Published in final edited form as:

Chem Rev. 2011 October 12; 111(10): 6299–6320. doi:10.1021/cr200273u.

Regulation of mammalian physiology, development, and disease by the sphingosine 1-phosphate and lysophosphatidic acid receptors

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1. Introduction

The moniker lysophospholipid incorporates two broad families of membrane-derived lipids: the glycerophospholipids and the sphingolipids¹. Primary representatives of these two arms are lysophosphatidic acid (LPA) and sphingosine 1-phosphate (S1P), the majority of whose activities are mediated via multiple G-protein-coupled receptors with a high degree of specificity for either LPA or S1P^{2,3}. Unlike S1P, which is a single molecular species (2S-amino-1-(dihydrogen phosphate)-4E-octadecene-1,3R-diol), LPA (1-O-acyl-2-hydroxy-sn-glycero-3-phosphate) is actually a diverse group of molecules consisting of either a saturated (e.g. 16:0; 18:0) or unsaturated (e.g. 16:1; 18:1; 18:2; 20:4) fatty acid chain esterified at the sn-1 or sn-2 position of a glycerol backbone^{4,5}. This can also be modified to alkyl or alkenyl at the sn-1 position⁶. Other bioactive members of the LP family include the LPA analogues cyclic phosphatidic acid (cPA), sphingosylphosphatidylcholine (SPC), lysophosphatidylcholine (LPC), lysophosphatidylserine (LPS), and lysophosphatidylinositol (LPI)^{1,5,7}. Numerous emerging reports indicate roles in mammalian biology for other, less well-characterized LP members, as well⁸. In the past decade, our understanding of LP biology has expanded exponentially, fueled by the identification of LP receptors, generation and analysis of LPA and S1P-receptor knockout mice, and small molecule compounds functioning as receptor-specific agonists or antagonists^{3,9,10}.

Specific receptors for LPA and S1P were cloned and characterized beginning only about 15 years ago. The description of the first LPAR was reported in 1996, and the orphan receptor EDG-1, which was cloned as an immediate-early gene from endothelial cells, was identified as the first S1P receptor in 1998¹¹⁻¹³. Since then, four more S1P receptors and at least five more LPA receptors have been identified³. The two families have been best characterized in specific organ systems; namely, LPARs in the nervous system and cancer, and S1PR in vascular biology and immunology. That is not to say that their contributions in other fields are minor, only that they are incompletely understood.

2. Synthesis and degradation of LPs

The synthesis of LPA and S1P occurs both intracellularly and extracellularly^{14-16,17}. S1P and LPA can be found in high nanomolar to low micromolar concentrations in the blood and lymph and low concentrations in normal tissue¹⁸⁻²⁰. This LP gradient is utilized by some

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cell types, especially hematopoietic/immune cells, as a migratory cue and will be described later.

A significant fraction of the plasma and lymph concentrations of both S1P and LPA have been attributed to production by erythrocytes and platelets, respectively²¹⁻²⁵. Vascular and lymphatic endothelial cells also contribute to the production of plasma and lymph S1P, respectively, and neural cells and adipocytes significant quantities of LPA²⁶⁻²⁹. Both LPA and S1P can also be found in ovarian cancer ascites at 5- to 10-fold the typical plasma concentration, although the specific cell types responsible for this production are unknown^{30,31}. Synthesis of LPs by neutrophils, mast cells, mononuclear cells, intestinal, lung, and uterine epithelium, testis, ovaries, and kidneys has also been reported³²⁻³⁹. It is likely the production by these, and other as yet unidentified cell types, is responsible for managing the recruitment of specific cells to sites in the microenvironment within certain tissue structures, or to stimulate growth in isolated areas, such as the LPA-mediated neurogenesis of post-mitotic cortical neurons⁴⁰. Tissue-specific knockouts of the synthetic enzymes and gene silencing studies should help to expand this list or to confirm its exclusivity.

2.1 Synthetic enzymes- autotaxin, and sphingosine kinases

Lysophospholipase D, also referred to as autotaxin (ATX), is a secreted enzyme. Phospholipase A1 and A2 are responsible for the de novo synthesis of the precursor LPC⁴¹. Deletion of the ATX gene is embryonic lethal in the mouse at e 8.5-9.5^{18,42}. The production of S1P from sphingosine is mediated by two kinases, Sphk1 and 2 (Figure 2)^{43,44}. Mice carrying deletions of only Sphk1 or 2 do not have gross abnormalities, but global deletion of both isoforms results in embryonic lethality at e10.5, similar to ATX knockouts, implying that in single knockouts, compensatory mechanisms exist⁴⁵. Additionally, no S1P was detected in *Sphk1/Sphk2* double-knockout embryos, suggesting that there are no alternative pathways for S1P synthesis, at least at the embryonic development stage. Studies have utilized “triple allele” knockouts of Sphk1 and 2, where both copies of one kinase and one copy of the other kinase have been deleted, allowing for some analyses to be performed in a partial loss-of-function scenario, as well as studies using knockouts of either isoform⁴⁶⁻⁵⁰.

2.2 Degradative mechanisms

Degradation of either LPA or S1P can occur in either a reversible or irreversible fashion. Dephosphorylation is mediated by a number of lipid phosphate phosphohydrolases (LPPs), all of which are integral membrane proteins, but one of which, LPP1, localizes with its active site facing the extracellular space^{51,52}. This indicates the possibility that it functions to dephosphorylate extracellular LPA or S1P close to membrane receptors, and thereby regulate signaling⁵². For S1P, this results in conversion to sphingosine, which can then be converted back to S1P. LPA, however, is irreversibly dephosphorylated to monoacylglycerol. What could be termed “reversible degradation” of LPA occurs via lysophosphatidic acid acyltransferase beta (AGPAT2), which converts LPA to PA, which can then reenter the lipid cycle to be converted to the LPA precursor LPC⁵³. Irreversible degradation of S1P can occur intracellularly by the lysophospholipase S1P lyase (SPL), an enzyme found in every mammalian tissue examined thus far, with the exception of platelets and erythrocytes⁵⁴⁻⁵⁶. In many immune cells, stimulation with endotoxin can modulate the expression of multiple lysophospholipid metabolizing enzymes, including SPP, Sphk1, Sphk2, SPL⁵⁷.

3. Receptor-ligand affinities and extracellular transport

Although collectively known as lysophospholipids, the S1PR and LPAR ligand recognition is highly specific, with dissociation constants (K_d) in the nanomolar range^{58,59}. Activation can occur across family lines; for example, LPA can activate S1P₁, but with a K_d one to two logs greater than that of S1P and thus unlikely to be physiologically relevant^{60,61}. This low level promiscuity likely reflects the shared ancestry of these divergent receptors⁶²⁻⁶⁴.

If synthesized intracellularly, how is S1P transported to the extracellular environment to exert autocrine and paracrine effects? In vitro studies using rat and human mast cell lines postulated that the ATP-binding cassette transporter protein ABCC1 was responsible for the transport of S1P from the site of synthesis in the cytoplasm or endoplasmic reticulum, through the membrane, and into the extracellular space where it could exert its influence via the GPCRs⁶⁵. Further in vitro studies also pointed to ABCA7 as a transporter in platelets and erythrocytes^{66,67}. However, although studies using pharmacological antagonists of either ABCA1 (glyburide) or ABCC1 (MK571) demonstrated significant suppression of endogenous S1P release, in vivo studies utilizing ABCA1-, ABCA7-, or ABCC1-null mice failed to demonstrate decreases in either plasma or lymph S1P concentrations⁶⁸.

It is important to note that such conclusions are made largely from the use of chemical inhibitors which are not specific at the doses employed^{69,70}. Importantly, the Spns2 (Spns2) protein was identified in zebrafish as an S1P transporter necessary for S1P-mediated migration of myocardial precursors⁷¹. Recently, it was shown that human Spns2 transfected into CHO cells could transport S1P and other phosphosphingolipids (e.g. DH-S1P, phyto-S1P), as well as FTY720-P, making it the first transporter shown to have such activity⁷². In the same study, Hisano, et al. demonstrated the inability of ABCA1, ABCB1, ABCC1, or ABCG2 to effectively transport S1P or FTY-P from cells over-expressing each transporter. Several possibilities exist for these conflicting data. In vivo, compensatory mechanisms, by these or other, as of yet unknown, transporters might exist that are not otherwise evident in an in vitro, single-cell system. Similarly, results for different transporters could be cell-type specific^{73,74}. Additionally, data obtained using MK-571 as an inhibitor of ABCC1 are unreliable, as its ability to antagonize cysteinyl leukotriene receptors and the downstream signaling pathways (e.g., calcium flux, cellular proliferation) is evident at concentrations one to two logs below the typical concentration used to block ABCC1 transport activity, a fact particularly relevant in mast cell studies^{75,76}. Thus far, Spns2 is the only bona fide S1P transporter, as demonstrated by both in vitro and in vivo studies⁷⁷.

4. Chaperones and carriers

Much effort has focused on determining how these lipids can arrive at the appropriate cell at the appropriate time to initiate specific signaling sequences. Lipoproteins such as high-density lipoprotein (HDL) or low-density lipoprotein (LDL) are known to be critically important for modulating vascular homeostasis⁷⁸. Whereas oxidized LDL stimulates platelet and endothelium activation and can have cytotoxic effects, HDL exerts cytoprotective and anti-atherogenic effects⁷⁸⁻⁸⁰. Several studies using aortae from pigs, rabbits, or endothelial cell culture demonstrated that the critical component mediating this cytotoxic effect was LPC in oxLDL (the precursor of LPA) activating a GPCR⁸¹⁻⁸³. Conversely, the vasoprotective qualities of HDL were not due solely to its ability to induce cholesterol efflux from cells, but also the presence of high concentrations of S1P found bound in HDL⁸⁴⁻⁸⁶. In fact, HDL-bound S1P accounts for approximately 65% of plasma S1P, but it was unknown how S1P was bound to this carrier, since HDL is composed of several apolipoproteins, as well as phospho- and other lipids⁸⁷.

4.1 S1P and ApoM

A recent study has shown that the apolipoprotein (apo) M is the HDL constituent responsible for the transport of S1P⁸⁸. HDL from mice lacking apoM contained no S1P, whereas over-expressing apoM resulted in higher concentrations of bound S1P that was not a result of increased HDL-cholesterol or other HDL components, as these were not significantly affected. In vitro internalization studies using cells transfected with GFP-tagged S1P₁ illustrated the ability of apoM-bound S1P to activate downstream signaling cascades, whereas functional assays of endothelial cell migration and adherens junction formation determined that S1P in complex with either apoM alone or HDL was necessary for full cellular responses. Finally, an in vivo assay of vascular barrier function confirmed this, finding that mice lacking apoM demonstrated increased vascular leakage as compared to wild-type controls. This result mirrors those in plasma S1P-deficient mice and supports the idea that apoM-, not albumin-bound S1P is the necessary functional pairing for vascular barrier maintenance in vivo²¹. As to how apoM mediates delivery of S1P to S1P₁, and possibly to other receptors is not known, the most likely scenario involves membrane docking of the HDL particle, either directly or via binding of scavenger receptor class B type 1 (SR-B1)⁸⁹. Additionally, how and where S1P is loaded onto apoM is unknown, since many cells possess the capacity for S1P synthesis. Future studies are needed to explore the transfer mechanism and its specificity with regard to different S1P receptors.

4.2 LPA and autotaxin

Autotaxin's property as a transporter is ancillary to its enzymatic activity in the biosynthesis of LPA. In vitro binding assays showed ATX binding to lymphocytes or activated platelets in an integrin-dependent fashion, and in both instances, ATX was found in association with these cells in vivo, in high endothelial venules or thrombi, respectively^{14,90}. Both of these cell types respond to LPA and express multiple LPAR, making it likely that ATX is able to bind numerous cell types via integrin associations⁹¹. Nishimasu, et al. state that the crystal structure of ATX suggests the intimate association of the ATX hydrophobic channel with the plasma membrane, subsequently allowing for the directed capture of LPA by its receptors on the cell surface⁹². It is not known if the ATX-LPA-LPA receptor association allows for cell- or receptor subtype-specific delivery of agonist, and if so, what its involvement in homeostasis or disease pathogenesis may be, and how might it be manipulated.

5. Lymphatic and cardiovascular systems

Effects on the vasculature often “read-out” as effects in other systems. Changes in vessels, large and small, can have far-reaching effects throughout an organism. Thus, to a great extent, the S1PR and LPAR can collectively modulate a multitude of responses just by altering vasculature. In these systems, the most characterized is the role of S1P₁ in barrier function and vessel maturation.

5.1 S1P receptors

S1P₁, originally named Edg1, was cloned from phorbol 12-myristate 12-acetate (PMA)-stimulated HUVEC in the search for immediate early genes regulating endothelial cell (EC) differentiation, thus the name “endothelial differentiation gene”¹². The importance of endothelial cell-expressed S1P₁ is now well-appreciated; *S1p1*^{-/-} embryos die in utero at e12.5- E14.5 as a result of edema and hemorrhage, despite development of a vascular network⁹³. Close examination of *S1p1*^{-/-} embryos revealed defective coverage of vessels by pericytes and vascular smooth muscle cell (VSMC) in several large vessels, including the dorsal aorta and the cerebral artery^{93,94}. Further studies with EC- or VSMC-specific deletion of S1P1 in vivo revealed that EC-intrinsic S1P1 is required for vessel coverage by

VSMC⁹⁵. It is now known that S1P1 regulates both vessel maturation by regulating recruitment of mural cells needed for blood vessel stabilization, and the integrity of vessel barrier function by regulating adherens junctions (AJ).

5.1.1 S1PR in vascular barrier function and contractility—The signaling mechanisms involved in both cell migration and cell-cell junction formation are complex, involving the coordinated signaling of many synergistic and sometimes opposing pathways. Both junctional assembly and migratory responses are positively regulated by S1P₁, and to some extent, S1P₃, whereas destabilization and migration inhibition are mediated by S1P₂ and possibly S1P₃ signaling⁹⁶⁻⁹⁸. Engagement of S1P₁ activates solely G_{ai}, but the downstream signaling outcome can be cell-specific⁹⁹⁻¹⁰². Thus, S1P₁ ligation can lead to increased intracellular Ca²⁺ mobilization and PI3K activation leading to Akt and/or ERK 1/2 phosphorylation, or Rac activation, subsequently inducing actin rearrangement, lamellapodia formation, and redistribution of junctional proteins, such as Zonula Occludens-1 (ZO-1), VE-cadherin, α - and β -catenin, and p-120 catenin to lipid rafts in the plasma membrane or the cell surface, culminating in the formation of endothelial AJ or TJ, or the migration of VSMC or EC. This can lead to decreased vascular permeability or increased vessel stabilization/angiogenesis^{96,103-106}.

The contribution of S1P₃ to vascular barrier function has been more difficult to dissect, as it couples not only to G_i, but also to G_q and G_{12/13}⁹⁹. Mice deficient in S1P₃ do not display any gross phenotypic abnormalities^{107,108}, and when *S1p1*^{-/-}*S1p3*^{-/-} embryos were generated, the bleeding phenotype was only slightly more severe than the *S1p1*^{-/-} single mutant, likely indicating a supportive role for S1P₃ in the maintenance of vascular barrier homeostasis and vascular development. Early in vitro studies demonstrated that S1P₃-mediated RhoA activation was specifically required for the formation of stress fibers, in concert with S1P₁-induced cortical actin assembly, and mouse embryonic fibroblasts (MEF) from *S1p3*^{-/-} mice had defective activation of PLC and intracellular Ca²⁺ mobilization^{96,107,108}. Recent reports in other systems support this earlier work, showing that RhoA-mediated mechanical force and activated Rac are necessary for the formation and stability of AJ^{109,110,111}.

In vivo studies utilizing a model of acute stress, LPS administration, demonstrated that S1P₃/RhoA/ROCK activation dramatically increased vascular permeability¹¹². This was mediated by suppression of activated protein C (apC), a clotting regulatory factor that protects from endotoxin-induced lethality by countering the resultant thrombin-mediated vascular leak. Mice deficient in apC were more susceptible to LPS-induced vascular leak than wild-type mice, a phenotype that could be countered by the delivery of an S1P₁ agonist or mimicked by S1P₃ deficiency¹¹³. The proposed mechanism for the barrier disruptive action is the stimulation of S1P₃ activating RhoA, and its subsequent association with ROCK1, which is known to destabilize endothelial barrier function, and has also been demonstrated in vitro with LPS stimulation or TNF α administration to increase ROCK1 in lung EC^{114,115}.

Although results of these studies are seemingly at odds with work suggesting the cooperative effort of S1P₁ and S1P₃, it is not difficult to imagine a model taking into account the necessity of a dynamic barrier with the ability to withstand constant fluctuations in mechanical forces of homeostatic stress with relatively constant concentration of S1P present in the circulation. Under these conditions, S1P₁ and S1P₃ can function coordinately to allow the maintenance of barrier integrity while ensuring the passage of macromolecules into the interstitial compartment and the sampling of the blood by tissue-resident immune cells in the extravascular compartment. Under inflammatory conditions, possibly with increased S1P concentrations and altered concentrations of other inflammatory mediators in

plasma, this balance could be manipulated. For example, splice variants of CD44, the hyaluronic acid receptor, differentially associated with and transactivated S1P₁ or S1P₃, depending upon the inflammatory environment ¹¹⁶. Consistent with this premise, whereas many studies show barrier-protective effects of S1P, high S1P concentrations can be barrier-disruptive in concert with LPS administration in vivo, an affect that can be attenuated by S1P₃ knockdown or S1P₂ knockout ¹¹⁷. Additionally, prolonged agonism of S1P₁ has recently been shown to induce pulmonary vascular leakage and exacerbate lung injury in a bleomycin-induced injury model ¹¹⁸.

S1P₂ appears to oppose many of the actions in the vasculature resulting from S1P₁ signaling, due to the ability to couple with G_{12/13}, initiating activation of the RHO/ROCK/PTEN signaling cascade ^{99,119,120}. Although its biological functions are not as well-characterized, *S1p2*^{-/-} mice have no obvious defects; however, they become deaf by one month of age due to defective vascularization of the stria vascularis, the tissue responsible for maintaining the endocochlear potential essential for audition ^{121,122}. Evidence of the cooperative functions of S1P₂ with S1P₁ or S1P₃ is found through studies using double- or triple-null mice. Whereas the S1P₂ deficiency alone has little effect, *S1p1*^{-/-}*S1p2*^{-/-} embryos demonstrated earlier hemorrhage at E10.5 and dramatically increased perinatal lethality as compared to the *S1p1*^{-/-} single gene deletion ^{94,108}. *S1p2*^{-/-}*S1p3*^{-/-} double deletion also resulted in increased perinatal lethality compared to either single null, with few pups surviving to weaning age, presumably due to defective RhoA activation, as demonstrated using MEFs in vitro ¹⁰⁸.

Although *S1p2*^{-/-} deficiency does not have the developmental effects of *S1p1*^{-/-} deletion, the counter-regulation of S1P₁ versus S1P₂ has been well-illustrated using models of endothelial or vascular smooth muscle cell migration, vascular permeability, or vasoconstriction. In vitro, stimulation of EC S1P₂/Rho/ROCK/PTEN disrupted AJ while stimulating stress fiber formation and increasing paracellular permeability ¹²⁰. In vivo, H₂O₂-induced pulmonary vascular permeability was inhibited in mice treated with the S1P₂ antagonist, JTE-013. Similarly, JTE-013 administration protected against S1P- and histamine-induced venular leakage in the cremaster muscle, in accordance with the enhancement of barrier function resulting from stimulation of S1P₁ with FTY720, or the S1P₁-specific agonist SEW2871 ¹²³. Similarly, whereas S1P₁ agonism or S1P₂ deficiency protected against LPS-induced pulmonary vascular leak, S1P₁ heterozygosity resulted in significant vascular barrier disruption, with or without exogenously administered LPS ¹¹⁷. However, another study challenged these conclusions, stating that platelet activating factor (PAF)-induced vascular permeability, which could be prevented by exposure to S1P, could only be reversed by S1P₁ antagonism, and not antagonism of S1P₂ or S1P₃ ¹²⁴.

The discrepancies between these studies may suggest specific actions of different vascular stress stimuli. PAF receptor signaling occurs primarily via G_i, which could affect S1P₁ signaling heterologously, whereas the histamine receptors may signal using several heterotrimeric G proteins ^{125,126}. H₂O₂ stimulation induces not only PAF signaling, but VEGF and eNOS production and PLD activation, which could have diverse effects on S1P receptor signaling ¹²⁷. Additionally, whereas S1P₁/G_i activation can induce Akt and Rac-regulated eNOS phosphorylation and cell motility, S1P₂/G_{12/13} or /G_q can inhibit chemotaxis by activating Rho ^{98,128}. Thus, it would be expected that these different inducers of vascular permeability might engage signaling by different S1P receptors. In vivo, these would all be working in concert to determine the level of barrier permissivity. Additionally, vascular beds from different organs can exhibit differential responsiveness to S1P and other vasculature modifiers ¹²⁹⁻¹³¹.

This is certainly true with regards to vascular contractility, which appears to vary depending upon the level of expression of S1P receptor subtypes. Activation of G_i/Akt induced eNOS-dependent vasorelaxation via agonism of S1P₃ in isolated rat aortae or S1P₁ in bovine lung EC^{104,132}. S1P₃-deficient mice demonstrated significantly blunted vasodilatory responses after intraarterial administration of HDL or isolated sphingolipids¹³². Conversely, coronary flow was decreased, and cerebral, mesenteric, or renal arteries were shown to contract ex vivo and in vivo in response to S1P signaling via S1P₁ or S1P₃, with S1P₃ possibly antagonizing eNOS induction by S1P₁ in some systems^{131,133-136}. Basilar arteries from *S1p3*^{-/-} mice were refractory to S1P-induced contractile responses, whereas arteries from *S1p2*^{-/-} mice responded with increased contractile sensitivity in response to S1P¹³¹. Interestingly, this same study demonstrated that cerebral arteries, but not arteries isolated from the periphery, contracted in a dose-dependent manner in response to phospho-FTY720¹³¹. Another study demonstrated that lungs from wild-type mice treated ex vivo with JTE-013 and lungs from S1P₂-deficient mice demonstrated decreased S1P-induced pulmonary vasoconstriction, while treatment with the S1P₃ antagonist VPC23019 had no effect¹³⁷. However, the specificity of both JTE-013 and VPC23019 has been called into question^{131,138}. These studies further highlight the differential responsiveness of vascular beds from various organ systems, as well as the complexities encountered when employing pharmacological tools.

5.1.2 S1PR regulation of angiogenesis—Cardiovascular and lymphatic angiogenesis are also regulated by S1P receptors, mediated primarily through regulation of cell motility, migration, and proliferative responses. Lymphangiogenesis may be positively regulated by S1P₁/PLC-induced Ca²⁺ increases, as S1P₁ RNA interference or S1P₁ antagonism prevented S1P₁-mediated lymphatic EC migration and proliferation in vitro¹³⁹. Many studies have documented the ability of S1P₁ signaling to induce the migration of vascular EC and SMC, both of which are required for the development of new vessels and their stabilization^{95,140-142}. For example, in vitro antagonism of S1P₁ inhibits vessel tube formation, whereas in vivo antagonism prevents angiogenesis in several model systems¹⁴²⁻¹⁴⁵. Embryos with EC-specific S1P₁ deletion display hemorrhage phenocopying that of the global *S1p1*^{-/-} embryos^{94,95}. S1P₂ may also play a role in the recruitment of cardiomyocyte progenitors: in zebrafish, the mutation of the S1P₂ orthologue *miles apart* (*mil*) results in defective primitive heart tube formation evident as cardia bifida¹⁴⁶.

5.2 LPA receptors

The role of LPARs in the vasculature is not as well-defined, despite the fact that studies of ATX-deficient mice clearly demonstrated severely impaired embryonic vasculogenesis, and LPA induced decreased vascular barrier integrity via Rho/RhoA kinase signaling in HUVEC^{18,42,147}. Signaling via chicken orthologues of LPA₁₋₃ in the chorio-allantoic membrane (CAM) assay demonstrated that LPA was angiogenic, most likely through LPA₃¹⁴⁸. Although agonism of HUVEC LPA₁ and LPA₃ induced adhesion molecule and pro-inflammatory cytokine up-regulation, LPA₁, LPA₂, or LPA₃ deficiency do not result in the dramatic lethality evident in *S1p1*^{-/-} mice, thus originally making it difficult to determine the relevance of these receptors in the vascular system¹⁴⁹⁻¹⁵². No defects in blood or lymph vessel formation were grossly apparent in any of these mutant mice, although a small percentage of *Lpa1*^{-/-} pups demonstrated frontal hematomas, a phenotype that was compounded in double deletants *Lpa1*^{-/-}*Lpa2*^{-/-}^{150,151}. Interestingly, zebrafish with LPA₁ knocked down did not have abnormal blood vessel development, but rather defective embryonic lymphatic vasculogenesis¹⁵³. More recently, it was reported that almost 20% of *Lpa4*^{-/-} embryos at E14.5 demonstrated increased perinatal lethality, with embryos displaying edema and hemorrhage at numerous anatomical sites¹⁵⁴. These phenotypes were the result of dilated blood vessels, defective lymphatic vessel development, and impaired

mural cell recruitment. Interestingly, a previous study had shown that LPA induced VSMC migration and proliferation via receptor(s) that couple to G_q , although LPA₄ had not yet been discovered and demonstrated to couple to G_q ^{155,156}. This contrasts with findings from another group using *Lpa4*^{-/-} MEFs, which demonstrated increased Akt/Rac signaling-mediated migratory potential¹⁵⁷; however, these cells were derived from mice with *Lpa4*^{-/-} on a mixed genetic background, which did not display the same perinatal lethality of *Lpa4*^{-/-} on a pure genetic background. How the genetic background affects vascular effects of LPA₄, or indeed, any effects of the LP receptors, illustrates the need for cautious investigation.

Despite the lack of dramatic vascular defects in *Lpa1-3*^{-/-} mice, studies have shown the importance of these receptors for maintenance of vascular homeostasis and modulation of inflammatory tone. Most recently, antagonism of LPA₃ alone, or both LPA₁ and LPA₃, demonstrated that these receptors could positively regulate angiogenesis by down-regulating MVEC expression of CD36, the thrombospondin receptor and a potent anti-angiogenic signal¹⁵⁸. In other models of vascular dysfunction, such as neointima formation, expression of LPA₁ and LPA₂ by SMC modulates their migration to sites of vascular injury and neointimal lesions, whereas LPA₃ in rat ASMC appears to mediate LPA-induced dedifferentiation^{159,160}. Additionally, bleomycin-induced pulmonary vascular leakage was dramatically reduced in *Lpa1*^{-/-} mice¹⁶¹.

LPA₅ and LPA₆ have only recently been described and determined to be bona fide LPA receptors, and thus far they do not appear to play any significant role in the regulation of endothelial cells¹⁶²⁻¹⁶⁴. However, LPA₅ was recently shown to regulate platelet activation, which can have dramatic effects on the cardiovascular system overall¹⁶⁵.

6. Immune system

Many of the SIP and LPA receptors have been shown to play important roles in immune cell biology, especially with regard to the modulation of cell recruitment^{30,166-169}. In fact, some of the receptors are expressed with near exclusivity on cells or in organs of the immune system. Perhaps the most comprehensively explored role of an LP receptor in regulating immunity is the ability of SIP₁ to regulate T cell migration into and out of the lymph node¹⁶⁷. However, LPAR and S1PR appear to play key roles in modulating the activity and migration of both innate and adaptive immune cells. Thus, in this review we will address the innate immune system first, as the first stage of the immune response.

6.1 Innate immunity

6.1.1 Neutrophils—Neutrophils are considered the first responders in most inflammatory responses. Because these granulocytes possess the ability to produce potent lipids, chemotactic factors, catabolic enzymes, and reactive oxygen and nitrogen species, their recruitment and activation are tightly controlled¹⁷⁰. Multiple studies have found LPAR and S1PR mRNA expression by human neutrophils, but the ability of LP to modulate neutrophils via GPCRs appears to depend upon their activation status¹⁷¹⁻¹⁷³. Incubation with LPA or SIP in isolation is relatively ineffective for inducing changes in cell morphology, chemotaxis, or activation status^{171,172}. However, the response of neutrophils exposed to inflammatory or chemotactic stimuli, such as IFN γ or IL-8, is modulated by treatment with LPA or SIP through G protein signaling^{173,174}. Additionally, neutrophil expression of LPA₁ and SIP₃ were dramatically increased in neutrophils isolated from peripheral blood of patients with pneumonia, and were more responsive to chemotactic stimuli than those from healthy subjects¹⁷³. These differences are intriguing in light of the recent study demonstrating that neutral sphingomyelinase, a modulator upstream of SIP synthesis, polarizes the distribution of Rac1/2 versus RhoA at the leading edge of elongated neutrophils¹⁷⁵. Additional evidence of the effect of S1PR on neutrophil migration was

demonstrated in studies using *Sgpl*^{-/-} mice¹⁷⁶: S1P lyase deficiency increased neutrophil numbers in circulation by impairing homeostatic chemotaxis into tissues. As this phenotype was partially normalized by S1P₄ deficiency, it is possible that S1P₄ is responsible for regulating neutrophil migration during inflammatory responses that increase S1P concentrations.

6.1.2 Eosinophils—Eosinophils (EO) and mast cells (MC), granulocytes important for anti-parasite and anti-bacterial immunity, are thought to play important roles in the development and propagation of many inflammatory conditions where S1P or LPA are implicated, such as airway and skin inflammation¹⁷⁷⁻¹⁸¹. Although treatment with FTY720 or S1P₁-specific antagonists can reduce airway inflammation, in some instances this can be attributed to reduced inflammatory status of the endothelium, and subsequently decreased chemokine production or inhibition of lymphocyte migration^{182,183}. In fact, a recent report states that administration of S1P can aggravate airway inflammation in a mouse model of allergen challenge¹⁸⁴. Thus, the many players of the inflammatory response and the models used can complicate understanding of LPAR and S1PR on EO and MC.

In IL-5 transgenic mice, the resultant eosinophilia can be reversed by treatment with S1P receptor modulators FTY720 or SEW2871¹⁸⁵. EO from these mice express S1P₄ < S1P₁ < S1P₃, and migrate toward S1P in vitro, a response that can be largely attenuated by pre-treatment of the mice with FTY720 or SEW2871. Additionally, human EO isolated from patients with allergic rhinitis also expressed S1P₁ and S1P₃₋₅, and this expression was up-regulated upon allergen challenge¹⁸⁶. S1P alone induces chemotaxis of human EO, but also induces up-regulation of other chemotaxis-related proteins, such as CCR3 and RANTES¹⁸⁷. Human EO also express LPA receptors, and it was reported that LPA induced migration, CD11b up-regulation, and induction of respiratory burst in vitro via LPA₁ and LPA₃¹⁸⁸. However, in studies using a collagen matrix, although LPA induced significant activation of RhoA, it was unable to induce EO migration through the 3D matrix¹⁸⁹.

6.1.3 Mast cells—The role of S1PR and LPAR in the regulation of mast cell biology has been extensively characterized. Both LPA and S1P have both been described as modulating mast cell activation state upon FcεRI ligation^{190,191}. Human MC express LPA₁₋₅, and their development from monocyte precursors can be accelerated by addition of LPA to growth medium, increasing granule development and tryptase content, *Kit* expression, and proliferative capacity via LPA₁ and/or LPA₃ in a G_i-dependent manner^{192,193}. However, histamine release and chemokine production by human MC in response to LPA was IL-4-dependent, and regulated by LPA₂ signaling¹⁹⁴. This was also true in rat and mouse MC, where LPA induced histamine release in a ROCK dependent-mechanism¹⁹⁵. Most recently, it was reported that LPA₅ was the most abundantly expressed LPA receptor on human MC, and production of the chemokine MIP-1β was almost entirely due to LPA₅ agonism¹⁹³.

S1PR appear to play a similar role in the activation of MC, including inducing the synthesis of the ligand via activation of sphingosine kinase, supporting an autocrine feedback loop¹⁹⁶. In vitro studies found that FcεRI ligation resulted in Sphk1 translocation and production of S1P; however, a more recent study using Sphk1 and Sphk2 knockout cells and mice suggested that Sphk2 was necessary for intracellular S1P signaling, regulating FcεRI Ca²⁺-dependent effector responses, such as LTB₄ and IL-13 release, whereas Sphk1-derived S1P from sources other than MC was necessary for S1PR activation of histamine release and the anaphylactic response^{46,197}. Exogenous S1P also induced the production of several CC chemokines, including MIP-1β, and induced cortical actin rearrangement and migration in bone marrow-derived mouse MC¹⁹⁷. In both human and rodent MC, S1P₁ was able to induce cell migration, whereas S1P₂ signaling regulates other activities, such as degranulation and chemokine release^{197,198}. Interestingly, whereas S1P₂ was necessary for

the induction of passive systemic anaphylaxis, induced by sensitization and subsequent challenge with IgE, it was subsequently found to be necessary for recovery from histamine-induced anaphylaxis, which obviates mast cell degranulation, leading to the hypothesis that S1P₂ is necessary for histamine clearance, possibly via modulation of renal vascular tone^{47,198}.

6.1.4 Macrophages—Less is known about the role of LP receptors on macrophages, but much of what we understand of the actions of S1P and LPA in these cells have been determined through the use of autoimmune and infectious disease models or the use of the agonist/functional antagonist FTY720. Mouse macrophage expression of S1PR mRNA is dependant upon their origin; bone marrow macrophages express S1P₁ and S1P₂, whereas thioglycolate-elicited macrophages express S1P₁₋₄, with S1P₁ and S1P₂ being expressed at equally high levels¹⁹⁹⁻²⁰¹. Expression by alveolar macrophages from rat and mouse differed, with S1P₂ and S1P₄ being the only S1PR mRNA expressed by rat, but mouse alveolar macrophages expressed S1P₁₋₄, with S1P₂ predominating^{202,203}. Human peripheral blood monocytes (PBM) and PBM-derived macrophages produced varying levels of mRNA for S1P₁₋₅ and LPA₁₋₅ expression which could be altered dependent upon phenotype, i.e., differentiation to foam cells could increase or decrease expression of certain receptors, and human alveolar macrophages also exhibited a different receptor pattern^{81,204,205}. Differences in receptor expression can likely be explained by cell location and isolation methods, as monocytes and macrophages are notoriously susceptible to alterations in activation status and protein expression caused by manipulation^{206,207}.

S1P₂ negatively affects macrophage migration in vitro in response to C5a complement component or CXCL12, as *S1p2*^{-/-} macrophages did not display the increased cAMP or decreased Akt phosphorylation evident in WT cells¹⁹⁹. This is evident in models of atherosclerosis, where *S1P2*^{-/-}*ApoE*^{-/-} mice demonstrate reduced plaque formation and lipid deposition possibly due to an increased ability of macrophages to exit atherosclerotic vessels and/or regulation of the inflammatory phenotype, as *S1p2*^{-/-} mice had decreased levels of serum pro-inflammatory mediators^{200,208}. Furthermore, S1P₃ deficiency had no affect atherosclerosis plaques size, but significantly decreased macrophage recruitment to lesions and impaired chemotaxis and chemokine production in vitro; however, another study utilizing the NOD mouse model found that *S1P3*^{-/-} monocytes were not defective in their ability to adhere to aortic endothelium ex vivo, and rather, S1P₁ was responsible for this interaction, as demonstrated using the S1P₁ agonist SEW2871^{209,210}.

Interestingly, apoptotic cell-derived S1P was shown to induce an anti-inflammatory “M2” phenotype in RAW264.7 macrophages via S1P₂, as demonstrated by siRNA knockdown or JTE-013 antagonist²¹¹. Primary human macrophages were also themselves protected from apoptosis, presumably via S1P₁, as pre-incubation with phospho-FTY720 blocked this protection²¹². In another study, however, S1P decreased LPS-induced pro-inflammatory cytokine production through S1P₁ agonism, thus inducing an M2 phenotype through this receptor²⁰¹. Some of these differences might be due in part to the inflammatory stimulus, and have implications for the mechanism of continued monocyte/macrophage activation in atherosclerosis and chronic inflammatory disease, as the number of necrotic versus apoptotic cells increases with the severity of atherosclerotic disease²¹³. S1P₂ also appears to modulate the macrophage response to infectious agents, as *S1p2*^{-/-} alveolar macrophages expressed reduced levels of Fc receptors, and were compromised in their ability to phagocytose the fungal pathogen *Cryptococcus neoformans*²⁰³.

Although LPA has long been known to act as a macrophage survival factor, it also modulates the activity of mouse and human macrophages²⁰². Through a G_i-dependent pathway, LPA up-regulated ROS production in mouse and human alveolar macrophages as

well as Ca^{2+} mobilization, IL-1 β and TNF α transcription and protein expression in the mouse J774A.1 macrophage cell line and human monocyte-derived macrophages^{81,202,204,214}. Cell migration was also controlled by LPA₁ and/or LPA₃, as monocytic cells appeared to migrate toward concentrations of LPA < 1 μM , whereas migration is inhibited above this concentration in vitro²¹⁵. The potential relevance of these receptors to the modulation of monocyte and macrophage recruitment during disease states is evident in models of atherosclerosis, where it was recently shown that macrophages in human atherosclerotic plaques expressed both of these receptors²¹⁶. Thus, LPA participates in a neatly organized amplification feedback loop: with LPAR expressed by both endothelial cells and monocytes/macrophages, LPA can activate both cell types, activating endothelial cells to produce myeloid recruiting chemokines, as well as LPA itself. These chemokines then coordinately recruit myeloid cells, which along with LPA, can also activate these cells to produce pro-inflammatory mediators, such as IL-1 β and TNF α , further activating the nearby endothelium, and amplifying the response^{149,216,217}.

6.1.5 Dendritic cells—Despite evidence that large amounts of both S1P and LPA are produced during some immune and inflammatory responses, the role of LPA in dendritic cells has not been widely explored. Immature dendritic cells (DC) derived from human peripheral blood monocytes express mRNA for LPA₁₋₅, and this expression is modulated upon maturation, with LPA₃ becoming significantly down-regulated²¹⁸⁻²²⁰. Accordingly, LPA differentially affects the activity of these different sets of DC: in immature DC, unsaturated LPA induces intracellular Ca^{2+} mobilization, actin polymerization, and chemotaxis^{218,220}. When added to maturation medium, those DCs matured in the presence of LPA induced a Th₂ phenotype in T cells, and augmented their proliferative capacity in vitro^{218,219}. Thus, LPA may play an important role in determining the tone of the nascent acquired immune response, but more study is needed to see how it specifically affects DC biology.

Many of the activities of S1P on DCs are reminiscent of those of LPA, with important roles in migration and phenotype. In fact, S1P₄ was cloned from mature human DC²²¹. Human and mouse immature and mature DC express mRNA and protein for S1P₁₋₅²²²⁻²²⁴. Similar to LPA, S1P induces intracellular Ca^{2+} mobilization, actin polymerization, and migration in immature DC, and appears to induce a shift from Th₁ to Th₂ phenotype in DC that are matured in the presence of LPS²²². Perhaps one of the more intriguing differences is the ability of S1P to induce the migration of DCs that are matured with TNF α and PGE₂ versus those matured with LPS, and DC that originate from the peripheral blood, bone marrow, or skin^{222,224-226}. For instance, the migration of LPS-matured DC appears to be dependent upon S1P₃ and not S1P₁, as demonstrated using cells from S1P₃ knockout mice, but TNF α /PGE₂-matured DC appeared dependent upon S1P₁; however, the mRNA of both receptors is up-regulated upon stimulation^{223,225,226}. In vivo studies using FITC-dextran recruitment or adoptive transfer of *S1p1*^{-/-} or *S1p3*^{-/-} DC illustrated the differential dependence of in vitro matured DC versus mature skin DC on both S1P_{1/3} or S1P₁ alone, respectively, in their migration to lymph nodes²²⁶.

Tissue-resident DC, such as Langerhans cells (LC) or lamina propria DCs, are phenotypically different from other DC, and as such might be expected to be differentially affected by S1P²²⁷. In fact, whereas SEW2871 prevented LC migration to draining LN, implying that LC require only S1P₁ for migration, lamina propria DC appear to require both S1P₁ and S1P₃ to exit the gut and migrate into mesenteric LN, as S1P₃^{-/-} mice have impaired DC migration from LP in response to LPS stimulation, but LC migration is unaltered^{225,226}. A separate study stated that skin DC were impaired in their ability to chemotax in response to various chemokines in the presence of the S1P₁-specific agonist SEW2871²²⁴.

Interestingly, S1P₁-mediated LN migration is negatively regulated by CD69, which performs the same role in T cells, a regulatory mechanism specific for S1P₁ ^{228,229}.

S1P mediates other aspects of DC biology besides migration, although it does not appear to be necessary for maturation ²²⁶. LC that had been exposed to FITC-dextran and migrated to LN expressed higher levels of mRNA for the S1P₁ and S1P₃ than did LC still resident in the skin ²²⁵. Although partially reflective of LC dependence on the S1PR for migration, it was also reported that S1P₃ was necessary for endocytosis, a step that would naturally occur before these cells traveled to the LN for antigen presentation to T cells: phospho-FTY720 impaired FITC-dextran endocytosis, whereas SEW2871 did not ²²³. Studies using S1P₃-deficient mice demonstrated that S1P₃-regulated IL-1 β production by DC is also a critical regulator of systemic inflammation induced by sepsis, illustrating that S1P₃ signaling likely plays a role in modulating the production of various DC-derived cytokines ^{113,222}.

6.1.6 Natural killer cells—Natural killer (NK) cells are innate immune cells important for killing transformed cells, primary defense against infectious organisms, especially viruses, and directing the acquired immune response through the production of interferons and other cytokines and chemokines ²³⁰. Not much is known about how these cells are regulated by LPAR and S1PR, although human and mice NK cells do express the mRNA for LPA₁₋₃, and S1P₁₋₅ ²³¹⁻²³³. As in many other immune cells, LPA induces intracellular Ca²⁺ mobilization, F-actin polymerization, and migration in a concentration- and G_i-dependent manner ^{231,234}. Curiously, although LPA increases NK cell IFN γ production, it decreases perforin production and cytotoxic activity through LPA₂ agonism and subsequent cAMP/PKA activation ^{231,234}.

Although NK cells appear to express all of the known S1P receptors, the receptor known to have any major role in the regulation of NK activity is S1P₅ ²³⁵. S1P₅ expression in NK is regulated by T-bet, a transcription factor critical for the decision between CD4 and CD8 cell fate ^{236,237}. NK cells migrate in response to S1P, and the expression of S1P₅ regulates bone marrow and lymph node egress of NK cells ^{232,233,237}. Other roles for S1P₅ or the other S1PR have not yet been reported.

6.2 Acquired immunity

6.2.1 Natural killer T cells—Natural killer T cells are a cellular bridge between the innate and acquired immune systems. They are thymocytes; however, they express the NK receptor and are characterized by a restricted T cell receptor (TCR) which recognizes only glycolipid antigens presented on the MHCI-like CD1d ²³⁸. Little is known about how the expression of LP receptors might affect these cells; indeed, it is unknown whether they express any of the LPAR, although pulsing antigen-presenting cells with LPC induced increased cytokine production by NKT clones and primary polyclonal human peripheral blood NKT cells ²³⁹. Although it has been demonstrated that NKT express S1P_{1, 2, & 4} mRNA, S1P₁ is thus far the only receptor whose role has been examined ²⁴⁰. Using inducible S1P₁ knockout mice, it was demonstrated that S1P₁ is not necessary for NKT precursor development and migration to the thymus, but is required for their normal distribution throughout the tissues ²⁴⁰. Although a subsequent paper reported that FTY720 treatment did not impair NKT migration but did alter cytokine responses, these responses occurred in a G_i-dependent manner, indicating that other S1PR, including S1P₅, could be involved ²⁴¹. It is curious that, despite the important role of S1P₅ in regulating NK migration, this receptor has not yet been examined in NKT cells.

6.2.2 T cells—A great deal is known about the role of S1PR in the regulation of T cell responses. The predominant S1P receptors on T cells appear to S1P₁ and S1P₄, although

mRNA for the other receptors can be found, albeit at low levels²⁴². T cell surface expression of S1P₁ is perhaps the major determinant of T lymphocyte egress from the thymus and secondary lymphoid organs, overriding the retention signals transduced by CCR7 signaling, and varies in a cyclical manner, inversely correlating with the S1P concentration gradient^{19,242-248}. Whereas single-positive (SP) CD4⁺ or CD8⁺ T cell S1P₁ expression is high in the thymus, it is low in the blood, up-regulated once again upon entry into the LN, and then decreases once again upon exit into the lymph²⁴⁵. This cyclical expression is orchestrated, in part, by CD69 expression, which is up-regulated immediately upon entry into the LN, and negatively regulates S1P₁ through direct interaction with the receptor, facilitating the retention of cells within the lymphoid organs^{229,249,250}. Forced expression of S1P₁ on double positive (DP) and immature SP cells, which under normal circumstances is directly controlled by the transcription factor Kruppel-like factor (KLF)-2, is sufficient to induce premature egress from the thymus^{251,252}. Specifically in differentiated Th₂ cells within the LN, extracellular matrix protein-1 (ECM1) drives S1P₁ re-expression after antigen education via KLF-2 induction, permitting LN egress²⁵³. Additionally, expression of S1P₁ modulates the ability of effector T cells to migrate out of inflamed tissues into afferent lymphatics, and is a factor in the decreased chemotactic capacity of T cells from old versus young human subjects²⁵⁴⁻²⁵⁶.

S1P₁ expression is also important for T cell maneuvering within the LN and tissue-specific homing. Entry into LYVE1⁺ cortical sinuses is also regulated by S1P₁ expression, as S1P₁-deficient cells display sinus probing behavior similar to that of WT cells, but their entry into sinuses was $\leq 20\%$ that of WT controls²⁵⁷. S1P₁ signals determine the migration of conventional SP T cells to become intraepithelial residents of the intestine, and T cell rolling and sticking in peripheral LN high endothelial venules (HEV) was reduced in S1P₁^{-/-} mice, but was unaffected in the Peyer's patches (PP)^{258,259}. Interestingly, Halin, et al. also indicated the potential involvement of other S1PR in the modulation of PP T cell trafficking, as treatment with FTY720 enhanced PP T cell retention in both WT and S1P₁^{-/-} mice.

S1P₁ can also modulate T cell subsets, which is especially evident in S1P₁ T cell-specific Tg mice²⁶⁰. Transduction of S1P₁ into a non-expressing T cell line lead to S1P-mediated reductions in IFN γ and IL-4 production by murine splenic CD4⁺ cells and inhibition of proliferative responses to anti-CD3/CD28 stimulation²⁶¹. In mice bearing both the OTII OVA-specific TCR Tg and the T cell-specific S1P₁ Tg, the Th₁₇ response was enhanced, with increased numbers of Th₁₇ cells producing greater concentrations of IL-17 as compared to single Tg OTII OVA mice²⁶². Similarly, WT T cell development of the Th₁₇ lineage in vitro was induced by S1P to the same extent as that induced by IL-23, and this differentiation could be recapitulated by SEW2871, or prevented by phospho-FTY720, indicating that S1P₁ may be an important modulator of the Th₁₇ inflammatory response²⁶³.

T regulatory (T_{reg}) cell development also appears to be dependent upon S1P₁ signaling. Administration of FTY720 increased the number and suppressive activity of splenic CD4⁺CD25⁺Foxp3⁺ T_{regs}, which express lower levels of S1P₁ and S1P₄ mRNA, and higher levels of S1P₃ mRNA than do CD4⁺ effector cells^{264,265}. Recent studies have demonstrated that S1P₁ blocks the thymic generation of CD4⁺CD25⁺Foxp3⁺ T_{regs} by activating the AKT-mTOR pathway, preventing the differentiation of CD4⁺CD25⁺Foxp3⁺-precursor cells²⁶⁶. Sustained stimulation of mTOR and suppression of TGF β -Smad3 signaling by S1P₁ in peripheral T cells leads to the preferential generation of Th₁ effectors over induced CD4⁺CD25⁺Foxp3⁺ T_{regs}²⁶⁷. Although both conventional SP cells and T_{regs} lose expression of S1P₁ upon TCR complex engagement, T_{regs} appear to down-regulate S1P₁ more slowly²⁶⁶. This elegant regulatory system prevents immune suppression from occurring too early during the immune response, but leads to its eventual termination.

LPAR also have a role in regulating T cell responses, although it is not as well understood as that of S1PR. Human CD4⁺ and CD8⁺ SP express predominantly LPA₁ and LPA₂, and low levels of LPA₃^{268,269}. LPA suppressed Bax-mediated apoptosis in a human T lymphoblastoma line, inhibited activation-induced CD4⁺ IL-2 production, and augmented IL-13 production via LPA₂ in human peripheral blood T cells^{180,268,270,271}. LPA₂ also stimulated CD4⁺ cell migration, but LPA₁ blocked migration of Jurkat T cells toward RANTES (CCL5)²⁷². The LPA₅ receptor is the most highly expressed LPAR in CD8⁺ cells of the gut epithelium; however, no further studies have been reported with regards to LPAR in this group of cells¹⁶². The effects of LPAR on T cell immunity are likely to be highly significant, as ATX is expressed on HEV of LN, and administration of an enzymatically inactive form of ATX impaired lymphocyte homing to secondary lymphoid organs (SLO)⁹⁰.

6.2.3 B cells—The role of S1PR and LPAR in the regulation of B cell responses has not been explored as thoroughly as in T cells. In fact, very little is known about the role of LPAR in normal B cells, as most research has been conducted using B cell cancer lines. LPA₁ is up-regulated in and protects from apoptosis chronic lymphocytic leukemia (CLL) cell lines or primary patient CLL²⁷³. LPA₁, 3, & 4 are expressed in a human B cell line, which also expressed functional ATX, and mouse primary splenic and bone marrow B cells mobilize Ca²⁺ in response to LPA, although it is unknown which receptor this occurs through^{274,275}.

More information is available regarding the role of S1PR in B cells. Expression of both S1P₁ and S1P₄ increases with maturation stage, whereas S1P₃ is most highly expressed in immature B cells^{276,277}. These changes in S1PR subtype expression may underlie the differential responsiveness of B cells at various stages of maturation. S1P₁ is involved in the migration of immature B cells into the bone marrow vascular compartment and subsequently into the peripheral blood^{21,276}. B cell-specific knockout of *S1p1* resulted in fewer immature B cells in the circulation and spleen without a concomitant increase in the number of immature cells present in the bone marrow parenchyma, although *S1p1*^{-/-} B cells were less efficient at egress from the bone marrow^{276,277}. This lack of reciprocity was evident in S1P₁-deficient mice, as well as WT mice treated with FTY720, and was explained by increased apoptosis of immature B cells that could contact the bone marrow vasculature, but not enter^{276,277}. Interestingly, *S1p1*^{-/-} immature B cells chemotax toward S1P, whereas *S1p3* deletion rendered immature B cells unresponsive to S1P in vitro, and *S1p3*^{-/-} mice had significantly fewer circulating immature B cells²⁷⁶. Additionally, S1P₃ expression by marginal zone (MZ) MAdCAM-1⁺ endothelial cells was required for their organization around B cell follicles, whereas migration and localization of the B cells themselves necessitated S1P₁ expression^{278,279}. Curiously, despite several studies demonstrating reduced migratory capacity of *S1p3*^{-/-} bone marrow and MZ B cells in chemotaxis assays, expression of this receptor has not yet been shown to have an intrinsic, in vivo effect on B cells^{276,278,279}.

Upon differentiation of mature activated B cells to IgG plasma cells, or antibody-secreting cells (ASC), differential S1P₁ expression is regulated by KLF2²⁸⁰. S1P₁ then determines whether a specific ASC will be retained in the SLO, or migrate into other organs, such as the BM or possibly the mucosa, an effect seen with both *S1p1*^{-/-} mixed bone marrow chimeras and FTY720 treatment²⁸⁰. With regard to the intestinal mucosa, studies using FTY720 treatment demonstrate that S1P regulates the trafficking of peritoneal B cells and Peyer's Patch plasmablasts (immature plasma cells) into the intestinal lamina propria, thus regulating IgA production^{281,282}. Although no receptor-specific agonists, antagonists or genetically-modified mice were used in the studies examining ASC migration in the gut

mucosa, the above-referenced study by Kabashima, et al. imply that S1P₁ will likely play a role in the retention of these cells.

7. Stem cells

Hematopoietic stem and progenitor cells (HPC) have been shown to constitutively migrate through the blood, BM, peripheral tissue, and lymph²⁸³. During an inflammatory response to zymosan or mobilization induced by G-CSF, S1P levels in peripheral blood increased, due in part to activation of the complement cascade, mobilizing HPC from the bone marrow²⁸⁴. Other reports demonstrated that S1P-induced homing of CD34⁺ HPC was partially regulated by S1P₃-mediated modulation of the CXCR4-JAK/STAT pathway, leading to increased SDF-1 responsiveness^{285,286}. Mononuclear cells from S1P₃-deficient mice were unable to home to sites of ischemic injury, and FTY720 pre-treatment increased homing of transferred CD34⁺ HPC injected via the tail vein or peritoneum to the bone marrow and sites of ischemic injury^{285,286}. Administration of FTY720 could improve neovascularization by patient-derived endothelial progenitors, as well as support proliferation and homing of HPC by drawing them out of the out of the lymph and blood into extramedullary tissues^{283,285,286}.

S1PR are also specifically involved in the migration of osteoclast precursors. SEW2871 treatment stimulated migration of osteoclast precursors from bone marrow into the blood, and mice lacking S1P₁ expression by osteoclast precursors demonstrated increased osteoporosis and osteoclast attachment to bone surfaces²⁸⁷. Additionally, FTY720 administration ameliorated bone loss in a mouse model of post-menopausal osteoporosis²⁸⁷. Conversely, JTE-013 blocked S1P₂ opposition S1P₁-mediated chemotaxis of RAW264.7 cells (used as a model of osteoclast precursors) at high S1P concentrations²⁸⁸. In vivo, JTE-013 administration largely reversed bone density loss induced by RANK ligand administration, an effect that was absent in *S1p2*^{-/-} mice²⁸⁸. Thus, osteoclast precursor mobilization in the marrow spaces appears to be reciprocally regulated by S1P₁ and S1P₂, as S1P₁ attracts osteoclast precursors to the blood and S1P₂ serves to repulse precursors from blood and maintain them in the bone marrow^{287,288}.

Early studies demonstrated that LPA₁ expression correlated with HPC ability to invade stromal cell layers in vitro²⁸⁹. Interestingly, LPAR also have a role in regulating bone morphogenesis, but do not appear to regulate migration. Rather, the LPA_{1/3} antagonist Ki16425 inhibited human mesenchymal stem cell (MSC) differentiation to osteoblasts, and as these cells did not express LPA₃ mRNA, this was attributed to LPA₁, whereas shRNA against LPA₄ allowed for osteogenesis, indicating a negative role for this receptor²⁹⁰. This was evident in *Lpa4*^{-/-} mice, which had increased bone volume and density²⁹⁰. Additionally, mice lacking LPA₁ exhibited skeletal abnormalities and decreased bone content, due to decreased osteoblast differentiation from precursors²⁹¹. Additional roles for LPAR and S1PR in SC differentiation have been proposed, based upon data from nervous system and will be discussed in that context.

8. Nervous system

Of the LP receptors, LPAR predominate in regulation of nervous system biology. LPA₁ was cloned from a neuronal cell line derived from the ventricular zone (VZ), and in the mouse embryonic neocortical VZ displayed enriched expression correlating with the stage of neuronal development, and was therefore originally named ventricular zone gene-1 (*vzg-1*)¹¹. Since that time, expression of LPA receptors by most cell types of the nervous system has been reported, with pleiotropic effects on the different neuronal cell types, including their development, migration, and signaling^{63,292}.

8.1 Neural progenitors

Neural progenitor cells (NPC) express high levels of LPA₁, LPA₂, and LPA₄ mRNA, and therefore are capable of transducing diverse signals⁶³. The embryonic telencephalon, as a whole, expresses LPA₁₋₅ mRNA²⁹³. LPA induces neurite retraction, nuclear rounding, and positioning of VZ neuroblast nuclei to the apical surface in vivo, and stimulates NPC survival in vitro through LPA₁/Rho activation²⁹⁴⁻²⁹⁷. Interestingly, the ability of LPA₁ and LPA₂ to coordinately inhibit VZ NPC cell death in the cerebral cortex are illustrated by the enhanced cortical growth and folding which results from exogenous LPA administration to cerebral explants, and the lack of folding in cortices of *Lpa1*^{-/-}*Lpa2*^{-/-} mice²⁹⁸. NPC fate decision between neuronal and oligodendroglial is also determined by LPA, with LPA₁/G_{ai} signaling leading to induction of neuronal differentiation while suppressing glial differentiation, as demonstrated using pertussis toxin or Ki16425²⁹⁹. Many of these responses are dependent in part or in whole, upon Ca²⁺ mobilization or Ca²⁺ influx, and both LPA₁ and LPA₂ have been shown to evoke Ca²⁺ responses in NPC in vitro²⁹³. Stable expression of LPA₄ allowed for LPA-mediated, Ca²⁺-dependent morphological changes, including cell rounding, neurite retraction, aggregation, and N-cadherin-mediated cell-cell adhesion via the Rho/ROCK pathway³⁰⁰. In differentiated mouse neuroblastoma cells intrinsically expressing or with stable transfection of LPA₁, neurite retraction was mediated by LPA₁ induction of PLC/Pyk2/GSK-3 β or PKA signaling pathways^{301,302}.

S1P also plays an important role in the developing nervous system and NPC recruitment, as *S1p1*^{-/-} embryos demonstrate significantly increased apoptosis and decreased mitosis in the neuroepithelial layers of the developing telencephalon⁴⁵. NPC expressed mRNA for S1P₁₋₅ and respond to S1P by induction of Ca²⁺ mobilization similar to LPA stimulation^{293,303}. NPC also responded to S1P by forming aggregates and undergoing proliferation and differentiation, which could be prevented by PTX or inhibition of ROCK in vitro³⁰³. In vivo, shRNAi silencing of S1P₁ prevented the migration of transferred NPC to sites of spinal cord injury³⁰⁴. Because of their known function in the regulation of hematopoietic stem cells, future studies are likely to uncover more functions of the S1PR in the developing nervous system.

8.2 Astrocytes

Astrocytes are cells that contribute to information processing and regulation of neural activity by networking with neurons and regulating brain metabolism³⁰⁵. These cells express LPA₁₋₅, as well as S1P₁, S1P₃₋₅^{306,307}. Although some debate exists as to the ability of LP to induce proliferation in vitro, in vivo injection of either LPA or S1P leads to astrogliosis³⁰⁷. Induction of proliferation in vitro occurs through activation of G_i-coupled pathways, and both LPA and S1P can induce Ca²⁺ mobilization³⁰⁷⁻³⁰⁹. Incubation with LPA mediated DNA synthesis and astrocyte proliferation while inhibiting their ability to uptake glutamate; the former was mediated by LPA₁, as demonstrated by LPA₁^{-/-} cells; however, the LPAR responsible for the regulation of glutamate uptake was not identified^{306,310}. Coordinated LPA₁ and LPA₂ signaling in astrocytes also induced the production of a factor that encouraged neuronal differentiation, as medium from WT astrocytes primed with LPA induced astrocyte differentiation, whereas the medium from similarly primed, *Lpa1*^{-/-}*Lpa2*^{-/-} astrocytes did not³¹¹.

Information regarding the role of S1P in these cells is not abundant; however it is known that S1P signaling via Rho/ROCK inhibits primary striatal astrocyte gap junction formation in vitro³¹². Additionally, a recent study utilizing CNS cell lineage-specific deletion of S1P₁ reported that non-immune mediated efficacy of FTY720 in models of EAE could be mediated through astrocyte-expressed S1P₁³¹³. Further studies will likely be aimed at

determining the precise mechanism by which FTY720 modulates astrocyte function and autoimmune inflammation in the CNS.

8.3 Microglia

Another glial cell type, microglia, are the tissue resident macrophages derived from primitive myeloid precursor cells, and are likely important for normal brain development³¹⁴. The level of expression of the LPAR varies between species; human microglial cell lines are able to express LPA₁₋₃^{315,316}. LPS stimulation of rat microglia increased production of LPA₃ mRNA, implicating a role for this receptor in inflammatory responses³¹⁷. LPA administration to microglia in vitro can induce Ca²⁺ signaling, proliferative responses, ATP release, ERK signaling, and membrane ruffling^{315,317-319}.

Microglia also express S1P_{1-3, 5} mRNA, and upon stimulation with LPS or agonism with S1P, S1P₁ and S1P₃ mRNA are decreased³¹⁷. S1P dose-dependently induced TNF α release from rat microglia, activated p38 signaling, and also altered expression of the mRNA for LPAR, demonstrating the interconnectedness of these two signaling pathways³¹⁷. Other studies on the role of S1PR in microglia have specifically examined the effects of FTY720. Although FTY720 induced down-regulation of phospho-ERK with a concomitant increase in phospho-MLC II in DC and macrophages, microglia p-ERK and p-MLC II were unaffected³¹⁹. FTY720 also increased the numbers of microglia in an ex vivo model of demyelination while decreasing their phagocytic capacity via S1P₁ and S1P₅, as determined by the use of SEW2871 and a Novartis S1P₅-specific agonist³²⁰.

8.4 Oligodendrocytes and Schwann cells

Oligodendrocytes and Schwann cells perform the task of myelination in different branches of the nervous system, CNS and PNS, respectively³²¹. The myelin sheath is composed approximately 70:30 of lipid, and LPA₁ expression correlates with oligodendrocyte maturation stage, and subsequently, myelination status³²². Differentiated oligodendrocytes express LPA₁, LPA₃ and very low levels of LPA₂, and LPA-initiated Ca²⁺ signaling and p-ERK are induced by MAPK/PLC signaling in a PTX-insensitive manner^{323,324}. LPA was also found to increase myelin basic protein (MPB) mRNA and the production of myelin sheets in vitro, although evidence for a direct effect on myelination by oligodendrocytes in vivo is lacking³²⁴.

The first description of S1P₅ stated that it was highly expressed in the white matter of the brain, implying expression in oligodendrocytes³²⁵. Subsequently, studies utilizing cells from *S1p5*^{-/-} mice demonstrated that S1P-mediated induction of process retraction and inhibition via Rho/ROCK of immature oligodendrocyte migration, and promotion of mature oligodendrocyte survival via G_i/AKT signaling occurred via S1P₅ signaling^{326,327}. Administration of FTY720 increased proliferation of mature oligodendrocytes and induced phosphorylation of AKT and ERK via S1P₁ signaling, whereas it promoted the maturation of oligodendrocyte precursors, which express high levels of S1P₅ and low levels of S1P₁, indicating that S1P₅ might play a role in the beneficial effects of FTY720 by promoting the oligodendrocyte differentiation³²⁸.

Although it does not affect oligodendrocyte survival, LPA is a critical Schwann cell survival factor, signaling via LPA₁/G_i/PI3K/AKT³²⁹. LPA₁/Rho/ROCK signaling also mediates Schwann cell actin cytoskeletal changes, induces focal adhesion assembly, and N-cadherin-based cell-cell adhesion^{330,331}. The role of LPA₁ in vivo was confirmed in *Lpa1*^{-/-} mice, which demonstrated increased apoptosis of sciatic nerve Schwann cells¹⁵⁰. S1P, however, has not been shown to play a prominent role in Schwann cell biology, despite their expression of S1P₁ and possibly S1P₂³²⁹. S1P also induced major morphological changes

due to actin cytoskeletal rearrangement, although this was reportedly due to activation of Rac1³³¹.

8.5 Neurons

Many of the neuronal effects of LPA, such as growth cone turning, neurite retraction, and chemorepulsion maybe be mediated by receptors other than LPA₁, as cortical neurons from *Lpa1*^{-/-} mice were responsive to LPA^{40,302,332}. LPA₃ is expressed on motor neurons of the spinal cord and the cortex and is up-regulated upon spinal or brain injury³³³. LPA₁ has, however, been shown to play an important role in nociception. Studies in *Lpa*^{-/-} mice demonstrated that the LPA₁/Rho/ROCK pathway mediated allodynia and hyperalgesia induced by LPA, as well as demyelination and neuropathic pain initiated by substance P production, sciatic nerve injury or compression of the trigeminal ganglion³³⁴⁻³³⁷. Interestingly, LPA₁ is involved in behavioral alterations unrelated to pain, including the suckling impulse of neonates, possibly regulated by LPA₁-mediated development of the cerebral cortex or olfactory bulb, and regulation of exploratory behavior, spatial learning, and working memory^{150,338}.

There have been few reports claiming an important role for specific S1PR signaling in adult neurons. Dorsal root ganglion and sensory neurons express mRNA for S1P₁₋₄, and S1P augments action potential of specific neurons by suppressing outward potassium current³³⁹. In another study, in vitro agonism of S1P₁ with SEW2871 increased the excitability of roughly half of the rat adult sensory neurons examined, also implicating other S1PR in the determination of neuronal sensitization³⁴⁰. In *Xenopus* retinal ganglion cells (RGC), anti-S1P₅ blocking antibody studies illustrated that the S1P₅/RhoA/Lim kinase signaling cascade mediated growth cone chemorepulsion to S1P, and ex vivo S1P was an axonal guidance cue in retinal explant cultures³⁴¹. S1P also elicited a cation current mediated by PLC activation and iCa²⁺ in chicken Amacrine cells (retinal interneurons), possibly via both S1P₁ and S1P₃, as treatment with SEW2871 induced current, whereas suramin, a S1P₃ antagonist, reversibly blocked current induction³⁴². However, caution is required when examining data obtained using suramin, as numerous other, non-S1PR-mediated effects have been reported^{343,344}.

9. Reproductive system

LPAR and S1PR have important roles in the process of reproduction under normal and disease conditions, in the parents as well as the offspring. Although early reports describe fluctuations in the concentrations of LPA, LPC, and S1P in the reproductive organs reflecting reproduction receptivity and capability, there is not much known about the contribution of specific receptors³⁴⁵.

9.1 Female reproductive biology and gestation

Embryo implantation in the endometrium is a critical milestone in the pregnancy timeline and can be a primary contributor to infertility³⁴⁶. Delayed embryonic development and increased embryonic death due to abnormal implantation and spacing resulted from maternal LPA₃ deletion, consequently down-regulating COX-2¹⁵². Pregnant *Lpa3*^{-/-} mice administered exogenous PGE₂ and PGI₂, COX-2 products required for implantation and decidualization, demonstrated increased successful embryo implantation^{152,346}. Studies in pigs indicated that LPA₃ expression may be spatially and temporally regulated, dependent upon the region of the uterus and phase of the estrus cycle³⁴⁷. Interestingly, although LPA₃ is highly expressed in oviduct, placenta, and uterus, it is absent from the ovary, where LPA₄ is highly expressed in both human and mouse tissues^{152,348}. In humans, endometrial stroma and first trimester trophoblasts expressed only LPA₁ mRNA, and LPA signaling induced in vitro IL-8 production, angiogenesis, and immune cell migration^{349,350}. These studies

demonstrate that both maternal and fetal LPAR signaling cooperate to ensure successful pregnancy.

Signaling via S1PR also plays an important role in pregnancy. S1P₁ was up-regulated in sheep endometrium and localized to areas of angiogenesis³⁵¹. S1P₁, 2, & 3 mRNA were up-regulated in murine decidua and in uterine stromal cells, and immunohistochemistry demonstrated that S1P₁ and S1P₂ colocalized with COX-2 at various points of the maternal-fetal interface, indicating that S1P signaling may participate with LPA₃ in regulating COX-2 expression in the uterus³⁵². However, a subsequent paper reported early pregnancy loss in *Sphk1*^{-/-}*Sphk2*^{+/-} mice due to defective decidualization, but no differences in uterine COX-2 expression nor PGE₂ were detected³⁵³. During late stages of pregnancy, S1P₁, S1P₂, and S1P₃ were also detected in human decidua, with S1P₃ expression altered according to gestational age³⁵⁴. Additionally, S1P metabolism increased with gestational age, with highest decidual activity at term, which might prepare the decidua for parturition³⁵¹. This is an interesting notion, as COX products are critical for the induction of labor, and therefore S1PR may be important for inducing COX regulation of later stages of pregnancy³⁴⁶.

9.2 Male reproductive biology

The male reproductive system is also regulated by LP receptors. High mRNA expression of LPA₁₋₃ and low expression of LPA₄ and LPA₅ were detected in mouse testis, as well as the germ cells themselves³⁵⁵. Single knockout males demonstrated a small decrease in sperm count at 6 months of age, and double knockout further decreased sperm numbers. LPA_{1/2/3} triple knockout males exhibited numerous reproductive deficiencies, including decreased mating activity, age-related testicular degeneration and decreased sperm counts related to increased germ cell apoptosis³⁵⁵.

S1P is also a regulator of the male reproductive organs, although little is known about the role of specific receptors. S1P inhibits male germ cell apoptosis, and *Sphk1* is expressed in the acrosomes of spermatozoa^{356,357}. Mature spermatozoa also express mRNA for S1P₁₋₃ and S1P₅, and S1P₁ is highly expressed in seminiferous tubules and spermatids in the testis³⁵⁸. S1PR may also play a role in male sexual function, by virtue of eNOS phosphorylation. Human corpus cavernosum expresses S1P₁₋₃, and S1P signaling potentiates acetylcholine-induced eNOS activation, which in turn regulates the hemodynamics required for erectile function³⁵⁹. Thus, S1PR and LPAR regulate the development and maintenance of both male and female fertility and reproduction.

10. Inhibitors, agonists, and antagonists

Development of S1PR- and LPAR-modifying compounds with in vivo utility has been hindered by numerous challenges, such as the lack of ligand-bound GPCR crystal structures, carrier/chaperone affinities, off-target effects, and conformational changes^{360,361}. Despite these difficulties, these receptors remain attractive targets, due to their disease state correlation, potent functional effects in numerous organs/systems, and prior amenability to modulation by pharmacological compounds. There are numerous agonists and antagonists of LPAR and S1PR, a few of which will be discussed herein according to receptor specificity/preference.

10.1 ATX inhibitors

Because of the proposed ability of autotaxin to deliver LPA directly to LPAR, some of the newest pharmacological tools, the autotaxin inhibitors, must be addressed in the discussion about LP receptor-modulating drugs. Previously, studies of ATX/LPA had been hindered by the lack of ATX inhibitors with in vivo potency^{362,363}. PF-8380 was obtained from a screen of the Pfizer compound collection and subsequent structure-function studies. Maximal

inhibition of LPA synthesis in plasma occurred 15-30 minutes after administration, and a 30 mg/kg dose in rats showed efficacy equal to the same dose of naproxen in a model of adjuvant-induced arthritis³⁶⁴. Small molecule screening and derivative optimization resulted in the synthesis of HA130, a boronic acid-based inhibitor targeted to the T210 active site of ATX. This compound, administered IV at 1 mol/g resulted in approximately a 75% decrease in plasma LPA within 10 minutes; however, no in vivo functional assays were performed in this report³⁶⁵.

The most recent advance in autotaxin inhibitor development was the design of stable analogues (compounds 22 and 30b) of S32826 [4-(tetradecanoylamino) benzyl] phosphonic acid, a molecule that demonstrated an IC₅₀ in the nanomolar range in a cell-free system, but poor stability precluded in vivo analyses^{366,367}. Both compounds 22 and 30b were highly specific for ATX, exhibited long plasma half-lives of approximately 10.5 hours, inhibited ATX-dependent invasion of HUVEC monolayers by rat hepatoma in vitro, and decreased metastatic foci in the B16-F10 melanoma model³⁶⁷. The authors suggest that continuing compound evolution originating from the same benzyl- and naphthylphosphonic acid scaffold may yield ATX inhibitors with extended half-lives, and thus greater utility in the tumor microenvironment, characteristics which would likely prove useful in other disease models.

10.2 LPAR agonists and antagonists

10.2.1 LPAR antagonists—AM095 is a LPA₁-specific antagonist from with efficacy in mouse models of fibrosis, scleroderma and bleomycin-induced pulmonary fibrosis without effects on wound healing^{368,369}. This compound led to the development of AM152, a LPA₁ antagonist that has recently completed Phase I clinical trials for the treatment of systemic sclerosis (<http://www.amirapharm.com/articles/AmiraAM152PhaseI.html>), demonstrated efficacy in numerous mouse models of fibrosis and was granted Orphan Drug Status by the FDA for the treatment of idiopathic pulmonary fibrosis^{370,371}. AM152 is the only LPA₁ antagonist currently in clinical trials.

Ki16425 is a LPA_{1/3} receptor antagonist that demonstrates specificity for LPA_{1/3}, little to no action on LPA₂, with no antagonism of S1PR³⁷². It is widely used in in vitro studies of LPAR function in different disease states, including various cancers, formation of neointima and atherosclerosis development, and fibrosis^{161,216,373-375}.

VPC12249 is a competitive LPA_{1/3} antagonist, the development of which resulted from a series of LPAR agonists and antagonists evolving from an agonist parent compound, *N*-acyl ethanolamide phosphate (NAEPA) and involves the 2-substitution of a bulky benzyl-4-oxybenzyl substituent in the *S* configuration³⁷⁶. This compound blocked Ca²⁺ mobilization and inhibited forskolin-induced cAMP accumulation in HEK293T cells³⁷⁶. It demonstrates similar effects to Ki6425, inhibiting fibroblast chemotaxis after bleomycin administration and chemotaxis of human pancreatic cancer cells^{161,377}.

10.2.2 LPAR agonist—OMPT (1-oleoyl-2-*O*-methyl-*rac*-glycerophosphothionate) is a LPA 18:1 analogue with the hydroxy group converted to an *O*-methoxy group, and the phosphate to a phosphothionate, resulting in potent and selective agonism of LPA₃³⁷⁸. Administration of OMPT to mice before induction of renal ischemia/reperfusion injury resulted in exacerbated pathology and was able to block the in vivo protective effects of VPC12249 administration³⁷⁹.

10.3. S1PR agonists and antagonists

10.3.1 S1PR agonists—FTY720 (Fingolimod/Gilenya; Novartis) is the first LP receptor modulator approved for clinical use. It is a novel oral drug approved by the FDA in September 2010 for the treatment of relapsing-remitting multiple sclerosis^{10,380}. FTY720 is phosphorylated *in vivo* by Sphk2 to the active form, FTY720-P (FTY-P)³⁸¹⁻³⁸³. Although FTY-P is an agonist of S1P₁ and S1P₃₋₅, binding of FTY-P to S1P₁ results in down-modulation and degradation of this receptor, amounting to functional antagonism^{145,384}.

Originally, FTY720 was targeted for use as an anti-rejection treatment in renal transplants; however, clinical trials were halted when it was determined that it provided no increase in prophylaxis of transplant rejection, but did lead to adverse side effects in some patients, including macular edema, bradycardia, reduced renal arterial function, and effects on the pulmonary vasculature³⁸⁵⁻³⁸⁷. The apparently immunosuppressive actions of the drug prompted researchers to examine efficacy in experimental autoimmune encephalomyelitis (EAE), a model of human MS, and found that it prevented pathology at concentrations several fold lower than those required for prevention of transplant allografts³⁸². The precise mechanism of FTY720 in MS is still debated: the sequestration of autoreactive cells in the LN has been considered the primary mechanism of action. However, some studies support the involvement of direct effects on the nervous system itself, including discrepancies between the timing of lymphopenia and amelioration/restoration of symptoms and the accumulation of FTY720 and FTYP in brain tissue^{388,389}. S1P₁ and S1P₃ protein up-regulation was found on lesional astrocytes of MS patients, and cell-specific deletion of S1P₁ in astrocytes ameliorated EAE pathology to a similar extent as FTY720 administration^{313,390}. For in-depth discussion of the putative mechanisms of FTY720 in EAE/MS, we refer you to several excellent reviews on the subject that have recently been published:^{10,391-393}.

FTY720 has shown efficacy in the prevention or amelioration in other models of disease, such as airway inflammation, atherosclerosis, renal ischemia/reperfusion injury, dermal allergic responses, models of Type-1 and -2 diabetes, and autoimmune uveitis^{183,185,209,265,394-396}. Future studies will likely examine the ability of FTY720 to modulate many more disease pathologies, as well as help determine the potential role of the S1PR in such systems.

AUY954 is a selective S1P₁ receptor modulator and an aminocarboxylate analog of FTY720, effective at low nanomolar concentrations³⁹⁷. Thus, it is a useful tool to dissect the effects of FTY720, or to attribute outcomes specifically to agonism of S1P₁^{113,182,246,313}. However, it also induces receptor internalization and degradation and therefore also acts as a functional antagonist³⁹⁸. Administration of AUY954 in EAE and models of airway inflammation or autoimmune neuritis recapitulates the effects seen with FTY720^{182,313,399}.

SEW2871 is a reversible, moderate-affinity, S1P₁-specific agonist that binds to the same site as S1P^{400,401}. Because of this specificity, unlike FTY720, it does not induce deleterious side effects that result from agonism of S1P₃, such as bradycardia¹³³. It also resembles S1P in that it induces signaling and receptor internalization without degradation, also unlike FTY720⁴⁰¹. Although long-term administration results in lymphopenia resembling that induced by FTY720 treatment, the potency and duration of action of SEW2871 are not as prolonged as the effects of FTY720⁴⁰².

10.3.2 S1PR antagonist—As the only known compound with S1P₂ specificity, agonistic or antagonistic, JTE-013 has been a critical tool for the dissection of S1P₂ inhibitory effects on migration and the complex G-protein signaling pathways involved⁴⁰³. It has also

allowed for assessing the relative contribution of S1P₂ activation in vivo, such as the regulation of B cell homeostasis and in models of atherosclerosis^{200,208,288,404}. Although JTE-013 has been regarded as a S1P₂-specific antagonist, the S1P₂ specificity of the compound has recently been questioned^{138,405}. Ex vivo studies utilizing basilar arteries demonstrated that JTE-013 inhibited vascular contraction induced by not only S1P, but also KCl and a thromboxane analogue, a result obtained using arteries from both wild-type and *S1p2*^{-/-} mice¹³¹. Additionally, Long, et al. reported that JTE-013 inhibited S1P-induced ERK-1/2 phosphorylation in MDA-MB-453 breast cancer cells, a result reiterated by S1P₄, but not S1P₂ knockdown, indicating that JTE-013 may be better classified as an S1P_{2/4} dual antagonist⁴⁰⁶.

11. Receptor-independent actions of S1P and LPA

Although the signaling events downstream of S1PR and LPAR ligand binding have been extensively examined, S1P and LPA were originally proposed to transduce non-receptor-mediated signals. Early studies indicated that S1P and precursor molecule ceramide were important second messengers in multiple mitogenesis and apoptosis pathways^{407,408,409,410,411}. Likewise, LPA was a known inducer of platelet activation and vasopression, via signaling of intracellularly synthesized LPA or modulation of cellular membrane architecture, respectively^{412,413}. Although later studies would demonstrate the role of GPCR signaling in these effects, others appear to be initiated, in part or whole, directly by intracellular LPs^{56,101,412,414,415,416}.

One such effect is the release of intracellular Ca²⁺ stores. Although activation of some LPAR and S1PR can lead to intracellular Ca²⁺ mobilization, intracellular LPA or S1P are capable of eliciting Ca²⁺ flux via store-operated or non-store-operated Ca²⁺ entry channels in diverse cell types, such as neutrophils, mast cells, macrophages, vascular smooth muscle cells, and pulmonary endothelial cells^{46,171,172,417-420}. Whether such effects are direct signaling function of the LPs on channel proteins or indirectly through membrane effects is not known. Recent in vitro studies have identified intracellular S1P as a negative regulator of histone deacetylase activity, a necessary component of the NF- κ B activation cascade via activation of the E3 ubiquitin ligase TRAF2, a modulator of Rhodopsin 1 endosomal trafficking, and is necessary for assembly of the electron transport chain and mitochondrial function⁴²¹⁻⁴²⁴. PPAR γ (peroxisome proliferator-activated receptor γ) agonism as a result of binding LPA was demonstrated in RAW264.7 monocytic cells and CHO cells, and in vivo using LPAR knockout mice in a model of chemically-induced arterial wall remodeling⁴²⁵⁻⁴²⁷. The relevance of such interactions to physiology and disease is not clear at present.

In some cases, the physiological contributions ascribed to non-GPCR-mediated S1P or LPA signaling are still unclear, due to the difficulties in differentiating between true second messenger signaling versus intracellular synthesis and subsequent autocrine or transcellular GPCR activation. For instance, mast cells produce and secrete S1P, which is thought to bind S1PR to modulate Fc ϵ R-mediated degranulation and inflammatory mediator synthesis, and in vivo studies using *Sphk1*^{-/-} or *Sphk2*^{-/-} mice yield conflicting results^{46,428,429}. Similarly, in studies utilizing preadipocytes, LPA binding to LPA₁ repressed PPAR γ in vitro, and mice with adipocyte-specific deletion of ATX demonstrated increased adiposity with a concomitant increase in PPAR γ expression, implying that the LPA that affects PPAR γ is produced extracellularly and signals via LPAR^{430,431}. Additionally, there have been reports of intracellular expression of LPA₁ and S1P₅^{432,433}. Thus, disentangling the relative contributions of GPCR- versus non-GPCR-mediated regulation of some cellular responses to either LPA or S1P will require meticulous dissection in both in vitro and in vivo systems.

12. Conclusions and perspectives

In the current review, we have attempted to comprehensively address the current state of our understanding of all bona fide members of the S1P and LPA receptor families, with emphasis on their contribution to the regulation of specific cell types in various systems. The past decade has seen a vigorous expansion in the study of S1P and LPA receptors. An increasing repertoire of agonists and antagonists, as well as the development of knockout and knockin animal models, has lead to our appreciation of these receptors as critical players in the regulation of homeostatic and disease states of most of the major body systems. Although the initial foci of S1PR and LPAR studies were the vascular or nervous systems, respectively, oncology, reproduction, immunology, and stem cell biology are just a few of the other fields that these receptors have been shown to dramatically influence.

Despite our increasing knowledge, the complexity and heterogeneity of LP receptor expression and signaling patterns demands intense investigation to fully comprehend the regulatory networks controlled by these receptors. New pathways and networks will likely emerge with further interrogation of the less-well characterized receptors, S1P₄ and S1P₅, and LPA₄₋₆, expanding the known overlapping or unique functionality of each family and individual receptor. The recent FDA approval of the S1PR modulator FTY720 as a first-line treatment for MS has heightened interest in the field of lysophospholipids and their receptors, and is likely a prelude to increased research and the future introduction of other LP receptor modulators into therapeutic use.

Acknowledgments

We gratefully acknowledge the support of the NIH, grants F32CA142117 (V.A.B.) and HL89934 and HL70694 (T.H.).

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Biographies

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Figure 1. Metabolism of LPA, LPAR G-protein coupling and down-stream signaling pathways
 PC, phosphatidylcholine; PE, phosphoethanolamine; PS, phosphatidylserine; PLD, phospholipase D; PLA_{1/2}, phospholipase A_{1/2}; PA, phosphatidic acid; LPC, lysophosphatidylcholine; LPA, lysophosphatidic acid; MAG, monoacylglycerol; LPP, lipid phosphate phosphatase; LDL, low-density lipoprotein; ATX/lysoPLD, autotaxin/lysophospholipase D; AC, adenylyl cyclase; cAMP, cyclic adenosine monophosphate; ROCK, RhoA-associated kinase; SRF, serum response factor; PLC, phospholipase C; PKC, protein kinase C; PI3K, phosphoinositide 3-kinase; MAPK, mitogen-activated protein kinase; eNOS, endothelial nitric oxide synthase. Dotted red line indicates suggested LPAR-G-protein coupling pathway.

Figure 2. Metabolism of S1P, S1PR G-protein coupling, and down-stream signaling pathways SMase, sphingomyelinase; SMS, sphingomyelin synthase; CDase, ceramidase; CerS, ceramide synthase; Sphk1/2, sphingosine kinase 1 and 2; SPP, sphingosine 1-phosphate phosphatase; LPP, lipid phosphate phosphatase; S1P, sphingosine 1-phosphate; Spns2, spinster 2 protein; ApoM, apolipoprotein M; ROCK, Rho-associated kinase; PTEN, phosphatase and tensin homolog; PLC, phospholipase C; PKC, protein kinase C; PI3K, phosphoinositide 3-kinase; MAPK, mitogen-activated protein kinase; eNOS, endothelial nitric oxide synthase.