Published in final edited form as:

Exp Cell Res. 2015 May 1; 333(2): 178–182. doi:10.1016/j.yexcr.2014.11.023.

Imaging S1P1 Activation In Vivo

Mari Kono and Richard L. Proia*

Genetics of Development and Disease Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda MD, 20892, USA

Abstract

Sphingosine-1-phosphate receptor 1 (S1P1) is a G protein-coupled receptor that is activated by the sphingolipid ligand sphingosine-1-phosphate (S1P). S1P1 is widely expressed across tissues and, when activated, has broad functions in the immune, vascular and nervous systems. In several diseases in which inflammation plays a critical role, S1P1 activation has been found to be involved in pathogenesis. However, the details of S1P1 activation in vivo under different physiologic conditions are not well understood. Here we describe how a new in vivo methodology to identify S1P1 activation has helped increase understanding of the manner in which this signaling molecule functions both in homeostasis and during inflammation.

Keywords

signaling; sphingolipid; sphingosine-1-phosphate; GPCR; inflammation; homeostasis

Introduction

Sphingosine-1-phosphate receptor 1 (S1P1) is the prototype for the sphingosine-1-phosphate (S1P) family of G protein-coupled receptors (GPCRs) (reviewed in [1]). Isolated as an abundant transcript in differentiating endothelial cells, S1P1 was originally named Edg1 and structurally related receptors were grouped into the “Edg” family of GPCRs. When S1P was identified as the endogenous high-affinity ligand for Edg1, it and 4 other members of the Edg family were reclassified as S1P receptors (S1P1-5).

S1P1 is one of the most abundant and widely expressed members of the entire GPCR superfamily [2]. During embryonic development, it is highly expressed in the developing vasculature and nervous system [3]. In adult tissues, S1P1 is highly expressed in the lung, brain, and discrete regions of immune organs, such as the marginal zone of the spleen [4–6]. In addition, many different cell types express S1P1 [6]. Endothelial cells in particular highly

*To whom correspondence should be addressed: Richard L. Proia, richardp@intr.niddk.nih.gov, Genetics of Development and Disease Branch, National Institute of Diabetes and Digestive and Kidney Diseases, Building 10, Room 9D-06, 10 Center DR MSC 1821, Bethesda, MD 20892-1821.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

express S1P1, which is a contributing factor to its wide tissue distribution [2, 4]. S1P1 is also expressed on parenchymal cells in many major organ systems [6].

S1P1's ligand, S1P, is produced during sphingolipid metabolism, which occurs in all mammalian cells to synthesize plasma-membrane lipids and signaling molecules (reviewed in [7]). During the catabolism of ceramide-containing sphingolipids, sphingosine is liberated and serves as a substrate for sphingosine kinase 1 and 2, which catalyze the intracellular formation of S1P through ATP-dependent phosphorylation [8]. S1P is secreted to the extracellular space via both dedicated and broad specificity lipid transporters. Particular cell types (such as red blood cells, endothelial cells, and, upon their activation, platelets) have distinctive metabolic machinery and transporters that make them highly efficient in S1P secretion [9].

Extracellular S1P is compartmentalized in the circulating blood and lymph at micromolar and high nanomolar concentrations, respectively [10]. In interstitial fluids, the S1P concentration is estimated to be 100-fold lower than that in blood and lymph. S1P is normally sparingly soluble in the aqueous milieu; however, in blood, it is bound to protein chaperones, enhancing its solubility and potentially diversifying its signaling activities. The major S1P protein carriers in blood are apolipoprotein M (ApoM, which is in turn bound to high-density lipoprotein [HDL]) and albumin [11, 12].

S1P1 Functions

The function of S1P1 differs according to the cell type on which it is expressed. On endothelial cells, S1P1 is needed for the proper development of the vascular system in the embryo [3]. In adult organisms, endothelial-cell S1P1 prevents a leaky vasculature by sensing blood-derived S1P and then promoting tightened junctions between cells (under both homeostatic and leak-inducing conditions) [13].

On lymphocytes, S1P1 serves to direct their trafficking from areas of low S1P concentration, found within most lymphoid tissues, into the circulation, blood, and lymph, where substantially higher levels of S1P are present [10]. Maturing thymocytes express S1P1 in order to exit from the thymus and enter the blood circulation. In a parallel fashion, maturing B cells require S1P1 expression to move from bone marrow to sinusoids and ultimately into the blood. Mature T and B cells require S1P1 in order to egress from lymph nodes and enter lymph. Plasma cells utilize S1P1 to leave the spleen to transit to the bone marrow via the blood circulation. In the splenic marginal zone, where blood with a high S1P concentration empties into the spleen, specialized marginal-zone B cells utilize S1P1 to cycle between follicles and the marginal zone in order to deliver antigens captured from the blood. S1P1 is also utilized by other cells, including hematopoietic progenitor cells, osteoclast precursors, and platelets, to move into the circulation [14–16].

S1P1 in Inflammatory Disease

S1P1 signaling has been linked to pathology in a number of inflammatory diseases. In multiple sclerosis (MS), S1P1 has been implicated in pathogenesis at multiple levels, including immune-cell trafficking and activation, astrocyte proliferation, microglia

activation, and potentially in blood-brain barrier function [17, 18]. The key role of S1P1 in MS is underscored by the efficacy of the FDA-approved compound FTY720 in treating relapsing-relapsing MS. FTY720, while an S1P1 agonist, is believed to exert its disease ameliorating effects through “functional antagonism” by down-modulation of S1P1 expression [17].

In cancer models, S1P1 activation has been linked to persistent Stat3 activation and inflammation that promote tumorigenesis [19, 20]. S1P1 signaling has also been shown to contribute to the inflammatory-disease component of both colitis and influenza [20–22].

Development and Validation of a Mouse Model to Track S1P1 Signaling In Vivo (S1P1 GFP Signaling Mice)

Tracking the activation of S1P1 or any other GPCR in vivo presents a challenge, because individual cells express many receptors that may couple to the same or overlapping intracellular pathways, confounding linkage to a specific activated receptor. We sought to engineer a synthetic signaling pathway that was S1P1-specific with an unambiguous and easily detectable read-out. The model was based on a GPCR: β -arrestin interaction assay [23]. GPCR activation by agonist results in the phosphorylation of the receptor C-terminal tail by GPCR kinases enabling recognition of the activated receptor by β -arrestin. In the mouse model for S1P1 activation, both the S1P1 and β -arrestin are modified as fusion proteins: S1P1 is linked to the tetracycline transcriptional activator (tTA) via a peptide linker containing the tobacco etch virus (TEV) protease cleavage site, and β -arrestin is fused to the TEV protease (Figure 1, left panel) [6]. The S1P1 and β -arrestin fusion genes, connected as a single transcriptional unit, are knocked-in to the endogenous S1P1 locus under control of the endogenous S1P1 promoter. Mice carrying the knockin gene are then bred with mice carrying the histone-green fluorescent protein (GFP) reporter gene driven by a tTA-responsive promoter to derive “S1P1 GFP signaling mice” (Figure 1, right panel) [6]. In this mouse model, activation of the modified S1P1 by agonist induces β -arrestin-TEV protease binding to the activated receptor and subsequent proteolytic release of tTA, which then enters the nucleus to drive transcription of the histone-GFP reporter (Figure 1, left panel). Cells with activated S1P1 can then be identified by the appearance of green fluorescent nuclei.

Cells from these mice respond to physiologically relevant concentrations of S1P by expression of a nuclear GFP signal on a time scale consistent with a transcriptional activation of the reporter gene after receptor activation [6]. The response is specific; that is, related lipids that do not activate the receptor do not induce a fluorescent signal [6].

Previous expression and functional genetic studies have provided compelling evidence that S1P1 activation within the cardiovascular and nervous systems is critical for their embryonic development [3, 24, 25]. As a way of testing the S1P1 activation model, we used S1P1 GFP signaling mice to examine developing embryos. We found that mid-gestation embryos exhibited specific GFP fluorescent signal in the developing heart, vessels, and brain [6], verifying this earlier work.

As a further validation of the model, administration of the S1P1 agonist FY720 into adult S1P1 GFP signaling mice activated S1P1 in endothelial cells in several tissues. In addition, hepatocyte S1P1 was highly activated by FTY720 [6].

Homeostatic S1P1 Activation

Under homeostatic conditions, the S1P1 GFP signaling mice identify endothelial cells as a major cell type with activated S1P1 [6]. Lymphoid tissues in particular have a high frequency of endothelial cells with activated S1P1 [6]. Specialized high endothelial venules (HEVs), which form the portals in lymph nodes for the entry of lymphocytes from the blood, are intensely positive for activated S1P1, which may reflect the recently described role of S1P signaling in maintaining vascular integrity in the face of the massive passage of lymphocytes from the blood through these endothelial cell-lined portals [26]. The high degree of S1P1 activation in endothelial cells in other vascular beds of lymphoid tissues may similarly reflect the extensive lymphocyte transit out of tissues and the use of S1P1 signaling to maintain endothelial-barrier integrity during homeostasis. The marginal-zone region of spleen, which is exposed to open blood flow and high S1P concentrations, contains a high density of cells with activated S1P1. These include marginal-zone macrophages and B cells, which may indicate that S1P signaling is needed for their positioning in the marginal zone. In non-lymphoid tissues, such as liver, lung, heart, and brain, endothelial cells with activated S1P1 are present, but to a lesser extent [6].

S1P1 Activation during Inflammation

Lipopolysaccharide (LPS) administration induces systemic inflammation [27]. In S1P1 GFP signaling mice, this inflammatory stimulation produces a substantial increase in the number and intensity of GFP-positive cells within several tissues, including liver, lung, lymphoid tissues, brain, and heart, indicative of heightened S1P1 activation [6]. Many of the cells with increased S1P1 activation are endothelial cells of the vasculature and the lymphatics. Hepatocytes also display activated S1P1 after systemic inflammation [6].

S1P1 GFP signaling mice made deficient in S1P in their circulation by genetically deleting hematopoietic-cell sphingosine kinases [13] have been used to reveal the source of the endogenous S1P signaling pool that activates S1P1 during LPS-induced systemic inflammation. In these “plasma S1P-less” S1P1 GFP signaling mice, LPS exposure induces substantially less S1P1 activation in liver and lung endothelial cells. S1P1 activation is also markedly decreased in hepatocytes in plasma S1P-less S1P1 GFP signaling mice during LPS-induced inflammation. These results show that, during systemic inflammation, a large fraction of cellular S1P1 activation is mediated through a hematopoietic source of S1P [6].

Model for S1P1 Activation during Inflammation

As previously described, under homeostatic conditions, S1P1 activation is present in some endothelial cells [6] (Figure 2, left panel). This finding is consistent with models positing that S1P in the circulation serves to maintain vascular-barrier integrity through S1P1 signaling on the endothelium [13]. Although parenchymal cells of most tissues express S1P1, S1P1 activation is minimal in these cells during homeostasis [6], indicating that

extracellular S1P levels are insufficient or incapable of inducing S1P1 activation in this context.

Upon systemic inflammation, endothelial cells in many tissues and some parenchymal cells (notably in liver) demonstrate substantially increased S1P1 activation (Figure 2, right panel). Vascular leakage, a key feature of inflammation, may raise extracellular levels of S1P in tissues and stimulate S1P1 activation. The form of S1P associated with this inflammatory signaling pathway is not known, but it may be delivered from the plasma by ApoM-HDL or albumin [11, 12]. S1P might also be carried by hematopoietically derived cells (such as red blood cells, platelets, and leukocytes) that extravasate into tissues during inflammation.

While the increased S1P1 activation of parenchymal cells during inflammation is presumably due to elevated levels of S1P signaling pools (which are normally very low) in the interstitial fluids [10], the basis for the increased S1P1 activation in endothelial cells (which are continuously exposed to high S1P in the plasma and lymph at steady-state conditions) is not known. Possibilities include mechanisms that limit S1P1 exposure to luminal S1P but allow interaction with basally deposited S1P during inflammation [13], expression of a co-receptor after inflammatory stimulus that may allow interaction with chaperone-bound S1P, or the controlled release of cell-compartmentalized S1P as a result of inflammation.

Implications for S1P1 Signaling in Inflammatory Disease

As described above, S1P1 signaling has been implicated in several diseases that involve an important inflammatory component, including MS, colitis, and cancer. The sources of endogenous S1P that stimulate the S1P1 signaling pathways in these pathologies still remain to be identified, but may be a hematopoietic source similar to the proposed model (Figure 2, right panel). In MS, S1P1 signaling on astrocytes promotes astrogliosis [28]. It is possible that hematopoietically derived S1P enters the central nervous system from the blood after breakdown of the blood-brain barrier during MS, raising levels of S1P and stimulating S1P1 on astrocytes within the central nervous system. Through a similar mechanism, leaky blood vessels, which are characteristic of tumors [29], can expose stromal cells to high levels of S1P. Increased S1P can activate S1P1 and downstream Stat3 signaling, a pathway that sustains inflammation and promotes tumor growth [19, 20].

Future Directions

When activated, S1P1 has been shown to have diverse physiological effects that can impact a number of disease processes. However, our understanding of when and where S1P1 activation occurs, as well as the nature of the relevant S1P signaling pool, is only beginning to be understood. Although hematopoietically derived S1P is an important signaling pool, the relevant forms of S1P used for S1P1 activation—whether chaperone-bound or in a cellular context—are not known. Non-hematopoietic sources of S1P also may exist and remain to be identified. Whereas S1P1 activation in endothelial cells is believed to promote barrier integrity under homeostatic and leak-inducing conditions, the function of S1P1 activation during inflammation in many types of tissue parenchymal cells that express S1P1 is largely unknown.

A model with a transcription read-out for activation, as exemplified by the S1P1 GFP signaling mouse, can track past cellular activation but cannot provide information about S1P1 activation in real time. This greatly limits our understanding of S1P1 activation, especially in cells that are migratory and may undergo rapid, transient S1P1 signaling and then travel to different locations. Models that report signaling in real time in living animals will be needed to understand the events that underlie critical S1P1 functions during homeostasis and disease.

Acknowledgments

We thank Linda Raab for editing and helpful comments on the manuscript. This research was supported by the Intramural Research Program of the National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases.

Abbreviations

S1P1	sphingosine-1-phosphate receptor 1
S1P	sphingosine-1-phosphate
GPCR	G protein-coupled receptor
ApoM	apolipoprotein M
HDL	high-density lipoprotein
HEV	high endothelial venules
MS	multiple sclerosis
tTA	tetracycline transcriptional activator
TEV	tobacco etch virus
GFP	green fluorescent protein
LPS	lipopolysaccharide

References

1. Blaho VA, Hla T. An update on the biology of sphingosine 1-phosphate receptors. *Journal of lipid research*. 2014
2. Regard JB, Sato IT, Coughlin SR. Anatomical profiling of G protein-coupled receptor expression. *Cell*. 2008; 135:561–571. [PubMed: 18984166]
3. Liu Y, Wada R, Yamashita T, Mi Y, Deng CX, Hobson JP, Rosenfeldt HM, Nava VE, Chae SS, Lee MJ, Liu CH, Hla T, Spiegel S, Proia RL. Edg-1, the G protein-coupled receptor for sphingosine-1-phosphate, is essential for vascular maturation. *The Journal of clinical investigation*. 2000; 106:951–961. [PubMed: 11032855]
4. Cahalan SM, Gonzalez-Cabrera PJ, Sarkisyan G, Nguyen N, Schaeffer MT, Huang L, Yeager A, Clemons B, Scott F, Rosen H. Actions of a picomolar short-acting S1P(1) agonist in S1P(1)-eGFP knock-in mice. *Nature chemical biology*. 2011; 7:254–256.
5. Chae SS, Proia RL, Hla T. Constitutive expression of the S1P1 receptor in adult tissues. *Prostaglandins & other lipid mediators*. 2004; 73:141–150. [PubMed: 15165038]
6. Kono M, Tucker AE, Tran J, Bergner JB, Turner EM, Proia RL. Sphingosine-1-phosphate receptor 1 reporter mice reveal receptor activation sites in vivo. *The Journal of clinical investigation*. 2014; 124:2076–2086. [PubMed: 24667638]

7. Merrill AH Jr. Sphingolipid and glycosphingolipid metabolic pathways in the era of sphingolipidomics. *Chemical reviews*. 2011; 111:6387–6422. [PubMed: 21942574]
8. Maceyka M, Spiegel S. Sphingolipid metabolites in inflammatory disease. *Nature*. 2014; 510:58–67. [PubMed: 24899305]
9. Olivera A, Allende ML, Proia RL. Shaping the landscape: metabolic regulation of S1P gradients. *Biochimica et biophysica acta*. 2013; 1831:193–202. [PubMed: 22735358]
10. Cyster JG, Schwab SR. Sphingosine-1-phosphate and lymphocyte egress from lymphoid organs. *Annual review of immunology*. 2012; 30:69–94.
11. Argraves KM, Argraves WS. HDL serves as a S1P signaling platform mediating a multitude of cardiovascular effects. *Journal of lipid research*. 2007; 48:2325–2333. [PubMed: 17698855]
12. Christoffersen C, Obinata H, Kumaraswamy SB, Galvani S, Ahnstrom J, Sevvana M, Egerer-Sieber C, Muller YA, Hla T, Nielsen LB, Dahlback B. Endothelium-protective sphingosine-1-phosphate provided by HDL-associated apolipoprotein M. *Proceedings of the National Academy of Sciences of the United States of America*. 2011; 108:9613–9618. [PubMed: 21606363]
13. Camerer E, Regard JB, Cornelissen I, Srinivasan Y, Duong DN, Palmer D, Pham TH, Wong JS, Pappu R, Coughlin SR. Sphingosine-1-phosphate in the plasma compartment regulates basal and inflammation-induced vascular leak in mice. *The Journal of clinical investigation*. 2009; 119:1871–1879. [PubMed: 19603543]
14. Zhang L, Orban M, Lorenz M, Barocke V, Braun D, Urtz N, Schulz C, von Bruhl ML, Tirniceriu A, Gaertner F, Proia RL, Graf T, Bolz SS, Montanez E, Prinz M, Muller A, von Baumgarten L, Billich A, Sixt M, Fassler R, von Andrian UH, Junt T, Massberg S. A novel role of sphingosine 1-phosphate receptor S1pr1 in mouse thrombopoiesis. *The Journal of experimental medicine*. 2012; 209:2165–2181. [PubMed: 23148237]
15. Juarez JG, Harun N, Thien M, Welschinger R, Baraz R, Pena AD, Pitson SM, Rettig M, DiPersio JF, Bradstock KF, Bendall LJ. Sphingosine-1-phosphate facilitates trafficking of hematopoietic stem cells and their mobilization by CXCR4 antagonists in mice. *Blood*. 2012; 119:707–716. [PubMed: 22049516]
16. Ishii M, Egen JG, Klauschen F, Meier-Schellersheim M, Saeki Y, Vacher J, Proia RL, Germain RN. Sphingosine-1-phosphate mobilizes osteoclast precursors and regulates bone homeostasis. *Nature*. 2009; 458:524–528. [PubMed: 19204730]
17. Brinkmann V, Billich A, Baumruker T, Heining P, Schmouder R, Francis G, Aradhye S, Burtin P. Fingolimod (FTY720): discovery and development of an oral drug to treat multiple sclerosis. *Nature reviews. Drug discovery*. 2010; 9:883–897.
18. Chun J, Hartung HP. Mechanism of action of oral fingolimod (FTY720) in multiple sclerosis. *Clinical neuropharmacology*. 2010; 33:91–101. [PubMed: 20061941]
19. Lee H, Deng J, Kujawski M, Yang C, Liu Y, Herrmann A, Kortylewski M, Horne D, Somlo G, Forman S, Jove R, Yu H. STAT3-induced S1PR1 expression is crucial for persistent STAT3 activation in tumors. *Nature medicine*. 2010; 16:1421–1428.
20. Liang J, Nagahashi M, Kim EY, Harikumar KB, Yamada A, Huang WC, Hait NC, Allegood JC, Price MM, Avni D, Takabe K, Kordula T, Milstien S, Spiegel S. Sphingosine-1-phosphate links persistent STAT3 activation, chronic intestinal inflammation, and development of colitis-associated cancer. *Cancer cell*. 2013; 23:107–120. [PubMed: 23273921]
21. Montrose DC, Scherl EJ, Bosworth BP, Zhou XK, Jung B, Dannenberg AJ, Hla T. S1P(1) localizes to the colonic vasculature in ulcerative colitis and maintains blood vessel integrity. *Journal of lipid research*. 2013; 54:843–851. [PubMed: 23296878]
22. Teijaro JR, Walsh KB, Cahalan S, Fremgen DM, Roberts E, Scott F, Martinborough E, Peach R, Oldstone MB, Rosen H. Endothelial cells are central orchestrators of cytokine amplification during influenza virus infection. *Cell*. 2011; 146:980–991. [PubMed: 21925319]
23. Barnea G, Strapps W, Herrada G, Berman Y, Ong J, Kloss B, Axel R, Lee KJ. The genetic design of signaling cascades to record receptor activation. *Proceedings of the National Academy of Sciences of the United States of America*. 2008; 105:64–69. [PubMed: 18165312]
24. Allende ML, Yamashita T, Proia RL. G-protein-coupled receptor S1P1 acts within endothelial cells to regulate vascular maturation. *Blood*. 2003; 102:3665–3667. [PubMed: 12869509]

25. Mizugishi K, Yamashita T, Olivera A, Miller GF, Spiegel S, Proia RL. Essential role for sphingosine kinases in neural and vascular development. *Molecular and cellular biology*. 2005; 25:11113–11121. [PubMed: 16314531]
26. Herzog BH, Fu J, Wilson SJ, Hess PR, Sen A, McDaniel JM, Pan Y, Sheng M, Yago T, Silasi-Mansat R, McGee S, May F, Nieswandt B, Morris AJ, Lupu F, Coughlin SR, McEver RP, Chen H, Kahn ML, Xia L. Podoplanin maintains high endothelial venule integrity by interacting with platelet CLEC-2. *Nature*. 2013; 502:105–109. [PubMed: 23995678]
27. Juskewitch JE, Platt JL, Knudsen BE, Knutson KL, Brunn GJ, Grande JP. Disparate roles of marrow- and parenchymal cell-derived TLR4 signaling in murine LPS-induced systemic inflammation. *Scientific reports*. 2012; 2:918. [PubMed: 23213355]
28. Choi JW, Gardell SE, Herr DR, Rivera R, Lee CW, Noguchi K, Teo ST, Yung YC, Lu M, Kennedy G, Chun J. FTY720 (fingolimod) efficacy in an animal model of multiple sclerosis requires astrocyte sphingosine 1-phosphate receptor 1 (S1P1) modulation. *Proceedings of the National Academy of Sciences of the United States of America*. 2011; 108:751–756. [PubMed: 21177428]
29. Hashizume H, Baluk P, Morikawa S, McLean JW, Thurston G, Roberge S, Jain RK, McDonald DM. Openings between defective endothelial cells explain tumor vessel leakiness. *The American journal of pathology*. 2000; 156:1363–1380. [PubMed: 10751361]

Highlights

- S1P1 is a widespread GPCR involved in key physiology and inflammatory diseases.
- A novel mouse model enables imaging of activated S1P1.
- Activated S1P1 has been mapped in vivo during homeostasis and inflammation.
- Model provided for S1P1 activation during homeostasis and inflammation.

Figure 1.

Design and derivation of S1P1 GFP signaling mice. Left panel: Design to monitor S1P1 activation. Based on the “Tango assay” [23], the S1P1 C-terminus is linked to tTA via a peptide linker containing the TEV protease cleavage sequence, and the TEV protease is fused to β -arrestin. Upon agonist binding to S1P1 and its activation, the intracellular C-terminal tail of S1P1 is phosphorylated, allowing recognition by β -arrestin-TEV protease and cleavage of the linker to release tTA. In the nucleus, the released tTA activates a histone-GFP reporter gene, fluorescently identifying nuclei of cells with activated S1P1. PM, plasma membrane. Right panel: Derivation of S1P1 GFP signaling mice. A transcriptional unit containing the S1P1-tTA fusion linked to the β -arrestin-TEV protease fusion by an internal ribosome entry sequence (IRES) is knocked-in to the endogenous S1P1 locus. Mice carrying the knockin gene are crossed with mice with a histone-GFP reporter gene under control of a tTA-responsive promoter. Mice carrying both the knockin and the reporter gene are termed “S1P1 GFP signaling mice”.

Figure 2.

Model of in vivo S1P1 activation during homeostasis and inflammation. S1P (red dots) is compartmentalized in the circulation. Under homeostasis, S1P1 activation occurs in some endothelial cells and is largely absent from parenchymal cells. Under inflammatory conditions, vascular leakage can expose S1P1 on endothelial cells and parenchymal cells to hematopoietically-derived S1P, to induce S1P1 activation.