Lysophospholipid receptors in vertebrate development, physiology, and pathology

Athanasia Skoura and Timothy Hla

Center for Vascular Biology, University of Connecticut Health Center, Farmington, CT 06030

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.

Supplementary key words sphingosine 1-phosphate • lysophosphatidic acid • G protein-coupled receptor

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, LPA1, that bound to the ligand, induced cell rounding and Gi-dependent cAMP suppression. Independent studies from our laboratory demonstrated that the G protein-coupled receptor EDG-I is a Gi-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, LPA4 and LPA5, are divergent in primary sequence from LPA1-3. 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 (SphKs) 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 (LPPs) or be irreversibly degraded into hexadecanal and phosphoethanolamine by S1P lyase (9). Products of the SPL are used in membrane phospholipid synthesis (for example, phosphatidyl 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 (PLA1 and PLA2) 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
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 lyospholipid mediators and evaluate potential novel therapeutic applications for the treatment of human diseases.

CARDIOVASCULAR SYSTEM

SIP 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 SIP 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 SIP is able to promote endothelial cell barrier integrity through SIP1 receptor function. Indeed, intravenously delivered SIP attenuated vascular barrier dysfunction in murine and canine models of acute lung injury (13). Furthermore, FTY720, a high affinity agonist for SIP 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 SIP1 receptor is essential for normal lung physiology because systemic antagonism of SIP1 receptor under basal physiological conditions enhanced pulmonary leakage (16).

Evidence for the functional role of SIP1 receptor in vasculature is derived from in vivo animal models where genetic deletion of SIP1 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, SIP1 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, SIP1 receptor is highly expressed in angiogenic tumor vessels in vivo and siRNAs targeted specifically to mouse SIP1 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 SIP (Sphingomab™, which is 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 SIP is unclear. In addition, it will be important to validate these results with independent studies without commercial conflicts.

Interestingly, Slp1/Slp2 double null embryos showed a more severe phenotype than Slp1 single null embryos, suggesting that SIP2 receptor is also significant during embryonic vascular development (22). In addition, Slp2 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 (Milh), an Slp2 ortholog, result in cardiac developmental defects (cardia bifida) due to defective migration of cardiomyocyte precursors, revealing an important function of SIP2 receptor in zebrafish heart organogenesis (24). In endothelial cells, SIP2 receptor activation results in disruption of adherens junctions and increased paracellular permeability, whereas JTE013 (SIP2 receptor antagonist) significantly inhibited H2O2-induced permeability in the rat lung perfused model (25). Hypoxic mouse retinas that lack SIP2 receptor present significantly decreased inflammatory cell infiltration and substantially enhanced revascularization of the retina tissue, indicating that SIP2 receptor activates inflammatory pathways that facilitate vascular permeability and pathological angiogenesis (26). SIP3 receptor has been reported to play a protective role against vascular endothelial injury. Specifically, the vasodilatory effect of HDL in SIP3 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 SIP1 and SIP3 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, SIP3 receptor also confers cardioprotection in a mouse model for ischemia-reperfusion, caused by coronary artery occlusion followed by reperfusion. In this model, SIP2,3 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 SIP 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 SIP receptor deficient mice, individual LPA receptors (LPA1,3) 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.
Sphk1
2
Sphk2
1
2
Lysophospholipid receptors

NERVOUS SYSTEM

The role of S1P in the nervous system is not well characterized. Several reports suggest that S1P₁ receptor regulates astrocyte motility, neurite extension and oligodendrocyte growth / survival. In contrast, S1P₂ receptor inhibits neurite extension and glioblastoma motility, whereas S1P₃ receptor appears to negatively control neurite extension. S1P₅ receptor inhibits oligodendrocyte progenitor migration, whereas it induces survival in oligodendroglial cells.

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 S1P₂ null mice (38). In addition, recent evidence suggest a role for LPA receptors in LPA-mediated myocardial hypertrophic 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.

LPA induces neurite and oligodendrocyte precursor cell retraction, whereas LPA₁ and LPA₂ 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 LPA₃ 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 LPA₁ 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 lysosphospholipids 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 genitlia 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 LPA₃ receptor in mice leads to uneven embryo spacing, possibly due to abnormal uterine contraction and also delayed implantation, due to abnormal uterine receptivity. Moreover, LPA₃ receptor null mice exhibit delayed
embryonic development, prolonged pregnancy and finally increased embryonic lethality. Interestingly, delivery of the prostaglandin E₂ (PGE₂) and an analog of prostaglandin I₂ (PGL₂) could rescue the delayed implantation phenotype, implicating the important role of prostaglandins in LPA₃ 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 S1P₁ 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 S1P₁ receptor specifically deleted in hematopoietic cells were generated. These mice showed that loss of S1P₁ receptor blocks thymocyte and lymphocyte egress from the thymus and lymphoid organs into blood due to defects in chemotactic response to S1P (52). Moreover, S1P₁ 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 S1P₁ receptor in thymocytes is regulated by the transcription factor KLF2 (55). Importantly, the cell surface glycoprotein CD69 interacts with S1P₁ 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 S1P₁ 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 S1P₃ receptor cross-talk in dendritic cells determines the progress of inflammation in a sepsis syndrome. Mechanistically, inhibition of protease-activated receptor 1–S1P₃ 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 outstanding questions. Better understanding of LP biology is warranted as a few of the first generation LP receptor modulators enter the therapeutic era.

REFERENCES

1. Tokumura, A., K. Fukuzawa, Y. Akamatsu, S. Yamada, T. Suzuki, and H. Tsukatani. 1978. Identification of vasopressor phospholipid in crude soybean lecithin. Lipids. 13: 468–472.
2. Zhang, H., N. N. Desai, A. Olivera, T. Seki, G. Brooker, and S. Spiegel. 1991. Sphingosine-1-phosphate, a novel lipid, involved in cellular proliferation. J. Cell Biol. 114: 155–167.
3. van Corven, E. J., A. Groenink, K. Jalink, T. Eichholtz, and W. H. Moolenaar. 1989. Lysosphatidate-induced cell proliferation: identification and dissection of signaling pathways mediated by G proteins. Cell. 59: 45–54.
4. Hecht, J. H., J. A. Weiner, S. R. Post, and J. Chun. 1996. Ventricular zone gene-1 (vsg-1) encodes a lysosphosphatic acid receptor expressed in neurogenic regions of the developing cerebral cortex. J. Cell Biol. 135: 1071–1083.
5. Lee, M. J., J. R. Van Brocklyn, S. Thangada, C. H. Liu, A. R. Hand, R. Menzeleev, S. Spiegel, and T. Hla. 1998. Sphingosine-1-phosphate as a ligand for the G protein-coupled receptor EDG-1. Science. 279: 1552–1555.
6. Rivera, R., and J. Chun. 2008. Biological effects of lysosphospholipids. Rev. Physiol. Biochem. Pharmacol. 160: 25–46.
7. Mizugishi, K., T. Yamashita, A. Olivera, G. F. Miller, S. Spiegel, and R. L. Proia. 2005. Essential Role for Sphingosine Kinases in Neural and Vascular Development. Mol. Cell. Biol. 25: 11113–11121.
8. Clair, T., J. Aoki, E. Koh, R. W. Bandle, S. W. Nam, M. M. Ptasynska, G. B. Mills, E. Schiffmann, L. A. Liotta, and M. L. Stracke. 1989. Autotaxin hydrolyzes sphingolipid to produce the regulator of migration, sphingosine-1-phosphate. Cancer Res. 63: 545–545.
9. Spiegel, S., and S. Milstien. 2003. Sphingosine-1-phosphate: an enigmatic signalling lipid. Nat. Rev. Mol. Cell Biol. 4: 897–907.
10. Dobrosotskaya, I. Y., A. C. Seegmiller, M. S. Brown, J. L. Goldstein, and R. B. Rawson. 2002. Regulation of SREBP processing and membrane lipid production by phospholipids in Drosophila. Science. 296: 879–883.
11. Hla, T., K. Venkataraman, and J. Michaud. 2008. The vascular S1P gradient–cellular sources and biological significance. Biochim. Biophys. Acta. 1781: 477–482.
12. Moolenaar, W. H., L. A. van Meeteren, and B. N. Giepmans. 2004. The ins and outs of lysosphosphatic acid signaling. BioEssays. 26: 870–881.
25. Sanchez, T., A. Skoura, M. T. Wu, B. Casserly, E. O. Harrington, and K. Kono, M., I. A. Belyantseva, A. Skoura, G. I. Frolenkov, M. F. Starost, et al. 2003. Validation of an anti-sphingosine-1-phosphate antibody as a potential therapeutic in reducing growth, invasion, and angiogenesis in mice. [see comment] Nat. Chem. Biol. 9: 235–244.

26. Liu, Y., R. Wada, T. Yamashita, Y. Mi, C. X. Deng, J. P. Hobson, H. M. Rosenfeld, V. E. Nava, U. J. Tietge, A. Godecke, I. Ishii, B. Kleuser, et al. 2006. Enhancement of capillary leakage and restoration of vascular endothelial cell adherens junction assembly and morphogenesis induced by sphingosine-1-phosphate. Cell. 99: 301–312.

27. Sanchez, T., T. Estrada-Hernandez, J.-H. Paik, M.-T. Wu, K. Venkataраман, V. Brinkmann, K. Casserly, and T. Hla. 2003. Phosphorylation and action of the immunomodulator FTY720 inhibits vascular endothelial cell growth factor-induced vascular permeability. J. Biol. Chem. 278: 47291–47299.

28. Samma, M. G., S. K. Wang, P. J. Gonzalez-Cabrera, A. Don, D. Marsolais, M. P. Matheu, S. H. Wei, I. Parker, E. Jo, W. C. Cheng, et al. 2006. Enhancement of capillary leakage and restoration of lymphocyte egress by a chiral S1P1 antagonist in vivo. [see comment] Nat. Chem. Biol. 2: 454–441.

29. Liu, Y. R., Wada, T. Yamashita, Y. Mi, C. X. Deng, J. P. Hobson, H. M. Rosenfeld, V. E. Nava, S. S. Chae, M. J. Lee, et al. 2000. Edg-1, the S1P2 receptor mediates vascular maturation. [see comment] J. Biol. Chem. 275: 22403–22405.

30. Argraves, K. M., B. A. Wilkerson, W. S. Argraves, P. A. Fleming, L. M. Obeid, and C. J. Drake. 2004. Sphingosine-1-phosphate signaling promotes critical migratory events in vasculogenesis. J. Biol. Chem. 279: 50580–50590.

31. Ye, J.-C., D. Ha, M. K. Aslam, J. Xie, M. S. Belyantseva, J. Wang, et al. 2000. Sphingosine-1-phosphate receptor agonists. Cell. 99: 301–312.

32. Skoura, A., T. Sanchez, K. Casserly, C. End, C. Shen, J. Chun, and H. Ueda. 2004. Sphingosine-1-phosphate receptor signaling of N-Cadherin mediates vascular stabilization. Genes Dev. 18: 2392–2403.

33. Chae, S. S., J. H. Paik, H. Furneaux, and T. Hla. 2004. Requirement for sphingosine-1-phosphate receptor-1 in tumor angiogenesis demonstrated by in vivo RNA interference. J. Clin. Invest. 114: 1082–1089.

34. Le Montagne, K., A. Littlewood-Evans, C. Schnell, T. O'Reilly, L. Wyder, T. Sanchez, B. Probst, J. Butler, A. Wood, G. Liu, et al. 2006. Antagonism of sphingosine-1-phosphate receptors by FTY720 inhibits angiogenesis and tumor vascularization. Cancer Res. 66: 221–235.

35. Kono, M., Y. Mi, Y. Liu, T. Sasaki, M. L. Allende, Y. P. Wu, T. Yamashita, and R. L. Proia. 2004. The sphingosine-1-phosphate receptors S1P1, S1P2, and S1P3 function coordinately during embryonic angiogenesis. J. Biol. Chem. 279: 29367–29373.

36. Kono, M., I. A. Belantaseva, A. Skoura, G. I. Frolenkov, M. F. Starost, J. L. Dreier, D. Lidington, S. S. Bolz, T. B. Friedman, T. Hla, et al. 2007. Deafness and stria vascularis defects in S1P2 receptor null mice. J. Biol. Chem. 282: 10690–10696.

37. Proia, R. L., R. A. An, N. Osborne, S. Waldron, and D. Y. Stainier. 2000. A sphingosine-1-phosphate receptor regulates cell migration during vertebrate heart development. [see comment] Nature. 406: 192–195.

38. Sanchez, T., A. Skoura, M. T. Wu, B. Caserly, E. O. Harrington, and T. Hla. 2007. Induction of vascular permeability by the sphingosine-1-phosphate receptor-2 (S1P2R) and its downstream effectors ROCK and PLD. Arterioscler. Thromb. Vasc. Biol. 27: 1312–1318.

39. Skoura, A., T. Sanchez, K. Casserly, S. M. Mandala, R. L. Proia, and T. Hla. 2007. Essential role of sphingosine-1-phosphate receptor-2 in pathological angiogenesis of the mouse retina. J. Clin. Invest. 117: 2506–2516.

40. Nofer, J.-R., M. van der Giet, M. Toll, I. Wolinska, K. von Wunck Laskowska, H. A. Baba, U. J. Tietge, A. Godecke, I. Ishii, B. Kleuser, et al. 2004. HDL induces NO-dependent vasorelaxation via the lysophospholipid receptor S1P3. J. Clin. Invest. 113: 569–581.

41. Keul, P., M. Toll, S. Lucke, K. von Wunck Lipinski, G. Heusch, M. Schuchardt, M. van der Giet, and B. Levkau. 2007. The sphingosine-1-phosphate analogue FTY720 reduces atherosclerosis in apolipoprotein E-deficient mice. Arterioscler. Thromb. Vasc. Biol. 27: 607–613.

42. Thielmeier, G., C. Schmidt, J. Herrmann, P. Keul, M. Schafers, I. Herrgott, J. Mersmann, J. Larmann, S. Herrmann, J. Stephan, et al. 2006. High-density lipoproteins and their constituent, sphingosine-1-phosphate, directly protect the heart against ischemia/reperfusion injury in vivo via the S1P3 lysophospholipid receptor. Circulation. 114: 1403–1409.

43. Argraves, K. M., B. A. Wilkerson, W. S. Argraves, P. A. Fleming, L. M. Obeid, and C. J. Drake. 2004. Sphingosine-1-phosphate signaling promotes critical migratory events in vasculogenesis. J. Biol. Chem. 279: 50580–50590.
The immune modulator FTY720 targets sphingosine 1-phosphate receptors. *J. Biol. Chem.* **277**: 21453–21457.

52. Matloubian, M., C. G. Lo, G. Cinamon, M. J. Lesneski, Y. Xu, V. Brinkmann, M. L. Allende, R. L. Proia, and J. G. Cyster. 2004. Lymphocyte egress from thymus and peripheral lymphoid organs is dependent on S1P receptor 1. *Nature* **427**: 355–360.

53. Chi, H., and R. A. Flavell. 2005. Cutting edge: regulation of T cell trafficking and primary immune responses by sphingosine 1-phosphate receptor 1. *J. Immunol.* **174**: 2485–2488.

54. Schwab, S. R., and J. G. Cyster. 2007. Finding a way out: lymphocyte egress from lymphoid organs. *Nat. Immunol.* **8**: 1295–1301.

55. Carlson, C. M., B. T. Endrizzi, J. Wu, X. Ding, M. A. Weinreich, E. R. Walsh, M. A. Wani, J. B. Lingrel, K. A. Hogquist, and S. C. Jameson. 2006. Kruppel-like factor 2 regulates thymocyte and T-cell migration. *Nature* **442**: 299–302.

56. Shiow, L. R., D. B. Rosen, N. Brdickova, Y. Xu, J. An, L. L. Lanier, J. G. Cyster, and M. Matloubian. 2006. CD69 acts downstream of interferon-[alpha]/[beta] to inhibit S1P1 and lymphocyte egress from lymphoid organs. *Nature* **440**: 540–544.

57. Rivera, J., R. L. Proia, and A. Olivera. 2008. The alliance of sphingosine-1-phosphate and its receptors in immunity. *Nat. Rev. Immunol.* **8**: 753–763.

58. Niessen, F., F. Schaffner, C. Furlan-Freguia, R. Pawlinski, G. Bhattacharjee, J. Chun, C. K. Derian, P. Andrade-Gordon, H. Rosen, and W. Ruf. 2008. Dendritic cell PAR1–S1P3 signalling couples coagulation and inflammation. *Nature* **452**: 654–658.