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**Phospholipase A<sub>2</sub> Subclasses in Acute Respiratory Distress Syndrome**Eirini Kitsioulis<sup>1</sup>, George Nakos<sup>2</sup>, Marilena E. Lekka<sup>3</sup>

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**ABSTRACT**

Phospholipases A<sub>2</sub> (PLA<sub>2</sub>) catalyse the cleavage of fatty acids esterified at the *sn*-2 position of glycerophospholipids. In acute lung injury-acute respiratory distress syndrome (ALI-ARDS) several distinct isoenzymes appear in lung cells and fluid. Some are capable to trigger molecular events leading to enhanced inflammation and lung damage and others have a role in lung surfactant recycling preserving lung function: Secreted forms (groups sPLA<sub>2</sub>-IIA, -V, -X) can directly hydrolyze surfactant phospholipids. Cytosolic PLA<sub>2</sub> (cPLA<sub>2</sub>-IVA) requiring Ca<sup>2+</sup> has a preference for arachidonate, the precursor of eicosanoids which participate in the inflammatory response in the lung. Ca<sup>2+</sup>-independent intracellular PLA<sub>2</sub>s (iPLA<sub>2</sub>) take part in surfactant phospholipids turnover within alveolar cells. Acidic Ca<sup>2+</sup>-independent PLA<sub>2</sub> (aiPLA<sub>2</sub>), of lysosomal origin, has additionally antioxidant properties, (peroxiredoxin VI activity), and participates in the formation of dipalmitoyl-phosphatidylcholine in lung surfactant. PAF-AH degrades PAF, a potent mediator of inflammation, and oxidatively fragmented phospholipids but also leads to toxic metabolites. Therefore, the regulation of PLA<sub>2</sub> isoforms could be a valuable approach for ARDS treatment.

## INTRODUCTION

Acute lung injury (ALI) and its severe form Acute Respiratory Distress Syndrome (ARDS) is a life threatening situation with high mortality rates. The pathophysiology of ALI/ARDS is associated with an excessive and protracted inflammation, characterised by increased vascular permeability, extravasation of plasma and leucocytes infiltration. It is often systemic, resulting in multi-organ dysfunction and death. Since inflammation is thought to contribute to the pathogenesis of ALI /ARDS it is rational to explore modulating therapies in this context. Thus, the investigation and clarification of underlying mechanisms of the inflammatory processes may contribute to such therapeutic approaches [1, 2].

Clinically, ALI /ARDS is characterised by an abrupt onset of hypoxemic respiratory failure, pulmonary oedema and lung infiltrates. ALI /ARDS resulting from direct lung insults (e.g. aspiration, inhalation, or lung infection) is termed pulmonary, primary or direct ARDS and when the initial cause is systemic (e.g. sepsis or traumatic shock) it is termed extrapulmonary, secondary or indirect ARDS. In addition to this distinction, a classification into early, late, persistent and chronic phases is also used. The early ARDS/ALI (<2 days from the initiation) is characterized by lung flooding with proteinaceous fluid. Prominent features that can be visualized by electron microscopy are: swelling of endothelial cells, widening of intercellular junctions, increased numbers of pinocytotic vesicles, and disruption of the basement membrane. Within this period of time, pulmonary oedema is most pronounced. Over the ensuing days (2-7 days), in late phase, hyaline membrane formation in the alveolar spaces is prominent. During the persistent phase of ALI /ARDS interstitial inflammation is the main characteristic, while chronic phase is dominated by extensive pulmonary fibrosis [3, 4, 5, 6]. Patients of all ages have the probability to develop

ALI /ARDS, however, data indicate that genetic predisposition plays a role [7, 8, 9]. In the development of the syndrome, various inflammatory cells such as polymorphonuclear neutrophils, alveolar macrophages and platelets participate [for review see ref. 10].

ALI/ARDS is associated with lung surfactant disorders which can be observed soon after the initial injurious event and leads to increased surface tension, alveolar collapse, loss of liquid balance in the lung and deficiency of immune host defence [11, 12]. Surfactant is a lipoprotein complex with essential role in reducing surface tension at the air-liquid interface of lung epithelia; in addition, it is a critical component in lung host defence. It is mainly composed by phospholipids and especially dipalmitoyl-phosphatidylcholine and phosphatidylglycerol, while approximately a 10 % is comprised of specific proteins. Hydrophilic surfactant proteins SP-A and SP-D belong to the collectin family and they regulate innate immune functions in inflammatory cells like macrophages. They also modulate the adaptive immune response by interacting with antigen-presenting and T cells, thereby linking innate and adaptive immunity. The hydrophobic surfactant proteins SP-B and SP-C contribute to the surface properties of pulmonary surfactant [13, 14].

Surfactant can be readily hydrolysed by phospholipases A<sub>2</sub> (PLA<sub>2</sub>), which are produced during inflammation. The produced lyso-phospholipids and eicosanoids have been implicated in the pathogenesis of acute lung injury [15, 16, 17, 18, 19].

### **PHOSPHOLIPASES A<sub>2</sub> (PLA<sub>2</sub>s)**

PLA<sub>2</sub>s are esterases that catalyse the hydrolysis of acyl-groups at the *sn*-2 position of glycerophospholipids (PL) and produce free fatty acids and lyso-PL by an interfacial activation catalytic mechanism. To date, at least 26 genes that encode various types of proteins with PLA<sub>2</sub> activity have been identified in human. These enzymes can be classified

into groups according to their biochemical characteristics, properties and localisation [20, 21, 22, 23] (**Table 1**).

PLA<sub>2</sub>s are expressed and released by human inflammatory cells such as neutrophils, eosinophils, T-cells, monocytes, macrophages and mast cells [24]. Several studies have shown that various types of phospholipases A<sub>2</sub> can be implicated in the pathogenesis of ALI/ARDS through the production of potent lipid mediators [15, 25]. In particular, the liberated arachidonic acid (AA) is the precursor of eicosanoids, signalling molecules formed by cyclooxygenases, lipoxygenases or endoperoxidases. These bind to specific G-protein coupled receptors and exert complex control over inflammation or immunity. Lyso-PLs are precursors for lysophosphatidic acid (LPA) and platelet activating factor (PAF), which are associated with cardiovascular homeostasis, inflammation and immunity. Lyso-PLs modulate the expression of specific pro-inflammatory genes by mobilising the phagocyte receptor G2A [26]. Due to their tensioactive properties they can deteriorate the structure of cell membranes. On the contrary, others PLA<sub>2</sub>s participate in surfactant biosynthesis through phospholipid remodelling, contributing to lung integrity [27].

PLA<sub>2</sub>s can disturb pulmonary function either directly, by hydrolyzing lung surfactant phospholipids or indirectly, through the production of biologically active molecules. This leads to increased alveolar-capillary barrier permeability and lung oedema formation which characterise ALI/ARDS [28, 29].

Taking into account the complexity of this superfamily of enzymes, in this work we are going to focus on the function of various PLA<sub>2</sub> isotypes in lung parenchyma and their implication in the development of ALI/ARDS.



### Secretory PLA<sub>2</sub>s (sPLA<sub>2</sub>s)

sPLA<sub>2</sub>s are low molecular weight secretory proteins (14-57 kDa) which usually contain 5-8 disulfide bonds (protein data bank number 1DB4) [30]. They possess a histidine in their active site, require mM levels of Ca<sup>2+</sup> and show preference for anionic phospholipids as substrates, such as phosphatidylglycerol, a major component of surfactant phospholipids. Ten human genes encode the active secreted PLA<sub>2</sub> enzymes [31].

Total lung extracts express sPLA<sub>2</sub>-IB, -IIA, -V, and -X. The prominent implication of sPLA<sub>2</sub>-IB and sPLA<sub>2</sub>-IIA in inflammation and ALI/ARDS has been established long ago in experimental models [32, 33, 34]. However, a role for sPLA<sub>2</sub>-X and -V cannot be excluded, given that, these isoforms appear uniquely expressed in airway epithelium and can effectively hydrolyze lung surfactant [35].

#### *sPLA<sub>2</sub>-IB*

sPLA<sub>2</sub>-IB, (pancreatic phospholipase A<sub>2</sub>, gene PLA2G1B), has a widespread tissue distribution, including lung [36]. It is a low molecular weight (14 kDa), Ca<sup>2+</sup>-dependent enzyme, activated by proteolytic cleavage of a cytosolic inactive propeptide [37]. It modulates inflammatory, immune responses and chemotaxis in human neutrophils through leukotriene B<sub>4</sub> production [38]. Apart from its direct lipolytic activity it also acts through binding to the membrane receptor for secretory PLA<sub>2</sub>s (PLA<sub>2</sub>R), (**scheme1-1**), [39, 40].

sPLA<sub>2</sub>-IB has been detected in serum of patients with acute lung injury but not in healthy controls. It can be used as a diagnostic and prognostic marker since the presence of the sPLA<sub>2</sub>-IB propeptide showed a 100% sensitivity for patients with ALI and 93% specificity for future development of ALI [41]. The enzyme has also been implicated in complications of severe acute pancreatitis [42, 43], a recognized risk factor for ALI/ARDS.

A cross-talk between sPLA<sub>2</sub>-IB and sPLA<sub>2</sub>-IIA has been reported. Interaction of the mature sPLA<sub>2</sub>-IB with its specific receptor caused an elevation in prostaglandin E<sub>2</sub> synthesis, PLA<sub>2</sub>-II mRNA levels as well as in an increase in PLA<sub>2</sub>-II secretion in rat mesangial cells. Both PLA<sub>2</sub>-II expression and PGE<sub>2</sub> synthesis were completely suppressed after pretreatment of the cells with actinomycin D and cycloheximide, inhibitors of protein synthesis, or dexamethasone, a potent anti-inflammatory corticosteroid [44].

### *sPLA<sub>2</sub>-IIA*

sPLA<sub>2</sub>-IIA (gene PLA2G2A) is the most studied isoform. It is a Ca<sup>2+</sup>-dependent enzyme with optimum pH: 8.0-10.0. The amino acid sequence of the human protein consists of 124 amino acids, contains structural features common to all known phospholipases A<sub>2</sub>, and has a half-cystine pattern that is characteristic of the snake venom group II enzymes [45].

sPLA<sub>2</sub>-IIA is present in platelets and is highly expressed in alveolar macrophages of a model of ALI induced by LPS and TNF-alpha [46] (**scheme 1-2**). It is stored in the secretory granules of cells, and also expressed in various human tissues including lung [47]. It plays a role in normal cellular function such as lipid remodelling for cell membrane homeostasis, antimicrobial activity, anticoagulation and cell adhesion; in addition, it has been implicated in inflammatory diseases, septic shock and adult respiratory distress syndrome [for review see ref 48]. The bactericidal - antimicrobial activity of sPLA<sub>2</sub>-IIA is exerted against gram-positive and -negative bacteria [49, 50]. The action against gram-negative bacteria appears to require the presence of other agents, such as bactericidal/permeability-increasing protein, whereas this requirement is not necessary against gram-positive ones [51]. In addition, sPLA<sub>2</sub>-IIA is regarded as an acute phase protein [52, 53].

During the last years, the involvement of secretory phospholipases A<sub>2</sub> in ARDS and sepsis has been widely recognised [54]. In the bronchoalveolar lavage (BAL) of animal models with lung injury induced by oleic acid, a marked increase of PLA<sub>2</sub> activity in comparison with control animals was observed. ALI was associated with surfactant hydrolysis by sPLA<sub>2</sub> as well as by a perturbation of surfactant fluidity caused by lyso-phospholipids. These result to an increase in airway and capillary permeability and to altered alveolar epithelium barrier function [55]. Separate research groups reported the increase of sPLA<sub>2</sub>-IIA in BAL fluid of ARDS patients [56, 15] and these levels were found to correlate positively with the severity of ARDS [57]. The levels of sPLA<sub>2</sub>-IIA detected in BAL fluid or cells, was considered as a marker for surfactant degradation and associated with ongoing alveolar inflammation [58].

In ARDS patients, increased sPLA<sub>2</sub>-IIA levels were detected in BAL fluid, BAL cells and plasma [59]. Total PLA<sub>2</sub> activity was inversely correlated with PaO<sub>2</sub>/FIO<sub>2</sub> ratio and positively with the mortality rate. Patients with direct ARDS exhibited higher PLA<sub>2</sub> activity compared with patients with indirect ARDS. An interesting finding in this study was that in ARDS patients, PLA<sub>2</sub> activity was localized in the very small surfactant aggregates; on the contrary, in control patients the enzyme was localised predominately in the large surfactant aggregates. Very small surfactant aggregates represent utilized-decomposed surfactant with poor surface properties, providing an additional evidence for the role of PLA<sub>2</sub> in surfactant degradation in ALI and ARDS. Surfactant phospholipid alterations in BAL fluid from ARDS patients included an increase in lyso-phospholipids, that was attributed to secreted phospholipases A<sub>2</sub> [11, 60].

In BAL fluid from ARDS patients, phosphatidylcholine (PC) was markedly reduced, while lyso-PC, a product of PLA<sub>2</sub> action on PC, was increased. Interestingly, the level of PC was positively related with survival of ARDS patients [15]. Elevated levels of plasma PLA<sub>2</sub> were also detected in patients with ARDS and Multiple-Organ Failure [61]. It has been suggested that lung injury induced by intestinal ischemia–reperfusion, as well as by acute hepatic deficiency appears to be mediated by a mechanism that involves sPLA<sub>2</sub>-IIA activation [53, 62]. Therefore, sPLA<sub>2</sub>-IIA seems to play an important role in the development of acute lung injury since surfactant phospholipids are major targets for the enzyme (**scheme 1-3**).

#### *sPLA<sub>2</sub>-IIA regulation*

Arachidonic acid (AA) differentially affects the basal and lipopolysaccharide-induced sPLA<sub>2</sub>-IIA expression: In unstimulated alveolar macrophages from guinea pigs, AA directly inhibited sPLA<sub>2</sub>-IIA expression via a process involving the impairment of NF- $\kappa$ B activation, whereas in LPS-stimulated cells, AA effect is mediated via its oxidative metabolism to the COX metabolites and the subsequent PPAR- $\gamma$  activation (**scheme 1-4**). Alveolar macrophages represent the major pulmonary source of sPLA<sub>2</sub>-IIA in LPS-induced ALI. Therefore, it is suggested that PPAR- $\gamma$  ligands may be useful in preventing ALI [46, 63]. The transcription of sPLA<sub>2</sub>-IIA gene is induced by interleukin-1beta (IL-1B), IL-6 and tumor necrosis factor (TNF- $\alpha$ ), while it is partially inhibited by dexamethasone [64]. Moreover, IL-1B acts in concert with HMGB1 (amphoterin), an ubiquitous protein that plays various roles in the nucleus, and activates the sPLA<sub>2</sub>-IIA genes, suggesting the triggering of a regulatory pathway that may amplify inflammation [65].

### *Other sPLA<sub>2</sub> types*

Regarding other sPLA<sub>2</sub> isoforms discovered recently by sequence homology identification to sPLA<sub>2</sub>-IIA, the mammalian sPLA<sub>2</sub>s-IIC, IID, IIE, IIF, V, and X share similar three-dimensional structure to groups IB and IIA. A wide profile of sPLA<sub>2</sub>s have been found in primary human lung parenchyma macrophages, including -IIA, -IID, -IIE, -IIF, -V, -X and -XIIA. Some of them (-IIE, -IIF, -III, -V and -XIIB) were only detected at the protein level. Regarding these isoforms, only sPLA<sub>2</sub>-IIA was upregulated by LPS [66].

Alveolar macrophages express sPLA<sub>2</sub>-IID, -V and -X isoforms. However, only sPLA<sub>2</sub>-V, and -X were found to hydrolyze efficiently surfactant phospholipids in vitro, and this activity was inhibited by surfactant protein A (**scheme 1-5, -9**) [67].

In human bronchoepithelial (BEAS-2B) and nasal epithelial (RPMI 2650) cells, TNF- $\alpha$  and IFN- $\gamma$  were able to induce gene expression of cPLA<sub>2</sub>-IVC and sPLA<sub>2</sub>-IID, respectively. The above PLA<sub>2</sub> types may thus be involved in cytokine-induced inflammation in the respiratory tract [68]. Regarding sPLA<sub>2</sub>-V, it has a specific function related to phagocytosis. In tissue macrophages, the enzyme is present in a prominent juxtannuclear location in association with the Golgi network and the recycling endosome. In response to zymosan stimulation, the enzyme translocates to the phagosome. The ability of sPLA<sub>2</sub>-V to regulate phagocytosis is specific and not shared with cPLA<sub>2</sub> $\alpha$  nor sPLA<sub>2</sub>-IIA [for review, see ref. 69]. Moreover, it was shown that activated BEAS-2B cells secrete sPLA<sub>2</sub>-V which exert transcellular lipolytic activity on neighboring inflammatory cells (**scheme 1-6**) [70]. The elevation of sPLA<sub>2</sub>-V expression in mice lungs with severe inflammation can be associated with an ongoing surfactant hydrolysis often observed in lung dysfunction (**scheme 1-5**) [71]. Finally, the involvement of sPLA<sub>2</sub>-V in ALI has been

demonstrated from experiments with specific inhibition of the enzyme or gene targeted mice lacking sPLA<sub>2</sub>-V, in which ALI was blocked and an attenuation of neutrophilic inflammation caused by LPS was observed [72].

sPLA<sub>2</sub>-X is found in an inactive form in the cytosol of alveolar type II epithelial cells, under physiological conditions [73]. Its activation under particular situations such as inflammation, cleavage of an N-terminal propeptide is required (**scheme 1-7**). sPLA<sub>2</sub>-X can regulate alveolar type II epithelial cell functions by releasing fatty acids from the cell membranes or by modulating the composition of cell membrane phospholipids. Similar to sPLA<sub>2</sub>-V, it presents transcellular lipolytic activity liberating AA from neighbouring and distal cells (**scheme 1-8, -9**) [73]. Although the enzyme can hydrolyse lung surfactant monolayers, (**scheme 1-10**), overexpressed sPLA<sub>2</sub>-X transgenic neonate mice displayed normal alveolar architecture and surfactant composition [71]. Finally, recombinant human sPLA<sub>2</sub>-X has been shown to degrade in vitro platelet activating factor (PAF), a potent inflammatory lipid mediator, when it is incorporated into large unilamellar PC phospholipid vesicles [70, 74].

#### ***sPLA<sub>2</sub> Receptor (PLA<sub>2</sub>R)***

Although most of the biological effects of sPLA<sub>2</sub>s rely on the hydrolysis of membrane phospholipids, the neurotoxicity and myotoxicity caused by certain sPLA<sub>2</sub> isoforms in mammals are attributed to specific binding of sPLA<sub>2</sub> proteins to cell surface receptors (PLA<sub>2</sub>R). Accordingly, two distinct types of receptors have been identified, the N (neural)-type, expressed mainly in the brain, but a similar type was found in other tissues as well, and the M (muscular)-type, expressed in various mammalian tissues, including lung

[75, 76, 77]. Later, the M-type receptor, was cloned in various mammalian species including humans and different secretory PLA<sub>2</sub> types were investigated as ligands [78, 79].

A widely expressed PLA<sub>2</sub>R has been demonstrated in mice type II alveolar epithelial cells and a subset of splenic lymphocytes as well as on the surface of polymorphonuclear neutrophils, and in human alveolar macrophages, the first line in lung host defence. In addition, PLA<sub>2</sub>R is expressed in kidney, pancreas, amnion, choriodecidua and placenta [80, 81].

PLA<sub>2</sub>R is a type I transmembrane glycoprotein with an approximate molecular mass of 180 kDa. It belongs to the subgroup IV of a particular C-type animal lectin family that includes the macrophage mannose receptor, DEC in dendritic cells and endothelial cell lectin-like lamda protein. These receptors mediate phagocytosis, pinocytosis and antigen presentation [82].

Secreted PLA<sub>2</sub>-IB, -IIA and -X can bind to the cell surface PLA<sub>2</sub>R with high-affinity in a Ca<sup>2+</sup>-independent manner and induce a variety of biological responses. For example, binding of sPLA<sub>2</sub>-IB induces the activation of the mitogen-activated protein kinase (MAPK) cascade (**scheme 1-11**) leading to cell proliferation, production of lipid mediators and selective release of arachidonic acid in bone marrow-derived mast cells. In neutrophils, binding of sPLA<sub>2</sub>-IB can activate p38 MAPK, stimulates elastase release and cell adhesion. Another function of PLA<sub>2</sub>R is associated to the clearance of sPLA<sub>2</sub>s, by internalization and degradation (**scheme 1-12**) [83].

Finally, a soluble form of PLA<sub>2</sub>R (sPLA<sub>2</sub>R) is constitutively present in the circulation, acting as an endogenous inhibitor of sPLA<sub>2</sub>-IB, -IIA and -X (**scheme 1-13**). It is produced after cleavage by metalloproteinases of PLA<sub>2</sub>R and contains all the

extracellular domains but lacks the transmembrane and cytosolic regions [84].

PLA<sub>2</sub>R-deficient mice had longer survival, were more resistant and they exhibited lower LPS-induced cytokines in plasma compared with the wild-type mice [85]. This reveals a potential role of the sPLA<sub>2</sub>-IB/PLA<sub>2</sub>R in endotoxic shock and in the development of ARDS.

#### **Cytosolic PLA<sub>2</sub>s (cPLA<sub>2</sub>s)**

The cPLA<sub>2</sub>s are soluble cytoplasmic proteins with variable sizes, (61–95 kDa), [86], which preferentially catalyze the hydrolysis of arachidonic acid from the *sn*-2 position of phospholipids. The cPLA<sub>2</sub>-IVA or cPLA<sub>2</sub>α, (gene PLA2G4A), was the first to be identified. The enzyme is also expressed in the lung among other tissues, it additionally displays lysophospholipase activity and its catalytic mechanism involves a Ser/Asp dyad [87].

cPLA<sub>2</sub>α participates in signal transduction. It requires physiologically relevant submicromolar levels of intracellular Ca<sup>2+</sup>, which facilitate its translocation from the cytosol and nucleus to perinuclear membrane vesicles (**scheme 1-14**) [88]. Ca<sup>2+</sup> binds to the C2 domain and is not directly involved in the catalytic mechanism, in contrast to sPLA<sub>2</sub>s [89]. The enzyme is activated by phosphorylation that is catalysed by MAPK [90]. This conversion only stimulates the enzyme activity, indicating that translocation and phosphorylation are independent mechanisms of cPLA<sub>2</sub> regulation. However, other kinases, such as phosphoinositide 3-kinase (PI3K) in human eosinophils or SAPKs in platelets, seem to be involved in cPLA<sub>2</sub>-IVA phosphorylation and activation [91, 92].

So far, five additional cPLA<sub>2</sub> members based on sequence homology have been identified in human: cPLA<sub>2</sub>-IVB or cPLA<sub>2</sub>β (gene PLA2G4B), cPLA<sub>2</sub>-IVC or cPLA<sub>2</sub>γ



(gene PLA2G4C), cPLA<sub>2</sub>-IVD or cPLA<sub>2</sub>δ (gene PLA2G4D), cPLA<sub>2</sub>-IVE or cPLA<sub>2</sub>ε (gene PLA2G4E) and cPLA<sub>2</sub>-IVF or cPLA<sub>2</sub>ζ (gene PLA2G4F).

The cPLA<sub>2</sub>-IVB has a much weaker activity than cPLA<sub>2</sub>-IVA. Human lung epithelial cells (BEAS-2B) contain three RNA splice variants of cPLA<sub>2</sub>β (β1, β2, and β3). cPLA<sub>2</sub>β3 is constitutively associated with membrane in unstimulated BEAS-2B cells, localizing to mitochondria and early endosomes. cPLA<sub>2</sub>β3 is removed from the membrane by homogenizing BEAS-2B cells with excess EGTA, suggesting that Ca<sup>2+</sup> plays a role in membrane binding [93].

cPLA<sub>2</sub>-IVC acts in a Ca<sup>2+</sup>-independent manner as it lacks the C2 domain and appears to be constitutively associated with membranes. It is also expressed in lung epithelium [94]. Both cPLA<sub>2</sub>-IVB and cPLA<sub>2</sub>-IVC show a preference for arachidonic acid at the *sn*-2 position of phosphatidylcholine. cPLA<sub>2</sub>-IVC also possess lysophospholipase / transacylation activities and it is though suggested that it may have a protective role through clearance of lysophospholipids by its transacylation [95].

cPLA<sub>2</sub> is suggested to play a crucial role in the pathogenesis of acute lung injury. Disruption of PLA2G4 gene significantly reduced acute lung injury caused by either endotoxemia or acid aspiration. These observations indicated that PLA2G4 gene may mediate acute lung injury, probably through the production of thromboxanes and leukotrienes [96].

A Ca<sup>2+</sup>-dependent PLA<sub>2</sub> was detected in high levels in BAL fluid, BAL cells and plasma of patients with ARDS. cPLA<sub>2</sub> might act indirectly, through the synthesis of inflammatory lipid mediators, whereas the co-localisation of cPLA<sub>2</sub> and sPLA<sub>2</sub> in BAL indicates that these enzymes might act synergistically for the development of ALI .

***Crosstalk between sPLA<sub>2</sub> and cPLA<sub>2</sub>***

It has been shown that distinct sPLA<sub>2</sub>s act in concert with cPLA<sub>2</sub>s in regulating arachidonic acid metabolism and phospholipid turnover in stimulated mammalian cells [97, 98, 99, 100]. Stimulation of cells induces cPLA<sub>2</sub> $\alpha$  and sPLA<sub>2</sub>-V activation and crosstalk that lead to AA release and eicosanoid generation (**scheme 1-15**). sPLA<sub>2</sub>-V contributes to AA release and eicosanoid generation by inducing cPLA<sub>2</sub> $\alpha$  expression. In addition, sPLA<sub>2</sub>-V can amplify the activity of cPLA<sub>2</sub> $\alpha$  through the amplification in phosphorylation by ERK. cPLA<sub>2</sub> $\alpha$  may activate sPLA<sub>2</sub>-V through the generation of LTB<sub>4</sub> or 12/15 lipoxygenase products [101]. Both enzymes may be additive in providing arachidonic acid for eicosanoid generation [69].

sPLA<sub>2</sub>-V can be secreted and hydrolyse phosphatidylcholine at the same or neighbouring cells, (**scheme 1-6**). Apart from its catalytic activity, additional functions of the enzyme are the regulation of phagocytosis, foam cell formation, and antibacterial activities. sPLA<sub>2</sub>-V regulates innate immune responses synergistically with cPLA<sub>2</sub>- $\alpha$  downstream of TLR-2 [102].

**Ca<sup>2+</sup>- independent PLA<sub>2</sub> (iPLA<sub>2</sub>-VI)**

Ca<sup>2+</sup>-independent PLA<sub>2</sub>s (iPLA<sub>2</sub>-VI) are cytosolic enzymes which possess a serine in the active site similar to cPLA<sub>2</sub>s but are not specific regarding to the fatty acid moiety. Different sizes of enzymes (from 53 to 146 kDa) with distinct tissue distribution, cellular localization and enzyme activity can be produced by alternative splicing of the human iPLA<sub>2</sub> transcript of PLA2G6 gene [103, 104, 105]. The iPLA<sub>2</sub>-VIA (or iPLA<sub>2</sub> $\beta$ ) isoform which is mostly studied, contains several ankyrin repeats which mediate protein-protein interactions in very diverse families of proteins [106]. The ankyrin-iPLA<sub>2</sub> sequence can

function as a negative regulator of iPLA<sub>2</sub> activity. The isoforms VIA and VIB (or iPLA<sub>2</sub>γ) exhibit PLA<sub>2</sub> activity only, whereas the others, VIC (or iPLA<sub>2</sub>δ), VID (or iPLA<sub>2</sub>ε), VIE (or iPLA<sub>2</sub>ζ) and VIF (or iPLA<sub>2</sub>η), show additional lysophospholipase, adiponutrin-like, triglyceride lipase and transacylase activities, respectively [107, 108]. The iPLA<sub>2</sub>γ is membrane-bound, while iPLA<sub>2</sub>β is cytoplasmic [109].

The iPLA<sub>2</sub>β isoenzyme has been implicated in various cellular processes such as phospholipids remodeling (**scheme 1-16**), eicosanoid formation, cell proliferation, apoptosis, and activation of Ca<sup>2+</sup> influx [110, 111].

The iPLA<sub>2</sub> is moderately expressed in alveolar cells and macrophages, in A-549 bronchoepithelial cells and in normal and cancer human lung tissue [112]. The isoforms iPLA<sub>2</sub>β and cPLA<sub>2</sub>α are potential targets for anti-inflammatory strategies since they regulate monocyte migration to the site of inflammation, with iPLA<sub>2</sub>β acting as a critical regulator of the cellular compass [113]. Instead, iPLA<sub>2</sub>γ is widely expressed across species and tissues and is suggested to play a role in oxidant-induced cell injury [114] as well as to participate in the regulation of sPLA<sub>2</sub>-IIA expression induced by inflammatory cytokines and endotoxin (**scheme 1-17**) [108].

The most powerful inhibitor of iPLA<sub>2</sub> is BEL (bromo-enol lactone). The (S)- and (R)-enantiomers of BEL offer high specificity for the differentiation between iPLA<sub>2</sub>β and iPLA<sub>2</sub>γ isoforms of the enzyme, respectively. These enantiomers were applied to identify which isoform was implicated in arginine vasopressin-induced arachidonic acid release in A-10 smooth muscle cells [115].

*Acidic Ca<sup>2+</sup>-independent PLA<sub>2</sub> (aiPLA<sub>2</sub>)*

Acidic, Ca<sup>2+</sup>-independent PLA<sub>2</sub> is a 25-kDa multifunctional enzyme of lysosomal origin, with dual phospholipase A<sub>2</sub> and peroxiredoxin 6 (or non-selenium glutathione peroxidase activities, gene PRDX6). It has an optimum pH: 4.0, that differentiates it from other iPLA<sub>2</sub> enzymes [116, 117]. The active sites comprise a catalytic serine for phospholipase A<sub>2</sub> activity and a catalytic Cys-47 which is oxidized to Cys-SOH for the redox-activity (protein data bank number 1PRDX) [118]. Recent studies suggest that aiPLA<sub>2</sub> activity is regulated through phosphorylation in Thr-177 by MAPKs [119].

The enzyme has been isolated from rat lung and is insensitive to pBPB and AACOCFs which inhibit other cytosolic phospholipases. Hexadecyl-3-trifluoroethylglycero-sn-2-phosphomethanol (MJ33) has been proposed as a specific inhibitor for aiPLA<sub>2</sub> [120]. The activity of aiPLA<sub>2</sub> is inhibited by surfactant protein-A through direct protein-protein interaction with Prdx6 [121].

aiPLA<sub>2</sub> is expressed primarily in lung among all the major organs. It is found in the cytoplasm, cytoplasmic vesicles and lysosomes of Clara cells of the conducting airways, type II epithelial cells and alveolar macrophages [122]. Expression of aiPLA<sub>2</sub> is elevated in several lung diseases including lung cancer, mesothelioma and sarcoidosis, although the mechanism for these alterations is not known [123].

The unique properties of aiPLA<sub>2</sub> disclose an important role for lung cell function. The enzyme participates in the regulation of surfactant phospholipids turnover in type II alveolar epithelial cells (**scheme 1-18**) as well as in the protection of the lung against oxidative injury by reducing H<sub>2</sub>O<sub>2</sub>, using glutathione as an electron donor, short chain organic fatty acids, and phospholipid hydroperoxides [124]. In particular, it participates in

the remodelling of phosphatidylcholine to dipalmitoyl-phosphatidylcholine (DPPC) that maintains the physicochemical properties of lung surfactant, being its major constituent. aiPLA<sub>2</sub> utilises as substrate PC from the recycled small vesicular formations of lung surfactant which are internalized and processed by the lysosomes of pneumocytes (**scheme 1-18**) [125]. A recent proteomic analysis of lamellar bodies isolated from rat lungs revealed the presence of PRDX6. This finding strengthens the hypothesis for an involvement of the enzyme on the degradation and recycling of surfactant phospholipids [126].

The role of the enzyme as an antioxidant was recently evaluated in primary lung alveolar epithelial type II cells isolated from wild type, PRDX6 <sup>-/-</sup>, or PRDX6 overexpressing transgenic mice. It was observed that the protein facilitates the repair of damaged cell membranes via reduction of peroxidized phospholipids (**scheme 1-19**) [127].

The protective role of aiPLA<sub>2</sub> on lung function by promoting surfactant DPPC biosynthesis and by exerting antioxidant activity [125], consists a promising area for research.

#### **Lysosomal PLA<sub>2</sub> (PLA<sub>2</sub>-XV)**

Lysosomal phospholipase A<sub>2</sub> (PLA<sub>2</sub>-XV, gene LYPLA3) is a Ca<sup>2+</sup>- independent enzyme with acyl-ceramide synthase (ACS), transacylase and lysophospholipase (LLPL, LPLA<sub>2</sub>) activities. It possesses a sequence similarity to plasma lecithin:cholesterol acyltransferase (LCAT) and initially it was thought to be associated with high-density lipoprotein. Later-on it was found that LCAT and LPLA<sub>2</sub> were encoded by the same gene but in the case of LPLA<sub>2</sub> it was posttranslationally modified by both signal peptide

cleavage and N-glycosylation. The deglycosylated protein has a molecular mass of 45 kDa [128].

Human PLA<sub>2</sub>-XV shows the lipase motif with a catalytic triad of ser/his/asp, several N-linked glycosylation sites and a pH optimum at 4.5, suggesting that the enzyme is localised at lysosomes [129]. Rat and mice PLA<sub>2</sub>-XV is selectively expressed in alveolar macrophages but not in peritoneal macrophages, peripheral blood monocytes, or other tissues [130]. It recognizes disaturated phosphatidylcholine as a substrate and has a broad positional specificity for the acyl groups of both the 1<sup>st</sup> and 2<sup>nd</sup> position of phosphatidylcholine and phosphatidylethanolamine.

LPLA<sub>2</sub> is secreted from macrophages in response to phagocytotic stimuli, while its re-incorporation into alveolar macrophages is suggested to occur via a mannose or mannose phosphate receptor. Deficiency of LPLA<sub>2</sub> results in foam cells formation, surfactant lipid accumulation and phospholipidosis, suggesting that PLA<sub>2</sub>-XV may be a major enzyme of pulmonary surfactant phospholipid degradation by alveolar macrophages (**scheme 1-20**). This was concluded from a study with PLA<sub>2</sub>-XV knockout mice used as a model of pulmonary alveolar proteinosis, a disorder of impaired surfactant catabolism. Foam cell formation characterized by lamellar inclusion bodies of alveolar and peritoneal macrophages was shown. [131, 132]. Further evidence was provided by in vitro stimulation of mouse alveolar macrophages with zymosan which induced LPLA<sub>2</sub> release into the medium, while LPLA<sub>2</sub>(-/-) alveolar macrophages were characterized by a marked accumulation of phosphatidylcholine and phosphatidylethanolamine [133].

Lysosomal constituents are important for the inflammatory response: The released lysosomal enzymes can cause damage in lung tissue thus contributing in the pathogenesis of many diseases including complement activation-produced lung injury [134].

### **PAF acetylhydrolases**

Phospholipases A<sub>2</sub> with preference to short acyl chains at the *sn*-2 position of phospholipids are termed as platelet activating factor -acetylhydrolases (PAF-AH). The name was derived from platelet-activating factor, a potent inflammatory mediator, which was firstly identified as the substrate of the enzyme. However, it has been also shown that PAF-AH can readily hydrolyse short, oxidatively fragmented acyl-chains during oxidative stress [135, 136]. From the three distinct PAF-AH groups encountered in mammals, the first includes a secreted, single-subunit (45 kDa) isoform, the plasma PAF-AH or lipoprotein-associated PLA<sub>2</sub> (PLA<sub>2</sub>-VIIA) which is encoded by gene PLA2G7. It degrades not only PAF but also phospholipids with short (up to 9 carbon atoms) oxidatively fragmented acyl groups at the *sn*-2 position [137, 138].

Plasma PAF-AH (protein data bank number 3D5E) possesses ser/his/asp catalytic aminoacids and its activity decreases with increasing length of the fatty acyl chain esterified at the *sn*-2 position of the phospholipids [139]. It is ubiquitously expressed but the primary source of the enzyme is macrophages. PAF-AH activity is also detected in alveolar macrophages and epithelial type II cells [140].

The second group, cytosolic PAF-AHs IB (or PLA<sub>2</sub>-VIII) are multi-subunit enzymes formed of 45 kDa (alpha), 30 kDa (beta) and 29 kDa (gamma) subunits. Beta and gamma subunits possess catalytic activity, whereas the alpha subunit has regulatory function. Genes PAFAH1B1, PAFAH1B2 and PAFAH1B3 encode the alpha, beta and

gamma subunit, respectively. The third group is a cytoplasmic 40kDa single-subunit protein designated as PLA<sub>2</sub>-VIIB which is a serine-dependent phospholipase A<sub>2</sub> encoded by gene PAFAH2 and is broadly expressed in different tissues [141].

Among the inflammatory mediators which are involved in the pathogenesis of ARDS, platelet activating factor (PAF) has received increased attention in recent years. PAF is an autacoid molecule which acts via a cell surface receptor (PAFR) (**scheme 1-21**) and is considered as one of the most potent pro-inflammatory mediators. PAF is detected in high levels in lungs and plasma of animal models with ARDS, as well as in bronchoalveolar lavage fluid (BALF) of ARDS patients [15, 142]. PAF significantly activates neutrophils, enhances the release of various cytokines and chemokines and increases vascular permeability during the process of ARDS [143]. The important role of PAF in ARDS was confirmed in a study of lung injury caused by HCl aspiration: Overexpression of PAFR gene in transgenic mice enhanced lung injury, pulmonary oedema and deterioration of gas exchange, while mice carrying a targeted disruption of the PAFR gene presented significantly less expression of all the aspects of the injury [144].

Moreover, PAF-AH activity levels were elevated in BAL fluid, plasma and serum from patients with ARDS [145]. Of interest, in ARDS patients, PAF-AH activity was distributed mainly in the large surfactant aggregates, whereas in patients without ARDS the activity was localised in the very small surfactant aggregates indicating that this topology could be correlated with a role and function of PAF-AH to inactivate PAF and oxidized phospholipids. In a recent study, PAF-AH expression was demonstrated in alveolar macrophages and lung epithelial cells of animal models with oleic acid-induced ALI, suggesting a local production and secretion by alveolar macrophages [146].



Due to their substrate preference, PAF-AH has been characterised as anti-inflammatory. However, controversial views suggest that their function could be also associated with the progression of inflammation because it catalyses the generation of lyso-phospholipids and fatty acid hydroperoxides (ROS). Lyso-phospholipids are able to induce severe alveolar epithelial injury while lyso-PC has a dramatic and dose-dependent tissue-damaging effect [147]. Lung tissue is a major target of ROS which induce acute lung injury [148] (**scheme 1-22**). The lower levels of PAF-AH activity measured in plasma in severe sepsis, prompted a treatment with recombinant PAF-AH but this did not reduce the incidence of ARDS in patients with severe sepsis [149, 150]. Finally, recent clinical studies proposed this enzyme as a risk marker or risk factor of coronary heart disease [151, 152, 153].

## **Inhibitors of PLA<sub>2</sub>**

### *sPLA<sub>2</sub> inhibitors*

Chemical inhibitors are used as tools for the identification PLA<sub>2</sub> isotypes in biological samples and the investigation of PLA<sub>2</sub> properties and pathophysiological role in various inflammatory diseases. Many different classes of compounds have been found to inhibit sPLA<sub>2</sub>s at the active site. Today, specific inhibitors are available for most of the sPLA<sub>2</sub>s subtypes [154, 155] and some of them have been used in studies for the treatment of ALI/ARDS.

Indole derivatives appeared to be the most potent mammalian sPLA<sub>2</sub> inhibitors. Indoxam, LY311727 and Me-indoxam are potent sPLA<sub>2</sub> inhibitors. A number of studies in ARDS in which LY311727 was used as an inhibitor for sPLA<sub>2</sub>-IIA have also been published [46, 156, 157, 158]. However, indoxam was suggested to interfere with sPLA<sub>2</sub>-

IB and -X binding to its cell surface receptor [159]. Therefore, inhibition of cellular responses by this type of inhibitors is not always associated to the inhibition of enzymic activity of the sPLA<sub>2</sub>. Me-Indoxam has the highest potency among these inhibitors [160] but has the disadvantage that it is not cell permeable [161]. Recently, it has been proposed that substituted indole and indolizine sPLA<sub>2</sub> inhibitors may be useful in the study of the role of various mammalian sPLA<sub>2</sub>s in cellular and whole animal responses [155].

Prophylactic treatment with the human sPLA<sub>2</sub>-IIA inhibitor S-5920/LY315920Na significantly ameliorated oleic acid-induced ALI in rabbits, as evidenced by significant improvement of hypoxemia and reduced compliance, the prevention of pulmonary oedema, and a decrease in the leakage of proteins into alveolar airspaces [61]. Moreover, S-5920/LY315920Na completely prevented intestinal ischemia / reperfusion -induced lung capillary-alveolar permeability [52] and attenuated acute lung injury in an animal model of ARDS via surfactant protection [162, 163].

Another class of inhibitors tested very recently are those derived from YM-26734 and it was observed that nanomolar concentrations inhibited human, mouse and rat sPLA<sub>2</sub>-IIA and sPLA<sub>2</sub>-V but not sPLA<sub>2</sub>-X. These hydrophobic, cell-permeable inhibitors may be useful for mouse and rat cellular studies of sPLA<sub>2</sub> function [164].

The effort on inhibitors research is to optimize their selectivity among the sPLA<sub>2</sub> groups, for the implementation on treatment of ARDS patients.

### ***Endogenous inhibitors of sPLA<sub>2</sub>***

Surfactant-associated SP-A can act as an endogenous inhibitor of sPLA<sub>2</sub>-IIA and -X, but not of -V, by interacting with their carbohydrate recognition domain. It may exert protection by maintaining surfactant integrity during lung injury. This was concluded from

in vivo experiments where sPLA<sub>2</sub>-IIA induced more pronounced ARDS in SP-A deficient than in wild-type mice. In vitro, SP-A in surfactant preparations inhibits sPLA<sub>2</sub>-IIA-induced surfactant hydrolysis in a dose-dependent manner [67]. Experiments with purified SP-A confirmed the inhibitory properties against sPLA<sub>2</sub>-IIA. SP-A, is a member of the C-type lectin superfamily that contains a COOH-terminal carbohydrate recognition domain and plays an important role in pulmonary host defence by inhibiting inflammation during lung infection. Decreased SP-A levels in BALF are observed in patients with ARDS or at risk for developing ARDS [165]. Increased sPLA<sub>2</sub> activity is associated with decreased SP-A, while surfactant causes a downregulation of sPLA<sub>2</sub>-IIA expression in alveolar macrophages of an experimental model of ALI [166]. In vivo administration of a semisynthetic surfactant in an animal model of ALI reversed the increase in sPLA<sub>2</sub>-IIA expression in lung tissues caused by intratracheal instillation of LPS [167].

Phospholipid components of surfactant also play a role in the inhibition of sPLA<sub>2</sub>-II expression. Among surfactant phospholipids, phosphatidylglycerol, which is known to inhibit PAF [168], was the most effective in inhibiting the expression of sPLA<sub>2</sub>-II in alveolar macrophages [169].

### ***cPLA<sub>2</sub> inhibitors***

There are several commonly used inhibitors for cPLA<sub>2</sub> –IVA including methyl arachidonyl fluorophosphonate (MAFP) [170], arachidonyl trifluoromethylketone (ATFK or AACOCF<sub>3</sub>) and pyrrophenone [171]. In addition to cPLA<sub>2</sub>-IVA, MAFP also irreversibly inhibits iPLA<sub>2</sub>–VI [172], and PAF-AH activity [173]. ATFK also inhibits iPLA<sub>2</sub>–VI [174] and cyclooxygenase [175].

ML3176, which is also characterized as a specific inhibitor for cytosolic PLA<sub>2</sub> [176], showed to inhibit partially the overall Ca<sup>2+</sup>-dependent PLA<sub>2</sub> activity detected in BAL fluid, BAL cells and plasma of ARDS patients suggesting that cPLA<sub>2</sub> might participate in regulation of inflammation in ARDS.

Pharmacological interventions for cPLA<sub>2</sub>, by administering ATFK, showed a significant attenuation in lung injury in a sepsis murine model. [177]. Furthermore, ATFK reduced cPLA<sub>2</sub> and sPLA<sub>2</sub> activity, thromboxane and leukotriene formation, as well as the expression of PAF receptor in an isolated rat model of lung ischemia-reperfusion injury [178]. Pyrrolidine1 and AZ-1 also tested as inhibitors of cPLA<sub>2</sub>- $\alpha$  in stimulated primary cultures of human lung macrophages caused a reduction in AA release and PAF production, suggesting their possible application in the treatment of acute lung injury.

#### ***PAF-AHs inhibitors***

PAF-AHs are inhibited at the catalytic subunit by organophosphorus compounds. The most common chemical inhibitors of plasma PAF-AH in vitro are PMSF and DFP, active in mM concentrations [179, 180]. Plasma PAF-AH is also inhibited by the esterase inhibitors MAFP, its linoleoyl homolog, and 4-(2-Aminoethyl)-benzenesulfonyl fluoride hydrochloride (Pefabloc) [181]. Highly potent inhibitors of PAF-AH are also obtained with sub-nanomolar potency. Among them, darapladib (SB-480848), demonstrated the best in vitro and in vivo profiles and nowadays is being tested in clinical trials of coronary heart disease [182].

### *Glucocorticoids*

Glucocorticoids (GCs) are known for their inhibitory effect on PLA<sub>2</sub> expression, while glucocorticoid receptor (GR) activation correlates negatively with sPLA<sub>2</sub> and cPLA<sub>2</sub> expression. Due to their lipophilic nature, GCs pass freely through the cell membranes and then can bind to glucocorticoid receptor  $\alpha$  (GR $\alpha$ ) which is located in the cytosol in a resting state. Ligand binding induces a conformational change of the receptor which is released from its chaperones and translocates to the nucleus. The beneficial effects of activated GR are thought to be due to both a negative modulation of pro-inflammatory cytokines and to the positive regulation of anti-inflammatory genes. GCs inhibit the transcription factors NF-kB, activator protein-1 (AP-1) or cAMP response element-binding protein (CREB). The transcription factor NF-kB activates genes encoding numerous inflammatory molecules such as PLA<sub>2</sub>, interleukins etc. [183, 184].

GCs have multiple effects on fetal development. They play a role in promoting maturation of the lung and pulmonary surfactant. Mice with homozygous disruptions in the corticotropin-releasing hormone gene die at birth due to pulmonary immaturity. GCs enhance surfactant production by inducing enzymes of the “deacylation-reacylation pathway” involved in palmitate incorporation into phosphatidylcholine thereby contributing to the acceleration of dipalmitoyl-phosphatidylcholine biosynthesis [185]. The antenatal corticosteroid administration has reduced the morbidity associated with preterm birth [186].

Regarding inflammation, GCs influence non-specifically all types of inflammatory events. They induce the lipocortin-1 (annexin-1) synthesis, which then binds to cell membranes, preventing PLA<sub>2</sub> contact with its substrate. This leads to diminished eicosanoid production. Cyclooxygenase expression, (both COX-1 and COX-2), is also

suppressed [187]. Corticosteroids markedly reduce COX2 activity in human airway epithelial cells in vitro, exhibiting little effect on COX1, cPLA<sub>2</sub>-IV, or iPLA<sub>2</sub>-VII [188]. A major mechanism of GC action in preventing prostaglandin release is proposed to occur through the suppression of cPLA<sub>2</sub> and COX-2 mRNA levels in human epithelial cells [189].

A variety of synthetic GCs, some far more potent than cortisol, have been created for therapeutic use [190]. Pretreatment of whistar rats with indomethacin and dexamethazone diminishes the toxic effect of phospholipase A<sub>2</sub> administration in the lungs [191]. Furthermore, it is demonstrated that GCs suppress the enhanced tissue expression of the PLA<sub>2</sub>-IIA gene in endotoxic shock rat models [192].

In endotoxemic guinea pigs that underwent repeated administration of dexamethasone during 3 days before the LPS challenge, dexamethasone showed to prevent both early events such as the macrophage priming and the TNF-alpha appearance and late events such as extracellular PLA<sub>2</sub> release and neutrophils recruitment [193].

In a sheep lung injury model induced by Escherichia coli endotoxin, a reduced GR binding capacity was observed that was associated with an elevation of cortisol levels in plasma and an increase in PLA<sub>2</sub> activity. It was suggested that the glucocorticoid hypofunction accelerates the pathologic response of acute lung injury and indicates an implication of PLA<sub>2</sub> in lung damage [194]. In conclusion, GCs exert inhibitory effects in several stages of the inflammatory cascade, including PLA<sub>2</sub> attenuation. Therefore, they could be a rational choice for treatment of acute lung injury. However, clinical outcomes in trials on the role of GCs in ARDS treatment were ambiguous [195, 196]. The reduced response to GCs which is observed in unresolving ARDS maybe due to reduced GR

binding to cortisol and/or GR $\alpha$  overexpression which is recently reported to occur during ALI [197].

### **PLA<sub>2</sub>s AS TARGETS FOR ARDS TREATMENT**

PLA<sub>2</sub>s are obviously taking part in ARDS pathogenesis directly or via the generation of lipid mediators. Therefore, the inhibition of PLA<sub>2</sub> activities or modulation of PLA<sub>2</sub>s production and secretion constitutes a promising therapeutic goal. However, it is not clearly understood which isoform(s) are the suitable targets. Particular PLA<sub>2</sub>s exert anti-inflammatory properties, while the bactericidal properties of sPLA<sub>2</sub>-IIA place the function of sPLA<sub>2</sub>-IIA in another perspective [198]. Therefore, the systemic, non-selective inhibition of PLA<sub>2</sub>s might add to adverse outcomes and the complete elimination of certain isoforms, such as iPLA<sub>2</sub> and cPLA<sub>2</sub> with an essential role in cellular phospholipids metabolism, might be detrimental.

The specific functions of PLA<sub>2</sub> isoforms appear to be tissue-, organ- and disease-specific. The treatment with PLA<sub>2</sub> inhibitors should take into account their organ specificity and the ability of some of them to act as homeostatic and anti-inflammatory agents [199]. Issues such as solubility, timing and dose, systemic or local administration should be addressed. It has been suggested that a preferable strategy for controlling inflammatory lipid mediator production is to protect the cells from sPLA<sub>2</sub>, using cell-impermeable sPLA<sub>2</sub> inhibitors that would not interfere with the vital intracellular phospholipid metabolism [200].

Specific inhibition of cPLA<sub>2</sub>-IVA, sPLA<sub>2</sub>-IIA or sPLA<sub>2</sub>-V, may present a valuable advance in treating ARDS since these enzymes play essential role in the onset and development of the syndrome. However, continuous 7-days infusion of LY315920NA/S-

5920, a selective inhibitor of sPLA<sub>2</sub>-IIA had no beneficial effect on mortality among severe sepsis patients with at least two organ failure [201].

Modulation of PLA<sub>2</sub> secretion, production or activation could be another therapeutic approach in ARDS prevention and treatment. Therefore, the modulation of PLA<sub>2</sub> with preconditioning, for example ischemic preconditioning, could limit the use of potentially harmful inhibitors of PLA<sub>2</sub>. Ischemic preconditioning refers to an adaptational response to ischemia reperfusion injury that allows protection of briefly ischemic tissues against the harmful effects of subsequent, prolonged ischemia (risk factor for ARDS). In a recent study we tested the effect of ischemic preconditioning on the intestinal ischemia-reperfusion-induced ARDS in rats, with particular focus on PLA<sub>2</sub>s. It was observed that PLA<sub>2</sub>, as well PAF-AH were significantly lower in ischemic preconditioned rats. The PLA<sub>2</sub>s decrease was associated with significant reduction of neutrophils in BAL fluid and lung tissue infiltration. These results suggest that intestinal preconditioning protects intestinal ischemia-reperfusion-induced - lung injury by modulating PLA<sub>2</sub>s secretion [202].

Corticosteroids, anti-inflammatory agents and non-selective inhibitors of PLA<sub>2</sub> have been used in the prevention of acute respiratory distress syndrome in critically ill adults as well as for the treatment of established ARDS. However, a definitive beneficial effect has not been established: A recent systematic review failed to show a convincing treatment effect of steroids in ARDS, although a trend of reduction in odds of mortality was found when steroids were given after the onset of ARDS. On the contrary, preventive steroid therapy in critically ill patients was associated with detrimental effects on the incidence of ARDS and subsequent mortality. Although steroids did not increase the overall infection risk, a dose dependent effect of steroid therapy on infection rates seemed to exist.



Furthermore, the optimal dose, timing, and duration of steroid therapy have not been accurately defined [195].

## CONCLUSION

Several PLA<sub>2</sub>s are involved in pathogenesis of inflammation in ARDS either directly or indirectly, through the production of biologically active molecules, however, some PLA<sub>2</sub>s may have anti-inflammatory properties and others are responsible for surfactant production. All these clearly make a therapeutic target in ARDS based in PLA<sub>2</sub>s inhibition, a difficult and complex approach.

Elucidation of the biological roles of each group of PLA<sub>2</sub>s in every state of ARDS should be of great value for the development of subtype-specific inhibitors as therapeutic agents.

## Abbreviations

AA	arachidonic acid
AACOCFs	arachidonyl trifluoromethylketone
aiPLA <sub>2</sub>	acidic-Ca <sup>2+</sup> -independent phospholipase A <sub>2</sub>
ALI	Acute lung injury
ARDS	Acute respiratory distress syndrome
ATFK	arachidonyl trifluoromethylketone
BAL	bronchoalveolar lavage fluid
BEL	( <i>E</i> )-6-(bromomethylene)-3-(1-naphthalenyl)-2 <i>H</i> -tetrahydropyran-2-one
COX	cyclooxygenase
cPLA <sub>2</sub>	cytosolic phospholipase A <sub>2</sub>
DFP	diisopropylfluorophosphate
DPPC	dipalmitoyl phosphatidylcholine
ERK	extracellular signal-regulated kinases
GCs	Glucocorticoids
GR	glucocorticoid receptor
HMGB1	High mobility group protein 1
IFN	Interferon
IL-	Interleukine
iPLA <sub>2</sub>	Ca <sup>2+</sup> -independent phospholipase A <sub>2</sub>
IR	ischemia-reperfusion
LPA	lysophosphatidic acid
LPLA <sub>2</sub>	lysophospholipase A <sub>2</sub>
LPS	lipopolysaccharide
LY311727	3-[3-(2-amino-2-oxoethyl)-2-ethyl-1-(phenylmethyl)indol-5-yl]oxypropylphosphonic acid
MAFP	methyl arachidonyl fluorophosphonate
MAPK	mitogen-activated protein kinase
ML3176	1-[2-ethyl]-3-dodecanoylindole-2-carboxylic acid
NF-κB	Nuclear factor kappa B
PAF	platelet activating factor
PAF-AH	platelet activating factor acetylhydrolase
PaO <sub>2</sub> /FiO <sub>2</sub>	Partial arterial oxygen/Fraction of inspired oxygen
pBPB	4-bromophenacyl bromide
PC	phosphatidylcholine
PE	phosphatidylethanolamine
Pefabloc	4-(2-Aminoethyl)-benzenesulfonyl fluoride hydrochloride
PL	phospholipid
PLA <sub>2</sub>	phospholipase A <sub>2</sub>
PLA <sub>2</sub> R	Phospholipase A <sub>2</sub> receptor
PMSF	phenylmethanesulfonyl fluoride

PPAR- $\gamma$	peroxisome proliferator-activated receptor - gamma
ROS	Reactive oxygen species
s PLA <sub>2</sub> R	Soluble part of PLA <sub>2</sub> R
S-5920/ LY315920Na	[[3-(Aminooxoacetyl)-2-ethyl-1-(phenylmethyl)-1H-indol-4-yl]oxy]acetate
SAPK	Stress-Activated Protein Kinase
SB-480848	N-(2-diethylaminoethyl)-2-[2-[(4-fluorophenyl)methylsulfanyl]-4-oxo-6,7-dihydro-5H-cyclopenta[d]pyrimidin-1-yl]-N-[[4-[4-(trifluoromethyl)phenyl]phenyl]methyl]acetamide
SP-A	Surfactant protein A
SP-B	Surfactant protein B
SP-C	Surfactant protein C
SP-D	Surfactant protein D
sPLA <sub>2</sub>	Secreted phospholipase A <sub>2</sub>
TLR	Toll like receptor
TNF- $\alpha$	tumor necrosis factor alpha
YM-26734	4-(3,5-didodecanoyl-2,4,6-trihydroxyphenyl)-7-hydroxy-2-(4-hydroxyphenyl)chroman

**Figure legends**

**Scheme 1.** Involvement of PLA<sub>2</sub> isoforms in the evolution or prevention of acute lung injury (ALI). Direct actions leading to ALI include the hydrolysis of lung surfactant, while indirect ones, the generation of eicosanoids and lipid mediators. In contrast, a protective role is ascribed to isoforms that mediate phospholipids remodeling:

Secreted PLA<sub>2</sub> types IIA, V, and X exhibit a direct hydrolytic action on lung surfactant (**3, 5, 10**, respectively).

sPLA<sub>2</sub>-IIA or -IB can interact with specific receptors leading to genes expression and protein production through the MAPK cascade (**1, 11**). sPLA<sub>2</sub>-IIA expression is induced by COX metabolites through PPAR- $\gamma$  activation (**4, 2**).

sPLA<sub>2</sub>-V has an autocrine or paracrine action on membranes. Secretion of sPLA<sub>2</sub>-V leads to transcellular lipolytic action on inflammatory cells, liberating AA and other free fatty acids (**6**).

During inflammation sPLA<sub>2</sub>-X is activated in the cytosol by proteolytic cleavage and under this form it is secreted from the cell (**7**). It could induce ALI through the liberation of free fatty acids from membrane phospholipids, degradation of surfactant (**10**) and generation of lipid mediators (**8**). sPLA<sub>2</sub>-X could be internalized after binding with PLA<sub>2</sub>R and degraded (**12**).

Secreted PLA<sub>2</sub>-IB, IIA and -X can bind to the cell surface PLA<sub>2</sub>R with high-affinity inducing a variety of biological responses (**11**). Alternatively, a soluble, circulating form of


sPLA<sub>2</sub>R acts as a negative regulator of sPLA<sub>2</sub>: This binds to the enzymes, moves to the plasma membrane and mediates their internalisation and decomposition (13).

cPLA<sub>2</sub> $\alpha$  requires submicromolar levels of intracellular Ca<sup>2+</sup>, which facilitate its translocation from the cytosol and nucleus to perinuclear membrane vesicles (14). A cross-talk mechanism between cPLA<sub>2</sub> $\alpha$  and sPLA<sub>2</sub>-V leads to AA release and eicosanoid generation, leading to ALI (15).

The iPLA<sub>2</sub> $\beta$  has been implicated in cellular processes such as phospholipids remodeling (16), eicosanoids formation, cell proliferation, apoptosis, and activation of Ca<sup>2+</sup> influx. iPLA<sub>2</sub> $\gamma$  is suggested to participate in the regulation of sPLA<sub>2</sub>-IIA expression (17) induced by inflammatory cytokines and endotoxin.

aiPLA<sub>2</sub> participates in the regulation of surfactant phospholipids turnover (18) as well as in the protection of the lung against oxidative injury via reduction of peroxidized phospholipids (19). Lysosomal PLA<sub>2</sub>-XV may be a major enzyme of pulmonary surfactant phospholipid degradation (20).

PAF-AH degrades platelet-activating factor and is considered as anti-inflammatory enzyme. PAF is an autacoid molecule which acts via a cell surface receptor (PAFR) (21). However, PAF-AH function could be also associated with the progression of inflammation because it catalyses the generation of lyso-phospholipids and fatty acid hydroperoxides (FA(OOH)) which disrupt membrane integrity(22).

*Interrupted arrow: damage or degradation; Blue arrows: secretion;  : recycling of damaged membranes.*

**Table 1.** Classification and characteristics of human PLA<sub>2</sub>s.

ACCEPTED MANUSCRIPT

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Types	Protein name	Common designation	Names of the origin genes	Length (aa)	Calcium requirement	Subcellular localization
<b>Cytosolic PLA<sub>2s</sub> (cPLA<sub>2s</sub>)</b>	Phospholipase A2 group IVA	cPLA2-IVA or cPLA2 $\alpha$	PLA2G4A	749	yes (only for translocation to membrane vesicles)	Cytoplasmic vesicles; Nucleus
	Phospholipase A2 group IVB (isoform $\beta$ 1 / $\beta$ 2 / $\beta$ 4)	cPLA2 $\beta$ 1 / cPLA2 $\beta$ 2 / cPLA2 $\beta$ 4	PLA2G4B	781 / 661 / 482	yes	Cytosol
	Phospholipase A2 group IVB (isoform $\beta$ 3)	cPLA2 $\beta$ 3	PLA2G4B	656	yes	Cytosol; Mitochondria; Early endosomes
	Phospholipase A2 group IVC	cPLA2-IVC or cPLA2 $\gamma$	PLA2G4C	541	no	Endoplasmic reticulum; Mitochondria
	Phospholipase A2 group IVD	cPLA2-IVD or cPLA2 $\delta$	PLA2G4D	818	yes (for enzymic activity and translocation to membrane vesicles)	Cytosol; Cytoplasmic vesicle membrane; Peripheral membrane protein; Cytoplasmic side
	Phospholipase A2 group IVE	cPLA2-IVE cPLA2 $\epsilon$	PLA2G4E	838	yes (for enzymic activity and translocation to membrane vesicles)	Cytosol; Lysosome membrane; Peripheral membrane protein; Cytoplasmic side
	Phospholipase A2 group IVF	cPLA2-IVF or cPLA2 $\zeta$	PLA2G4F	849	yes (for enzymic activity and translocation to membrane vesicles)	Cytosol; Lysosome membrane; Peripheral membrane protein; Cytoplasmic side
<b>Secretory PLA<sub>2s</sub> (sPLA<sub>2s</sub>)</b>	Phospholipase A2 group IB	sPLA2-IB	PLA2G1B	148	yes	Secreted
	Phospholipase A2 group IIA	sPLA2-IIA	PLA2G2A	144	yes	Secreted; Membrane; Peripheral membrane; Secretory granules
	Phospholipase A2 group IID	sPLA2-IID	PLA2G2D	145	yes	Secreted
	Phospholipase A2 group IIE	sPLA2-IIE	PLA2G2E	142	yes	Secreted
	Phospholipase A2 group IIF	sPLA2-IIF	PLA2G2F	168	yes	Secreted
	Phospholipase A2 group III	sPLA2-III	PLA2G3	509	yes	Secreted
	Phospholipase A2 group V	sPLA2-V	PLA2G5	138	yes	Secreted; Golgi apparatus; Nuclear envelope; Plasma membrane
	Phospholipase A2 group X	sPLA2-X	PLA2G10	155	yes	Secreted
Phospholipase A2 group XIII	sPLA2-XIII	PLA2G12A	189	yes	Secreted; Cytoplasm	
Phospholipase A2 group XIIB (or XIII)	sPLA2-XIIB (or sPLA2-XIII)	PLA2G12B	194	yes	Secreted	
<b>Intracellular calcium-independent PLA<sub>2s</sub> (iPLA<sub>2s</sub>)</b>	Ca <sup>2+</sup> -independent phospholipase A2 group VIA (multiple short isoforms from transcript variants)	iPLA2-VIA or iPLA2- $\beta$	PLA2G6	752	no	Cytosol
	Ca <sup>2+</sup> -independent phospholipase A2 group VIB, isoform LH-iPLA2 (long isoform)	iPLA2-VIB or iPLA2- $\gamma$	PLA2G6	806	no	Endoplasmic, peroxisomal and mitochondrial membranes
	Phospholipase A2 group VIC or neuropathy target esterase or Patatin-like phospholipase domain-containing	iPLA2-VIC or iPLA2- $\delta$	PNPLA6	1366	no	Endoplasmic reticulum membrane; Single-pass type I membrane protein; Cytoplasmic side
	Phospholipase A2 group VID or Patatin-like phospholipase domain-containing protein 3 or Acylglycerol O-acyltransferase or Adiponutrin	iPLA2-VID or iPLA2 $\epsilon$	PNPLA3	481	no	Membrane; Single-pass type II membrane protein
	Phospholipase A2 group VIE or Patatin-like phospholipase domain-containing protein 2 or Adipose triglyceride lipase or Desnutrin or TTS2	iPLA2-VIE or iPLA2 $\zeta$	PNPLA2	504	no	Lipid droplet membrane; Single-pass type II membrane protein; Cell membrane
	Phospholipase A2 group VIF or Patatin-like phospholipase domain-containing protein 4 or GS2	iPLA2-VIF or iPLA2 $\eta$	PNPLA4	253	no	Cytoplasm
<b>Acidic calcium-independent PLA<sub>2</sub> (aiPLA<sub>2</sub>)</b>	Acidic calcium-independent phospholipase A2 (or Peroxiredoxin-6)	aiPLA2	PRDX6	224	no	Cytoplasm; Lysosome
<b>PAF acetylhydrolases (PAF-AH)</b>	Phospholipase A2 group VII or Platelet-activating factor acetylhydrolase or LDL-associated phospholipase A2	PAF-AH or LpPLA2	PLA2G7	441	no	Secreted
	Phospholipase A2 group VIII (multi-subunit enzymes formed of alpha, beta and gamma subunits)	PAF-AH IB or PLA <sub>2</sub> -VIII	PAFAH1B1 / PAFAH1B2 / PAFAH1B3	410 / 229 / 231	no	Cytoplasm
	Phospholipase A2 group VIIB	PLA <sub>2</sub> -VIIB	PAFAH2	392	no	Cytoplasm
<b>Lysosomal PLA<sub>2</sub></b>	Lysosomal phospholipase A2 or Lysophospholipase 3 or 1-O-acylceramide synthase (ACS) or LCAT-like	LPLA2 or LLPL	LYPLA3	412	no	Secreted; Lysosome

Source: NCBI database and www.uniprot.org/

