Published in final edited form as:

Curr Top Microbiol Immunol. 2014 ; 378: 85–105. doi:10.1007/978-3-319-05879-5_4.

S1P Control of Endothelial Integrity

Yuquan Xiong and Timothy Hla

Center for Vascular Biology, Department of Pathology and Laboratory Medicine, Weill Cornell Medical College, Cornell University, New York, NY 10165, USA

Timothy Hla: tih2002@med.cornell.edu

Abstract

Sphingosine 1-phosphate (S1P), a lipid mediator produced by sphingolipid metabolism, promotes endothelial cell spreading, vascular maturation/stabilization, and barrier function. S1P is present at high concentrations in the circulatory system, whereas in tissues its levels are low. This so-called vascular S1P gradient is essential for S1P to regulate much physiological and pathophysiological progress such as the modulation of vascular permeability. Cellular sources of S1P in blood has only recently begun to be identified. In this review, we summarize the current understanding of S1P in regulating vascular integrity. In particular, we discuss the recent discovery of the endothelium-protective functions of HDL-bound S1P which is chaperoned by apolipoprotein M.

1 Sphingosine 1-Phosphate Metabolism

Lysophospholipids (LPs) are minor lipid components compared to the major membrane phospholipids such as phosphatidylcholine (PC), phosphatidylethanolamine (PE), and sphingomyelin (SM). The LPs were originally presumed to be simple metabolic intermediates in the de novo biosynthesis of phospholipids. However, subsequent research demonstrated that the LPs exhibited significant biological activity by acting as extracellular growth factors or intercellular signaling molecules (Moolenaar and Hla 2012; Chun et al. 2010). As one of the most biologically significant LPs, Sphingosine 1-phosphate (S1P) has drawn considerable attention since the discovery that S1P is a signaling molecule that regulates multiple cell functions such as cell proliferation, differentiation, and migration (Olivera and Spiegel 1993; Lee et al. 1998; Lee et al. 1999; Hla et al. 2001; Paik et al. 2001).

The degradation of SM, a eukaryotic-specific phospholipid essential for the formation of membrane rafts and caveolae, is a major pathway involved in producing S1P. SM is metabolized by the sphingomyelinase pathway to produce progressively polar molecules: ceramide, sphingosine, and S1P (Hannun and Obeid 2008). Ceramide is catalyzed by ceramidase into sphingosine, which is phosphorylated by sphingosine kinase (Sphk) enzymes into S1P (Hait et al. 2006). There are two forms of Sphk, Sphk1 and Sphk2. Sphk1 is generally localized in the cytoplasm and translocates to the plasma membrane upon activation, while Sphk2 is primarily but not exclusively localized in nuclei (Ogawa et al.

2003; Igarashi et al. 2003; Venkataraman et al. 2006). S1P levels in cells are tightly regulated by the balance between its synthesis and degradation. Degradation of S1P occurs through the action of S1P lyase or by the specific S1P phosphatases (SPP1 and SPP2) as well as lysophospholipid phosphatase 3 (LPP3) (Le Stunff et al. 2002; Ogawa et al. 2003). The different S1P phosphatases regenerate sphingosine that can re-enter the sphingolipid metabolic pathway. S1P can also be irreversibly degraded by S1P lyase to yield hexadecenal and phosphoethanolamine, intermediates which are used as a substrate for phospholipid synthesis (Bandhuvula and Saba 2007). The degradation of S1P by the S1P lyase pathway serves as an important pathway for the conversion of sphingolipids into glycerolipids.

Although originally thought to be an intracellular second messenger (Olivera and Spiegel 1993), most of the biological effects of S1P were attributed to the signaling of its five ubiquitously expressed cell surface receptors, designated S1P₁₋₅, all of which bind to the ligand with nM affinity (Chun et al. 2002; Min et al. 2002; Blaho and Hla 2011). Although all S1PRs are G protein-coupled, each receptor subtype exhibits differential coupling efficacy to G protein alpha subunits. Although widely expressed, S1P receptors also display tissue-selective expression patterns as only three of the five S1P receptor subtypes (S1P₁, S1P₂, and S1P₃) are expressed in vascular tissues, whereas expression of the S1P₄ and S1P₅ receptors are largely confined to cells of the hematopoietic and nervous systems, respectively (Waeber 2013).

2 Sphingosine 1-Phosphate in the Blood

S1P is a pleiotropic lipid mediator capable of modulating the functions of many cell types. However, in mammalian systems S1P is found mainly in the blood and lymph in homeostasis (Pappu et al. 2007; Venkataraman et al. 2008; Hla et al. 2008; Yatomi et al. 2001). Thus, the functions of S1P in these two organ systems have been characterized most extensively. However, in the extravascular compartment, S1P can be produced in an inducible manner (Venkataraman et al. 2008; Hla et al. 2008). Interestingly, a significant concentration gradient of S1P exists between plasma and interstitial fluids: the concentration of S1P in plasma varies from 0.1 to 0.6 μ M (Yatomi et al. 1997b; Yatomi et al. 1997a; Caligan et al. 2000; Berdyshev et al. 2005), while tissue S1P levels are generally low (0.5–75 pmol/mg) (Edsall and Spiegel 1999; Allende et al. 2004; Le Stunff et al. 2002; Ancellin et al. 2002; Ogawa et al. 2003; Chun et al. 2002; Venkiteswaran et al. 2002; Min et al. 2002). This concentration gradient, termed the vascular S1P gradient appears to form as a result of substrate availability and the action of metabolic enzymes. The physiological significance of this S1P gradient is now becoming clear but how it is maintained is an active area of investigation.

Serum S1P levels are always higher than those in plasma, which was explained by the abundance of S1P in platelets and its extracellular release upon stimulation by platelet activators such as thrombin. Platelets were assumed to be the major source of S1P in plasma as they express a high activity of SphK1, lack the S1P lyase that irreversibly degrades S1P, and their activation causes the release of S1P (English et al. 2000; Yatomi et al. 1997b; Yatomi et al. 1997a; Tani et al. 2005). However, whether platelets contribute to plasma S1P in vivo was not examined critically until recently. The transcription factor *NF-E2*-deficient

had normal plasma S1P concentrations (Pappu et al. 2007), despite having virtually no circulating platelets (Shivdasani et al. 1995). In addition, circulating platelet depletion by infusion of an antibody against platelet glycoprotein GPIIb/IIIa (CD42b) also did not decrease plasma S1P levels (Venkataraman et al. 2008; Hla et al. 2008). These data suggest that platelets are unlikely to be the significant source of plasma S1P under physiological conditions, while recent reports suggest that red blood cells (RBC) and vascular endothelial cells (EC) may be the sources of S1P in plasma (Jessup 2008). However, a recent report suggested that during immune cell trafficking into high endothelial venules of lymph nodes, platelets extravasate into the basolateral surface and interact with fibroblast reticular cells via podoplanin/CLEC-2 interaction, resulting in stimulated formation of S1P and preservation of endothelial barrier function of this specialized endothelium (Herzog et al. 2013).

Significant progress toward identifying the cellular source(s) of S1P were made by using a novel *Sphk1/2* double gene-specific knockout mouse model. With this system, Pappu et al. showed that plasma S1P levels were reduced to undetectable levels in the mouse of *Sphk1/2* deleted by poly(I:C)-induced Mx1-Cre expression (pS1P less mice) (Pappu et al. 2007), although it is difficult to pinpoint the precise cellular source in this system since the Mx1-Cre transgene is responsive to IFN- α/β and is rapidly induced in multiple tissues including liver, hematopoietic cells, and variably in other cell types (Kuhn et al. 1995). However, by conducting adoptive transfer experiments, they demonstrated that wild-type red blood cells alone have the capacity to restore plasma S1P to normal levels in conditional *Sphk1/2*-double knockout mice after lethal whole-body irradiation, suggesting that red cells are capable of being a major source of S1P in plasma. Erythrocytes possess much weaker Sphk activity compared to platelets but lack the S1P-degrading activities of either S1P lyase or S1P phosphohydrolase and erythrocytes are much more abundant in blood than platelets. This combination suggests that the S1P provided by erythrocytes is much greater (Sanchez et al. 2007; Ito et al. 2007; Xu et al. 2007). The studies from group of Gräler also suggested that erythrocytes are the main blood cell population that is capable of incorporating, protecting, storing, and releasing S1P in vitro or ex vivo (Hanel et al. 2007; Bode et al. 2010).

However, our own findings suggest that hematopoietic cells are not the only cellular source that is capable of producing S1P to maintain high plasma levels (Venkataraman et al. 2008; Hla et al. 2008). Hematopoietic cell depletion experiments showed that mice that are severely anemic (>50 % reduction in hematocrit), thrombocytopenic (>90 % suppression of platelets), and irradiated (leukopenic, thrombocytopenic, and anemic) possessed wild-type S1P levels in plasma. We also found that plasma S1P bound to albumin turns over rapidly with a half-life of ≈ 15 min, which suggests that very active synthetic and degradative pathways of S1P metabolism exist in vivo, and that vascular endothelium might be a major contributor to plasma S1P (Venkataraman et al. 2008; Hla et al. 2008). Spinster 2 (*Spns2*), a member of the major facilitator superfamily of nonATP-dependent transporters, has been shown to be an S1P transporter (Kawahara et al. 2009). The plasma S1P levels of *Spns2*-deficient mice was reduced to approximately 60 % of wild-type, while analysis of the cells isolated from *Spns2*-deficient mice demonstrated that mammalian *Spns2* is the S1P

transporter in vascular ECs but not in erythrocytes and platelets (Hisano et al. 2012; Fukuhara et al. 2012). Indeed, in *Spns2* EC-specific knockout mice, plasma S1P concentration was decreased to the level observed in *Spns2*^{-/-} mice (Fukuhara et al. 2012; Mendoza et al. 2012).

Sphk enzyme activity is expressed in various cell types (Yatomi et al. 1997b; Yatomi et al. 1997a), suggesting there could be many cellular sources of blood S1P in addition to erythrocytes and ECs. Recently, Kurano et al. also reported that plasma S1P levels were decreased by hepatectomy, suggesting that liver may contribute to plasma S1P levels in vivo (Kurano et al. 2013). The brain is the organ containing the largest amount of S1P (4–40 nmol/g wet weight, corresponding to about 4–40 μ M) (Jiang and Han 2006; Murata et al. 2000b; Murata et al. 2000a), suggesting that neurons and astrocytes may represent a significant source of S1P, able to activate vascular S1P receptors. Indeed, high levels of *Sphk2* mRNA were detected by real-time reverse transcriptase polymerase chain reaction in glial cells and cortical neurons (Blondeau et al. 2007) and S1P has been shown to be released from cultured neural cells, such as astrocytes and granule cells, in response to stimuli (Anelli et al. 2005; Bassi et al. 2006). Whether S1P crosses the blood–brain barrier is not known.

3 Maintenance of Vascular Barrier Integrity by Sphingosine 1-Phosphate

The integrity of blood vessels is critical to vascular homeostasis, as failure of this system results in serious consequences such as hemorrhage, edema, inflammation, and tissue ischemia. Vascular integrity is tightly regulated by a number of factors that ensure proper functions of various components of the blood vessel wall, while S1P has been identified as a robust barrier-enhancing factor with great potential to serve as a novel and specific therapy for EC barrier dysfunction (Lee et al. 1999; Garcia et al. 2001; Sanchez et al. 2003; Jung et al. 2012; Gaengel et al. 2012). In general, the transport of fluids and solutes through the endothelial barrier of “generic” continuous endothelium is determined by two separate pathways: an active transcellular pathway for macromolecules larger than 3 nm, such as albumin, and a passive paracellular pathway for smaller molecules (Predescu et al. 2007; Michel and Curry 1999). This paracellular pathway is regulated by a complex balance of intracellular contractile forces generated by actin-myosin and tethering forces between adjacent ECs and between cells and the extracellular matrix. In various vascular beds, specific mechanisms such as the blood–brain barrier and fenestrae exist to either further increase or decrease the barrier functions, respectively.

3.1 S1P Signaling to Endothelial Cytoskeleton

Cytoskeleton, a complex network of actin microfilaments, microtubules, and intermediate filaments, which combine to regulate shape change and transduce signals within and between neighboring cells. Cytoskeletal rearrangement is a critical cellular event mediating endothelial barrier function (Bogatcheva and Verin 2008). Morphologic studies demonstrate that S1P at 1 μ M produces rapid and dramatic enhancement of polymerized F-actin and myosin light chain phosphorylation at the cell periphery, meanwhile, maximal barrier enhancement is observed with 1 μ M S1P that peaks after 10–20 min and is sustained for hours. Consistent with the conceptual framework that barrier regulation is intimately linked

to the cytoskeleton, changes in the actin cytoskeleton were essential for S1P-mediated barrier enhancement as cytochalasin B, an actin depolymerizing agent, and latrunculin B, which inhibits actin polymerization, both prevent the barrier-enhancing effects of S1P (Garcia et al. 2001). Recently, Arce et al. characterized the structural and mechanical changes in the cytoskeleton of cultured human pulmonary artery ECs in response to S1P, and they found the elastic modulus, an indicator of underlying structural force, is significantly elevated at the peripheral region of the cell by S1P treatment (Arce et al. 2008). These studies suggest a critical role of dynamic actin assembly/disassembly and subsequent cortical redistribution in mediating S1P-induced barrier enhancement.

The actin-associated cytoskeletal proteins, cortactin and myosin light chain kinase (MLCK), also play a role in the augmentation of EC barrier function induced by S1P. Cortactin is involved in stimulating actin polymerization and cortical actin rearrangement, and tyrosine phosphorylation of cortactin is seen after stimuli that cause cytoskeletal rearrangement (Owen et al. 2007; Belvitch and Dudek 2012). MLCK are a family of soluble protein kinases that function principally to phosphorylate myosin light chain (MLC). MLC phosphorylation enables actin-myosin interaction, leading to formation of stress fibers and cell contraction (Takashima 2009). Exposure of ECs to S1P produces rapid and significant translocation of cortactin from the cytoplasm to a peripheral cortical distribution. In ECs, cortactin depletion by antisense oligonucleotide techniques results in a 50 % inhibition of peak S1P barrier enhancement, whereas overexpressing wild-type cortactin showed enhanced transmonolayer electrical resistance (TER) after S1P treatment (Dudek et al. 2004). Similar to cortactin, when exposed to physiologic levels of S1P, MLCK is rapidly redistributed to areas of active membrane ruffling and directly bind to the cortactin Src homology 3 domain. The interaction of cortactin and MLCK appears to be necessary for optimal S1P-induced barrier enhancement since cortactin blocking peptide inhibits S1P-induced MLC phosphorylation and peak S1P-induced TER values.

The effect of S1P on these actin-dependent processes is mediated, in large part, by the Rho family of small GTPases. The Rho family of GTPases (Rho, Rac, and Cdc42) is a group of regulatory molecules that link surface receptors to downstream effectors regulating actin cytoskeletal structure (Spiering and Hodgson 2011). Rho, Rac, and Cdc42 function as molecular switches and promote the formation of stress fibers, lamellipodia, and filopodia, respectively, by catalyzing specific types of actin polymerization. The primary S1P receptors expressed in EC are S1P₁, S1P₂ and S1P₃ (Ancellin et al. 2002), which exhibit distinct coupling to Rho family GTPases. S1P₁ activates Rac through a mechanism that requires *Gai*-dependent activation of PI3-Kinase. In contrast, S1P₂ activates Rho through *Ga*12/13 and RGS family of Rho-specific guanine nucleotide exchange factors (GEFs) that interact with this G protein. S1P₃ can activate Rac through *Gai*, but also couples to *Gaq*/11, and activates Rho through the Trio family of Rho-GEFs (Oo et al. 2011; Blaho and Hla 2011; Sanchez et al. 2003; Hla 2003). Rac activity is required for S1P-induced adherens junction assembly and cytoskeleton rearrangement (Lee et al. 1999). S1P preferentially activates Rac via S1P₁ in a pertussis toxin-sensitive fashion and enhances barrier integrity. Overexpression of constitutively active Rac enhances peripheral actin polymerization in the cortical ring, and Rac activation induces lamellipodia formation, membrane ruffling, the

formation of cortical actin filaments, and the spreading of ECs (Garcia et al. 2001; Dudek et al. 2004). While inhibition of Rac GTPase leads to increased monolayer permeability and enhances the thrombin-mediated barrier dysfunction response through a variety of signaling proteins (Wojciak-Stothard et al. 2001). Microinjection of dominative negative Rac into ECs dramatically diminishes S1P-induced VE-cadherin and β -catenin enrichment at cell–cell junctions, while overexpression of active Rac reproduces changes in the cortical actin similar to those evoked by S1P (Lee et al. 1999). Moreover, reduction of Rac expression by siRNA significantly attenuates the S1P TER response (Dudek et al. 2007). The S1P-stimulated cytoskeletal rearrangement and barrier protection are highly dependent on the exact S1P concentrations utilized. Rac GTPases are rapidly activated by physiologic concentrations of S1P (10 nM–2 μ M) producing barrier enhancement, whereas higher concentrations of S1P result in S1P₃-dependent RhoA-mediated signaling and increased barrier permeability (Shikata et al. 2003).

3.2 S1P Signaling to Endothelial Junctions

In ECs, among the three types of intercellular junctions are adherens junctions (AJ or zonula adherens), tight junctions (TJ or zonula occludens), and gap junctions (GJ). In general, AJ and TJ contribute to the structural integrity of the endothelium (Dejana 2004; Bazzoni and Dejana 2004). Vascular endothelial cadherin (VE-cadherin) is the major structural protein of adherens junctions. Stability of VE-cadherin at adherens junctions, which is controlled by binding to catenins, especially to p120-catenin, is critical to the maintenance of endothelial permeability and integrity (Venkiteswaran et al. 2002). Targeted disruption of VE-cadherin gene or truncation of β -catenin binding domain of VE-cadherin in mice causes lethality at E9.5 days of gestation due to immature vascular development (Carmeliet et al. 1999). In a mouse model, injection of anti-VE-cadherin antibodies induces a marked increase in pulmonary vascular permeability, but a similar effect is not observed in the brain vasculature (Corada et al. 1999). Furthermore, endothelial-specific deletion of β -catenin is embryonic lethal starting at E11.5, presenting with vascular insufficiency, including reduced numbers of endothelial junctions, hemorrhage, and fluid extravasation (Cattellino et al. 2003).

Our studies showed that in confluent human umbilical vein endothelial cells (HUVEC), S1P significantly increases the abundance of VE-cadherin and β -catenin at the cell–cell contact regions and enhances AJ assembly. While the translocation of VE-cadherin to cell–cell contact regions in HUVEC after treatment of S1P was attenuated by microinjection of oligonucleotides designed to interrupt S1P₁ and S1P₃ receptor expression (Lee et al. 1999). Overexpression of S1P₁ in HEK293 cells markedly increases the expression level of P-cadherin and E-cadherin, but not α -catenin and β -catenin, and induces formation of well-developed adherens junctions in a manner dependent on S1P and the small guanine nucleotide binding protein Rho (Lee et al. 1998). Furthermore, S1P₁ silencing leads to a reduction in expression of both VE-cadherin and platelet-endothelial cell adhesion molecule-1 (PECAM-1), and the degree of S1P₁ knockdown was correlated with the extent of suppression of VE-cadherin and PECAM-1 (Krump-Konvalinkova et al. 2005). Recently, our studies demonstrate that in vivo S1P₁ function is required for adherens junction stability in the developing retinal vasculature. Lack of S1P₁ promoted junctional destabilization, as evidenced by increased tyrosine phosphorylation, trypsin sensitivity of the extracellular

domain of VE-cadherin (Jung et al. 2012). Gaengel et al. also reported that S1P₁ signaling positively and directly regulate the level of VE-cadherin at endothelial junctions, an effect that overrides the negative effect of VEGF on junctional VE-cadherin concentrations (Gaengel et al. 2012). However, the direct functional role of VE-cadherin in mediating S1P-induced endothelial barrier enhancement is complex and still poorly characterized. A recent study indicates that VE-cadherin may not be involved in the rapid and immediate effects of S1P on the barrier regulation as shown through TER studies but may play a role in the delayed onset-sustained effects of S1P on barrier enhancement (Pappu et al. 2007; Xu et al. 2007).

In addition to enhancing adherens junction assembly, there is also evidence that S1P assists in the formation of endothelial tight junctions. The TJ are positioned on the outer leaflets of the lateral membranes between adjacent cells. The TJ anchors into the actin cytoskeleton through the interaction and binding of the occludins, claudins, and junctional adhesion molecules (JAM) with the zona occludens proteins (ZO-1, ZO-2, or ZO-3) (Bazzoni and Dejana 2004). Following stimulation by S1P, ZO-1 is reassigned to the lamellipodia and to the cell-cell junctions via the S1P₁/Gi/Akt/Rac pathway, while the enhanced barrier function induced by S1P is attenuated by siRNA downregulation of ZO-1 expression (Lee et al. 2006). Thus, like the AJ, the TJ also plays an important role in S1P-mediated barrier regulation. Whether this mechanism is important in tissues with high vascular barrier, i.e., CNS, testis, retina is not known.

4 Sphingosine 1-Phosphate Regulates Vascular Leak in Disease Models

One of the main pathophysiological mechanisms involved in the genesis of various vascular disease conditions is endothelial dysfunction. Conditions ranging from inflammation, atherosclerosis, anaphylaxis, Ischemia-reperfusion injury, and cerebrovascular disease have a basis in endothelial barrier imbalance and dys-regulation (Mullin et al. 2005). As presenting at high concentrations in plasma, S1P is important to maintain vascular integrity and regulate vascular leak. Indeed, mice selectively lacking S1P in plasma (pS1Pless mice) and mice with degradation of S1P₁ in vivo exhibit basal vascular leak and increased local response to leak-inducing agents (Camerer et al. 2009; Oo et al. 2011; Karuna et al. 2011; Argraves et al. 2011; Lee et al. 2011).

4.1 Acute Lung Injury

Acute lung injury (ALI) is a devastating inflammatory lung disease characterized by a marked increase in vascular permeability which is often exacerbated by the mechanical ventilation (Matthay et al. 2012). Intratracheal administration of lipopolysaccharide (LPS) is a well-characterized experimental model to mimic the clinical presentation of ALI. In an isolated perfused murine lung model, intravenous administration of S1P significantly reduces the inflammatory histological changes produced by LPS and attenuates neutrophil infiltration in lung parenchyma. Similarly, intraperitoneal injection of FTY720, the S1P analog, significantly decreases LPS-induced pulmonary microvascular leakage (Peng et al. 2004; McVerry et al. 2004). Use of a large animal canine model allowed investigation of regional lung changes in ALI and the effect of S1P on these changes. In a canine model of ALI, Intravenously delivered S1P also significantly attenuated both alveolar and vascular

barrier dysfunction (Peng et al. 2004; McVerry et al. 2004). Moreover, S1P also protected against intrabronchial LPS-induced ALI in a canine model. S1P attenuated the formation of shunt fraction and both the presence of protein and neutrophils in bronchoalveolar lavage (BAL) fluid compared to vehicle controls (Szczepaniak et al. 2008). Recently, Zhao et al. also reported that inhibition of S1P lyase resulted in increased S1P levels in lung tissue and bronchoalveolar lavage fluids and reduced lung injury and inflammation (Zhao et al. 2011). However, there are differential effects for S1P receptors on airway and vascular barrier function in the murine model of ALI. At physiologically relevant concentrations, S1P is barrier protective via ligation of S1P₁, regardless of delivery via intratracheal or intravenous routes. The activation of S1P₂ and S1P₃ receptors, however, contributes to alveolar and vascular barrier disruption, whereas the targeted deletion or silencing of S1P₂ and S1P₃ was found to be beneficial (Sammani et al. 2010; Peng et al. 2004; McVerry et al. 2004; Zhang et al. 2013; Cui et al. 2013). It is also thought that S1P₂ and S1P₃ are responsible for the increased permeability that is observed after administration of higher doses of S1P or SEW2871 (Sammani et al. 2010).

4.2 Anaphylaxis

Anaphylaxis is a serious allergic response with a rapid onset. The release of inflammatory mediators like histamine subsequently impairs the function of multiple organ systems and increased vascular permeability and fluid extravasation is a known complication (De Bisschop and Bellou 2012). There is a strong association between the plasma concentration of S1P and the concentration of circulating histamine after the anaphylactic response (Olivera et al. 2007). Indeed, *Sphk1*-deficient mice had lower S1P concentrations in blood compared to wild-type mice, while increased S1P concentrations in blood of *Sphk2*-deficient mice resulted in a faster recovery from an anaphylactic shock due to enhanced histamine clearance in blood (Olivera et al. 2007). Furthermore, pS1Pless mice displayed increased vascular leak and impaired survival after anaphylaxis, administration of platelet-activating factor (PAF) or histamine, and exposure to related inflammatory challenges. Increased leak was associated with increased interendothelial cell gaps in venules and was reversed by transfusion with wild-type erythrocytes (which restored plasma S1P levels) and by acute treatment with an agonist for the S1P₁ (Camerer et al. 2009). Thus the plasma S1P-endothelial S1P₁ axis plays a central role in maintaining vascular barrier integrity. In contrast to S1P₁, it has been shown that pharmacologic blockade of S1P₂ reduced histamine-induced vascular leakage, and genetic deletion of S1P₂ attenuated vascular leakage and hypothermia in a mouse model of anaphylaxis after antigen challenge (Oskeritzian et al. 2010; Lee et al. 2009). Our studies also showed that S1P₂ activation in endothelial cells increases vascular permeability. The balance of S1P₁ and S1P₂ receptors in the endothelium may determine the regulation of vascular permeability by S1P (Sanchez et al. 2007). More recently, Zhang et al. also reported that inhibition of S1P₂ signaling dramatically decreased PLS-induced vascular permeability (Zhang et al. 2013; Cui et al. 2013). However, a recent study showed that S1P₂ protects mice from vascular barrier disruption elicited by either antigen challenge or PAF injection and that this protective effect of S1P₂ is mediated through suppression of anaphylaxis-associated endothelial nitric oxide synthase (eNOS) stimulation (Cui et al. 2013). Thus the in vivo role of S1P₂ in inflammatory conditions such as sepsis and anaphylaxis is not yet fully understood.

4.3 Ischemia-Reperfusion Injury

Ischemia-reperfusion (I/R) injury is encountered in a variety of settings from disease states such as transplantation, stroke, hemorrhage, cardiopulmonary bypass, and aneurysm repair. Microvascular dysfunction mediates many of the local and systemic consequences of I/R injury, while disruption of the endothelial barrier and increased hydraulic conductivity lead to compression of the vessel by interstitial edema (Seal and Gewertz 2005). In a rat model of orthotopic left lung transplantation, Okazaki et al. showed that S1P treatment of lung recipients just prior to graft reperfusion improves lung function. In comparison to vehicle-treated grafts, S1P-mediated preservation of lung graft function was associated with markedly less tissue injury as evidenced by significantly reduced vascular permeability, inflammatory cell infiltration, and EC apoptosis (Okazaki et al. 2007). Furthermore, in another report of lung I/R injury via pulmonary artery ligation and subsequent reperfusion, animals pretreated with S1P exhibited reduced BAL, inflammatory cells, BAL neutrophils, and BAL albumin content compared to controls (Moreno-Vinasco et al. 2008). Hepatic I/R injury that frequently complicates acute kidney injury (AKI) showed elevated inflammatory cytokines and increased vascular permeability during the perioperative period, while pretreatment with S1P resulted in an attenuation of systemic inflammation and endothelial injury, suggesting modulation of the S1P signaling might have some therapeutic potential in hepatic IR injury-induced kidney injury (Belvitch and Dudek 2012; Lee et al. 2011). Moreover, recent studies showed there is a highly significant inverse relationship between the level of S1P in the high-density lipoprotein (HDL) containing fraction of serum and the occurrence of ischemic heart disease (Argraves et al. 2011; Egom 2013). Oyama et al. showed that either local exogenous S1P administration or endogenous S1P overproduction promotes post-ischemic angiogenesis and blood flow recovery mouse ischemic hindlimbs (Oyama et al. 2008). Furthermore, our group reported that the S1P in the extracellular milieu, generated by the overexpression of Sphk1, induced angiogenesis and vascular maturation (Ancellin et al. 2002). All these studies suggest the potential usefulness of S1P as an angiogenic therapeutic agent in I/R injury.

4.4 FTY720 in the Pathology of Vascular Barrier Dysfunction

FTY720 is a sphingosine analog and is phosphorylated by sphingosine kinase-2–FTY720-phosphate, which is an agonist of 4 out of 5 S1P receptors. FTY720 has been approved by the United States Food and Drug Administration in 2010 for the treatment of multiple sclerosis (Pitman et al. 2012). The clinical availability of FTY720 makes it attractive as a potential mediator for patients with vascular barrier dysfunction. Indeed, a single intraperitoneal injection of FTY720 significantly attenuated murine pulmonary injury after LPS administration (Peng et al. 2004; McVerry et al. 2004). Similarly, low concentrations of FTY720 (0.1 mg/kg) reduced lung permeability in mechanically ventilated mice (Christoffersen et al. 2011; Muller et al. 2011). However, there are limitations to the therapeutic utility of FTY720 in vascular barrier dysfunction. Prolonged exposure to FTY720 resulted in the downregulation of S1P₁ on the EC surface and decreased responses to S1P (Krump-Konvalinkova et al. 2008). An administration of high concentrations FTY720 (0.5–5.0 mg/kg) to mice induced a dose-dependent S1P₁ degradation and an increase in vascular permeability (Shea et al. 2010). This in vivo barrier-disruptive effect of

high-dose FTY720 is in contrast to its barrier protective effect observed in vitro (Sanchez et al. 2003; Berdyshev et al. 2009).

5 Endothelium-Protective Function of Apolipoprotein M/HDL-Bound S1P

The concentration of S1P in the plasma ranges between 200 and 1,000 nM, and most of the S1P in plasma is protein-bound. The majority of plasma S1P (~60 %) is bound to HDL, whereas ~30 % is bound to albumin and a minor fraction to very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) (Murata et al. 2000b; Murata et al. 2000a; Karuna et al. 2011). It was unknown how S1P binds to HDL, until apolipoprotein M (ApoM) was revealed as a carrier of S1P on HDL particle (Christoffersen et al. 2011) and the linkage of ApoM/HDL-bound S1P and endothelial protection was explored. ApoM is a 25 kDa protein predominantly associated with HDL via a retained hydrophobic signal peptide (Christoffersen et al. 2008). The plasma ApoM concentration is approximately 0.9 $\mu\text{mol/l}$, and more than 95 % is bound to HDL (the remaining ApoM is bound to LDL and VLDL) (Christoffersen et al. 2006). *ApoM*^{-/-} mice have approximately 46 % reduced plasma S1P levels compared with wild-type mice, remarkably, S1P is absent in HDL from *ApoM*^{-/-} mice. Moreover, S1P is increased approximately by 71 and 267 % in mice models with either 2-fold or 10-fold increase of plasma ApoM, respectively. Also, the S1P content of HDL is confined to the ApoM-containing particles in human plasma (Christoffersen et al. 2011). Thus ApoM was defined as a carrier of S1P in HDL.

Growing evidence indicates that HDL-associated S1P is responsible for the beneficial effects on vascular integrity. HDL-associated S1P was reported to promote endothelial barrier via Gi-coupled S1P receptors and the Akt signaling pathway (Argaves et al. 2008). In addition, S1P was identified as one of the principal bioactive lysophospholipids in HDL which is responsible for about 60 % of the vasodilatory effect of HDL in isolated aortae ex vivo (Nofer et al. 2004). Moreover, the regulation of endothelial barrier by protein-bound S1P is carrier-dependent. Wilkerson et al. indicate that the duration of the barrier promotion elicited by HDL-S1P lasted longer than albumin-S1P, which may due to the specific effects of HDL-S1P on S1P₁ trafficking that prolong S1P-S1P₁ signaling involving persistent activation of Akt and eNOS (Wilkerson et al. 2012). ApoM was recently reported to mediate the S1P-dependent vascular protective effects of HDL by delivering S1P to the S1P₁ receptor (Christoffersen et al. 2011). S1P carried by ApoM in the HDL fraction has an important role in preserving vascular integrity, which proved that ApoM-containing HDL (with S1P) is better in activating and inducing EC migration, and formation of endothelial adherens junctions than ApoM-free HDL (without S1P). Interestingly, *ApoM*^{-/-} mice also display vascular leakage in the lungs, accompanying decreased plasma S1P (Christoffersen et al. 2011). Recently, the S1P₁ receptor was crystallized (Hanson et al. 2012). The structural information forms a new basis for understanding the interaction between ApoM-containing HDL and release of S1P from the calyx into the binding pocket of the S1P₁.

6 Conclusions and Future Perspectives

These studies have highlighted the generality of the regulation of endothelial cell barrier function by S1P. Future challenges include further defining molecular mechanisms which

are of fundamental importance in vascular biology and therapeutic application of this knowledge base to many pathological functions in which vascular barrier function is dysregulated, including sepsis, anaphylaxis, acute lung injury, inflammation, stroke, Dengue hemorrhagic fever, etc.

Abbreviations

AJ	Adherens junctions
AKI	Acute kidney injury
ALI	Acute lung injury
ApoM	Apolipoprotein M
BAL	Bronchoalveolar lavage
EC	Endothelial cells
eNOS	Endothelial nitric oxide synthase
GEFs	Guanine nucleotide exchange factors
GJ	Gap junctions
HDL	High-density lipoprotein
HUVEC	Human umbilical vein endothelial cells
I/R	Ischemia-reperfusion
JAM	Junctional adhesion molecules
LDL	Low-density lipoprotein
LPP3	Lysophospholipid phosphatase 3
LPs	Lysophospholipids
LPS	Lipopolysaccharide
MLC	Myosin light chain
MLCK	Myosin light chain kinase
PAF	Platelet-activating factor
PE	Phosphatidylethanolamine
PECAM-1	Platelet-endothelial cell adhesion molecule-1
RBC	Red blood cells
S1P	Sphingosine 1-phosphate
SM	Sphingomyelin
Spns2	Spinster 2
SPP	S1P phosphatases
TER	Transmonolayer electrical resistance

TJ	Tight junctions
VE-cadherin	Vascular endothelial cadherin
VLDL	Very low-density lipoprotein
ZO	Zona occludens proteins

References

- Allende ML, Sasaki T, Kawai H, Olivera A, Mi Y, van Echten-Deckert G, Hajdu R, Rosenbach M, Keohane CA, Mandala S, Spiegel S, Proia RL. Mice deficient in sphingosine kinase 1 are rendered lymphopenic by FTY720. *J Biol Chem.* 2004; 279(50):52487–52492. M406512200 [pii]. 10.1074/jbc.M406512200 [PubMed: 15459201]
- Ancellin N, Colmont C, Su J, Li Q, Mittereder N, Chae SS, Stefansson S, Liao G, Hla T. Extracellular export of sphingosine kinase-1 enzyme. Sphingosine 1-phosphate generation and the induction of angiogenic vascular maturation. *J Biol Chem.* 2002; 277(8):6667–6675. M102841200 [pii]. 10.1074/jbc.M102841200 [PubMed: 11741921]
- Anelli V, Bassi R, Tettamanti G, Viani P, Riboni L. Extracellular release of newly synthesized sphingosine-1-phosphate by cerebellar granule cells and astrocytes. *J Neurochem.* 2005; 92(5):1204–1215. JNC2955 [pii]. 10.1111/j.1471-4159.2004.02955.x [PubMed: 15715670]
- Arce FT, Whitlock JL, Birukova AA, Birukov KG, Arnsdorf MF, Lal R, Garcia JG, Dudek SM. Regulation of the micromechanical properties of pulmonary endothelium by S1P and thrombin: role of cortactin. *Biophys J.* 2008; 95(2):886–894. S0006-3495(08)70263-7 [pii]. 10.1529/biophysj.107.127167 [PubMed: 18408039]
- Argaves KM, Gazzolo PJ, Groh EM, Wilkerson BA, Matsuura BS, Twal WO, Hammad SM, Argaves WS. High density lipoprotein-associated sphingosine 1-phosphate promotes endothelial barrier function. *J Biol Chem.* 2008; 283(36):25074–25081. M801214200 [pii]. 10.1074/jbc.M801214200 [PubMed: 18606817]
- Argaves KM, Sethi AA, Gazzolo PJ, Wilkerson BA, Remaley AT, Tybjaerg-Hansen A, Nordestgaard BG, Yeatts SD, Nicholas KS, Barth JL, Argaves WS. S1P, dihydro-S1P and C24:1-ceramide levels in the HDL-containing fraction of serum inversely correlate with occurrence of ischemic heart disease. *Lipids Health Dis.* 2011; 10:70. 1476-511X-10-70 [pii]. 10.1186/1476-511X-10-70 [PubMed: 21554699]
- Bandhuvula P, Saba JD. Sphingosine-1-phosphate lyase in immunity and cancer: silencing the siren. *Trends Mol Med.* 2007; 13(5):210–217. S1471-4914(07)00060-3 [pii]. 10.1016/j.molmed.2007.03.005 [PubMed: 17416206]
- Bassi R, Anelli V, Giussani P, Tettamanti G, Viani P, Riboni L. Sphingosine-1-phosphate is released by cerebellar astrocytes in response to bFGF and induces astrocyte proliferation through Gi-protein-coupled receptors. *Glia.* 2006; 53(6):621–630. 10.1002/glia.20324 [PubMed: 16470810]
- Bazzoni G, Dejana E. Endothelial cell-to-cell junctions: molecular organization and role in vascular homeostasis. *Physiol Rev.* 2004; 84(3):869–901. 84/3/869 [pii]. 10.1152/physrev.00035.2003 [PubMed: 15269339]
- Belvitch P, Dudek SM. Role of FAK in S1P-regulated endothelial permeability. *Microvasc Res.* 2012; 83(1):22–30. S0026-2862(11)00158-0 [pii]. 10.1016/j.mvr.2011.08.012 [PubMed: 21925517]
- Berdyshev EV, Gorshkova I, Skobeleva A, Bittman R, Lu X, Dudek SM, Mirzapoiazova T, Garcia JG, Natarajan V. FTY720 inhibits ceramide synthases and up-regulates dihydrosphingosine 1-phosphate formation in human lung endothelial cells. *J Biol Chem.* 2009; 284(9):5467–5477. M805186200 [pii]. 10.1074/jbc.M805186200 [PubMed: 19119142]
- Berdyshev EV, Gorshkova IA, Garcia JG, Natarajan V, Hubbard WC. Quantitative analysis of sphingoid base-1-phosphates as bisacetylated derivatives by liquid chromatography-tandem mass spectrometry. *Anal Biochem.* 2005; 339(1):129–136. S0003-2697(04)00975-3 [pii]. 10.1016/j.ab.2004.12.006 [PubMed: 15766719]

- Blaho VA, Hla T. Regulation of mammalian physiology, development, and disease by the sphingosine 1-phosphate and lysophosphatidic acid receptors. *Chem Rev.* 2011; 111(10):6299–6320.10.1021/cr200273u [PubMed: 21939239]
- Blondeau N, Lai Y, Tyndall S, Popolo M, Topalkara K, Pru JK, Zhang L, Kim H, Liao JK, Ding K, Waeber C. Distribution of sphingosine kinase activity and mRNA in rodent brain. *J Neurochem.* 2007; 103(2):509–517. JNC4755 [pii]. 10.1111/j.1471-4159.2007.04755.x [PubMed: 17623044]
- Bode C, Sensken SC, Peest U, Beutel G, Thol F, Levkau B, Li Z, Bittman R, Huang T, Tolle M, van der Giet M, Graler MH. Erythrocytes serve as a reservoir for cellular and extracellular sphingosine 1-phosphate. *J Cell Biochem.* 2010; 109(6):1232–1243.10.1002/jcb.22507 [PubMed: 20186882]
- Bogatcheva NV, Verin AD. The role of cytoskeleton in the regulation of vascular endothelial barrier function. *Microvasc Res.* 2008; 76(3):202–207. S0026-2862(08)00095-2 [pii]. 10.1016/j.mvr.2008.06.003 [PubMed: 18657550]
- Caligan TB, Peters K, Ou J, Wang E, Saba J, Merrill AH Jr. A high-performance liquid chromatographic method to measure sphingosine 1-phosphate and related compounds from sphingosine kinase assays and other biological samples. *Anal Biochem.* 2000; 281(1):36–44. S0003-2697(00)94555-X [pii]. 10.1006/abio.2000.4555 [PubMed: 10847608]
- 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. *J Clin Invest.* 2009; 119(7):1871–1879. [PubMed: 19603543]
- Carmeliet P, Lampugnani MG, Moons L, Breviario F, Compernelle V, Bono F, Balconi G, Spagnuolo R, Oosthuysen B, Dewerchin M, Zanetti A, Angellilo A, Mattot V, Nuyens D, Lutgens E, Clotman F, de Ruiter MC, Gittenberger-de Groot A, Poelmann R, Lupu F, Herbert JM, Collen D, Dejana E. Targeted deficiency or cytosolic truncation of the VE-cadherin gene in mice impairs VEGF-mediated endothelial survival and angiogenesis. *Cell.* 1999; 98(2):147–157. S0092-8674(00)81010-7 [pii]. [PubMed: 10428027]
- Cattellino A, Liebner S, Gallini R, Zanetti A, Balconi G, Corsi A, Bianco P, Wolburg H, Moore R, Oreda B, Kemler R, Dejana E. The conditional inactivation of the beta-catenin gene in endothelial cells causes a defective vascular pattern and increased vascular fragility. *J Cell Biol.* 2003; 162(6):1111–1122. jcb.200212157 [pii]. 10.1083/jcb.200212157 [PubMed: 12975353]
- Christoffersen C, Ahnstrom J, Axler O, Christensen EI, Dahlback B, Nielsen LB. The signal peptide anchors apolipoprotein M in plasma lipoproteins and prevents rapid clearance of apolipoprotein M from plasma. *J Biol Chem.* 2008; 283(27):18765–18772. M800695200 [pii]. 10.1074/jbc.M800695200 [PubMed: 18460466]
- Christoffersen C, Nielsen LB, Axler O, Andersson A, Johnsen AH, Dahlback B. Isolation and characterization of human apolipoprotein M-containing lipoproteins. *J Lipid Res.* 2006; 47(8):1833–1843. M600055-JLR200 [pii]. 10.1194/jlr.M600055-JLR200 [PubMed: 16682745]
- 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. *Proc Natl Acad Sci USA.* 2011; 108(23):9613–9618. 1103187108 [pii]. 10.1073/pnas.1103187108 [PubMed: 21606363]
- Chun J, Goetzl EJ, Hla T, Igarashi Y, Lynch KR, Moolenaar W, Pyne S, Tigyi G. International Union of Pharmacology. XXXIV. Lysophospholipid receptor nomenclature. *Pharmacol Rev.* 2002; 54(2):265–269. [PubMed: 12037142]
- Chun J, Hla T, Lynch KR, Spiegel S, Moolenaar WH. International Union of Basic and Clinical Pharmacology. LXXVIII. Lysophospholipid receptor nomenclature. *Pharmacol Rev.* 2010; 62(4):579–587.10.1124/pr.110.003111 [PubMed: 21079037]
- Corada M, Mariotti M, Thurston G, Smith K, Kunkel R, Brockhaus M, Lampugnani MG, Martin-Padura I, Stoppacciaro A, Ruco L, McDonald DM, Ward PA, Dejana E. Vascular endothelial-cadherin is an important determinant of microvascular integrity in vivo. *Proc Natl Acad Sci USA.* 1999; 96(17):9815–9820. [PubMed: 10449777]
- Cui H, Okamoto Y, Yoshioka K, Du W, Takuwa N, Zhang W, Asano M, Shibamoto T, Takuwa Y. Sphingosine-1-phosphate receptor 2 protects against anaphylactic shock through suppression of endothelial nitric oxide synthase in mice. *J Allergy Clin Immunol.* 2013 S0091-6749(13)01151-2 [pii]. 10.1016/j.jaci.2013.07.026

- de Bisschop MB, Bellou A. Anaphylaxis. *Curr Opin Crit Care*. 2012; 18(4):308–317.10.1097/MCC.0b013e3283557a63 [PubMed: 22732436]
- Dejana E. Endothelial cell-cell junctions: happy together. *Nat Rev Mol Cell Biol*. 2004; 5(4):261–270. nrm1357 [pii]. 10.1038/nrm1357 [PubMed: 15071551]
- Dudek SM, Camp SM, Chiang ET, Singleton PA, Usatyuk PV, Zhao Y, Natarajan V, Garcia JG. Pulmonary endothelial cell barrier enhancement by FTY720 does not require the S1P1 receptor. *Cell Sig*. 2007; 19(8):1754–1764. S0898-6568(07)00098-8 [pii]. 10.1016/j.cellsig.2007.03.011
- Dudek SM, Jacobson JR, Chiang ET, Birukov KG, Wang P, Zhan X, Garcia JG. Pulmonary endothelial cell barrier enhancement by sphingosine 1-phosphate: roles for cortactin and myosin light chain kinase. *J Biol Chem*. 2004; 279(23):24692–24700. M313969200 [pii]. 10.1074/jbc.M313969200 [PubMed: 15056655]
- Edsall LC, Spiegel S. Enzymatic measurement of sphingosine 1-phosphate. *Anal Biochem*. 1999; 272(1):80–86. S0003-2697(99)94157-X [pii]. 10.1006/abio.1999.4157 [PubMed: 10405296]
- Egom EE. Sphingosine-1-phosphate signalling as a therapeutic target for patients with abnormal glucose metabolism and ischaemic heart disease. *J Cardiovasc Med (Hagerstown)*. 2013 in press. 10.2459/JCM.0b013e3283639755
- English D, Welch Z, Kovala AT, Harvey K, Volpert OV, Brindley DN, Garcia JG. Sphingosine 1-phosphate released from platelets during clotting accounts for the potent endothelial cell chemotactic activity of blood serum and provides a novel link between hemostasis and angiogenesis. *FASEB J*. 2000; 14(14):2255–2265. 14/14/2255 [pii]. 10.1096/fj.00-0134com [PubMed: 11053247]
- Fukuhara S, Simmons S, Kawamura S, Inoue A, Orba Y, Tokudome T, Sunden Y, Arai Y, Moriwaki K, Ishida J, Uemura A, Kiyonari H, Abe T, Fukamizu A, Hirashima M, Sawa H, Aoki J, Ishii M, Mochizuki N. The sphingosine-1-phosphate transporter Spns2 expressed on endothelial cells regulates lymphocyte trafficking in mice. *J Clin Invest*. 2012; 122(4):1416–1426. 60746 [pii]. 10.1172/JCI60746 [PubMed: 22406534]
- Gaengel K, Niaudet C, Hagikura K, Lavina B, Muhl L, Hofmann JJ, Ebarasi L, Nystrom S, Rymo S, Chen LL, Pang MF, Jin Y, Raschperger E, Roswall P, Schulte D, Benedito R, Larsson J, Hellstrom M, Fuxe J, Uhlen P, Adams R, Jakobsson L, Majumdar A, Vestweber D, Uv A, Betsholtz C. The sphingosine-1-phosphate receptor S1PR1 restricts sprouting angiogenesis by regulating the interplay between VE-cadherin and VEGFR2. *Dev Cell*. 2012; 23(3):587–599. S1534-5807(12)00373-5 [pii]. 10.1016/j.devcel.2012.08.005 [PubMed: 22975327]
- Garcia JG, Liu F, Verin AD, Birukova A, Dechert MA, Gerthoffer WT, Bamberg JR, English D. Sphingosine 1-phosphate promotes endothelial cell barrier integrity by Edg-dependent cytoskeletal rearrangement. *J Clin Invest*. 2001; 108(5):689–701.10.1172/JCI12450 [PubMed: 11544274]
- Hait NC, Oskeritzian CA, Paugh SW, Milstien S, Spiegel S. Sphingosine kinases, sphingosine 1-phosphate, apoptosis and diseases. *Biochim Biophys Acta*. 2006; 1758(12):2016–2026. S0005-2736(06)00313-0 [pii]. 10.1016/j.bbamem.2006.08.007 [PubMed: 16996023]
- Hanel P, Andreani P, Graler MH. Erythrocytes store and release sphingosine 1-phosphate in blood. *FASEB J*. 2007; 21(4):1202–1209. fj.06-7433com [pii]. 10.1096/fj.06-7433com [PubMed: 17215483]
- Hannun YA, Obeid LM. Principles of bioactive lipid signalling: lessons from sphingolipids. *Nat Rev Mol Cell Biol*. 2008; 9(2):139–150. nrm2329 [pii]. 10.1038/nrm2329 [PubMed: 18216770]
- Hanson MA, Roth CB, Jo E, Griffith MT, Scott FL, Reinhart G, Desale H, Clemons B, Cahalan SM, Schuerer SC, Sanna MG, Han GW, Kuhn P, Rosen H, Stevens RC. Crystal structure of a lipid G protein-coupled receptor. *Science*. 2012; 335(6070):851–855. 335/6070/851 [pii]. 10.1126/science.1215904 [PubMed: 22344443]
- 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 (7469):105–109.10.1038/nature12501 [PubMed: 23995678]
- Hisano Y, Kobayashi N, Yamaguchi A, Nishi T. Mouse SPNS2 functions as a sphingosine-1-phosphate transporter in vascular endothelial cells. *PLoS One*. 2012; 7(6):e38941. PONE-D-12-07568 [pii]. 10.1371/journal.pone.0038941 [PubMed: 22723910]

- Hla T. Signaling and biological actions of sphingosine 1-phosphate. *Pharmacol Res.* 2003; 47(5):401–407. S104366180300046X [pii]. [PubMed: 12676514]
- Hla T, Lee MJ, Ancellin N, Paik JH, Kluk MJ. Lysophospholipids–receptor revelations. *Science.* 2001; 294(5548):1875–1878. 294/5548/1875 [pii]. 10.1126/science.1065323 [PubMed: 11729304]
- Hla T, Venkataraman K, Michaud J. The vascular S1P gradient–cellular sources and biological significance. *Biochim Biophys Acta.* 2008; 1781(9):477–482. S1388-1981(08)00132-7 [pii]. 10.1016/j.bbailip.2008.07.003 [PubMed: 18674637]
- Igarashi N, Okada T, Hayashi S, Fujita T, Jahangeer S, Nakamura S. Sphingosine kinase 2 is a nuclear protein and inhibits DNA synthesis. *J Biol Chem.* 2003; 278(47):46832–46839. M306577200 [pii]. 10.1074/jbc.M306577200 [PubMed: 12954646]
- Ito K, Anada Y, Tani M, Ikeda M, Sano T, Kihara A, Igarashi Y. Lack of sphingosine 1-phosphate-degrading enzymes in erythrocytes. *Biochem Biophys Res Commun.* 2007; 357(1):212–217. S0006-291X(07)00607-9 [pii]. 10.1016/j.bbrc.2007.03.123 [PubMed: 17418101]
- Jessup W. Lipid metabolism: sources and stability of plasma sphingosine-1-phosphate. *Curr Opin Lipidol.* 2008; 19(5):543–544. 00041433-200810000-00017 [pii]. 10.1097/MOL.0b013e32830f4a90 [PubMed: 18769238]
- Jiang X, Han X. Characterization and direct quantitation of sphingoid base-1-phosphates from lipid extracts: a shotgun lipidomics approach. *J Lipid Res.* 2006; 47(8):1865–1873. D600012-JLR200 [pii]. 10.1194/jlr.D600012-JLR200 [PubMed: 16682747]
- Jung B, Obinata H, Galvani S, Mendelson K, Ding BS, Skoura A, Kinzel B, Brinkmann V, Rafii S, Evans T, Hla T. Flow-regulated endothelial S1P receptor-1 signaling sustains vascular development. *Dev Cell.* 2012; 23(3):600–610. S1534-5807(12)00334-6 [pii]. 10.1016/j.devcel.2012.07.015 [PubMed: 22975328]
- Karuna R, Park R, Othman A, Holleboom AG, Motazacker MM, Sutter I, Kuivenhoven JA, Rohrer L, Matile H, Hornemann T, Stoffel M, Rentsch KM, von Eckardstein A. Plasma levels of sphingosine-1-phosphate and apolipoprotein M in patients with monogenic disorders of HDL metabolism. *Atherosclerosis.* 2011; 219(2):855–863. S0021-9150(11)00853-7 [pii]. 10.1016/j.atherosclerosis.2011.08.049 [PubMed: 21944699]
- Kawahara A, Nishi T, Hisano Y, Fukui H, Yamaguchi A, Mochizuki N. The sphingolipid transporter spns2 functions in migration of zebrafish myocardial precursors. *Science.* 2009; 323(5913):524–527. 1167449 [pii]. 10.1126/science.1167449 [PubMed: 19074308]
- Krump-Konvalinkova V, Chwalla I, Siess W. FTY720 inhibits S1P-mediated endothelial healing: relationship to S1P1-receptor surface expression. *Biochem Biophys Res Commun.* 2008; 370(4):603–608. S0006-291X(08)00637-2 [pii]. 10.1016/j.bbrc.2008.03.144 [PubMed: 18402775]
- Krump-Konvalinkova V, Yasuda S, Rubic T, Makarova N, Mages J, Erl W, Vosseler C, Kirkpatrick CJ, Tigyi G, Siess W. Stable knock-down of the sphingosine 1-phosphate receptor S1P1 influences multiple functions of human endothelial cells. *Arterioscler Thromb Vasc Biol.* 2005; 25(3):546–552. 01.ATV.0000154360.36106.d9 [pii]. 10.1161/01.ATV.0000154360.36106.d9 [PubMed: 15618544]
- Kuhn R, Schwenk F, Aguet M, Rajewsky K. Inducible gene targeting in mice. *Science.* 1995; 269(5229):1427–1429. [PubMed: 7660125]
- Kurano M, Tsukamoto K, Ohkawa R, Hara M, Iino J, Kageyama Y, Ikeda H, Yatomi Y. Liver involvement in sphingosine 1-phosphate dynamism revealed by adenoviral hepatic overexpression of apolipoprotein M. *Atherosclerosis.* 2013; 229(1):102–109. S0021-9150(13)00266-9 [pii]. 10.1016/j.atherosclerosis.2013.04.024 [PubMed: 23664237]
- Le Stunff H, Peterson C, Thornton R, Milstien S, Mandala SM, Spiegel S. Characterization of murine sphingosine-1-phosphate phosphohydrolase. *J Biol Chem.* 2002; 277(11):8920–8927. M109968200 [pii]. 10.1074/jbc.M109968200 [PubMed: 11756451]
- Lee JF, Gordon S, Estrada R, Wang L, Siow DL, Wattenberg BW, Lominadze D, Lee MJ. Balance of S1P1 and S1P2 signaling regulates peripheral microvascular permeability in rat cremaster muscle vasculature. *Am J Physiol Heart Circ Physiol.* 2009; 296(1):H33–42. 00097.2008 [pii]. 10.1152/ajpheart.00097.2008 [PubMed: 19011048]
- Lee JF, Zeng Q, Ozaki H, Wang L, Hand AR, Hla T, Wang E, Lee MJ. Dual roles of tight junction-associated protein, zonula occludens-1, in sphingosine 1-phosphate-mediated endothelial

- chemotaxis and barrier integrity. *J Biol Chem*. 2006; 281(39):29190–29200. M604310200 [pii]. 10.1074/jbc.M604310200 [PubMed: 16891661]
- Lee MJ, Thangada S, Claffey KP, Ancellin N, Liu CH, Kluk M, Volpi M, Sha'afi RI, Hla T. Vascular endothelial cell adherens junction assembly and morphogenesis induced by sphingosine-1-phosphate. *Cell*. 1999; 99(3):301–312. S0092-8674(00)81661-X [pii]. [PubMed: 10555146]
- Lee MJ, Van Brocklyn JR, Thangada S, Liu CH, Hand AR, Menzeleev R, Spiegel S, Hla T. Sphingosine-1-phosphate as a ligand for the G protein-coupled receptor EDG-1. *Science*. 1998; 279(5356):1552–1555. [PubMed: 9488656]
- Lee SY, Kim DH, Sung SA, Kim MG, Cho WY, Kim HK, Jo SK. Sphingosine-1-phosphate reduces hepatic ischaemia/reperfusion-induced acute kidney injury through attenuation of endothelial injury in mice. *Nephrology (Carlton)*. 2011; 16(2):163–173. 10.1111/j.1440-1797.2010.01386.x [PubMed: 21272128]
- Matthay MA, Ware LB, Zimmerman GA. The acute respiratory distress syndrome. *J Clin Invest*. 2012; 122(8):2731–2740. 60331 [pii]. 10.1172/JCI60331 [PubMed: 22850883]
- McVerry BJ, Peng X, Hassoun PM, Sammani S, Simon BA, Garcia JG. Sphingosine 1-phosphate reduces vascular leak in murine and canine models of acute lung injury. *Am J Respir Crit Care Med*. 2004; 170(9):987–993. 200405-684OC [pii]. 10.1164/rccm.200405-684OC [PubMed: 15282202]
- Mendoza A, Breart B, Ramos-Perez WD, Pitt LA, Gobert M, Sunkara M, Lafaille JJ, Morris AJ, Schwab SR. The transporter Spns2 is required for secretion of lymph but not plasma sphingosine-1-phosphate. *Cell Rep*. 2012; 2(5):1104–1110. S2211-1247(12)00325-7 [pii]. 10.1016/j.celrep.2012.09.021 [PubMed: 23103166]
- Michel CC, Curry FE. Microvascular permeability. *Physiol Rev*. 1999; 79 (3):703–761. [PubMed: 10390517]
- Min JK, Yoo HS, Lee EY, Lee WJ, Lee YM. Simultaneous quantitative analysis of sphingoid base 1-phosphates in biological samples by o-phthalaldehyde precolumn derivatization after dephosphorylation with alkaline phosphatase. *Anal Biochem*. 2002; 303(2):167–175. S000326970295579X [pii]. 10.1006/abio.2002.5579 [PubMed: 11950216]
- Moolenaar WH, Hla T. SnapShot: bioactive lysophospholipids. *Cell*. 2012; 148(1–2):378–378. e372.10.1016/j.cell.2012.01.013 [PubMed: 22265422]
- Moreno-Vinasco L, Jacobson JR, Bonde P, Sammani S, Mirzapoiazova T, Vigneswaran WT, Garcia JGN. Attenuation of rodent lung ischemia-reperfusion injury by sphingosine 1-phosphate. *J Organ Dysfunction*. 2008; 4(2):106–114. 10.1080/17471060701505289
- Muller HC, Hocke AC, Hellwig K, Gutbier B, Peters H, Schonrock SM, Tschernig T, Schmiedl A, Hippenstiel S, N'Guessan PD, Rosseau S, Suttrop N, Witzernath M. The Sphingosine-1 Phosphate receptor agonist FTY720 dose dependently affected endothelial integrity in vitro and aggravated ventilator-induced lung injury in mice. *Pulm Pharmacol Ther*. 2011; 24(4):377–385. S1094-5539(11)00039-3 [pii]. 10.1016/j.pupt.2011.01.017 [PubMed: 21419858]
- Mullin JM, Agostino N, Rendon-Huerta E, Thornton JJ. Keynote review: epithelial and endothelial barriers in human disease. *Drug Discov Today*. 2005; 10(6):395–408. S1359644605033799 [pii]. 10.1016/S1359-6446(05)03379-9 [PubMed: 15808819]
- Murata N, Sato K, Kon J, Tomura H, Okajima F. Quantitative measurement of sphingosine 1-phosphate by radioreceptor-binding assay. *Anal Biochem*. 2000a; 282(1):115–120. S0003-2697(00)94580-9 [pii]. 10.1006/abio.2000.4580 [PubMed: 10860507]
- Murata N, Sato K, Kon J, Tomura H, Yanagita M, Kuwabara A, Ui M, Okajima F. Interaction of sphingosine 1-phosphate with plasma components, including lipoproteins, regulates the lipid receptor-mediated actions. *Biochem J*. 2000b; 352(Pt 3):809–815. [PubMed: 11104690]
- Nofer JR, van der Giet M, Tolle M, Wolinska I, von Wnuck Lipinski K, Baba HA, Tietge UJ, Godecke A, Ishii I, Kleuser B, Schafers M, Fobker M, Zidek W, Assmann G, Chun J, Levkau B. HDL induces NO-dependent vasorelaxation via the lysophospholipid receptor S1P3. *J Clin Invest*. 2004; 113(4):569–581. 10.1172/JCI18004 [PubMed: 14966566]
- Ogawa C, Kihara A, Gokoh M, Igarashi Y. Identification and characterization of a novel human sphingosine-1-phosphate phosphohydrolase, hSPP2. *J Biol Chem*. 2003; 278(2):1268–1272. M209514200 [pii]. 10.1074/jbc.M209514200 [PubMed: 12411432]

- Okazaki M, Kreisel F, Richardson SB, Kreisel D, Krupnick AS, Patterson GA, Gelman AE. Sphingosine 1-phosphate inhibits ischemia reperfusion injury following experimental lung transplantation. *Am J Transplant*. 2007; 7(4):751–758. AJT1710 [pii]. 10.1111/j.1600-6143.2006.01710.x [PubMed: 17391120]
- Olivera A, Mizugishi K, Tikhonova A, Ciaccia L, Odom S, Proia RL, Rivera J. The sphingosine kinase-sphingosine-1-phosphate axis is a determinant of mast cell function and anaphylaxis. *Immunity*. 2007; 26(3):287–297. S1074-7613(07)00174-4 [pii]. 10.1016/j.immuni.2007.02.008 [PubMed: 17346996]
- Olivera A, Spiegel S. Sphingosine-1-phosphate as second messenger in cell proliferation induced by PDGF and FCS mitogens. *Nature*. 1993; 365(6446):557–560.10.1038/365557a0 [PubMed: 8413613]
- Oo ML, Chang SH, Thangada S, Wu MT, Rezaul K, Blaho V, Hwang SI, Han DK, Hla T. Engagement of S1P(1)-degradative mechanisms leads to vascular leak in mice. *J Clin Invest*. 2011; 121(6):2290–2300. 45403 [pii]. 10.1172/JCI45403 [PubMed: 21555855]
- Oskeritzian CA, Price MM, Hait NC, Kapitonov D, Falanga YT, Morales JK, Ryan JJ, Milstien S, Spiegel S. Essential roles of sphingosine-1-phosphate receptor 2 in human mast cell activation, anaphylaxis, and pulmonary edema. *J Exp Med*. 2010; 207(3):465–474. jem.20091513 [pii]. 10.1084/jem.20091513 [PubMed: 20194630]
- Owen KA, Pixley FJ, Thomas KS, Vicente-Manzanares M, Ray BJ, Horwitz AF, Parsons JT, Beggs HE, Stanley ER, Bouton AH. Regulation of lamellipodial persistence, adhesion turnover, and motility in macrophages by focal adhesion kinase. *J Cell Biol*. 2007; 179(6):1275–1287. jcb.200708093 [pii]. 10.1083/jcb.200708093 [PubMed: 18070912]
- Oyama O, Sugimoto N, Qi X, Takuwa N, Mizugishi K, Koizumi J, Takuwa Y. The lysophospholipid mediator sphingosine-1-phosphate promotes angiogenesis in vivo in ischaemic hindlimbs of mice. *Cardiovasc Res*. 2008; 78(2):301–307. cvn002 [pii]. 10.1093/cvr/cvn002 [PubMed: 18187460]
- Paik JH, Chae S, Lee MJ, Thangada S, Hla T. Sphingosine 1-phosphate-induced endothelial cell migration requires the expression of EDG-1 and EDG-3 receptors and Rho-dependent activation of alpha vbeta3-and beta1-containing integrins. *J Biol Chem*. 2001; 276(15):11830–11837. M009422200 [pii]. 10.1074/jbc.M009422200 [PubMed: 11150298]
- Pappu R, Schwab SR, Cornelissen I, Pereira JP, Regard JB, Xu Y, Camerer E, Zheng YW, Huang Y, Cyster JG, Coughlin SR. Promotion of lymphocyte egress into blood and lymph by distinct sources of sphingosine-1-phosphate. *Science*. 2007; 316(5822):295–298. 1139221 [pii]. 10.1126/science.1139221 [PubMed: 17363629]
- Peng X, Hassoun PM, Sammani S, McVerry BJ, Burne MJ, Rabb H, Pearse D, Tudor RM, Garcia JG. Protective effects of sphingosine 1-phosphate in murine endotoxin-induced inflammatory lung injury. *Am J Respir Crit Care Med*. 2004; 169(11):1245–1251. 200309-1258OC [pii]. 10.1164/rccm.200309-1258OC [PubMed: 15020292]
- Pitman MR, Woodcock JM, Lopez AF, Pitson SM. Molecular targets of FTY720 (fingolimod). *Curr Mol Med*. 2012; 12(10):1207–1219. CMM-EPUB-20120723-1 [pii]. [PubMed: 22834825]
- Predescu SA, Predescu DN, Malik AB. Molecular determinants of endothelial transcytosis and their role in endothelial permeability. *Am J Physiol Lung Cell Mol Physiol*. 2007; 293(4):L823–842. 00436.2006 [pii]. 10.1152/ajplung.00436.2006 [PubMed: 17644753]
- Sammani S, Moreno-Vinasco L, Mirzapourzadeh T, Singleton PA, Chiang ET, Evenoski CL, Wang T, Mathew B, Husain A, Moitra J, Sun X, Nunez L, Jacobson JR, Dudek SM, Natarajan V, Garcia JG. Differential effects of sphingosine 1-phosphate receptors on airway and vascular barrier function in the murine lung. *Am J Respir Cell Mol Biol*. 2010; 43(4):394–402. 2009-0223OC [pii]. 10.1165/rcmb.2009-0223OC [PubMed: 19749179]
- Sanchez T, Estrada-Hernandez T, Paik JH, Wu MT, Venkataraman K, Brinkmann V, Claffey K, Hla T. Phosphorylation and action of the immunomodulator FTY720 inhibits vascular endothelial cell growth factor-induced vascular permeability. *J Biol Chem*. 2003; 278(47):47281–47290. M306896200 [pii]. 10.1074/jbc.M306896200 [PubMed: 12954648]
- Sanchez T, Skoura A, Wu MT, Casserly B, Harrington EO, Hla T. Induction of vascular permeability by the sphingosine-1-phosphate receptor-2 (S1P2R) and its downstream effectors ROCK and PTEN. *Arterioscler Thromb Vasc Biol*. 2007; 27(6):1312–1318. ATVB AHA.107.143735 [pii]. 10.1161/ATVB AHA.107.143735 [PubMed: 17431187]

- Seal JB, Gewertz BL. Vascular dysfunction in ischemia-reperfusion injury. *Ann Vasc Surg.* 2005; 19(4):572–584.10.1007/s10016-005-4616-7 [PubMed: 15981128]
- Shea BS, Brooks SF, Fontaine BA, Chun J, Luster AD, Tager AM. Prolonged exposure to sphingosine 1-phosphate receptor-1 agonists exacerbates vascular leak, fibrosis, and mortality after lung injury. *Am J Respir Cell Mol Biol.* 2010; 43(6):662–673. 2009-0345OC [pii]. 10.1165/rcmb.2009-0345OC [PubMed: 20081052]
- Shikata Y, Birukov KG, Garcia JG. S1P induces FA remodeling in human pulmonary endothelial cells: role of Rac, GIT1, FAK, and paxillin. *J Appl Physiol.* 2003; 94(3):1193–1203. 00690.2002 [pii]. 10.1152/japplphysiol.00690.2002 [PubMed: 12482769]
- Shivdasani RA, Rosenblatt MF, Zucker-Franklin D, Jackson CW, Hunt P, Saris CJ, Orkin SH. Transcription factor NF-E2 is required for platelet formation independent of the actions of thrombopoietin/MGDF in megakaryocyte development. *Cell.* 1995; 81(5):695–704. 0092-8674(95)90531-6 [pii]. [PubMed: 7774011]
- Spiering D, Hodgson L. Dynamics of the Rho-family small GTPases in actin regulation and motility. *Cell Adh Migr.* 2011; 5(2):170–180. 14403 [pii]. [PubMed: 21178402]
- Szczepaniak WS, Zhang Y, Hagerty S, Crow MT, Kesari P, Garcia JG, Choi AM, Simon BA, McVerry BJ. Sphingosine 1-phosphate rescues canine LPS-induced acute lung injury and alters systemic inflammatory cytokine production in vivo. *Transl Res.* 2008; 152(5):213–224. S1931-5244(08)00225-9 [pii]. 10.1016/j.trsl.2008.09.002 [PubMed: 19010292]
- Takashima S. Phosphorylation of myosin regulatory light chain by myosin light chain kinase, and muscle contraction. *Circ J.* 2009; 73(2):208–213. JST.JSTAGE/circj/CJ-08-1041 [pii]. [PubMed: 19110504]
- Tani M, Sano T, Ito M, Igarashi Y. Mechanisms of sphingosine and sphingosine 1-phosphate generation in human platelets. *J Lipid Res.* 2005; 46(11):2458–2467. M500268-JLR200 [pii]. 10.1194/jlr.M500268-JLR200 [PubMed: 16061940]
- Venkataraman K, Lee YM, Michaud J, Thangada S, Ai Y, Bonkovsky HL, Parikh NS, Habrukowich C, Hla T. Vascular endothelium as a contributor of plasma sphingosine 1-phosphate. *Circ Res.* 2008; 102(6):669–676. CIRCRESAHA. 107.165845 [pii]. 10.1161/CIRCRESAHA.107.165845 [PubMed: 18258856]
- Venkataraman K, Thangada S, Michaud J, Oo ML, Ai Y, Lee YM, Wu M, Parikh NS, Khan F, Proia RL, Hla T. Extracellular export of sphingosine kinase-1a contributes to the vascular S1P gradient. *Biochem J.* 2006; 397(3):461–471.10.1042/BJ20060251 [PubMed: 16623665]
- Venkiteswaran K, Xiao K, Summers S, Calkins CC, Vincent PA, Pumiglia K, Kowalczyk AP. Regulation of endothelial barrier function and growth by VE-cadherin, plakoglobin, and beta-catenin. *Am J Physiol Cell Physiol.* 2002; 283(3):C811–821.10.1152/ajpcell.00417.2001 [PubMed: 12176738]
- Waeber, C. Lysophospholipid receptors. John Wiley & Sons, Inc; New York: 2013. Sphingosine 1-Phosphate (S1P) signaling and the vasculature; p. 313-347.
- Wilkerson BA, Grass GD, Wing SB, Argraves WS, Argraves KM. Sphingosine 1-phosphate (S1P) carrier-dependent regulation of endothelial barrier: high density lipoprotein (HDL)-S1P prolongs endothelial barrier enhancement as compared with albumin-S1P via effects on levels, trafficking, and signaling of S1P1. *J Biol Chem.* 2012; 287(53):44645–44653. M112.423426 [pii]. 10.1074/jbc.M112.423426 [PubMed: 23135269]
- Wojciak-Stothard B, Potempa S, Eichholtz T, Ridley AJ. Rho and Rac but not Cdc42 regulate endothelial cell permeability. *J Cell Sci.* 2001; 114 (Pt 7):1343–1355. [PubMed: 11257000]
- Xu M, Waters CL, Hu C, Wysolmerski RB, Vincent PA, Minnear FL. Sphingosine 1-phosphate rapidly increases endothelial barrier function independently of VE-cadherin but requires cell spreading and Rho kinase. *Am J Physiol Cell Physiol.* 2007; 293(4):C1309–1318. 00014.2007 [pii]. 10.1152/ajpcell.00014.2007 [PubMed: 17670896]
- Yatomi Y, Igarashi Y, Yang L, Hisano N, Qi R, Asazuma N, Satoh K, Ozaki Y, Kume S. Sphingosine 1-phosphate, a bioactive sphingolipid abundantly stored in platelets, is a normal constituent of human plasma and serum. *J Biochem.* 1997a; 121(5):969–973. [PubMed: 9192741]
- Yatomi Y, Ozaki Y, Ohmori T, Igarashi Y. Sphingosine 1-phosphate: synthesis and release. *Prostaglandins Other Lipid Mediat.* 2001; 64(1–4):107–122. [PubMed: 11324700]

- Yatomi Y, Welch RJ, Igarashi Y. Distribution of sphingosine 1-phosphate, a bioactive sphingolipid, in rat tissues. *FEBS Lett.* 1997b; 404(2–3):173–174. S0014-5793(97)00121-X [pii]. [PubMed: 9119058]
- Zhang G, Yang L, Kim GS, Ryan K, Lu S, O'Donnell RK, Spokes K, Shapiro N, Aird WC, Kluk MJ, Yano K, Sanchez T. Critical role of sphingosine-1-phosphate receptor 2 (S1PR2) in acute vascular inflammation. *Blood.* 2013; 122(3):443–455. blood-2012-11-467191 [pii]. 10.1182/blood-2012-11-467191 [PubMed: 23723450]
- Zhao Y, Gorshkova IA, Berdyshev E, He D, Fu P, Ma W, Su Y, Usatyuk PV, Pendyala S, Oskouian B, Saba JD, Garcia JG, Natarajan V. Protection of LPS-induced murine acute lung injury by sphingosine-1-phosphate lyase suppression. *Am J Respir Cell Mol Biol.* 2011; 45(2):426–435. 2010-0422OC [pii]. 10.1165/rcmb.2010-0422OC [PubMed: 21148740]