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Phospholipases A2 as biomarkers in acute respiratory distress syndrome

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ABSTRACT

Acute respiratory distress syndrome (ARDS) is a multifactorial life-threatening lung injury, characterized by diffuse lung inflammation and increased alveolocapillary barrier permeability. The different stages of ARDS have distinctive biochemical and clinical profiles. Despite the progress of our understanding on ARDS pathobiology, the mechanisms underlying its pathogenesis are still obscure. Herein, we review the existing literature about the implications of phospholipases 2 (PLA2s), a large family of enzymes that catalyze the hydrolysis of fatty acids at the *sn*-2 position of glycerophospholipids, in ARDS-related pathology. We emphasize on the versatile way of participation of different PLA2s isoforms in the distinct ARDS subgroup phenotypes by either potentiating lung inflammation and damage or by preserving the normal lung. Current research supports that PLA2s are associated with the progression and the outcome of ARDS. We herein discuss the transcellular communication of PLA2s through secreted extracellular vesicles and suggest it as a new mechanism of PLA2s involvement in ARDS. Thus, the elucidation of the spatiotemporal features of PLA2s expression may give new insights and provide valuable information about the risk of an individual to develop ARDS or advance to more severe stages, and potentially identify PLA2 isoforms as biomarkers and target for pharmacological intervention.

Phospholipases A2 (PLA2s) compose a superfamily of enzymes hydrolyzing, through surface-activated catalysis, esterified fatty acids from the *sn*-2 position of glycerophospholipids producing free fatty acids and lyso-phospholipids [1]. Phospholipolysis is also required for the established bactericidal activity of certain PLA2 members and host defense activity [2]. Recently, PLA2 activity has even gained attention as a key player in Covid-19 pathogenesis [3]. Furthermore, experimental data indicate that certain functions of PLA2s are

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mediated independently of their catalytic activity by interaction/binding with membrane receptors [4,5].

Their classification depends on topology, size, amino acid sequence homology, Ca^{2+} and substrate requirements [6]. A rough classification of PLA2s expressed in lung is presented below:

Cytosolic PLA2s (cPLA2, PLA2G4)

This group includes a family of soluble cytoplasmic proteins showing μM Ca^{2+} requirement and preference for arachidonate [7]. They are expressed in human lung macrophages and contributes to monocyte migration to sites of inflammation in a variety of pathophysiological conditions [8]. cPLA2s are post-transcriptionally regulated, whereas its biological function is post-translationally controlled by phosphorylation and Ca^{2+}-dependent translocation to perinuclear compartments [9].

Calcium-independent PLA2s (iPLA2s, PLA2G6)

This Ca^{2+}-independent group participates in membrane remodelling and arachidonic acid generation. iPLA2 isoforms show distinct cellular localization exerting key roles in apoptosis during ER stress [10], regulate sPLA2IIA induction [11] and the speed and directionality of human monocytes(MC) during chemotaxis towards inflamed areas [12].

Lysosomal PLA2s (aiPLA2 and LPLA2, PLA2G15)

The acidic, Ca^{2+}-independent aiPLA2 (called peroxyredoxin-6, prdx6) has an additional glutathione-peroxidase activity. It is expressed by alveolar macrophages(AM) and airway epithelium, identified in lamellar bodies, cytosol and extracellular fluids. Bronchoalveolar fluid (BALF)-iaPLA2 levels are significantly higher in acute respiratory distress syndrome (ARDS) patients compared to control patients [13]. The other member, LPLA2, possessing also transacylase activities, is highly expressed in AM and in the lung tissue overall, regulating lung surfactant(LS) degradation [14,15].

Secretory PLA2s (sPLA2s, gene name varies depending on the group)

They are small molecular weight, Ca^{2+}-dependent proteins, encompassing 11 isoforms. This category has attracted translational research interest due to their multiple roles in lung host defense [16], especially in antimicrobial and anticoagulation activities [17,18], tissue injury [19], eicosanoid generation [20], inflammation [21,22] and cell adhesion [23]. Post translational modifications might regulate the sPLA2IIA shuttling and compartmentalization [24,25]. In recent years, there is an increasing interest on sPLA2IIA as a regulator of various cell functions.

Finally, **PAF-Acetyl Hydrolase (PAF-AH, PLA2G7)** show preference for glycerophospholipids containing short or oxidatively truncated acyl-chains. Its beneficial effect in sepsis is disputed by the fact that injection of PAF-AH did not bring the expected results, possibly due to the toxicity of liberated products [26].

Certain sPLA2 members have been characterized as biomarkers in lung diseases, ensuing consideration as pharmacological targets. However, in most cases, the term biomarker is used mainly because of their high abundance in inflammatory diseases, rather than that they follow the requirements of systematic validation procedures [27]. Concerning PLA2s, meta-analysis of original published data regarding biomarkers in lung fluid for ARDS of different etiologies, included PAF-AH as a biomarker [28]. In another study, sPLA2IIA was characterized as a biomarker for sepsis [29] and early diagnosis of bacteremia [30]. Apart from secretory, other PLA2 groups are present in inflamed areas, potentially demonstrating an emerging interplay between different PLA2 groups [31]. Considering functional relationships, interactions of PLA2s with newly identified agents of the immune response, like neutrophil extracellular traps should be considered.

In this review, we will focus on PLA2 isoenzymes associated with lung pathology or are characterized as biomarkers. Moreover, we will provide additional information regarding interplay, synergies, temporal fluctuation, topology and shuttling through extracellular vesicles. Finally, we will emplace sPLA2s in the frame of distinct phenotypes of ARDS subgroups.

The diversal complexity of the inflammatory response in ARDS

ARDS is a multifactorial syndrome associated with high morbidity and mortality rates, which are reflected in heterogeneous phenotypes. It is characterized by acute onset and dispersed inflammation, accompanied by increased alveolocapillary barrier permeability, presented in mild, moderate, or severe forms [32]. It is known that LS deficiency is associated with ARDS pathogenesis [33,34]. LS is a tensioactive material, of specific phospholipid-protein composition, spread over the liquid film covering the alveolar epithelium. It equilibrates the pressure in the alveoli, prevents the formation of alveolar oedema and consequently, protects alveoli from collapsing. Primary LS deficiency is encountered in preterm neonates, while in adults, LS can be destroyed secondarily, by direct or indirect insults. Surfactant replacement therapy is one of the first line of treatments in respiratory distress syndrome originating from primary LS deficiency. However, the effectiveness of this treatment is hindered in individuals with secondary LS deficiency possibly by its degradation into the lung by PLAs expressed in the pulmonary system. Indeed, experimental evidence in pediatric RDS revealed that all the sPLA2 isoforms tested, sPLA2IIA predominating, were significantly higher in the alveolar fluid of neonates with various subtypes of RDS as compared to controls [35], prolonging the duration of mechanical ventilation and morbidity of the subjects.

Various PLA2 types including sPLA2 members such as sPLA2IIA, sPLA2IID, sPLA2IB, and sPLA2V [36–38] have been identified in chronic and acute lung diseases [39,40]. Up-to-date knowledge has implicated PLA2s in the modulation of immune status in the lung through two different mechanisms:

A) Through their catalytic activity: They can hydrolyze bacterial membranes [16,41] lung cell membranes and LS as well. The produced arachidonic acid can be metabolized into eicosanoids mainly by lipoxygenases, cyclo-oxygenases and cytochromes P450, and has been extensively studied in the context of lung inflammation [42]. The specific catalytic action of PLA2s highlights its involvement in inflammation and indicates PLA2s as immune response modifiers through the initiation of eicosanoid signaling pathway. In addition, lysoPAF-acetyltransferases or transacetylases can catalyze PAF production [43,44]. These molecules can elicit inflammatory reactions and allergic responses in the lung such as increased leukocyte adhesion, chemotaxis, respiratory burst, and increased vascular permeability, leading to lung dysfunction and adverse clinical outcomes, both in adults and children and eventually to ARDS. The resulting bioactive lipid mediators exert their biological action in second-wave responses. B) Through the concomitant production of the tensioactive lysophospholipids, leading inevitably to disruption of alveolar physicochemical homeostasis. In fact, lysophosphatidylcholine (Lyso-PC) levels are increased in BALF, especially from patients with late ARDS. Lyso-PC levels could potentially be used as a marker for the late phase of the syndrome, distinguishing between the different phases of ARDS. Lyso-phosphatidylcholine levels are increased in BALF, especially from patients with late ARDS [45]. Recent review on sepsis [46] refers that serum LPC concentrations may have utility in predicting sepsis severity. In human patients with sepsis, serum and plasma LPC concentrations increase overtime in survivors but not in nonsurvivors, while persistently lower plasma LPC levels associate with 28- and 90-day mortality [47]. However, Lyso-PC levels are tightly regulated not only by PLA2 activity but also by several other isoenzymes of lysophosphatidylcholine acyl-transferase (LPCAT) which are responsible for the reacylation of LPC. Alternatively, LPC can be further hydrolyzed by phospholipase D to produce lysophosphatidic acid (LPA). More studies are needed in order to support the above hypothesis.

PLA2s in phenotypes of ARDS subtypes

Several systematic scientific reviews and meta-analysis data depict our current knowledge on biomarkers in ARDS phenotypes [48–50] [Fig. 1]. Data concerning PLA2 isoforms are presented below:

Mechanical ventilation

Critically ill patients usually need mechanical ventilation to confront severe hypoxemia and impairment in lung mechanics. This manipulation can cause lung injury due to shear stress that activates the extracellular cell matrix/integrin/cytoskeleton pathway [51]. Recent studies showed that sPLA2IIA specifically binds to integrins [52]. Patients with ventilator-associated lung injury express increased PAH-AH levels in BALF compared to controls [53,54]. In the lung epithelial cell line A549 even mild stretch can activate post-translational phosphorylation of cPLA2 through the MEK/ERK and PI3K pathways ending up to dipalmitoyl-glycerophosphatidylcholine synthesis [55,56]. The association of sPLA2V with acute lung injury was shown *in vitro* and *in vivo* in mice ventilated with high tidal volumes [57]. Other studies provided evidence that the LPS-induced sPLA2V is considered responsible for the alveolar-capillary barrier deterioration [58].

Direct vs indirect ARDS

ARDS can be generated either directly, from local insults, including bacterial or viral pneumonia, inhalation of harmful substances such as smoke, acid aspiration, mechanical ventilation, near drowning, lung contusion, or indirectly, as a result of acute systemic inflammatory response, usually due to sepsis [59]. In direct ARDS the severe injury is expressed primarily in the alveolar epithelium and less in endothelium, whereas in indirect ARDS, endothelial injury prevails [60]. Differentiation of direct from indirect ARDS based on PLA2s expression is quite limited. Considering PAF-AH, studies showed that it was higher in patients with direct compared to indirect ARDS [61], and more prominent in BALF than in plasma, a fact that is consistent with PAF-AH levels in BALF from patients with fat embolism syndrome [62] and in parenteral nutrition using medium and long chain triglycerides in early direct ARDS patients [63]. The positive correlation of sPLA2IIA and cPLA2 with the severity of lung injury has been known for several years [64]. In sheep animal models, smoke inhalation caused an increase in cPLA2 [65]. Animal studies showed that cPLA2 participates in LPS-induced lung injury, rendering this isoform an appropriate target for pharmacological interventions [66]. The combination of CD64 and sPLA2IIA has been proposed as an early biomarker for distinguishing sepsis from bacterial infections in ARDS [67]. Furthermore, studies in ischemia-reperfusion animal models showed increased PLA2 activities in BALF. Interestingly, intestinal ischemic preconditioning used to improve intestinal tolerance to subsequent sustained ischemia, reduced PLA2 levels and ameliorated the accompanying markers characteristic for lung injury [68]. The association of oxidative stress-induced lung injury with lung tissue-PLA2 activity, measured as a marker of inflammation, is supported by animal studies showing that infusion of the iron chelator desferrioxamine, results in alleviated oxidative stress with a concomitant decrease in PLA2 expression [69]. The synergistic effect between different members of PLAs has also gained attention [11].

Phases of ARDS

In the acute exudative phase of ARDS, or early phase, non-cardiogenic pulmonary edema is accumulated, while defense cells are recruited in the alveoli from the circulation. After 7–14 days a proliferative phase follows, in which repairment procedures and fibroblasts proliferation occurs. Finally, a fibrotic phase can ensue. Studies suggest that fibrosing mechanisms start from the early phase of ARDS and influences the patients' outcome [70]. Late ARDS is frequently associated with multiple systems injury [71]. Despite the different pathophysiological background between the two phases, the mortality rates are not significantly different [72]. At the early phase, a significant increase in biomarkers of epithelial and endothelial injuries, pro- and anti-inflammatory agents, and markers of

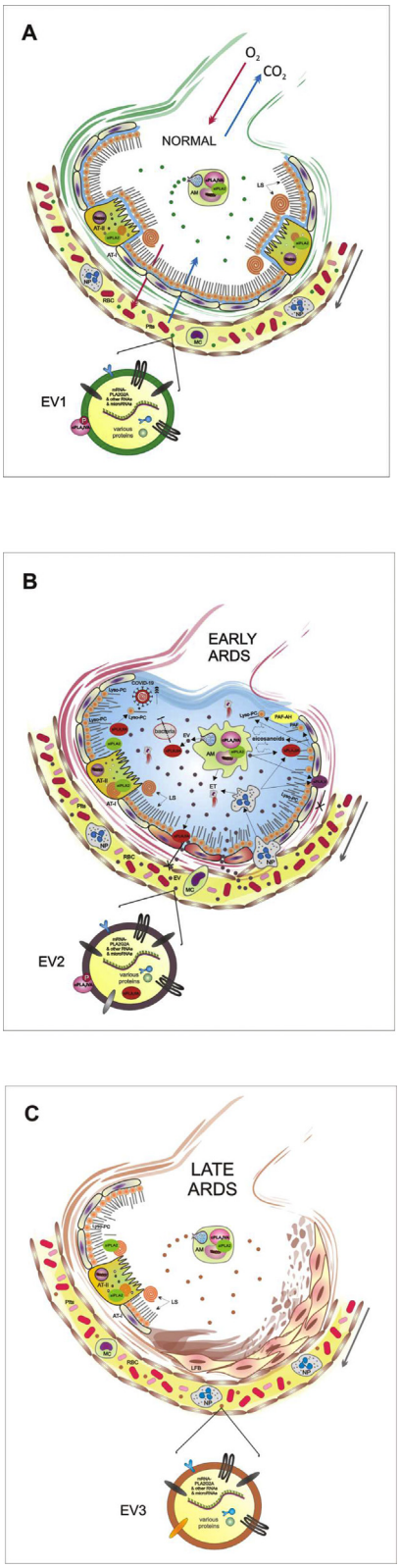

Fig. 1 **Phospholipases A2 in ARDS.** ***Normal alveolus (Panel A)***. In the normal alveoli the epithelium is intact. Alveolar Type I cells permit gas exchange, while Alveolar Type II cells produce lung surfactant. Resident alveolar macrophages under normal conditions express protein levels of cPLA2-IVA and aiPLA2 but not sPLA2IIA. EVs contain sPLA2IIA mRNA but not sPLA2IIA as a functional protein. ***Alveolus in early phase of ARDS (Panel B)***. Alveolar-capillary damage leads in alveoli flooding with a proteinaceous liquid (blue color). Activated alveolar macrophages and recruited monocytes and neutrophils (NP) from the circulation secrete sPLA2IIA and extracellular traps which participate in the clearance of microbial intruders. At the same time, sPLA2IIA can deteriorate lung surfactant. Lyso-PC, PAF and eicosanoids production is an on-going process at this stage of ARDS. sPLA2V and sPLA2IIA are linked with epithelial and endothelial damage. EVs1 containing sPLA2IIA at both mRNA and protein levels, as well as pcPLA2 are secreted into the extracellular space. EVs containing sPLA2IIA mediate transcellular communication locally or in distal locations. ***Alveolus in late phase of ARDS (Panel C)***. A proliferative phase follows the early phase of ARDS during which repairment procedures and proliferation of fibroblasts is taking place. Finally, a fibrotic phase may ensue contributing to chronic inflammation. Lyso-PC levels are yet detectable at this phase. EVs contain sPLA2IIA mRNA but not sPLA2IIA as a functional protein.

coagulation/fibrinolysis are under investigation [28,44,45,73,74]. Analysis of BALF from patients with ARDS demonstrated an increase in total PLA2 activity in the differential centrifugation subfractions containing inactivated surfactant vesicles and small-dense EVs. Most of this activity was Ca^{2+}-dependent, as in plasma [75]. sPLA2IIA circulating levels, one of the major components involved in innate host defense against bacteria, increase significantly during infection or inflammation [76,77]. AM play a central role in innate immunity and are the first line of defense against inhaled pathogens. Studies from Touqui's lab in animals, documented that AMs are the major pulmonary source of sPLA2-IIA IIA in acute lung injury [78,79]. A potential involvement of PLA2s to the inflammatory response can be inferred by the fact that sPLA2IIA acts as an acute phase protein induced by a variety of pro-inflammatory cytokines and LPS through the NF-κB pathway [78,80]. The suppression of LPS-induced sPLA2IIA expression in various cell types treated with dexamethasone, or the antibiotic azithromycin, supports the idea of sPLA2IIA utilization as a biomarker [81,82].

Immunoparalysis

Immunoparalysis is a common complication in septic severely ill patients characterized by downregulated innate and adaptive immune responses and is accompanied by decreased pro-inflammatory cytokines release from defense cells, significant apoptosis rates and increased mortality rates [83]. Immunoparalysis is defined as the reduced (<30%) HLA-DR expression in MC. A fine-tuning between pro- and anti-inflammatory factors in ARDS is of utmost importance. Therefore, standard therapeutic strategies targeting to reduce the excessive inflammatory response in ARDS may not be appropriate, since their unbalanced administration can lead to secondary opportunistic infections. Primary cultures of BALF cells from ARDS-immunoparalysed patients showed attenuation in BALF-PLA2, but treatment with Interferon-γ, a macrophage primer, restored the HLA-DR expression in BALF macrophages within 3 days after the treatment initiation, followed by increased PAF-AH activity [84].

PLAs in extracellular vesicles (EVs)

Small (30–150 nm) and dense EVs of endosomal origin are engaged in intercellular communication delivering proteins, bioactive lipids, RNAs, miRNAs and transcription factors to recipient cells, constitutively or after induction [85,86] and have attracted scientific interest as biomarkers platforms for ARDS [87,88]. It has been demonstrated in BRL cells that various PLA2 groups are associated with EVs, each one having distinct contribution on EVs formation, loading with bioactive lipid metabolites and release [89,90]. The inhibition of secreted sPLA2IIA from rat cells by brefeldin A, an inhibitor of the protein trafficking, suggests its association with EVs [91]. An interesting study by Papadopoulos et al. provided evidence that sPLA2IIA is a cargo of BALF-EVs in ARDS patients. More specifically, data showed that while sPLA2IIA mRNA was detected in exosomal type EVs from BALF, independently of the presence of ARDS, the functional protein was detectable only in vesicles of early ARDS. These findings provide a relationship between sPLA2IIA and the evolution of ARDS through a novel mechanism involving exosomes and specific time window in ARDS [92]. Thus, EV-derived sPLA2IIA from BALF can serve not only as a marker of early-phase ARDS, but also as a tracer of spatiotemporal events characterizing the propagation and exacerbation of the syndrome.

Conclusions

The increased expression of PLA2s in ARDS and their response to pharmacological treatments strongly support that they can serve as biomarkers. However, dedicated clinical studies are required for the establishment of a proof of concept. Overall, we suggest that spatiotemporal features of PLA2s expression, their shuttling to distal locations in conjunction with their interaction with sets of functional (extra)cellular components, in a context of network biology, could give new insights into the monitoring mechanisms of propagation and dissemination of ARDS and new drug discovery.

Conflicts of interest

The authors declare no conflict of interest.

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