Lysophospholipid receptors in vertebrate development, physiology, and pathology

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Abstract Lysophospholipid (LP) research has experienced a period of renaissance with the discovery of the lysophosphatidic acid (LPA) and sphingosine 1-phosphate (S1P) receptors in the late 1990s. Vertebrate LP receptors regulate embryogenesis, vascular development, neurogenesis, uterine development, oocyte survival, immune cell trafficking and inflammatory reactions. LP signaling is important in cancer, autoimmunity and inflammatory diseases. Research on LP biology has contributed to the development of a first-generation S1P receptor modulator that has entered phase III clinical trials for the treatment of multiple sclerosis. Further basic research on LP signaling is anticipated to lead to novel therapeutic tools to combat various human diseases.—Skoura, A., and T. Hla. **Lysophospholipid receptors in vertebrate development, physiology, and pathology. *J. Lipid Res.* 2009.** 50: **S293–S298.**

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Historically, the term lysophospholipid (LP) referred to trace phospholipids with detergent-like properties. Lysophosphatidic acid (LPA), the earliest known LP, was shown to regulate blood pressure, platelet aggregation, and cell proliferation (1). Sphingosine 1-phosphate (S1P) was identified as a lipid metabolite that induced intracellular calcium rises and NIH3T3 cell proliferation (2). However, the notion that LPs are bona fide lipid mediators was met with considerable skepticism. A major advance in the LP field occurred with the demonstration that the effects of LPA required the action of heterotrimeric G proteins (3).

We now know that LPs act via membrane-bound G protein-coupled receptors. Hecht et al. (4) described the first LPA receptor, LPA_1, that bound to the ligand, induced cell rounding and G_i-dependent cAMP suppression. Independent studies from our laboratory demonstrated that the G protein-coupled receptor EDG-1 is a G_i-coupled high affinity S1P receptor (5). Currently, five LPA receptors and five S1P receptors have been described (6). Among the LPA receptors, two recent additions, LPA_4 and LPA_5, are divergent in primary sequence from LPA_{1-3}. In general, LPA and S1P receptors are widely expressed and many cells express more than one subtype of LP receptors.

S1P is produced from the metabolism of sphingomyelin, which is synthesized at the cytosolic face of the endoplasmic reticulum. Ceramide, formed by the hydrolysis of sphingomyelin, is further metabolized by ceramidase to produce sphingosine, which in turn is phosphorylated by sphingosine kinases (SphK)s to generate S1P. Analysis of knockout mice for *Sphk1* and *Sphk2* suggests that S1P is formed exclusively from this pathway in vivo (7). However, in vitro studies suggest a theoretical possibility that S1P may be produced by the autotaxin-mediated degradation of sphingosylphosphoryl choline (8). Once formed, S1P can be dephosphorylated to sphingosine by specific phosphatases or broad-spectrum lipid phosphate phosphatases (LPP)s or be irreversibly degraded into hexadecanal and phosphoethanolamine by S1P lyase (9). Products of the SPL are used in membrane phospholipid synthesis (for example, phosphotidyl ethanolamine) (10). S1P is found abundantly in vertebrate blood and lymph. SphK1 may be a major enzyme involved in the production of extracellular S1P. Mechanisms involved in the secretion of S1P are poorly understood (11).

LPA production involves the activity of multiple highly regulated enzymes such as phospholipases (PLA_1 and PLA_2) through deacylation of phosphatidic acid, lysophospholipase D (lysoPLD, autotoxin), which converts lysophosphatidylcholine into LPA, and monoacylglycerol kinase. However, extracellular LPA is thought to be produced primarily by the autotaxin pathway. On the other hand, LPA degradation is mediated by LPPs through hydrolysis of LPA to monoacylglycerol and through acylation by acyltransferases (12).

LP receptor genomes are found only in vertebrates, suggesting that extracellular signaling of LPs coevolved with vertebrate phyla (11). Indeed, recent development of

Abbreviations: LP, lysophospholipid; LPA, lysophosphatidic acid; LPP, lipid phosphate phosphatase; S1P, sphingosine 1-phosphate; SphK, sphingosine kinase.

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genetic and pharmacologic tools has led to a plethora of previously unrecognized aspects of receptor-driven LP function in development and pathology of the cardiovascular, nervous, immune, and reproductive systems of vertebrates. This review intends to highlight these recent findings on the biological role of lysophospholipid mediators and evaluate potential novel therapeutic applications for the treatment of human diseases.

CARDIOVASCULAR SYSTEM

S1P receptors regulate important physiological functions of the vascular system, such as vascular morphogenesis and maturation, cardiac function, vascular permeability, and tumor angiogenesis (13). Indeed, the endothelium is highly responsive to S1P stimulation in vitro, resulting in the induction of endothelial cell proliferation, migration, survival, and vascular morphogenesis into capillary-like networks (14). It is well established that S1P is able to promote endothelial cell barrier integrity through $S1P_1$ receptor function. Indeed, intravenously delivered S1P attenuated vascular barrier dysfunction in murine and canine models of acute lung injury (13). Furthermore, FTY720, a high affinity agonist for S1P receptors, induced adherens junction assembly in endothelial cell monolayer, whereas oral FTY720 administration in mice potently blocked VEGF-induced dermal vascular permeability in vivo (15). Moreover, the $S1P_1$ receptor is essential for normal lung physiology because systemic antagonism of $S1P_1$ receptor under basal physiological conditions enhanced pulmonary leakage (16).

Evidence for the functional role of $S1P_1$ receptor in vasculature is derived from in vivo animal models where genetic deletion of $S1P_1$ receptor in mice blocks vascular maturation, the phenomenon whereby mural cells (SMCs and pericytes) cover and stabilize newly formed endothelial tubes. Specifically, null embryos die due to hemorrhage at E12.5-14.5 days of gestation. Mechanistically, $S1P_1$ receptor in the endothelial compartment promotes the formation of N-cadherin-based junctions between endothelial and vascular smooth muscle cells which is needed for vessel stability (17, 18). Postnatally, $S1P_1$ receptor is highly expressed in angiogenic tumor vessels in vivo and siRNAs targeted specifically to mouse $S1P_1$ receptor potently suppressed tumor growth by inhibiting vascular stabilization (19). In addition, FTY720 treatment of mice led to strong inhibition of angiogenesis in the in vivo Matrigel plug assay, reduced tumor size, and significantly inhibited metastatic spread of melanoma (20). Furthermore, it has been reported that monoclonal antibody to S1P (Sphingomab™, which is being developed as a human therapeutic) blocked endothelial cell migration, capillary morphogenesis, and reduced tumor growth in murine xenograft models (21). However, the mechanism of how this antibody interacts with S1P is unclear. In addition, it will be important to validate these results with independent studies without commercial conflicts.

Interestingly, *S1p1/S1p2* double null embryos showed a more severe phenotype than *S1p1* single null embryos, suggesting that $S1P_2$ receptor is also significant during embryonic vascular development (22). In addition, *S1p2* null mice are profoundly deaf due to vascular abnormalities in the stria vascularis of the inner ear and degeneration of sensory hair cells in the organ of Corti (23). Moreover, mutations in the zebrafish gene *miles-apart* (*Mil*), an *S1p2* ortholog, result in cardiac developmental defects (cardia bifida) due to defective migration of cardiomyocyte precursors, revealing an important function of $S1P_2$ receptor in zebrafish heart organogenesis (24). In endothelial cells, $S1P_2$ receptor activation results in disruption of adherens junctions and increased paracellular permeability, whereas JTE013 ($S1P_2$ receptor antagonist) significantly inhibited H_2O_2-induced permeability in the rat lung perfused model (25). Hypoxic mouse retinas that lack $S1P_2$ receptor present significantly decreased inflammatory cell infiltration and substantially enhanced revascularization of the retina tissue, indicating that $S1P_2$ receptor activates inflammatory pathways that facilitate vascular permeability and pathological angiogenesis (26). $S1P_3$ receptor has been reported to play a protective role against vascular endothelial injury. Specifically, the vasodilatory effect of HDL in $S1P_3$ receptor-deficient thoracic aortic rings was significantly reduced, likely by compromising Akt/ endothelial nitric oxide synthase signaling pathway and downstream nitric oxide release (27). In addition, FTY720 (which is phosphorylated into a $S1P_1$ and $S1P_3$ receptor agonist), a potent immunosuppressive reagent and eNOS activator, was able to significantly reduce the atherosclerotic lesion formation in apolipoprotein E deficient mice (28). Besides its atheroprotective effect, $S1P_3$ receptor also confers cardioprotection in a mouse model for ischemia-reperfusion, caused by coronary artery occlusion followed by reperfusion. In this model, $S1P_{2,3}$ receptor double null mice display significantly increased infarct size and compromised survival of endothelial cells and cardiomyocytes (29). These studies suggest that cooperative and/or antagonistic signaling between S1P receptor subtypes influence pathological angiogenesis, permeability, wound healing, and other clinical syndromes associated with cancer, sepsis, stroke, and heart disease.

LPA also exerts potent effects on the endothelial physiology, including promotion of cell migration and invasion that are essential events during vascular morphogenesis and angiogenesis, whereas its role in regulating endothelial monolayer integrity may be vascular-bed specific. In addition, LPA facilitates vascular network establishment of mouse allantois explants (30). In sharp contrast to S1P receptor deficient mice, individual LPA receptors (LPA_{1-3}) appear to be dispensable for mouse embryonic vascular cardiovascular development (31). However, autotoxin deficient mice die at early embryonic development due to impaired blood vessel formation in both yolk sac and embryo, suggesting functional redundancy among the LPA receptors expressed in the mouse vascular system (32).

Several reports suggest a crucial role for LPA in the development and progression of atherosclerotic disease. LPA content is substantially increased in the atherosclerotic plaque since oxidized low-density lipoproteins promote the production of LPA, suggesting that LP could be used as a potential biomarker for atheromatic vascular diseases

(33). Indeed, LPA promotes monocyte and neutrophil adhesion to activated endothelial cells, enhancing pro-inflammatory events that lead to atheroma development (34). In addition, LPA that originates from homogenates of lipid-rich atheromatic lesions promotes activation of different cell types involved in the initiation and progression of the disease such as platelets, endothelial cells, vascular smooth muscle cells and macrophages (35). LPA-mediated platelet aggregation could lead to arterial thrombus formation, a major cause of adverse cardiovascular pathologies such as coronary artery disease, cerebrovascular, and other peripheral arterial diseases. Mouse carotid artery ligation experiments in LPA_1 and LPA_2 receptor deficient mice suggest that double-null animals were partially protected from neontima hyperplasia, whereas LPA_1 null mice developed increased vascular injury (36). In addition, recent evidence suggest a role for LPA receptors in LPA-mediated myocardial hypetrophic growth (37). However, the specific mechanistic pathways underlying the LPA-induced vascular phenotypes are still poorly understood and further studies are necessary in order to demonstrate the role of the LPA receptors in these phenomena.

NERVOUS SYSTEM

The role of S1P in the nervous system is not well characterized. Several reports suggest that $S1P_1$ receptor regulates astrocyte motility, neurite extension and oligodendrocyte growth / survival. In contrast, $S1P_2$ receptor inhibits neurite extension and glioblastoma motility, whereas $S1P_3$ receptor appears to negatively control neurite extension. $S1P_5$ receptor inhibits oligodendrocyte progenitor migration, whereas it induces survival in oligodendroglial cells (38).

In vivo studies showed that embryos lacking sphingosine kinase enzymes show exencephaly, a cranial neural tube defect due to impaired neural tube closure (7). More importantly, the significance of S1P in nervous system physiology has been revealed by behavioral studies of $S1P_2$ receptor deficient mice. Indeed, mice that lack the receptor exhibit spontaneous, sporadic, and occasionally lethal seizures, which could be explained by defects in neuronal excitability (23). The observations suggest that S1P might play a critical role during the development of the mouse central nervous system.

Multiple sclerosis is an autoimmune condition in which the immune system attacks the central nervous system, leading to oligodendrocyte demyelination. The sphingosine analog, FTY720 (Fingolimod™, which is currently in clinical trials), exhibits a therapeutic effect in human patients with relapsing-remitting multiple sclerosis (39). The action of FTY720-phosphate has been attributed to the dramatic inhibitory effect on lymphocyte egress from secondary lymphoid organs, thus the antiinflammatory activity of FTY720 could allow oligodendrocyte remyelination to take place (40). However, the drug has the potential to cross the blood brain barrier and have further implications in the nervous system physiology distinct from its immunosuppressive action.

LPA induces neurite and oligodendrocyte precursor cell retraction, whereas LPA_1 and LPA_2 receptors are the major LPA receptors expressed in the central nervous system. In addition, LPA regulates apoptosis and mitosis of the neural progenitor cell population. Experiments with ex vivo cortical cultures suggest its involvement in cortical development (41). Although studies on individual LPA receptor null mice suggest that the receptors are dispensable for neural development, growing evidence indicate that LPA and its receptors are involved in chronic neuropathic pain (42). Indeed, LPA injection into the animals leads to LPA_1 and small RhoGTPase activity-dependent thermal hyperalgesia and mechanical allodynia, suggesting that LPA signaling is implicated in nerve injury and neuropathic pain development. Moreover, mice deficient for LPA_1 receptor show significant changes in chemical metabolites such as taurine and aspartate as well as abnormalities of sensorimotor gating, the transmission of sensory information to a motor system (43). Importantly, such defects are noted in patients suffering from illnesses such as schizophrenia and Alzheimer's disease.

REPRODUCTIVE SYSTEM

Recent in vivo studies have demonstrated the essential role of lysophospholipids in the physiology of the mammalian reproductive system. Indeed, S1P treatment is able to protect the female germ cells from apoptosis after irradiation or chemotherapy. In addition, S1P-treated irradiated female mice produced normal oocytes, suggesting that S1P can promote ovarian function and fertility in vivo (44). Moreover, detailed examination of the $Sphk1^{-/-}Sphk2^{+/-}$ female mouse reproductive system indicates abnormalities in the process of uterine decidualization, a phenomenon involving the transformation of the endometrial stroma into decidua. This structure controls trophoblast invasion, protection of the embryo from the maternal immune system and provides nutrition and gas exchange. Indeed, membranous cytoplasmic bodies containing high levels of sphingoid bases accumulated within the decidual and endothelial cells of the $Sphk1^{-/-}Sphk2^{+/-}$ decidua, leading to uterine hemorrhage and early embryonic lethality, potentially due to nonreceptor mediated events (45).

LPA also regulates the mammalian reproductive system. Indeed, LPA is present in human follicular fluid and increased during pregnancy due to enhanced autotoxin activity (46). In vitro studies suggest that LPA signaling facilitates oocyte nuclear and cytoplasmic maturation, whereas mice that overexpress LPP1 exhibit major defects in male genitalia and spermatogenesis (46, 47). In agreement with these results, a recent report suggests that deletion of the major LPA receptors in mice leads to reduced mating activity and diminished sperm counts, suggesting an antiapoptotic role for LPA signaling in male germ cells (48). More importantly, loss of LPA_3 receptor in mice leads to uneven embryo spacing, possibly due to abnormal uterine contraction and also delayed implantation, due to abnormal uterine receptivity. Moreover, LPA_3 receptor null mice exhibit delayed

embryonic development, prolonged pregnancy and finally increased embryonic lethality. Interestingly, delivery of the prostaglandin E_2 (PGE_2) and an analog of prostaglandin I_2 (PGI_2) could rescue the delayed implantation phenotype, implicating the important role of prostaglandins in LPA_3 receptor signaling during implantation (31).

Finally, LPA signaling has been implicated in the pathogenesis of various cancers because LPA levels were reported to be increased in the plasma and ascites of patients with ovarian, endometrial, cervical and also prostate cancer (49).

IMMUNE SYSTEM

A breakthrough in the field occurred when S1P was shown to regulate immune cell trafficking. FTY720, a sphingosine analog, acted as a prodrug and activated S1P receptors to induce lymphopenia (50, 51). Indeed, the essential role of $S1P_1$ receptor in lymphocyte trafficking and migration was unraveled in vivo when mice with conditional deletion of the receptor in the T-cell and B-cell lineage and chimeric mice with the $S1P_1$ receptor specifically deleted in hematopoietic cells were generated. These mice showed that loss of $S1P_1$ receptor blocks thymocyte and lymphocyte egress from the thymus and lymphoid organs into blood due to defects in chemotactic response to S1P (52). Moreover, $S1P_1$ receptor overexpressing T-cells show increased T-cell egress from the lymph nodes and attenuated humoral immunity (53). In addition, in vivo studies show that the highly regulated activities of S1P metabolic enzymes such as sphingosine kinases and lyase determine the establishment of an S1P gradient between lymphoid organs (low S1P concentration) and circulation (high S1P concentration), which controls the mechanism of lymphocyte egress and immune surveillance (54). The expression of $S1P_1$ receptor in thymocytes is regulated by the transcription factor KLF2 (55). Importantly, the cell surface glycoprotein CD69 interacts with $S1P_1$ receptor in immune cells and downregulates its cell surface expression, suggesting that internalization and retention of the complex inside the cell accounts for the impaired lymphocyte egress (56). Although better understanding of the detailed mechanism that determines S1P-driven lymphocyte egress needs to be established, it is evident that $S1P_1$ receptor regulation by therapeutic drugs could potentially be applied for the treatment of immunological diseases such as multiple sclerosis, systemic lupus erythematosus, and arthritis.

Furthermore, S1P levels are significantly increased in the airways of asthmatic patients following allergen stimulation, whereas S1P through its receptors regulates migration and degranulation of mast cells/eosinophils, crucial events in asthma, allergic dermatitis and other allergic inflammatory diseases (57). Importantly, a recent report showed that protease-activated receptor 1 and $S1P_3$ receptor cross-talk in dendritic cells determines the progress of inflammation in a sepsis syndrome. Mechanistically, inhibition of protease-activated receptor 1–$S1P_3$ receptor signaling attenuates systemic inflammation by sequestering dendritic cells and inflammation (58).

CONCLUSIONS

Since the discovery of LP receptors over 10 years ago, the pace of progress in this field has accelerated. It is now established that S1P and LPA are important lipid mediators in many organ systems, including the cardiovascular, nervous, reproductive, and immune systems. The use of receptor specific agonists and antagonists as well as receptor null mice has revealed the specific functions regulated by receptor subtypes. However, multiple critical questions remain to be answered to achieve a thorough understanding of the logic of LP signaling and biology. For example, how is the ligand produced and secreted under specific biological contexts, how are the receptors regulated, and how is LP signaling coordinated with other growth factors, cytokines, and lipid mediators to achieve specific biological outputs are but a few of the oustanding questions. Better understanding of LP biology is warranted as a few of the first generation LP receptor modulators enter the therapeutic era.

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