Role of the Lysophospholipid Mediators Lysophosphatidic Acid and Sphingosine 1-Phosphate in Lung Fibrosis

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Aberrant wound healing responses to lung injury are believed to contribute to fibrotic lung diseases, such as idiopathic pulmonary fibrosis (IPF). The lysophospholipids lysophosphatidic acid (LPA) and sphingosine 1-phosphate (S1P), by virtue of their ability to mediate many basic cellular functions, including survival, proliferation, migration, and contraction, can influence many of the biological processes involved in wound healing. Accordingly, recent investigations indicate that LPA and S1P may play critical roles in regulating the development of lung fibrosis. Here we review the evidence indicating that LPA and S1P regulate pulmonary fibrosis and the potential mechanisms through which these lysophospholipids may influence fibrogenesis induced by lung injury.

Keywords: lysophospholipids; lysophosphatidic acid; sphingosine 1-phosphate; lung fibrosis

The response to lung injury involves a complex series of biological responses which, if appropriate in timing, magnitude, and balance, lead to resolution of injury and restoration of normal lung structure and function. When dysregulated or overexuberant, however, these responses can result in excessive scar formation, or fibrosis, characterized by the pathological accumulation of fibroblasts and myofibroblasts and the extracellular matrix that they synthesize. Such dysregulated wound healing responses to injury have been implicated in the pathogenesis of idiopathic pulmonary fibrosis (IPF) and the fibroproliferative phase of acute respiratory distress syndrome (ARDS). Notably in IPF, the replacement of normal tissue with fibrosis is progressive and eventually leads to end-stage organ dysfunction and death (1). Because lung fibrosis may be difficult to reverse once established, strategies aimed at preventing fibrosis by interrupting the upstream biological processes that contribute to fibrogenesis are likely to have the greatest therapeutic impact.

The biological responses to injury that may contribute to the development of lung fibrosis include: (1) epithelial cell death/apoptosis; (2) increased vascular permeability; (3) inflammatory cell recruitment; (4) extravascular coagulation; (5) activation of transforming growth factor- β (TGF- β) and other profibrotic mediators; (6) fibroblast recruitment, proliferation, persistence, and activation to myofibroblasts; and (7) synthesis of collagen

and other extracellular matrix proteins (2–5). In individuals in whom these processes are dysregulated or overexuberant, devastating diseases such as IPF may result from commonly occurring injurious insults to the lung, such as inhaled particulates, viral infections, or aspiration of refluxed gastric acid (6–8). Better understanding of the molecular mediators and pathways that regulate these wound-healing responses should therefore lead to novel therapeutic targets for fibrotic diseases. In support of this approach, multiple investigations in animal models of lung fibrosis have demonstrated that targeting one or more of these wound-healing responses can prevent the development of fibrosis and in some cases reverse established fibrosis (9–15).

Lysophosphatidic acid (LPA) and sphingosine 1-phosphate (S1P) are bioactive lysophospholipids that signal through distinct G protein-coupled receptors (GPCRs), LPA₁₋₅ and S1P₁₋₅ (16). Both of these lysophospholipids mediate many basic cellular functions, including cell migration, survival, contraction, proliferation, gene expression, and cell–cell interactions (17, 18). Particularly relevant to wound healing responses to lung injury, LPA and S1P have been shown to regulate epithelial cell and fibroblast apoptosis, fibroblast migration and myofibroblast differentiation, vascular permeability, and TGF- β signaling *in vitro* (19–26). Furthermore, recent studies have demonstrated important roles for LPA and S1P in regulating the development of lung fibrosis *in vivo*. Targeting the LPA and S1P pathways therefore may represent effective therapeutic strategies for fibrotic diseases such as IPF. Here we review the relevant literature demonstrating important roles for LPA and S1P in lung fibrosis, focusing on animal models of this disease and on the cellular mechanisms through which these two lysophospholipids regulate its development.

LPA AND S1P STRUCTURE, METABOLISM, AND RECEPTORS

LPA and S1P Chemical Structure

LPA is the common name for 1-acyl-2-hydroxy-sn-glycero-3-phosphates, which consist of a glycerol phosphate backbone esterified with a single fatty acid (27). Various species of LPA differ only in the identity of the fatty acid moiety. S1P is the phosphorylation product of sphingosine (2-amino-4-octadecene-1,3-diol), a long-chain, unsaturated amino alcohol, which is a backbone component of membrane sphingolipids (28). The chemical structures of LPA species and S1P are illustrated in Figure 1.

LPA and S1P Metabolism

LPA has been demonstrated to be produced in response to injury and to promote wound healing in multiple tissues, including the skin, the gastrointestinal tract, and the cornea (14, 29–34). LPA can be produced from membrane phospholipids of cells or platelets, and from surfactant phospholipids in the lung, by at least three pathways (Figure 2) (35). Of these, conversion of lysophosphatidylcholine to LPA by the enzyme autotaxin appears

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Figure 1. Chemical structures of lysophosphatidic acid (LPA) and sphingosine 1-phosphate (S1P).

to account for the majority of extracellular LPA *in vivo* (36, 37). LPA can also be degraded in several ways (38). Of these, hydrolysis of LPA by the lipid phosphate phosphatases is likely the major pathway for clearance of LPA *in vivo* (39).

S1P is a bioactive sphingolipid derived from sphingosine. There are hundreds of known sphingolipid species; the metabolism of some of the more commonly studied sphingolipids is depicted in Figure 3. Phosphorylation of sphingosine by sphingosine kinases results in the synthesis of S1P, which can either be converted back to sphingosine by the action of S1P phosphatase or the lipid phosphate phosphatases, or it can be degraded by S1P lyase (40, 41). Given the pleiotropic effects of LPA and S1P, altering their levels by targeting the enzymes involved in their metabolism holds great therapeutic potential for human diseases.

LPA and S1P Receptors

Although LPA and S1P were initially identified as phospholipid precursors and metabolites in cell membrane biosynthesis, their subsequently appreciated ability to regulate diverse biological processes stems from their ability to activate specific GPCRs (42, 43). Currently, five accepted LPA receptors (LPA₁-LPA₅) and five accepted S1P receptors (S1P₁-S1P₅) have been identified (16). Eight of these receptors, LPA₁₋₃ and S1P₁₋₅, share substantial sequence homology. Other GPCRs with less homology have also been identified as LPA receptors, including the accepted LPA receptors LPA₄ and LPA₅, as well as P2Y₅, a lower-affinity receptor that is likely to join the LPA receptor family as LPA₆ (16), indicating that LPA receptors have evolved through two distinct lineages in the GPCR family (16).

LPA AND LUNG FIBROSIS

The role of LPA as a potent mediator of lung fibrosis was recently demonstrated. LPA signaling through the LPA₁ receptor was found to be critically required for the development of

Figure 2. Lysophosphatidic acid (LPA) metabolism. LPA can be produced by several different pathways. Phospholipids such as phosphatidylcholine (PC) can be converted to lysophospholipids such as lysophosphatidylcholine (LPC) by members of the phospholipase A₁ (PLA₁) and phospholipase A₂ (PLA₂) families of enzymes. These lysophospholipids can be converted to LPA by the lysophospholipase D activity of autotaxin (ATX). Alternatively, LPA can be generated from phospholipids by the sequential actions of lecithin cholesterol acyltransferase (LCAT) and ATX. Last, PLA₁ and PLA₂ enzymes can produce LPA directly by hydrolysis of phosphatidic acid (PA). Several PLA₁ and PLA₂ enzymes have been implicated in extracellular LPA production, including mPA-PLA₁α/Lipase H (LIPH), mPA-PLA₁β, PS-PLA₁, and sPLA₂-IIA. LPA can also be degraded in several ways. Of these, hydrolysis of LPA to monoacylglycerol (MAG) by lipid phosphate phosphatases (LPP-1, LPP-2, and LPP-3) is likely the major pathway for clearance of LPA *in vivo*.

Figure 3. Sphingosine 1-phosphate (S1P) metabolism. Ceramide is considered to be the central molecule in sphingolipid metabolism. It can be generated *de novo* from serine and palmitoyl-CoA through several intermediate steps, or it can be formed from the catabolism of sphingomyelin and complex glycosphingolipids. Ceramide can be metabolized via several different pathways, one of which is the conversion by ceramidases to sphingosine, which can in turn be phosphorylated by sphingosine kinases (Sphk) to synthesize S1P. S1P can either be converted back to sphingosine by the action of S1P phosphatase or the lipid phosphate phosphatases, or it can be degraded by S1P lyase.

pulmonary fibrosis induced in mice by bleomycin lung injury: LPA levels increased in bronchoalveolar lavage (BAL) fluid after bleomycin challenge, and mice deficient for LPA₁ (LPA₁ knockout [KO] mice) were found to be dramatically protected from lung fibrosis and mortality in this model (14). Similarly, treatment with a specific LPA₁ antagonist protected mice from the development of fibrosis induced by bleomycin (44). Studies in patients with IPF have implicated LPA-LPA₁ signaling in the development of lung fibrosis in humans as well. LPA levels were increased in BAL samples from patients with IPF, LPA₁ was highly expressed by fibroblasts recovered from these samples, and inhibition of LPA₁ markedly reduced fibroblast responses to the chemotactic activity of those BAL samples (14). These data suggest that LPA signaling through LPA₁ contributes to the excessive accumulation of fibroblasts in the lungs of patients with IPF. The LPA-LPA₁ pathway appears to mediate numerous wound-healing responses which may contribute to the development of lung fibrosis, including epithelial cell apoptosis, increased vascular permeability, and fibroblast migration and persistence (Figure 4).

LPA-LPA₁ Signaling Promotes Epithelial Cell Apoptosis

Increased lung epithelial cell apoptosis in response to injury is now believed to play a central role in pulmonary fibrogenesis, and LPA-LPA₁ signaling appears to contribute to this process. Increased numbers of apoptotic cells have been observed in both the alveolar and bronchial epithelia of patients with IPF (45, 46), and induction of pulmonary epithelial cell apoptosis in mice is sufficient to result in the development of fibrosis (47–50). Epithelial cell apoptosis is also prominent in the bleomycin model of pulmonary fibrosis, in which intratracheal bleomycin challenge leads to the rapid appearance of apoptosis in alveolar and bronchial epithelial cells (48, 49, 51). The number of apoptotic cells present in both the alveolar and bronchial epithelia of LPA₁ KO mice was significantly reduced compared with wild-type mice early post-bleomycin challenge, suggesting that LPA-LPA₁ signaling promotes epithelial apoptosis after lung injury (52). Consistent with these *in vivo* results, we found that LPA signaling through LPA₁ induced apoptosis in culture of both primary human bronchial epithelial cells and a rat alveolar epithelial cell line (52). In these *in vitro* studies, LPA-LPA₁ signaling appeared to specifically mediate anoikis, the apoptosis of anchorage-dependent cells induced by their detachment (53).

LPA-LPA₁ Signaling Promotes Increased Vascular Permeability

Vascular leak is another hallmark of tissue injury (54, 55). Increased alveolar-capillary permeability has been demonstrated to be present in the lungs of patients with pulmonary fibrosis and to predict worse outcomes (56, 57). Tissue injury can

Figure 4. Lysophosphatidic acid (LPA) and sphingosine 1-phosphate (S1P) regulation of lung fibrosis. Schematic representation of the biological processes implicated in lung fibrosis that appear to be regulated by LPA and S1P. Evidence reviewed in this article indicates that LPA contributes to the development of fibrosis after lung injury through multiple mechanisms (solid green arrows), including the induction of: (1) epithelial cell apoptosis; (2) increased vascular permeability, resulting in increased intraalveolar coagulation; (3) fibroblast recruitment into the injured airspaces and their resistance to apoptosis; and (4) activation of latent TGF-β. Mechanisms 1 to 3 appear to be mediated by LPA signaling through LPA₁, whereas mechanism 4 appears to be mediated by LPA signaling through LPA₂. S1P may have both anti- and profibrotic effects in the lung. Evidence reviewed in this article indicates that S1P inhibits the development of pulmonary fibrosis through attenuation of lung vascular leak (red line). This mechanism appears to be mediated by S1P signaling through S1P₁ expressed on endothelial cells. Potential profibrotic activities of S1P, which have not yet been investigated in the lung (dashed green arrows), include the induction of: (1) epithelial cell apoptosis, (2) fibroblast recruitment, and (3) myofibroblast differentiation.

directly disrupt blood vessels, but beyond the immediate effects of vascular disruption, tissue injury also results in the elaboration of bioactive mediators that cause an increase in vascular permeability that persists throughout the early phases of tissue repair (58). In the lung, LPA appears to be one of these mediators contributing to persistent vascular leak after injury.

Increased transport of fluid and macromolecules across the endothelium under pathologic conditions primarily occurs through paracellular gaps formed by the disruption of endothelial intercellular junctions (59). LPA is known to disrupt endothelial monolayers *in vitro* through activation of RhoA/Rho kinase within endothelial cells, which results in the formation of intracellular actin stress fibers and the opening up of paracellular gaps (60). This process also appears to be mediated by LPA₁, as we have found that LPA-induced endothelial barrier disruption *in vitro* is inhibited by AM095 (unpublished data), a specific LPA₁ receptor antagonist (61). LPA₁ KO mice exhibit decreased vascular leak after bleomycin lung injury, indicating that LPA-LPA₁ signaling participates in endothelial barrier dysfunction induced by lung injury *in vivo* (14). The LPA-LPA₁ pathway therefore may also contribute to lung fibrosis by increasing vascular permeability in the lung after injury.

Persistent vascular leak appears to contribute to the development of fibrosis after lung injury through the promotion of intra-alveolar coagulation (62, 63). Increased vascular permeability results in extravasation of coagulation cascade proteins into the airspaces, where they are activated by tissue procoagulants. Intra-alveolar coagulation can, in turn, promote lung fibrosis via at least two potential mechanisms: (1) promotion of fibroblast accumulation in response to the presence of fibrin clot in the airspaces, and (2) direct profibrotic effects of the coagulation proteinases. Fibrin in the airspaces may contribute to fibroblast accumulation by providing a provisional matrix into which fibroblasts migrate during tissue repair and by promoting epithelial-to-mesenchymal transition (54, 64, 65). Intra-alveolar fibrin deposition does not appear to be absolutely required for fibrosis to occur, however, as fibrinogen-null mice are capable of developing lung fibrosis after bleomycin lung injury despite being unable to form fibrin clots (66, 67). Thrombin and other coagulation proteinases have now been shown to have profibrotic effects that are independent of their ability to generate fibrin, through activation of the proteinase-activated receptors on epithelial cells and fibroblasts. Activation of these receptors can promote fibrosis through the induction of mediators such as platelet-derived growth factor and CCL2, and/or through the activation of latent TGF- β (12, 63, 68). LPA-LPA₁-mediated endothelial barrier disruption therefore could contribute to the development of fibrosis after lung injury by augmenting activation of the coagulation cascade and its proteases in the airspaces. Consistent with this hypothesis, LPA₁ KO mice exhibited decreased intra-alveolar coagulation in addition to decreased vascular leak after bleomycin lung injury (14). Based on the data linking intra-alveolar coagulation to fibrosis, the decreased intra-alveolar coagulation observed in LPA₁ KO mice would therefore be predicted to contribute to their protection from pulmonary fibrosis.

LPA-LPA₁ Signaling Promotes Fibroblast Migration

The pathologic accumulation of fibroblasts in the lung is another hallmark of pulmonary fibrosis and may result from a combination of increased fibroblast recruitment and proliferation and decreased fibroblast apoptosis. After cutaneous injury, fibroblast migration into the provisional fibrin matrix of the wound clot is a central step in granulation tissue formation (54, 55). Fibroblasts analogously migrate into the fibrin-rich exudates that

develop in the airspaces after lung injury in IPF (69). Several lines of evidence suggest that this fibroblast migration is a critical step in the development of lung fibrosis. Fibroblast chemoattractant activity is generated in the airspaces in IPF, and the extent of this activity correlates with disease severity (70). Genes related to cell migration are up-regulated in the lungs of patients with an accelerated variant of IPF, and BAL samples from these “rapid progressors” induce significantly greater fibroblast migration than BAL from “slow progressors” (71). Last, several studies in animal models have found that the inhibition of fibroblast migration can attenuate the development of pulmonary fibrosis, whereas the promotion of fibroblast migration can exaggerate fibrosis (13, 72–75).

LPA is a potent inducer of migration of multiple cell types, including fibroblasts (20). As noted above, LPA levels increased in BAL fluid recovered from mice after bleomycin challenge. LPA₁ is highly expressed on mouse lung fibroblasts, and chemotaxis induced by BAL from bleomycin-challenged mice was attenuated by greater than 50% when the responding cells were lung fibroblasts isolated from LPA₁ KO mice (14), suggesting that LPA-LPA₁ signaling is predominantly responsible for fibroblast recruitment to sites of lung injury in the bleomycin model of lung fibrosis. Consistent with this hypothesis, LPA₁ KO mice exhibited diminished fibroblast accumulation in the lungs after bleomycin injury, suggesting that diminished fibroblast migration may contribute to the attenuated lung fibrosis seen in bleomycin-challenged LPA₁ KO mice. Furthermore, analysis of the fibroblast chemoattractant activity in BAL fluid obtained from patients with IPF indicated that LPA-LPA₁ signaling also directs fibroblast migration into the injured airspaces in IPF, as described above (14).

LPA-LPA₁ Signaling Promotes Fibroblast Persistence

The LPA-LPA₁ pathway also promotes fibroblast resistance to apoptosis, which has been hypothesized to also contribute to the pathologic accumulation of fibroblasts in lung fibrosis (76). Compared with lung fibroblasts from control subjects, lung fibroblasts isolated from patients with IPF have been demonstrated to be resistant to apoptosis induced *ex vivo* (77). LPA has been reported to prevent apoptosis in various fibroblast cell lines (78). Our laboratory has found that LPA signaling specifically through LPA₁ can completely suppress the apoptosis of primary mouse lung fibroblasts induced by serum deprivation (52), suggesting that suppression of fibroblast apoptosis is another mechanism through which LPA-LPA₁ signaling may promote pulmonary fibrosis.

LPA-LPA₁ signaling therefore appears have divergent effects on the apoptotic behaviors of epithelial cells and fibroblasts, promoting apoptosis in the former but suppressing apoptosis in the latter. Consistent with these findings, LPA's effects on apoptosis have previously been demonstrated to be cell-specific, promoting apoptosis of certain cell types but inhibiting apoptosis of others (19). Increased epithelial cell apoptosis coupled with fibroblast resistance to apoptosis has been referred to as an “apoptosis paradox” in IPF (76). The molecular pathways responsible for the divergent susceptibilities of epithelial cells and fibroblasts to apoptosis in IPF have yet to be fully identified, but our data suggest that LPA signaling through LPA₁ may contribute (52). There is precedent for both epithelial cell apoptosis and fibroblast resistance to apoptosis being induced by the same mediator. As we found with LPA, TGF- β induces apoptosis of lung epithelial cells (79, 80) but induces resistance to apoptosis in lung fibroblasts (81, 82). Prostaglandin E₂ (PGE₂) has also been shown to mediate divergent effects on cell survival, although its effects appear to oppose those of LPA and TGF- β :

PGE₂ was found to protect lung epithelial cells against apoptosis while promoting the apoptosis of lung fibroblasts (83). Therefore, increased LPA levels, increased TGF- β activity, and decreased PGE₂ levels in the lung may each contribute to the increased epithelial cell but reduced fibroblast apoptosis observed in IPF.

LPA-LPA₂ Signaling Promotes TGF- β Activation

In addition to the mechanisms described above by which LPA-LPA₁ signaling may contribute to lung fibrosis, LPA signaling through another one of its receptors, LPA₂, may contribute to fibrosis by activating the central profibrotic cytokine TGF- β . Through interaction with its cell-surface receptors, TGF- β influences a wide variety of cellular behaviors, many of which are directly involved in tissue remodeling and repair (84). TGF- β overexpression in mice has been shown to be sufficient to cause pulmonary fibrosis, and numerous studies have shown that inhibition of TGF- β activation and/or signaling is protective in mouse models of pulmonary fibrosis (10, 85–87).

TGF- β activity is primarily regulated by the post-translational conversion of latent TGF- β complexes to active TGF- β (88), and LPA-LPA₂ signaling may contribute to this conversion. TGF- β is synthesized as part of a larger precursor molecule, which is cleaved post-translationally into mature TGF- β and a latency-associated peptide (LAP). LAP remains noncovalently bound to TGF- β on secretion and prevents TGF- β from binding to its receptors (89). TGF- β must therefore dissociate from, or alter its interactions with, LAP to exert its biological effects. Several processes can mediate this required activation of TGF- β , including conformational changes in LAP induced by interaction with one of four α_v -containing integrins, $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_v\beta_6$, or $\alpha_v\beta_8$ (90). Activation of TGF- β specifically by the $\alpha_v\beta_6$ integrin has been shown to be crucial for the development of lung fibrosis in several animal models, including the bleomycin model of pulmonary fibrosis (10). Activation of TGF- β by α_v -containing integrins requires that these integrins themselves become activated (91), and thrombin signaling through proteinase-activated receptor-1 has been demonstrated to produce active TGF- β by activating $\alpha_v\beta_6$ (68). More recently, LPA signaling through LPA₂ has similarly been demonstrated to induce $\alpha_v\beta_6$ -dependent activation of latent TGF- β by normal human bronchial epithelial cells (21), indicating that TGF- β activation may be another important mechanism through which LPA promotes pulmonary fibrosis. Activation of TGF- β by LPA may be a conserved profibrotic pathway in the lung; LPA has recently been demonstrated to also induce $\alpha_v\beta_5$ -dependent activation of latent TGF- β by human airway smooth muscle cells, which may contribute to the peribronchial fibrosis of asthmatic airway remodeling (92).

S1P AND LUNG FIBROSIS

Like LPA, S1P has been shown to play important roles in many basic cellular functions, including processes implicated in the development of lung fibrosis, such as cellular apoptosis, fibroblast migration and myofibroblast differentiation, vascular permeability, and TGF- β signaling (Figure 4). Furthermore, it has recently been shown that S1P signaling through the S1P₁ receptor limits development of pulmonary fibrosis in the bleomycin mouse model. This protective effect of S1P is likely mediated by its ability to limit vascular leak during the early, exudative response to lung injury.

S1P Signaling Limits Increased Vascular Permeability

S1P has been established as a key regulator of the increased vascular permeability characteristic of the early, exudative response

to lung injury. S1P's ability to regulate vascular permeability in the lung appears to be attributable to signaling through S1P₁. Activation of S1P₁ on endothelial cells by S1P or S1P₁ agonists induces cytoskeletal rearrangements that promote formation of intercellular adherens and tight junctions, resulting in enhancement of endothelial monolayer barrier function (23, 93–97). Mice that are genetically deficient for the S1P-producing enzyme sphingosine kinase 1 (Sphk1) develop exaggerated vascular leak after lung injury (98, 99). Genetic deletion of both sphingosine kinases (Sphk1 and Sphk2) results in a complete lack of circulating S1P and increased vascular permeability in multiple organ systems, even in the absence of tissue injury (100). Interrupting S1P-S1P₁ signaling with S1P₁ receptor antagonists similarly increases vascular leak (101). Conversely, short-term treatment with S1P or S1P₁ receptor agonists has been shown to prevent vascular leak in numerous animal models of acute lung injury as well as models of renal and cutaneous injury (95, 97, 101–107).

As described above, excessive or excessively persistent vascular leak is one of the aberrant wound-healing responses to lung injury believed to contribute to the development of pulmonary fibrosis. Attenuation of the early, exudative responses to lung injury by S1P-S1P₁ signaling may therefore also limit the subsequent development of fibrosis. In support of the ability of S1P-S1P₁ signaling to limit injury-induced lung fibrosis, S1P₁ inhibition has recently been shown to increase both pulmonary vascular leak and fibrosis after bleomycin-induced lung injury in mice (108). Given the evidence implicating extravascular coagulation in the development of lung fibrosis discussed above, the increase in lung fibrosis caused by S1P₁ inhibition was likely attributable to the increases in pulmonary vascular leak and intraalveolar coagulation observed in these mice (108). S1P₁ inhibition also altered leukocyte accumulation in the airspaces after bleomycin challenge, reducing T-cell but increasing neutrophil accumulation (108), which may have contributed to the exaggerated fibrotic response. Whether or not inflammatory leukocytes play a central role in the development of lung fibrosis after injury is unclear, however, as the fibrotic and inflammatory responses to bleomycin have been dissociated from each other in multiple studies (10, 14, 109–114).

S1P Signaling and Other Profibrotic Processes

In vitro studies have shown that S1P is capable of mediating other processes that may contribute to the development of lung fibrosis, although some of these studies have produced conflicting data. For example, S1P has been shown to potentiate fibroblast chemotaxis in some investigations but inhibit fibroblast chemotaxis in others (24, 115–117). S1P has also been shown to have cell-specific effects on apoptosis, either inducing apoptosis or promoting survival in different cell types (26, 118, 119). Similar to LPA, S1P could therefore potentially contribute to the divergent apoptotic behaviors of lung epithelial cells and fibroblasts observed in lung fibrosis. Last, also like LPA, S1P has been shown to play a role in TGF- β -induced differentiation of fibroblasts into myofibroblasts *in vitro*, another critical process in fibrogenesis (25, 120, 121).

CONCLUSIONS

LPA and S1P influence a wide array of basic cellular functions, including survival, proliferation, migration, and contraction. Many of the cellular processes regulated by LPA and S1P are fundamentally involved in wound-healing responses to lung injury. Recent studies indicate that LPA and S1P may be centrally involved when these wound-healing responses become aberrant and result in the development of lung fibrosis. LPA has been

shown to have mostly profibrotic effects in the lung, by promoting such processes as epithelial cell apoptosis, increased vascular permeability, fibroblast migration and resistance to apoptosis, and activation of latent TGF- β . S1P-S1P₁ signaling appears to protect against the development of lung fibrosis, likely through its ability to limit vascular leak during the early, exudative response to lung injury. Conversely, S1P acting on cells *in vitro* has also been shown to mediate processes that could promote lung fibrosis, but the contribution of S1P signaling to these processes in the lung *in vivo* has not yet been fully investigated.

Given the similarities between these two lipid mediators in terms of their chemical structures and metabolism, receptors and signaling pathways, and effects on cellular behavior, LPA and S1P likely exert overlapping effects on the myriad biological processes involved in fibrogenesis *in vivo*. In the case of vascular leak, they oppose each other, with LPA-LPA₁ signaling disrupting endothelial barrier function and S1P-S1P₁ signaling restoring barrier function. For processes such as vascular leak, it may be the balance of LPA-LPA₁ and S1P-S1P₁ signaling that is critical for regulating fibrotic responses to lung injury. Conversely, because both LPA and S1P have been shown to mediate cellular apoptosis and fibroblast migration, they may exert additive or even synergistic effects on these or other fibrosis-modulating pathways. Further study is needed to clarify the mechanisms and specific receptors involved in the pro- and anti-fibrotic effects of LPA and S1P and the potential interactions between these two lipids and their respective signaling pathways. What we believe has already become clear, however, is that targeting these lysophospholipids and their receptors, either to inhibit or enhance their signaling, holds great promise for the development of novel therapies for fibrotic lung diseases.

Author disclosures are available with the text of this article at www.atsjournals.org.

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