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REVIEW PAPER

Secretory Phospholipase A_2 Enzymes in Acute Lung Injury

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Abstract

The secretory phospholipase A_2 ($sPLA_2$) group of secreted enzymes hydrolyze phospholipids and lead to the production of multiple biologically active lipid mediators. $sPLA_2$s and their products (e.g., eicosanoids) play a significant role in the pathophysiology of various inflammatory diseases, including life-threatening lung disorders such as acute lung injury (ALI) and the Acute Respiratory Distress Syndrome (ARDS). The ALI/ARDS spectrum of severe inflammatory conditions is caused by direct (such as bacterial or viral pneumonia) or indirect insults (sepsis) that are associated with high morbidity and mortality. Several $sPLA_2$ isoforms are upregulated in patients with ARDS as well as in multiple ALI preclinical models, and individual $sPLA_2$s exert unique roles in regulating ALI pathophysiology. This brief review will summarize the contributions of specific $sPLA_2$ isoforms as markers and mediators in ALI, supporting a potential therapeutic role for targeting them in ARDS.

Keywords Inflammation · ARDS · Endothelial · $sPLA_2$-IIA · $sPLA_2$-V · $gVPLA_2$

Introduction

Phospholipase A_2 enzymes (PLA_2s) are a superfamily of proteins that hydrolyze the sn-2 position of glycerophospholipids to generate free fatty acids and lysophospholipids [1]. PLA_2s can be classified into several groups based on their biochemical characteristics, including the cytosolic PLA_2 ($cPLA_2$), the secretory PLA_2 ($sPLA_2$), and Ca^{2+}-independent PLA_2 ($iPLA_2$) groups [1]. These PLA_2 enzymes are functionally important due to their capacity to generate lipids that are critical signaling messengers and important regulators of inflammatory processes. PLA_2-catalyzed fatty acids, such as arachidonic acid (AA) and its metabolites (especially the eicosanoids), have been implicated in a diversity of inflammatory diseases [2–4]. Lysophospholipids, the other major products of PLA_2 catalysis, are a large and important family of bioactive lipid molecules with diverse biologic functions [5].

Early studies in the 1980s and 1990s identified PLA_2s as potential markers and mediators of acute lung injury (ALI) syndromes. Since that time, there has been extensive investigation into the roles of individual PLA_2s in regulating several aspects of pulmonary inflammation [6]. Acute Respiratory Distress Syndrome (ARDS) is a severe form of clinical ALI caused by direct lung injury such as pneumonia, acid aspiration, and mechanical ventilation, and also by indirect injury such as sepsis [7]. In both cases, inflammatory insults, including bacteria (e.g., *Staph aureus*, *Strep pneumoniae*), viruses (e.g., SARS-CoV-2, influenza), acid (e.g., due to aspiration), or injurious mechanical forces (e.g., during positive pressure ventilation), lead to the disruption of the alveolo-capillary permeability, dysfunction of pulmonary surfactant, and infiltration of neutrophils; all these processes represent key features of ALI [8]. The resulting protein-rich edema fluid and uncontrolled inflammation (e.g., cytokine storm) cause severe hypoxemia and respiratory failure [8]. Understanding the mechanisms underlying ALI, and identifying molecular targets that can be exploited therapeutically, are the major areas of research focus in the field. This brief review will discuss and summarize our current understanding of the roles of secreted PLA_2s in the regulation of lung injury and inflammation. We also will highlight knowledge gaps in the field and potential areas of research for future studies.

Overview of $sPLA_2$ Enzymes

The $sPLA_2$ family consists of low molecular weight (~14–19 kDa) enzymes that are secreted extracellularly.

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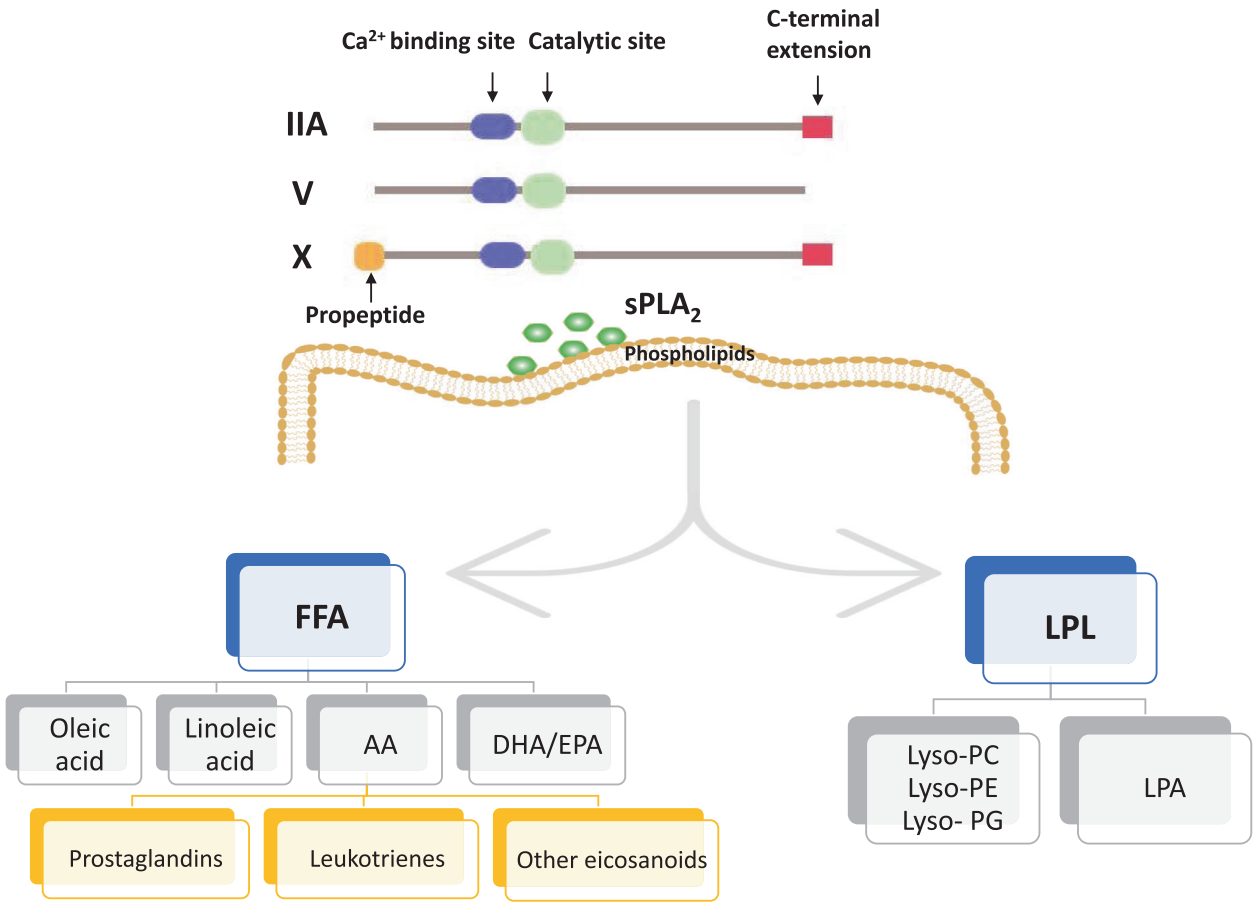

Fig. 1 Mammalian $sPLA_2$ structure and generation of lipid mediators. (Top) Simplified schema representing protein structures for the three mammalian $sPLA_2$ enzymes potentially relevant to acute lung injury syndromes. All active $sPLA_2$s have Ca^{2+}-binding (purple) and catalytic sites (light green), while a C-terminal extension (red) is present in $sPLA_2$-IIA and $sPLA_2$-X. $sPLA_2$-X also contains an N-terminal propeptide (orange), which is cleaved off for enzymatic activation. $sPLA_2$-V has the fewest domains, with no N-terminal propeptide or C-terminal extension, and contains only six disulfides. (Middle) During inflammatory conditions such as ALI/ARDS and sepsis, $sPLA_2$s hydrolyze phospholipids to produce free fatty acids (FFA) and lysophospholipids (LPL). (Bottom) FFAs include oleic acid, linoleic acid, arachidonic acid (AA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA). Through the subsequent actions of cyclooxygenase and 5-lipoxygenase enzymes, AA is converted into prostaglandins, leukotrienes, and other inflammatory eicosanoids. LPL products include the bioactive lipid mediators such as lysophosphatidylcholine (lyso-PC), lysophosphatidyl-ethanolamine (lyso-PE), lysophosphatidylglycerol (lyso-PG), and lysophosphatidic acid (LPA) (color figure online)

To date, at least 11 mammalian $sPLA_2$s (IB, IIA, IIC, IID, IIE, IIF, III, V, X, XIIA, and XIIB) have been identified, of which XIIB represents the only inactive isoform [9, 10]. For their catalytic activity, $sPLA_2$s require millimolar levels of Ca^{2+}, and their catalytic site contains a histidine-aspartic acid dyad (Fig. 1). In contrast to $cPLA_2$s and $iPLA_2$s, $sPLA_2$s act primarily on phospholipids that are exposed extracellularly, such as the outer plasma membrane of nearby cells, membranes of extracellular vesicles or bacteria, alveolar surfactant, etc. Each $sPLA_2$ member has a distinct biological role attributed to their unique substrate specificity, different cell and tissue distribution, and enzymatic properties [11]. Individual $sPLA_2$s can affect lung function directly by modifying the lipid composition of cell membranes or pulmonary surfactant, or indirectly through the generation of lipid mediators (Fig. 1). In addition to their biological functions, $sPLA_2$s have also emerged as potential biomarkers of ARDS [12], as their expression and secretion are significantly upregulated in patients with ARDS, and in animal and in vitro ALI models (as discussed below).

Role of $sPLA_2$ Group IIA and IID in ALI

Group II $sPLA_2$ enzymes (IIA, IIC, IID, IIE, IIF) exhibit a membrane binding preference for phosphatidylglycerol (PG), phosphatidylethanolamine (PE), and phosphatidylserine, while they have lower binding capacity for phosphatidylcholine (PC), the main phospholipid in the outer leaflet of mammalian plasma membranes.

Among the $sPLA_2$s, $sPLA_2$ group IIA (also defined as $sPLA_2$-IIA, PLA2G2A, or $gIIaPLA_2$) is the most well-studied

$sPLA_2$ and has been associated with a variety of diseases [13]. The circulating levels of $sPLA_2$-IIA are very low in healthy conditions but increase significantly during inflammation or infection [13, 14]. Several studies have highlighted the role of $sPLA_2$-IIA as a biomarker in sepsis and various infections [14–16]. $sPLA_2$-IIA is also well-recognized for its antimicrobial role, especially toward gram-positive bacteria since it can directly hydrolyze the membranes of these organisms, which are enriched in PG and PE, the preferred substrates for this specific isoform [17]. In addition, $sPLA_2$-IIA hydrolyzes membrane phospholipids of mitochondria that are released from activated platelets, which serves to promote neutrophil activation and amplify inflammation [18].

A role for $sPLA_2$-IIA in ALI/ARDS is supported by multiple clinical and preclinical studies [11, 19]. Independent investigations have demonstrated increased $sPLA_2$-IIA expression in the bronchoalveolar lavage (BAL) fluid and plasma of patients with ARDS [20–23], and also both $sPLA_2$-IIA protein and mRNA can be detected in extracellular vesicles in BAL from patients with early stage ARDS [24]. Recently, $sPLA_2$-IIA levels were found to be significantly elevated in the plasma of COVID-19 patients and correlated with disease severity [25]. In vitro, different cell types involved in ALI pathogenesis, such as alveolar macrophages and alveolar epithelial cells, express $sPLA_2$-IIA upon stimulation with inflammatory agents such as endotoxins and TNF-α [20, 26]. $sPLA_2$-IIA levels are also increased in multiple experimental models of ALI in vivo, while direct administration of $sPLA_2$-IIA to the lungs causes respiratory distress and hydrolysis of surfactant PG [27, 28]. This associative data linking $sPLA_2$-IIA to ALI pathophysiology led to studies that explored the therapeutic potential of inhibiting this enzyme. Although preclinical studies demonstrated that specific inhibition of $sPLA_2$-IIA by the small molecule LY315920Na/S-5920 was effective in attenuating lung injury in animal models of ALI and sepsis [29], clinical trials using this same inhibitor failed to provide survival protection in patients with sepsis and organ failure [30, 31]. One potential explanation for these disappointing results is that $sPLA_2$-IIA also has strong bactericidal effects, as mentioned earlier, and exerts protective roles against infections [14]. Therefore, although it could represent a useful marker of lung injury, $sPLA_2$-IIA inhibition might impair the host defense against bacterial infections, and thus worsen ALI caused by these organisms. Despite extensive research into $sPLA_2$-IIA, it is evident that new studies are needed to further explore the differential roles of $sPLA_2$-IIA in regulating ALI and understand the integrated effects of its pro- and anti-inflammatory roles.

Recently, another isoform in the $sPLA_2$-II group, $sPLA_2$-IID (or PLA2G2D), was identified as a potential important mediator in respiratory infections. $sPLA_2$-IID expression is increased in aged CD11c+ cells (alveolar macrophage and respiratory dendritic cells), while middle-aged mice lacking $sPLA_2$-IID are protected against SARS-CoV (i.e., SARS) infection [32]. In the absence of $sPLA_2$-IID, there was enhanced dendritic cell migration in the lymph nodes, augmented antiviral T-cell responses, reduced lung damage, and increased survival following viral infection. Increased $sPLA_2$-IID expression in lung CD11c+ cells resulted in upregulation of several eicosanoids that might be involved in the initial immune response [32]. Based on these findings, it will be intriguing to further explore the role of this specific $sPLA_2$ isoform in COVID-19 pathogenesis. Another in vivo study has demonstrated that $sPLA_2$-IID expression is increased in the lungs of LPS-treated mice, further implicating this specific isoform in ALI pathogenesis [33].

Role of $sPLA_2$-V in ALI

$sPLA_2$ group V (defined as $sPLA_2$-V, $gVPLA_2$, or PLA2G5) is less well-studied compared to $sPLA_2$-IIA; however, research in the last decade has provided strong evidence that it is involved in several inflammatory disease processes [34]. In contrast to $sPLA_2$-II, $sPLA_2$-V has a high capacity to bind PC, a major phospholipid in the outer leaflet of the mammalian plasma membrane and pulmonary surfactant. Some reports suggest that $sPLA_2$-V preferentially releases fatty acids with a low degree of unsaturation, such as oleic and linoleic acids [11]; however, a large body of evidence also implicates $sPLA_2$-V in AA mobilization and eicosanoid generation [11, 34–36].

One of the major effects of $sPLA_2$-V in the lung is the hydrolysis of lung surfactant. Surfactant, a complex mixture of phospholipids and proteins secreted by alveolar epithelial type II cells, functions to reduce the surface tension at the air–liquid interface in the lung [37]. Dysfunctional surfactant results in alveolar injury and is a major hallmark of ALI. $sPLA_2$-V contributes to disruption of surfactant activity by hydrolyzing PC, one of its major phospholipid components. This is evident in neonate mice overexpressing $sPLA_2$-V that die within 8 h from birth due to increased surfactant hydrolysis and resulting severe lung damage [38].

In addition to directly affecting lung function by altering the surfactant activity, preclinical work from our group and others has demonstrated additional mechanisms by which $sPLA_2$-V may participate in ALI pathogenesis (summarized in Fig. 2). In a direct model of LPS-induced ALI in mice, $sPLA_2$-V protein and mRNA expression were increased in lung tissues [39]. $sPLA_2$-V genetic deletion (i.e., knockout mice) or inhibition (after treatment with MCL-3G1, a specific $sPLA_2$-V antibody) resulted in significant decreases in LPS-induced lung edema and inflammation [39]. In ALI, lung endothelial barrier disruption is a key event toward edema formation and facilitation of inflammatory cell

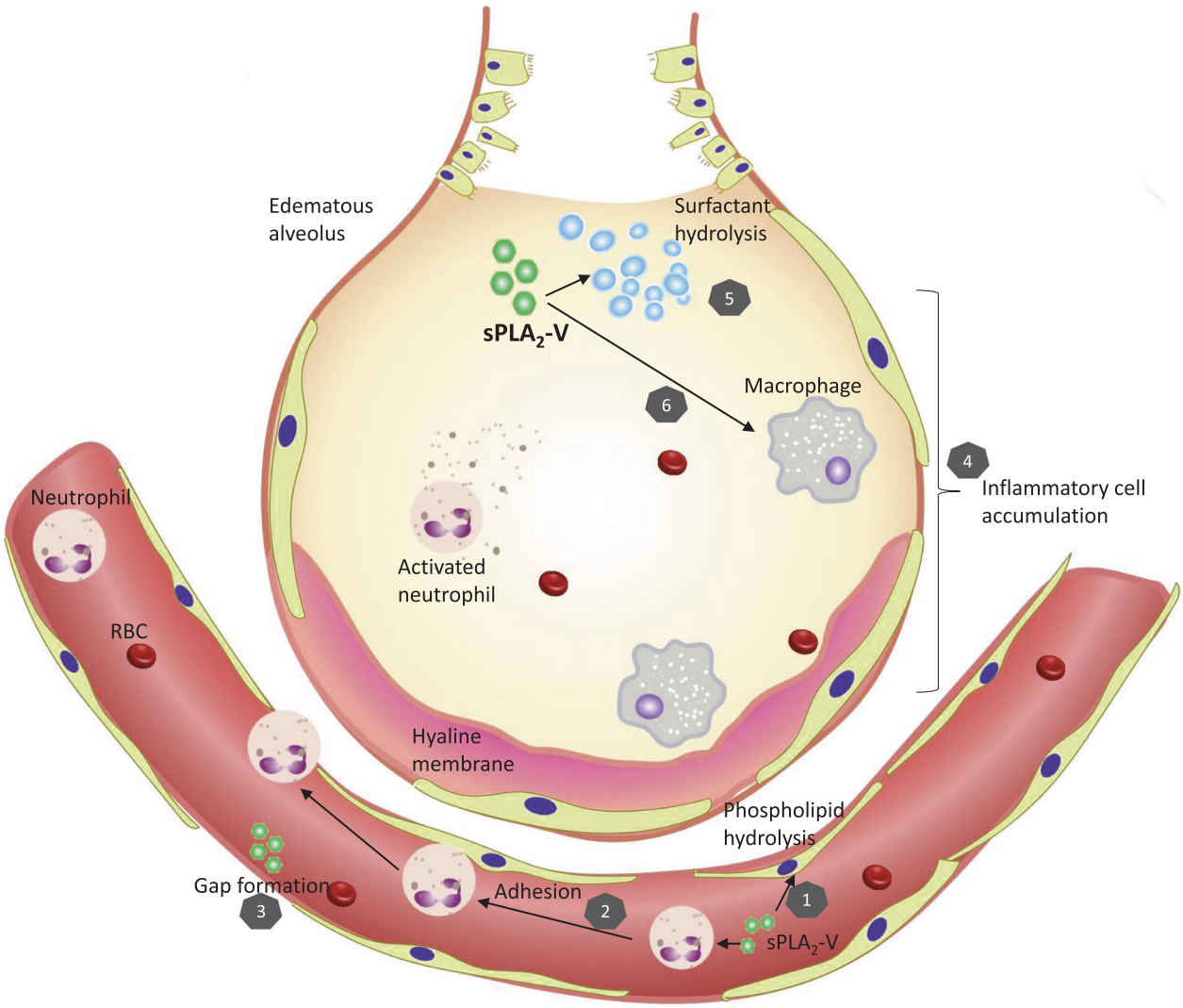

Fig. 2 $sPLA_2$-V mediates acute lung injury pathophysiology. In response to multiple ALI-relevant stimuli, $sPLA_2$-V expression (green hexagons) is increased in lung endothelium and directly hydrolyzes phospholipids to promote inflammatory processes (1). These include neutrophil activation, adhesion to lung EC, and migration to the interstitium and alveolar space to further drive inflammation (2). $sPLA_2$-V also directly causes lung EC dysfunction and leads to gap formation and increased vascular permeability (3). During ALI, $sPLA_2$-V is increased in the alveolar space where it hydrolyzes lung surfactant and contributes to alveolar injury, hyaline membrane formation, accumulation of edema fluid, and inflammatory cell recruitment (4, 5). In addition, $sPLA_2$-V may alter lung macrophage function, including phagocytosis (6) (color figure online)

accumulation into the alveolar space [40, 41]. Given the role of $sPLA_2$-V in regulating lung edema in vivo, our group further explored the contribution of $sPLA_2$-V in regulating lung endothelial cell (EC) function. These in vitro studies demonstrated that $sPLA_2$-V expression is constitutively expressed in unstimulated lung EC and is upregulated upon treatment with LPS [42]. LPS is a potent inflammatory stimulus that induces actin cytoskeletal remodeling and disruption of EC junctions, leading to gap formation and increased permeability. Specific blockade of $sPLA_2$-V by MCL-3G1 attenuates these cytoskeletal rearrangements and disruption of EC barrier by LPS [42]. Consistent with these findings, another group reported that increased mechanical forces (i.e., stretch) that occur during mechanical ventilation in patients with ARDS (i.e., ventilator-induced lung injury or VILI) also upregulate $sPLA_2$-V in lung EC [43]. Similar to LPS, high levels of mechanical stretch cause EC barrier dysfunction and induce pro-inflammatory signaling. Genetic deficiency or inhibition of $sPLA_2$-V (by the MCL-3G1) protects against lung vascular leak and inflammation in vivo in mice subjected to VILI [43]. In response to mechanical stretch, $sPLA_2$-V upregulates lung EC ICAM-1 expression and cytokine production, as well as increased neutrophil adhesion to activated EC [43]. These studies from our group and others provide strong evidence that $sPLA_2$-V is a critical mediator

of lung EC barrier dysfunction in ALI. However, this prior work employed relatively simplistic ALI models using only one stimulus that does not fully recapitulate the human disease. To advance these observations further, our laboratory recently has developed a clinically relevant model of bacterial infection-induced ALI, which employs the live pathogen methicillin-resistant *Staph aureus* (MRSA). MRSA-induced pneumonia is a common cause for sepsis and ARDS, and recent studies have described the injurious effects of *Staph aureus* on lung EC [44, 45]. In our preliminary studies, MRSA induces the expression of $sPLA_2$-V in lung EC, and its blockade or deficiency inhibits MRSA-induced EC barrier disruption in vitro and ALI in vivo (unpublished data). This work is ongoing.

Although these findings have established a functional role for $sPLA_2$-V in modulating endothelial injury and inflammation during ALI, the mechanisms underlying these effects are less well-understood. $sPLA_2$-V induction in lung EC has been associated with increased cell surface expression [42, 43], suggesting that inflammatory stimuli induce its translocation to the outer membrane, where it can directly modulate barrier function. Indeed, exogenous administration of recombinant $sPLA_2$-V causes significant disruption of the lung EC barrier [42]. Intracellular pathways activated by $sPLA_2$-V include F-actin rearrangement (cytoskeleton remodeling), stress fiber formation, and adherens junction disruption. These changes were inhibited after blocking $sPLA_2$-V activity using the MCL-3G1 antibody [42]. Notably, administration of the related $sPLA_2$-IIA enzyme has no effect on EC barrier function [42].

There are several possible mechanisms by which $sPLA_2$-V mediates EC barrier disruption, involving direct outer plasma membrane hydrolysis by $sPLA_2$-V, and/or indirect disruption caused by the $sPLA_2$-V-induced mediators/signaling [46]. As described above, $sPLA_2$-V activity on the outer membrane of cells generates multiple bioactive lipid mediators (Fig. 1). Our group explored the effects of these products on EC permeability induced by $sPLA_2$-V. Lysophosphatidyl-choline (lyso-PC), lysophosphatidyl-glycerol (lyso-PG), lysophosphatidic acid, and arachidonic acid all failed to cause significant barrier disruption in cultured lung EC [46]. Future studies may examine the role of other specific $sPLA_2$-V hydrolysis products on EC permeability, such as oleic and linoleic acids, which can mediate EC dysfunction [47–49]. Another mechanism by which $sPLA_2$-V may disrupt EC barrier function is by activating specific downstream intracellular signaling pathways. $sPLA_2$-V activates members of the MAP kinase family and $cPLA_2$ (group IV) in lung EC [46]. MAPK enzymes are known to regulate multiple EC functions [50], while $cPLA_2$ is an inflammatory mediator that can be activated by both ERK and $sPLA_2$-V enzymes [51, 52]. However, specific inhibition of these pathways did not attenuate the effects of $sPLA_2$-V on EC permeability, suggesting that their activation is not required for $sPLA_2$-V-mediated effects [46]. Whether other downstream signaling pathways activated by $sPLA_2$-V contribute to EC permeability remains to be determined. At a whole-organism level, EC disruption may be mediated by the effects of $sPLA_2$-V activity on neighboring cells. As one example, a recent study demonstrated that $sPLA_2$-V activates neutrophils to release VEGF, a potent endothelial barrier disrupting agent [53]. EC–neutrophil interactions play a critical role in the progression of ALI, and $sPLA_2$-V may mediate this cellular cross-talk to contribute to lung EC barrier failure.

To understand how $sPLA_2$-V mediates its responses, it is also important to characterize its interactions with the plasma membrane. $sPLA_2$-V binds to cells by interacting with cell surface heparan sulfate proteoglycans or by binding to phospholipids [54]. We demonstrated that heparinase treatment that results in degradation of the cell surface heparin sulfate moieties did not affect EC barrier disruption by $sPLA_2$-V; however, in this study, we did not assess whether heparinase efficiently blocks $sPLA_2$-V binding [46]. It is likely that binding of $sPLA_2$-V to heparan sulfate proteoglycans is an important mechanism for $sPLA_2$-V internalization and degradation, as discussed below. Whether $sPLA_2$-V binds to other cell surface molecules, such as receptors, to activate downstream signaling that mediates EC barrier disruption should also be considered. Indeed, a recent report suggested that $sPLA_2$-V mediates its effects in neutrophils by interacting with integrins or a PLA_2 receptor (PLAR1) [53].

The maintenance of lung endothelial function and its protection from barrier disrupting stimuli are critical for the prevention of ALI. Understanding these processes may also facilitate the development of endothelium-targeted therapeutics for ARDS. As $sPLA_2$-V appears to be an important regulator of endothelial barrier function and a promising therapeutic target, it is pivotal to understand not only the mechanisms by which this enzyme exerts its effects, but also how its expression, secretion, and clearance are regulated. Early work demonstrated that $sPLA_2$-V binding to cell surface heparan sulfate proteoglycans mediates its internalization and degradation in human neutrophils [54]. More recent studies from our laboratory have identified a new clearance mechanism, in which $sPLA_2$-V upon internalization into lung ECs associates with autophagosomes and is eliminated through a lysosome-related pathway [55]. Conditions in which this pathway is dysregulated could result in impaired clearance of this inflammatory enzyme and exaggeration of injurious effects during ALI.

In addition to the central role of $sPLA_2$-V in regulating the lung endothelial barrier, other studies have implicated this enzyme in host immune responses. Human macrophages activated by IL-4 (M2 phenotype) express increased levels of $sPLA_2$-V and exhibit increased phagocytosis of

yeast-derived zymosan and bacteria, a process mediated by lysophosphatidyl-ethanolamine (LPE), an $sPLA_2$-V product [56]. Macrophages lacking $sPLA_2$-V have impaired phagocytosis, which can be restored after the exogenous addition of LPE [56]. Alternatively, $sPLA_2$-V regulates phagocytosis by translocating to the phagosomes to mediate maturation of phagolysosomes [57]. Similar to macrophages, neutrophils are another component of the immune system that play a key role in phagocytosis and pathogen clearance. In a model of *E.coli* pneumonia, $sPLA_2$-V-deficient mice have higher bacterial loads in the alveolar space compared to wild-type mice [58]. Although the exact mechanisms by which $sPLA_2$-V affects bacterial loads remain unclear, this study suggested that the absence of $sPLA_2$-V may result in diminished accumulation of neutrophils in the alveolar space [58]. Notably, although $sPLA_2$-V is stored in neutrophil granules and may be liberated upon their activation to directly target bacteria, $sPLA_2$-V has no direct effect on *E.coli* viability [58]. Other studies have demonstrated that $sPLA_2$-V plays an important role in the generation of eicosanoids in macrophages and neutrophils [35, 54, 59]. Based on these prior investigations, future studies should explore the specific roles of $sPLA_2$-V in modulating immune cell functions and signaling in ALI.

Although there is strong evidence that $sPLA_2$-V modulates key functions of several cell types involved in ALI pathogenesis, in contrast to $sPLA_2$-IIA there is a paucity of data regarding the potential role of $sPLA_2$-V as a biomarker. One published study detected $sPLA_2$-V in the BAL of infants with ARDS [22], while we have observed significant amounts of the enzyme in BAL of mice treated with LPS, MRSA, or high-tidal volume mechanical ventilation (unpublished data). These observations suggest that $sPLA_2$-V may also have an important role as an ALI biomarker that warrants further study.

Role of $sPLA_2$-X in ALI

Group X $sPLA_2$ (defined as $sPLA_2$-X, $gXPLA_2$, PLA2G10, or GX-$sPLA_2$), which is structurally related to both group-I and -II $sPLA_2$, exhibits the most potent hydrolytic activity toward PC and induces the release of AA more efficiently than other $sPLA_2$s [13, 60]. $sPLA_2$-X is synthesized as a zymogen and requires proteolytic cleavage for activation [13]. Despite the high affinity of $sPLA_2$-X for PC, the major surfactant phospholipid, mice overexpressing $sPLA_2$-X display minimal abnormality of the respiratory tract function and have normal alveolar architecture and unaltered surfactant [38].This observation may be due to the fact that the $sPLA_2$-X remains inactive under physiologic conditions and only becomes activated after proteolysis triggered by inflammatory stimuli [38].

Several studies have investigated the role of $sPLA_2$-X as a potent regulator of airway inflammation underlying asthma (reviewed in refs. [13, 61]); however, its involvement in ALI is less well-understood. One study suggested that $sPLA_2$-X may be involved in acute inflammatory processes induced by influenza infection [62]. Following H1N1 infection, $sPLA_2$-X lung expression is increased, while targeted deletion of $sPLA_2$-X significantly increased survival. Mechanistically, $sPLA_2$-X produces several inflammatory lipid mediators at the early phase of inflammation and dysregulates the adaptive immune system [62]. It remains to be determined if inhibition of $sPLA_2$-X activity during bacterial or viral infections has therapeutic potential in ALI.

Other $sPLA_2$s and ALI

Knowledge is limited about other $sPLA_2$s and their potential involvement in ALI. $sPLA_2$-IB, similar to $sPLA_2$-X, exists as an inactive proenzyme that can be cleaved to become active. In vitro studies have demonstrated that $sPLA_2$-IB is released from alveolar epithelial cells upon *Pseudomonas aeruginosa* infection (a common cause of ALI) and that $sPLA_2$-IB causes PC efflux, a process mediated by ABCTA1, a lipid-export pump [63]. This and other studies that have demonstrated increased levels of $sPLA_2$-IB in ALI [22, 64] suggest that this isoform may play a functional role in ALI pathogenesis, but additional studies are needed.

Future Directions and Conclusion

Despite strong evidence that members of the $sPLA_2$ family are critical mediators of ALI, there remains a lack of effective therapeutic strategies to target these enzymes. This is due in part to existing knowledge gaps regarding $sPLA_2$ biology and its roles in regulating disease pathogenesis. In this review, we have summarized key findings about the regulation and distinct roles of individual $sPLA_2$ isoforms in ALI and discussed some specific research areas that would benefit from future studies. In addition to those mentioned above, some other suggested future directions are the following: (1) although $sPLA_2$s are secreted enzymes, they can also act intracellularly, through mechanisms that need to be elucidated. (2) In addition to their extracellular enzymatic activity, $sPLA_2$s also interact with receptors or other molecules on the cell surface (e.g., PLA_2 receptors, proteoglycans, integrins). New studies are needed to identify potential interacting or binding molecules for these enzymes and characterize the resultant downstream signaling. (3) Understanding the mechanisms underlying $sPLA_2$ expression, secretion, and clearance under normal and pathological conditions would

contribute to the development of new strategies to target the $sPLA_2$s. (4) Several mutations in $sPLA_2$ enzymes have been associated with diseases such as atherosclerosis, cardiovascular disease, and asthma [65]. Recently a rare mutation (R123H) was also identified in $sPLA_2$-IIA in two infants with ARDS [66]. New studies are needed to identify additional mutations or SNPs associated with ARDS, and to understand how these genomic alterations affect $sPLA_2$ function. (5) There is evidence that PLA_2 isoforms interact with each other, but a potential role for PLA_2 cross-talk in ALI is understudied. Finally, (6) improved understanding is needed regarding the effects of the lipid mediators produced by $sPLA_2$ activity on lung cells.

In summary, there is significant evidence demonstrating that in association with ALI pathophysiology, $sPLA_2$s are upregulated in different cell types in the lung compartment, their extracellular levels are highly elevated, and individual isoforms exert specialized biologic functions contributing to pro- or anti-inflammatory signaling. Due to their biological roles and extracellular presence during lung inflammation, $sPLA_2$s represent attractive targets for therapeutic and/or diagnostic biomarker development.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interest.

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