), or lysosomal PLA<sub>2</sub> (LPLA<sub>2</sub>). The latest classification, based on genetic structure, divides these enzymes into groups from I to XVI (in each one, the enzyme is represented by a capital letter) [1]. The characteristic features of each group are presented in Table 1. Table 2 includes information about the mechanism of action and function of particular subgroups of PLA<sub>2</sub>s concerning physiology and pathophysiology.
established that primary human lung mast cells constitutively express mRNA for the IB, IIA, IID, IIE, IIF, III, V, X, XIIA, and XIIB sPLA₂ groups and stimulation with anti-IgE antibodies can induce their secretion [10]. Hence sPLA₂ proteins are believed to belong to preformed mediators which are stored in mast cells granules. Cells stimulation by anti-IgE antibodies causes degranulation of mast cells, and sPLA₂ appears in the early phase of allergic reaction. Muñoz et al. have shown that sPLA₂V is not expressed in eosinophils in detectable amounts. However exogenous hPLA₂V can activate eosinophils, inducing the liberation of arachidonic acid (AA) and LTC₄ production [11]. Increased cPLA₂α phosphorylation and cPLA₂α activity was observed in eosinophils of asthmatics after allergen challenge [12].

Alveolar macrophages and neutrophils play a crucial role in the pathophysiology of COPD [13, 14]. Human macrophages express cPLA₂, iPLA₂, iPLA₂, and several sPLA₂s (IIA, IID, IIE, IIF, V, X, and XIIA, but not group IB and III enzymes). Higher expression of sPLA₂IIA is observed after LPS treatment [15]. Neutrophils stimulated in vitro by the tripeptide formyl-Met-Leu-Phe (fMLP) demonstrate mRNA and protein expression of sPLA₂V and sPLA₂X, where the sPLA₂V protein is found in azurophilic and specific granules, and sPLA₂X is found only in azurophilic granules. GIB, GIH, GIIE, GIIF, GIII, and GXII sPLA₂s are undetectable. Cell activation by fMLP or zymosan results in the release of GV but not GX sPLA₂ [16].

The BALF of patients with COPD demonstrates a three- to fivefold higher activity of sPLA₂ in comparison to a control BALF but the protein level shows no difference [17]. No differences in sPLA₂ IIIs serum levels exist between healthy smokers and nonsmokers. However, significantly greater levels of this enzyme are found in the BALF of smokers compared with nonsmokers [18]. Among sPLA₂s, sPLA₁ID is also considered as a molecule involved in the course of COPD. A change of Gly80Ser in the sPLA₁ID protein may be associated with body weight loss in patients suffering from COPD [19]. sPLA₁ID can be also involved in control of inflammation by inhibition of CD4+, CD8+ T cells proliferation and induction of regulatory T cell differentiation [20]. Cigarette smoke extract (CSE) can induce the production of cytokotic phospholipase A₂ in human pulmonary microvascular endothelial cells [21]. Moreover oxidative stress can increase the activity of cPLA₂ by promoting its phosphorylation [22]. cPLA₂ also participates in phosphodiesterase 4 signaling, whose inhibition attenuates neutrophilic inflammation in COPD [23]. The increased values of PLA₂VII in patients with long-standing pulmonary hypertension (severe complication in COPD) are related to severe endothelial dysfunction [24].

sPLA₂V plays a different role in the activation of eosinophils and neutrophils. Hence, its involvement in the pathogenesis of asthma and COPD can vary. Exogenous sPLA₂V can activate the production of AA and leukotrienes in both cell types. However, LTB₄ is preferentially produced in neutrophils, and LTC₄ in eosinophils [11]. The sPLA₂V-induced activation of neutrophils in contrast to eosinophils requires the presence and activation of cPLA₂ [25]. The inhibition of cPLA₂ may be more effective in diseases where neutrophils

![Figure 1: Phospholipases and their role in lipids metabolism.](image-url)
Table 1: Characteristics of structure and localization of human phospholipase A₂ enzymes. Adapted and modified from [1, 4]. The Roman numeral indicates the group, and the capital letter after the number indicates the subgroup.

<table>
<thead>
<tr>
<th>Name</th>
<th>Members (human)</th>
<th>Molecular mass (kDa)</th>
<th>Relationship with Ca²⁺</th>
<th>Catalytic site</th>
<th>Localization</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Secretory phospholipase A₂ (sPLA₂)</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IB (sPLA₂,IB)</td>
<td></td>
<td>13–15</td>
<td></td>
<td>Secreted</td>
<td>Secreted; membrane; secretory granules</td>
</tr>
<tr>
<td>IIA (sPLA₂,IIA)</td>
<td></td>
<td>13–15</td>
<td></td>
<td>Secreted</td>
<td></td>
</tr>
<tr>
<td>IID (sPLA₂,IID)</td>
<td></td>
<td>14-15</td>
<td></td>
<td>Secreted</td>
<td></td>
</tr>
<tr>
<td>IIE (sPLA₂,IIE)</td>
<td></td>
<td>14-15</td>
<td></td>
<td>Secreted</td>
<td></td>
</tr>
<tr>
<td>IIF (sPLA₂,IIF)</td>
<td></td>
<td>16-17</td>
<td></td>
<td>Secreted</td>
<td></td>
</tr>
<tr>
<td>III (sPLA₂,III)</td>
<td></td>
<td>55</td>
<td>Dependent Histidine/Aspartic acid</td>
<td>Secreted; Golgi apparatus; nuclear envelope; plasma membrane</td>
<td></td>
</tr>
<tr>
<td>V (sPLA₂,V)</td>
<td></td>
<td>14</td>
<td></td>
<td>Secreted</td>
<td></td>
</tr>
<tr>
<td>X (sPLA₂,X)</td>
<td></td>
<td>14</td>
<td></td>
<td>Secreted</td>
<td>Secreted; cytoplasm</td>
</tr>
<tr>
<td>XIIB (sPLA₂,XIIB, XIII)</td>
<td></td>
<td>20</td>
<td></td>
<td>Secreted</td>
<td></td>
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<tr>
<td><em>Cytosolic phospholipase A₂ (cPLA₂)</em></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>IVA (cPLA₂,α)</td>
<td></td>
<td>85</td>
<td>Dependent</td>
<td>Nucleus; cytoplasmic vesicles</td>
<td></td>
</tr>
<tr>
<td>IVB (cPLA₂,β)-three splice variants</td>
<td></td>
<td>114</td>
<td></td>
<td>Cytosol</td>
<td></td>
</tr>
<tr>
<td>IVC (cPLA₂,γ)</td>
<td></td>
<td>61</td>
<td>Independent</td>
<td>ER; Mitochondrion</td>
<td></td>
</tr>
<tr>
<td>IVD (cPLA₂,δ)</td>
<td></td>
<td>92-93</td>
<td>Serine/Aspartic acid/Arginine</td>
<td>Cytosol; Cytoplasmic vesicle membrane; peripheral membrane protein; cytoplasmic side</td>
<td></td>
</tr>
<tr>
<td>IVE (cPLA₂,ε)</td>
<td></td>
<td>96</td>
<td>Dependent</td>
<td>Cytosol; lysosome membrane; peripheral membrane protein; Cytosol; lysosome membrane; peripheral membrane protein; cytoplasmic side</td>
<td></td>
</tr>
<tr>
<td>IVF (cPLA₂,ζ)</td>
<td></td>
<td>95</td>
<td></td>
<td>Cytosol; lysosome membrane; peripheral membrane protein; cytoplasmic side</td>
<td></td>
</tr>
<tr>
<td><em>Ca²⁺-independent phospholipase A₂ (iPLA₂)</em></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>VIA-(iPLA₂,β)-five splice variants</td>
<td></td>
<td>84–90</td>
<td></td>
<td>Cytosol</td>
<td></td>
</tr>
<tr>
<td>VIB (iPLA₂,γ)-four splice variants</td>
<td></td>
<td>88–91</td>
<td></td>
<td>ER; peroxisomal and mitochondrial membrane</td>
<td></td>
</tr>
<tr>
<td>VIC (iPLA₂,δ, NTE)</td>
<td></td>
<td>146</td>
<td>Independent</td>
<td>ER; single-pass type I membrane protein; cytoplasmic side</td>
<td></td>
</tr>
<tr>
<td>VID (iPLA₂,ε, adiponutrin)</td>
<td></td>
<td>53</td>
<td></td>
<td>Membrane; single-pass type II membrane protein</td>
<td>Lipid droplet membrane; single-pass type II membrane protein; cell membrane</td>
</tr>
<tr>
<td>VIE (iPLA₂,ζ)</td>
<td></td>
<td>57</td>
<td></td>
<td>Cytosol</td>
<td></td>
</tr>
<tr>
<td>VIF (iPLA₂,η)</td>
<td></td>
<td>28</td>
<td></td>
<td>Cytosol</td>
<td></td>
</tr>
<tr>
<td><em>Acidic Ca²⁺-independent phospholipase A₂ (aiPLA₂)</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>aiPLA₂</td>
<td></td>
<td>26</td>
<td>Independent</td>
<td>Serine</td>
<td>Cytoplasm; Lysosome</td>
</tr>
<tr>
<td><em>Lysosomal phospholipase A₂</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>XV (LPLA₂, LLPL, ACS)</td>
<td></td>
<td>45</td>
<td>Independent</td>
<td>Serine/Histidine/Aspartic acid</td>
<td>Secreted; Lysosome</td>
</tr>
</tbody>
</table>
play a crucial role because they indirectly inhibit also the function of sPLA₂.

5. Role of PLA₂s in Asthma and COPD

The proposed mechanism of action of phospholipases A₂ (PLA₂s) in inflammatory diseases includes the liberation of arachidonic acid, generation of lysophospholipids, interaction between enzymes belonging to the A₂ superfamily, surfactant degradation, release of cytokines, and the impact on immunological and inflammatory cells (dendritic cells, T-cells, and leukocytes) [26].

5.1. The Enzymatic Activity of PLA₂s. The enzymatic properties of PLA₂s refer to their phospholipase, lysophospholipase, transacylase, adiponutrin-like, triglyceride lipase, peroxiredoxin 6, and acyl-ceramide synthase activities. Phospholipases A₂ play a pivotal role in eicosanoid production because they hydrolyze the ester bond at the sn-2 position of the glycerophospholipid membrane, releasing arachidonic acid (AA) and lysophospholipids [27]. Arachidonic acid plays a dual role. It can act as a signaling molecule that regulates the activity of protein kinase C (PKC) and phospholipase Cγ₁, influences Ca²⁺ concentration, and acts as an endogenous ligand for PPARγ receptors [28, 29]. AA is also a precursor of lipid inflammatory mediators (eicosanoids). In cyclooxygenase (COX) pathways, it is transformed to prostaglandins and thromboxane while in lipoxygenase (ALOX) pathways, it is converted to leukotrienes. These molecules are responsible for bronchial constriction, increased vessel permeability, and inflammatory cell recruitment [30]. AA is also a substrate for resolvins and lipoxins (LXs) which have anti-inflammatory properties. Lipoxins can block granulocyte chemotaxis, migration, degranulation, oxidative burst, cytokine-mediated signaling in eosinophils, and secretion of cytokines from bronchial epithelial cells [31]. Several independent studies have reported that significantly lower levels of LXs are observed in severe asthmatics compared to patients with non-severe asthma [32, 33]. Resolvins demonstrate endogenous anti-inflammatory, proresolving, antifibrotic, antiangiogenic, anti-infective, and antihyperalgesic activity [31].

Among cytosolic phospholipases A₂, it has been well documented that cPLA₂IVA (cPLA₂α) plays an important role in eicosanoid production. In patients with inherited cPLA₂ deficiency (loss-of-function mutations in both cPLA₂ alleles), a widespread decrease in eicosanoid concentrations has been observed [34]. S11P, R485H, and K65IR mutations in PLA2G4A gene are thought to play a crucial role in this condition. The functional consequences of localized mutations concerning cPLA₂ catalytic activity, Ca²⁺ recruitment, and affinity for the phospholipid membrane have been confirmed in vitro and in cell culture [35]. In patients with severe asthma, the microsatellite fragments (T)ₙ and (CA)ₙ in the promoter region of cPLA₂α gene (PLA2G4A) are shorter in comparison to healthy subjects [36]. In addition, asthmatic patients with shorter microsatellite sequences demonstrate greater expression of cPLA₂α mRNA, cPLA₂α protein, PGE₂ and 15-HETE, but not LTC₄ [37]. cPLA₂ participates in intracellular signaling, leading to allergen-induced production of inflammatory cytokines in the PBMC of asthmatics [38]. Hallstrand et al. [39] identified increased expression of three cPLA₂s, including cPLA₂α, cPLA₂β, and cPLA₂γ in induced sputum cells from subjects with asthma and exercise-induced bronchoconstriction. Both cPLA₂β and cPLA₂γ enzymes also participate in eicosanoids biosynthesis [40, 41]. Increased cPLA₂ expression and subsequent PGE₂ production are present in the asthma phenotype. The therapeutic decision to inhibit cPLA₂ in asthmatics may be unclear when considering the role of PGE₂ in airway inflammation. There is some evidence that PGE₂ can act as bronchodilator, as well as an inhibitor of both allergen-induced bronchoconstriction and inflammatory mediators production [42]. It should be noticed that PGE₂ acts through four different types of receptors (EP₁, EP₂, EP₃, and EP₄). Changes in expression and combination of receptor subtypes actions may affect the action of PGE₂ giving it proinflammatory or bronchoprotective outcomes [43–45]. The pleiotropic properties of PGE₂ make it difficult to establish the direct impact of PGE₂ deficiency which appears as a consequence of cPLA₂ inhibition [46]. Moreover, although cPLA₂ is a major enzyme, it is not the only one providing substrates for eicosanoids synthesis; hence it cannot be excluded that other existing pathways can also perform this function.


**Table 2: Mechanism of action and function of human phospholipase A₂ enzymes. Adapted and modified from [1, 4, 5].**

<table>
<thead>
<tr>
<th>Name</th>
<th>Mechanism of action</th>
<th>Function</th>
<th>Pathophysiology</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Secretory phospholipases A₂ (sPLA₂s)</strong></td>
<td>(i) Enzymatic (liberation of AA and lysophospholipids) (ii) Autocrine and paracrine action by binding to N-type and M-type receptors or by binding to integrins</td>
<td>(i) Lipid remodeling for membrane homeostasis (ii) Exocytosis (iii) Phagocytosis (iv) Anticoagulant activity (v) Antibacterial activity (Gram-positive and Gram-negative bacteria) (vi) Antifungal and antiadenoviral activity (vii) Parturition (viii) Spinal processing of nociception</td>
<td>(i) Inflammatory diseases (rheumatoid arthritis, adult respiratory distress syndrome, inflammatory bowel disease, and pancreatitis) (ii) Sepsis (iii) Atherosclerosis (foam cell formation) (iv) Cancer (v) Surfactant hydrolysis</td>
<td>Neutrophils, eosinophils, basophils, T-cells, monocytes, macrophages, platelets, mast cells, airway epithelial cells, alveolar type II epithelial cells,</td>
</tr>
<tr>
<td><strong>Cytosolic phospholipases A₂ (cPLA₂s)</strong></td>
<td>(i) enzymatic: lysophospholipase and transacylase activity</td>
<td>(i) AA releasing (ii) Cellular signaling (iii) Parturition (iv) Nociception</td>
<td>(i) Inflammation (ii) Intestinal ulceration (iii) Psoriasis (iv) Acute lung injury (v) Polyposis (vi) Brain injury (vii) Anaphylaxis</td>
<td>Every tissue</td>
</tr>
<tr>
<td><strong>Ca²⁺-independent phospholipases A₂ (iPLA₂s)</strong></td>
<td>VIA, VIB, VIC, VID, VIEVIF-phospholipase A₂ activity VIC-lysophospholipase activity VID-adiponutrin-like activity VIE-triglyceride lipase activity VIF-transacylase activity</td>
<td>(i) Remodeling of phospholipids (ii) AA releasing (iii) Protein expression (iv) Acetylcholine-mediated endothelium-dependent relaxation of the vasculature (v) Apoptosis (vi) Insulin secretion (vii) Bone formation (viii) Sperm development (ix) Cell proliferation (x) Activation of Ca²⁺ influx (xi) Axon regeneration in nerve injury (VIA) (i) Degradation and recycling of surfactant phospholipids (remodeling of phosphatidylcholine to dipalmitoyl-phosphatidylcholine (DPPC)) (ii) Antioxidative activity</td>
<td>(i) Wallerian degeneration (VIA) (ii) regulation of monocyte migration (VIB) (iii) Oxidant-induced cell injury (VIC) (iv) Ischemia-induced ventricular tachyarrhythmias (i) Lung cancer, mesothelioma, sarcoidosis</td>
<td>(i) Alveolar cells (ii) Macrophages (iii) Normal and cancer lung tissue (iv) Neurons</td>
</tr>
<tr>
<td><strong>aiPLA₂-phospholipase A₂ and peroxiredoxin 6 activity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Lyosomal phospholipase A₂</strong></td>
<td>(i) Acyl-ceramide synthase (ii) Transacylase activity (iii) Lyso phospholipase activity</td>
<td>(i) may be the crucial enzyme of pulmonary surfactant phospholipid degradation by alveolar macrophages</td>
<td>(i) Phospholipidosis (ii) Complement activation (iii) Induced lung injury</td>
<td>(i) Alveolar macrophages (ii) Peripheral blood monocytes</td>
</tr>
<tr>
<td><strong>PAF acetylhydrolases (PAF-AH) or Lipoprotein-associated phospholipases A₂</strong></td>
<td>(i) Phospholipase A₂ activity</td>
<td>(i) Anti-inflammatory properties by hydrolyzing platelet activating factor (PAF) (ii) Protection against oxidative stress (iii) Brain development</td>
<td>(i) Generation of lysophospholipids and fatty acid hydroperoxides (ROS) (ii) Acute respiratory distress syndrome (iii) Marker of coronary heart disease (iv) Miller-Diker lissencephaly</td>
<td>(i) Alveolar macrophages (ii) Epithelial type II cells</td>
</tr>
</tbody>
</table>
sPLA₂s and arachidonic acid accumulate in the BALF of asthmatics after allergen challenge [47, 48]. Despite being specific to the sn-2 bond, sPLA₂s play more of a supporting role in AA liberation. Only sPLA₂V and sPLA₂X can efficiently interact and hydrolyze phospholipids from the outer surface of the cell membrane [9]. In acute and chronic animal asthma models, a deficit of sPLA₂X diminishes the features of asthma (eosinophilia, airway hyperresponsiveness to methacholine, airway remodeling, eicosanoids, and Th2 cytokine production) [49].

Hallstrand et al. [50] showed that the expression of sPLA₂X predominates in the airway epithelium, and both sPLA₂X and sPLA₂IIA are the main phospholipases produced by BALF cells. The activity of the sPLA₂V protein was found to be greatly lowered and undetectable. They have suggested that sPLA₂X is most important among secretory phospholipases. Only sPLA₂X, not sPLA₂IIA, is correlated with asthma features such as lung function, recruitment of neutrophils in asthmatics [50]. sPLA₂X is responsible for production of cysteinyl leukotrienes (cysLTs) which are proinflammatory in asthma and can be responsible for observable features of asthma. Moreover, the level of prostaglandin E₂ (PGE₂) is also connected with sPLA₂X, which can be explained by the fact that sPLA₂X increases activity of cPLA₂IV which in turn leads to production of PGE₂. These results are consistent with earlier studies by the same authors in which gene expression of sPLA₂X and sPLA₂ XII was demonstrated to be elevated in induced sputum cells of patients with asthma. The level of sPLA₂X in induced sputum cells supernatant increased after exercise challenge among asthmatics with exercise-induced bronchoconstriction (EIB) [39]. Lai et al. [51] have confirmed the involvement of sPLA₂X. They demonstrated that recombinant sPLA₂X caused AA release and rapid onset of cysLT synthesis in human eosinophils.

Limited information suggests a possible anti-inflammatory role of sPLA₂X. However in asthma, sPLA₂X facilitates the polarization toward proasthmatic M2-macrophage phenotype [52]. It is possible that in a proinflammatory environment, that the sPLA₂X propeptide is more rapidly converted to an active form that might influence the Th1/Th2 balance [53]. All these factors may suppress its anti-inflammatory action.

Other sPLA₂s (IIA, IID, IIE) contain a heparin-binding domain which allows these enzymes to be taken into the cells and further directed to compartments enriched in AA and enzymes responsible for eicosanoid production [54].

In spite of the fact that several studies have confirmed the participation of iPLA₂β [55] and iPLA₂γ [56] in AA release and eicosanoid production, there is no data indicating that these enzymes play a direct role in asthma. By the induction of Ca²⁺ influx they can influence the translocation and activity of Ca²⁺-dependent PLA₂ isoforms.

Group VII and VIII PAF-AH hydrolyze the short sn-2 residue of PAF (platelet activating factor). As they lack activity against membrane phospholipids with long-chain sn-2 residues, they are unable to release arachidonic acid from membrane phospholipids [57]. They exhibit pro- and anti-inflammatory properties. On the one hand, they inactivate PAF—the proinflammatory mediator—by hydrolyzing it to inactive acetate and lysolipid but on the other hand, they assist in the generation of lysophospholipids and fatty acid hydroperoxides [4]. Stafforini et al. [58] have established that asthmatics have a decreased level of PAF-AH, and that asthma incidence and severity correlate to PAF-AH deficiency in the Japanese population. Also some PAF-AH gene polymorphisms (Ile198Thr and Ala379Val variants) are known to be a risk factors for developing atopy and asthma [59]. Despite positive effects in animal models [60], administration of human recombinant PAF-AH (rPAF-AH) does not reduce both early and late phase of asthmatic response in mild asthmatics challenged with allergens [61].

The enzymatic activity of PLA₂s embraces also lysophospholipid generation. Lysophospholipids are biologically active molecules acting through specific receptors. They are a precursor of platelet activating factor (PAF) and lysophosphatidic acid (LPA). LPA is involved in cell adhesion, motility, and survival. In animal models, lysophospholipid receptors are required for proper development and function of the cardiovascular, immune, respiratory, and reproductive systems [62]. Lysophosphocholine and polyunsaturated fatty acids, including AA, can activate cPLA₂ and 5-lipoxygenase by increasing Ca²⁺ and inducing cPLA₂ phosphorylation, which then leads to LTB₄ biosynthesis [25]. Lysophospholipid has nonspecific cytotoxic effect that depends on its concentration (critical micelle concentration). At concentration below their unspecific cytotoxic effect lysophospholipids can induce apoptosis by interrupting the synthesis of phosphatidylyceroline [63].

Phospholipases A₂ activity is also connected with disturbed lipid homeostasis in the lung. Asthma and other inflammatory lung diseases are characterized by impaired surfactant function [64]. Secretory phospholipases degrade phosphatidylcholine (PC), the main component of the surfactant responsible for maintenance of small airway patency. The generation of lysophospholipids and free fatty acids by sPLA₂-mediated PC hydrolysis has been implicated in small airway closure in asthma. sPLA₂ action is enhanced by
eosinophilic lysophospholipases that use lysophospholipids as a substrate [65–68]. The presence of iPLA2 proteins in alveolar macrophages suggests that they might play a role in surfactant degradation [69].

It should be mentioned that some PLA2s are involved in antibacterial defense thanks to their ability to hydrolyze the lipids of the bacterial membrane. sPLA2s IIa, V, X, and IB demonstrate bactericidal activity against gram-positive pathogens but the most effective is sPLA2-IIA. Group XII can directly kill E.coli, unlike the other sPLA2s that require cofactors [70]. This property of phospholipases can be important in bacterial exacerbations of asthma and COPD.

5.2. Nonenzymatic Activity of PLA2s. The secretory forms of many PLA2s exert a range of actions in airway inflammation. Apart from their enzymatic activity, they can act as extracellular mediators involved in chemotaxis, cytokine production, and induction of cellular signaling pathways. Mammalian N-type receptors have been identified for sPLA2, IB and IIA, X and M-type receptors for sPLA2, IB, IIA, IIE, IIF, V, and X [71]. N-type like receptors are present in lungs whereas M-type receptors have been identified in lung and myeloid cells [72]. The binding of sPLA2s to their M-type receptor deactivates their enzymatic properties [73].

sPLA2s are stored in intrinsic mast cell granulates and are released after cell activation by IgE and non-IgE stimuli [9]. After exocytosis, they can act in both autocrine and paracrine manners. By interacting with heparan sulphate proteoglycans and M-type receptors, they can induce PGD2 and LTC4 production and stimulate the subsequent degranulation of mast cells [74]. Granata et al. [17] delivered an evidence that sPLA2s can act as proinflammatory connections between mast cells and macrophages in the airway. They suggest that the activation of macrophages by sPLA2s leads to production of proinflammatory cytokines which sustain the inflammatory and immune response, chemokines responsible for recruitment of monocytes and neutrophils, as well as destructive lysosomal enzymes, NO, PGE2, and metalloproteinases involved with airway remodeling [17]. The sPLA2s induce β-glucuronidase release and production of IL-6 from human lung macrophages [75]. They influence the migration and adhesion of neutrophils as well as the release of elastase [76, 77]. In eosinophils, sPLA2 IA and IIA stimulate β-glucuronidase release and cytokine production (IL-6, IL-8) by AA and lysophospholipid generation, by interaction with membrane peptidoglycans via their heparin-binding site, and through binding with specific M-type or N-type receptors [78]. The functions of sPLA2s receptors require further studies because there are still some missing or unequivocal information [52].

5.3. Crosstalk between PLA2s. The phospholipases can cooperate in mechanism leading to eicosanoid production. sPLA2 and cPLA2 interaction is quite well documented [79, 80]. The effect of group IIa and V PLA2s on H2O2-induced AA release is dependent upon the presence of cPLA2 and the activation of PKC and ERK1/2 in murine mesangial cells. Offer et al. [81] have described negative feedback between sPLA2 and cPLA2 in eicosanoid production. sPLA2 activation induces production of bronchoconstrictor cysteinyi leukotrienes and suppresses cPLA2 expression and the subsequent production of bronchodilator PGE2. Recently it has been established that in human eosinophils, sPLA2 initiates Ser(505) phosphorylation of cPLA2α and stimulates leukotriene synthesis through involvement of p38 and JNK MAPK, cPLA2α, and 5-lipoxygenase activation, which may be an important process also in airways of asthmatics [51]. Also in bone-marrow-derived mast cells, sPLA2 mediates the selective release of AA by binding M-type receptors and then inducing MAPK signaling pathways that lead to cPLA2 activation [82].

5.4. PLA2s in the Exacerbation of Disease. Another aspect of phospholipases and the asthma/COPD relationship is the participation of these enzymes in the pathogenetic mechanisms of disease exacerbation caused by bacterial factors. This role relates to increased expression of selective PLA2s, modulation of their activity and involvement in cellular signaling. Elevated cPLA2α expression was found in human lung macrophages after LPS treatment [15, 83]. LPS stimulates expression of cPLA2 and COX-2 in macrophages, leading to increased production of AA and PGE2 [83]. LPS treatment was also followed by rapid changes in cPLA2 phosphorylation [84, 85]. This is one of the mechanisms of regulating enzyme activity [86]. The LPS-phosphorylated form of cPLA2 is present in induction of iNOS and TNF-α expression [87, 88] and metalloproteinase production [89]. Selective sPLA2 contributes to LPS-intracellular signaling in liver macrophages [84, 90, 91].

In mice with LPS-induced lung inflammation, the expression of sPLA2X remains the same before and after treatment. In this study, increased expression of sPLA2IID and sPLA2V has been observed, as well as decreased sPLA2IIE and sPLA2IIIF levels in the lungs. In rats, sPLA2IIA was seen to have the highest expression after LPS administration [92]. In msPLA2X−/− mice with knock-in of human sPLA2X (hsPLA2X), allergen-induced inflammatory cell recruitment into airways (eosinophils) was restored, as well as hyperresponsiveness to methacholine. The application of specific hsPLA2X inhibitor (RO 061606) significantly attenuates airway inflammation symptoms, mucous secretion, and hyperresponsiveness [93]. In sPLA2V−/− knock-out mice, sPLA2V has been proven to play a role in the development of lung injury and neutrophilic inflammation after bacterial stimulus (LPS) [94]. In addition, sPLA2V was seen to be connected with regulation of cell migration and generation of airway hyperresponsiveness after ovalbumin challenge [95]. In a murine allergen-challenged asthma model, administration of rPAF-AH is effective in blocking late-phase pulmonary inflammation [60].

6. The Clinical Significance of Studying the Participation of PLA2s in Airway Inflammatory Diseases

Taking into consideration the severe asthma phenotype, the difficulties related to obtain asthma control utilizing currently
available treatments and the progressive character of inflammation in patients with COPD that increases the morbidity, it seems reasonable to study the differences in pathogenesis of the diseases conditions, especially in relation to possible new therapies and drugs. The PLAs are an interesting object of study for several reasons. The superfamily of these enzymes contains approximately 30 members that have similar and isoform-specific properties. It has been confirmed that they are strictly connected with inflammation. The inhibitors of particular PLAs show the positive effect in treatment of inflammatory diseases [96] and they inhibit allergic reaction in vitro [38]. The cPLAα that evolved together with receptors for eicosanoids, present only in vertebrate, seems to play crucial role in course of inflammation. Its inhibitors such as efipladib [97] and ecopladib [98] successfully inhibit inflammation in rheumatoid arthritis and osteoporosis. The inhaled form of cPLAα inhibitor, the PLA-950, is considered as potential new treatment in asthmatic patients as well as other PLAs can influence the function of cPLAα or have similar effects. Recent studies report positive results of a preclinical evaluation of a cPLAα inhibitor [99]. The studies and analysis of protein involved in regulation of particular sPLA involved in inflammatory diseases could result in finding new target for drugs.

Since 1980, it has been known that glucocorticoids (GCs) can inhibit the activity of PLA2 [100]. The underlying mechanism concerns induction of mRNA and protein expression of lipocortin 1 (annexin 1) and the PLA2 inhibitory protein [101–104]. The structure, function, and mechanism behind the anti-inflammatory action of annexin 1 have been well described elsewhere [105]. Glucocorticoids can also suppress the production of sPLA2IIA by blocking mRNA synthesis and posttranslational expression in rats [106]. It is questionable whether therapeutic doses of glucocorticoids have sufficient power to satisfactorily inhibit the activity of PLA2. Juergens et al. [107] demonstrated that topical GCs at therapeutically relevant concentration (10^–8 M) inhibit the spontaneous activity of cPLA2 in the range of 8.6–17.3% depending on the type of GC. They suggest also that this effect may appear as a consequence of a decreased ability to binding the receptors by GCs present in airway in subtherapeutic doses. Although it has been established that treatment with GCs can indirectly inhibit cPLA2 and AA-derives production resistance to GCs in patients with asthma and COPD could also be problematic. Moreover the GCs have systemic effects and long-term application can cause the side effects. The approach to attack the inflammation process more precisely and downstream (inhibition the eicosanoids production) seems to be rationale.

Another aspect regarding annexin 1 and PLAs is their cell-specific manner of interactions [105]. Kwon et al. [108] demonstrated that cleavage of annexin 1 causes phosphorylation of cPLA2 during mast-cell activation. Hence it is not clear whether GCs-induced expression of annexin always leads to inhibition of cPLA2 activity. Posttranslational changes can dramatically influence the primary protein function. As previous studies indicate that GCs can stimulate expression of cPLA2 in amnion fibroblast it cannot be excluded that in some specific circumstances GCs may directly induce cPLA2 [109,110].

7. Conclusions

Previous studies confirm the involvement of phospholipases A2 in asthma and COPD although there are some gaps relating to the roles of specific enzymes. The participation of PLAs in asthma pathogenesis has been better investigated. The diagnostic problems concerning the overlap syndrome that shares the features of asthma and COPD demand further studies on the pathogenesis of these diseases. The phospholipases A2 through their involvement in the course of inflammation seem to be important aspects of this investigation. As they demonstrate pro- and anti-inflammatory properties, a detailed analysis of their role should act as a focus for further studies intended to bring new insights into the pathogenesis of the diseases and identify targets for new drugs.

Data from studies focused on role of PLAs in inflammatory diseases facilitate the understanding of molecular aspects of inflammation. It can be observed that cPLA2 plays a main role in eicosanoid production and other PLAs may influence their activity thanks to enzymatic properties or act as regulators of inflammation through their nonenzymatic activity. The pleiotropic properties of single phospholipase and their differential expression in many cells confirm that this is well-organized network of interaction, and further studies focused on this aspect may provide more useful knowledge. A comparison of how this network works in different inflammatory diseases, as well as in healthy subjects may indicate a key molecule, whose activity or presence will be a diagnostic parameter or whose activation or inhibition will have therapeutic value.

Asthma and COPD are heterogeneous diseases and current treatment gives only the possibility to obtain the phenotype of well-controlled diseases. Analysis of data regarding the involvement of PLAs in course of diseases arises the concept to use combined therapy rather than the treatment based on inhibition of one of them. The results from preclinical studies of cPLA2 inhibitors are promising but clinical trials will give concrete knowledge about the effectiveness and possible side effects.

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