S1P Control of Endothelial Integrity

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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.

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

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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

Contents

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 $S1P_{1\text{-}5}$, 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 ($S1P_1$, $S1P_2$, and $S1P_3$) are expressed in vascular tissues, whereas expression of the $S1P_4$ and $S1P_5$ 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 μM (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 SIP 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 GPIba (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 $\approx$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 μM) (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 μM 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 μM 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 $S1P_1$, $S1P_2$ and $S1P_3$ (Ancellin et al. 2002), which exhibit distinct coupling to Rho family GTPases. $S1P_1$ activates Rac through a mechanism that requires Gαi-dependent activation of PI3-Kinase. In contrast, $S1P_2$ activates Rho through Gα12/13 and RGS family of Rho-specific guanine nucleotide exchange factors (GEFs) that interact with this G protein. $S1P_3$ can activate Rac through Gαi, but also couples to Gαq/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 $S1P_1$ 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 μM) producing barrier enhancement, whereas higher concentrations of S1P result in $S1P_3$-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 (Cattelino 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 $S1P_1$ and $S1P_3$ receptor expression (Lee et al. 1999). Overexpression of $S1P_1$ 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, $S1P_1$ silencing leads to a reduction in expression of both VE-cadherin and platelet-endothelial cell adhesion molecule-1 (PECAM-1), and the degree of $S1P_1$ 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 $S1P_1$ function is required for adherens junction stability in the developing retinal vasculature. Lack of $S1P_1$ 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 $S1P_1$ 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 $S1P_1$/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 dysregulation (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 $S1P_1$ 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 $S1P_1$, regardless of delivery via intratracheal or intravenous routes. The activation of $S1P_2$ and $S1P_3$ receptors, however, contributes to alveolar and vascular barrier disruption, whereas the targeted deletion or silencing of $S1P_2$ and $S1P_3$ 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 $S1P_2$ and $S1P_3$ 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 $S1P_1$ (Camerer et al. 2009). Thus the plasma S1P-endothelial $S1P_1$ axis plays a central role in maintaining vascular barrier integrity. In contrast to $S1P_1$, it has been shown that pharmacologic blockade of $S1P_2$ reduced histamine-induced vascular leakage, and genetic deletion of $S1P_2$ 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 $S1P_2$ activation in endothelial cells increases vascular permeability. The balance of $S1P_1$ and $S1P_2$ 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 $S1P_2$ signaling dramatically decreased PLS-induced vascular permeability (Zhang et al. 2013; Cui et al. 2013). However, a recent study showed that $S1P_2$ protects mice from vascular barrier disruption elicited by either antigen challenge or PAF injection and that this protective effect of $S1P_2$ is mediated through suppression of anaphylaxis-associated endothelial nitric oxide synthase (eNOS) stimulation (Cui et al. 2013). Thus the in vivo role of $S1P_2$ 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 $S1P_1$ 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 $S1P_1$ 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 ($\sim$60 %) is bound to HDL, whereas $\sim$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 μmol/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 (Argraves 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 $S1P_1$ trafficking that prolong S1P-$S1P_1$ 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 $S1P_1$ 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 $S1P_1$ 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 $S1P_1$.

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.

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