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PROSPECTS

Endothelial Cell Barrier Regulation by Sphingosine 1-Phosphate

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Abstract Disruption of vascular barrier integrity markedly increases permeability to fluid and solute and is the central pathophysiologic mechanism of many inflammatory disease processes, including sepsis and acute lung injury (ALI). Dynamic control of the endothelial barrier involves complex signaling to the endothelial cytoskeleton and to adhesion complexes between neighboring cells and between cells and the underlying matrix. Sphingosine 1-phosphate (S1P), a biologically active lipid generated by hydrolysis of membrane lipids in activated platelets, organizes actin into a strong cortical ring and strengthens both intercellular and cell–matrix adherence. The mechanisms by which S1P increases endothelial barrier integrity remain the focus of intense basic research. The downstream structural changes induced by S1P interact to decrease vascular permeability to fluid and solute, which translates into a reduction lung edema formation in animal models of ALI, thus suggesting a potentially life-saving therapeutic role for vascular barrier modulation in critically ill patients. J. Cell. Biochem. 92: 1075–1085, 2004.

Key words: Rho GTPase; Rac GTPase; cadherin; endothelial permeability; cytoskeleton

Disruption of vascular barrier integrity results in marked increases in permeability to fluid and solute and is the central pathophysiologic mechanism of many inflammatory disease processes. Current concepts indicate that three main processes regulate the transport of fluid and macromolecules across the endothelium. Capillary beds such as in endocrine tissue or kidney contain fenestrae, small diaphragm-covered pores in the endothelial surface, which allow passive diffusion of fluid and particles from the luminal surface to the interstitium based upon ultrastructural features of the molecule such as size and charge. In contrast, transcytosis is an active process of albumin transport across the endothelium in which endocytic vesicles fuse with the luminal endothelial membrane in response to cell surface glycoprotein (gp60) receptor ligation [Minshall et al., 2002]. Through G_i protein mediated Src-family kinase activation, vesicles containing albumin bound to gp60 are endocytosed, transported across the cell, and deposited at the abluminal membrane where vesicle fusion allows depostion of albumin in the subendothelial compartment [Minshall et al., 2000, 2002]. Interestingly, transcytosis of albumin is dissociated from fluid permeability [Minshall et al., 2000]. However, the primary pathway involved in endothelial barrier permeability under pathological conditions is via paracellular transport of fluid and macromolecules through gaps which form following the disruption of intercellular adherens and focal adhesion (FA) complexes [Dudek and Garcia, 2001].

The lung is particularly sensitive to endothelial barrier disruption due to the extensive surface area of the microvascular network intimately associated with alveolar air spaces. The pulmonary microvessel endothelium is of the continuous, non-fenestrated type and is the site of the majority of fluid and solute exchange, a function of the enormous surface area available for both diffusion and filtration. Dynamic

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control of the lung endothelial barrier involves complex signaling to the endothelial cytoskeleton and adhesion complexes between neighboring cells and between cells and the underlying extracellular matrix, which consists of a complex array of molecules: laminin, type I and type IV collagen, proteoglycans, fibronectin, and vitronectin. The three-dimensional arrangement of matrix proteins provides a restrictive barrier to the transport of molecules and acts as a sieve, differentially filtering molecules of variable molecular weights. Therefore, both the endothelium and the extracellular matrix are barriers to solute transport. Increases in vascular permeability may be the result of alterations in one or both of these barriers, each intimately linked to the cytoskeleton [Dudek and Garcia, 2001]. Pro-inflammatory molecules, including thrombin and TNF-α, perturb the balance between contractile and tethering forces in favor of the formation of a complex network of actin stress fibers and subsequent cellular contraction, disruption of adhesion complexes, and paracellular gap formation. Additionally, angiogenic factors such as vascular endothelial growth factor (VEGF) and angiopoietin-2 have been demonstrated to increase vascular permeability, while angiopoietin-1 decreases endothelial permeability, suggesting a distinct connection between angiogenesis and vascular barrier regulation [Thurston et al., 2000].

Platelets have long been demonstrated to enhance the integrity of the endothelial barrier [Gimbrone et al., 1969]. Thrombocytopenia increases capillary permeability and accelerates fluid and protein extravasation, an effect that is reversible with infusion of platelets or platelet-released products [Lo et al., 1988]. Recently, our group identified sphingosine 1-phosphate (S1P), a biologically active lipid generated by hydrolysis of membrane lipids in activated platelets, as the major barrier-protective product of platelets [Dudek and Garcia, 2001; Schaphorst et al., 2003]. S1P has been demonstrated in vitro to organize actin into a strong cortical ring and to assemble and strengthen intercellular junctions and cell–matrix adherence. These structural changes correlate with decreased vascular permeability both in vitro and in vivo. Interestingly, S1P also serves as a potent chemo-attractant for endothelial cells and promoter of angiogenesis [Garcia et al., 2001]. The mechanisms by which S1P enhances the integrity of the endothelial barrier remain an active area of research.

The roles of S1P in endothelial cell cytoskeletal regulation, cellular locomotion, vascular maturation, and angiogenesis are well described in the literature. The details of the signaling cascades leading to such functions are active foci of intense research. Recently, a potential role for S1P in immune function has emerged in the literature with recent reports that FTY720, a structural analogue to sphingosine and, in its phosphorylated form, a potent agonist of the S1P-1 receptor alters lymphocyte trafficking and may serve as a potent immunosuppressant for clinical use in solid organ transplant recipients [Brinkmann et al., 2002; Mandala et al., 2002]. Furthermore, S1P has been demonstrated to decrease neutrophil chemotaxis, transendothelial migration, and tissue invasiveness in response to IL-8 [Kawa et al., 1997]. Thus, trafficking of immune cells may play a key role in modulation of inflammatory diseases by S1P. The effect of S1P on epithelial function remains an open area of investigation. Our lab has recently demonstrated induction of IL-8 production by S1P in immortalized airway epithelial cells [Cummings et al., 2002] but failed to identify any significant alteration in IL-8 secretion in primary human epithelium. In addition, we have identified an increase in transepithelial monolayer electrical resistance in A549 cells treated with S1P, suggesting a decreased permeability response in these cells (unpublished data). Given the paucity of evidence available to support a role for S1P in the modulation of the epithelial barrier, this review will summarize current literature regarding S1P endothelial barrier and enhancing mechanisms address ongoing controversies and unanswered questions with a focus on the clinical relevance of endothelial barrier regulation.

S1P SIGNALING

S1P is a biologically active sphingolipid that signals via cell surface G-protein coupled receptors (GPCR) eliciting a wide variety of tissue-specific biologic activities. Its role as an intracellular signal transducer is poorly defined at present, although recent evidence links intracellular S1P to store-operated calcium entry in neutrophils independent of intracellular calcium store depletion, G-protein activation, or phospholipase C (PLC) activation

[Itagaki and Hauser, 2003]. Furthermore, S1P appears to promote cell growth and to suppress apoptosis in fibroblasts independent of GPCR ligation [Olivera et al., 2003]. Intracellular molecular targets for S1P remain to be identified, and as such, its role as an intracellular messenger remains controversial. Specifically, intracellular S1P signaling in endothelial cells as it relates to barrier function remains completely undefined.

As noted elsewhere in this issue, S1P signaling is mediated through ligation of cell surface receptors belonging to the endothelial differentiation gene (*Edg*) family. Edg receptors are expressed on various cell types including neurons, cardiomyocytes, leukocytes, and endothelial cells (EC). Vascular EC primarily express Edg-1 (S1PR1) and Edg-3 (S1PR3) with significantly lower or absent expression of Edg-5 (S1PR2) [Garcia et al., 2001]. Activation of Edg-1, through the G-protein G_i, and Edg-3, through activation of G_i, G_q, and $G_{12/13}$, promote cellular motility, vascular maturation, focal contact formation, and decreased permeability through modulation of Rho family GTPases and mitogen activated protein (MAP) kinases [Liu et al., 2001; Hla, 2003]. Reduction in Edg-1 expression attenuates S1P-, platelet-, and platelet supernatant-mediated barrier enhancement [Schaphorst et al., 2003]. Edg-6, a recently cloned S1P-specific GPCR, is expressed predominantly in lymphoid, hematopoietic, and lung tissue. The role of Edg-6 ligation is unclear as it relates to endothelial barrier function or immune modulation, although signaling involves activation of the MAP kinase pathway and appears to alter cell migration in vitro [Kohno et al., 2003]. As we describe below, our group has focused on the predominant downstream effects of S1P ligation of cell surface GPCR via dramatic cytoskeletal reorganization and modulation of intercellular adhesion complexes. Ultimately, these processes combine to decrease endothelial permeability to fluid and solute, which may be important clinically in the management of a variety of inflammatory disease states such as acute lung injury (ALI) and sepsis.

ROLE OF THE CYTOSKELETON

Endothelial barrier function depends upon a complex dynamic inter-relationship between cytoskeletal elements. Contractile forces within the cell are balanced by tethering forces between cells and between the individual cell and the underlying matrix. Through its enzymatic cleavage of the PAR-1 receptor, thrombin induces a prominent calcium (Ca^{2+}) transient leading to increased levels of myosin light chain (MLC) phosphorylation. This involves dual coordinated actions of Ca^{2+}/Calmodulin dependent MLC kinase (MLCK) and inhibition of MLC phosphatase via activation of the small GTPase, Rho, and associated Rho-kinase [Dudek and Garcia, 2001]. Furthermore, thrombin activates p38 MAPK which results in MLCK-independent stress fiber formation downstream [Borbiev et al., 2003]. Thrombin stimulation of the EC contractile apparatus results in profound actin stress fiber formation, increased intracellular tension, paracellular gap formation, increased albumin clearance [Garcia et al., 1986], and a reduction in transendothelial monolayer resistance (TER) indicative of increased permeability [Garcia et al., 2001]. Our group was the first to demonstrate barrier enhancement by S1P utilizing in vitro measurements of TER to show dose-dependent S1P associated enhancement of EC monolayer barrier function [Garcia et al., 2001] and reversal of the barrier disruptive response to thrombin when administered subsequent to thrombin challenge.

The cellular mechanism underlying the barrier-enhancing effects of S1P remains an active area of investigation. Rearrangement of cytoskeletal elements represents the key cellular event affecting endothelial barrier function. Morphologic studies demonstrate rapid cortical actomyosin redistribution in EC monolayers treated with S1P. We speculate that this cytoskeletal rearrangement is essential for barrier enhancement function as TER fails to increase in EC monolayers treated with S1P in the presence of cytochalasin, an actin depolymerizing agent, or latrunculin, an inhibitor of actin polymerization [Garcia et al., 2001]. Several molecules and events appear to be important for the rearrangement of cytoskeletal elements and the formation of the cortical actin ring (Fig. 1). Evidence suggests a potential role for myosin light chain kinase (MLCK) as phosphorylated MLC colocalizes with cortical actin in EC monolayers stimulated with S1P [Garcia et al., 2001], and MLCK translocates to this area as well [Dudek et al., 2004]. Myosin light chain phosphorylation, in this spatially defined locale,

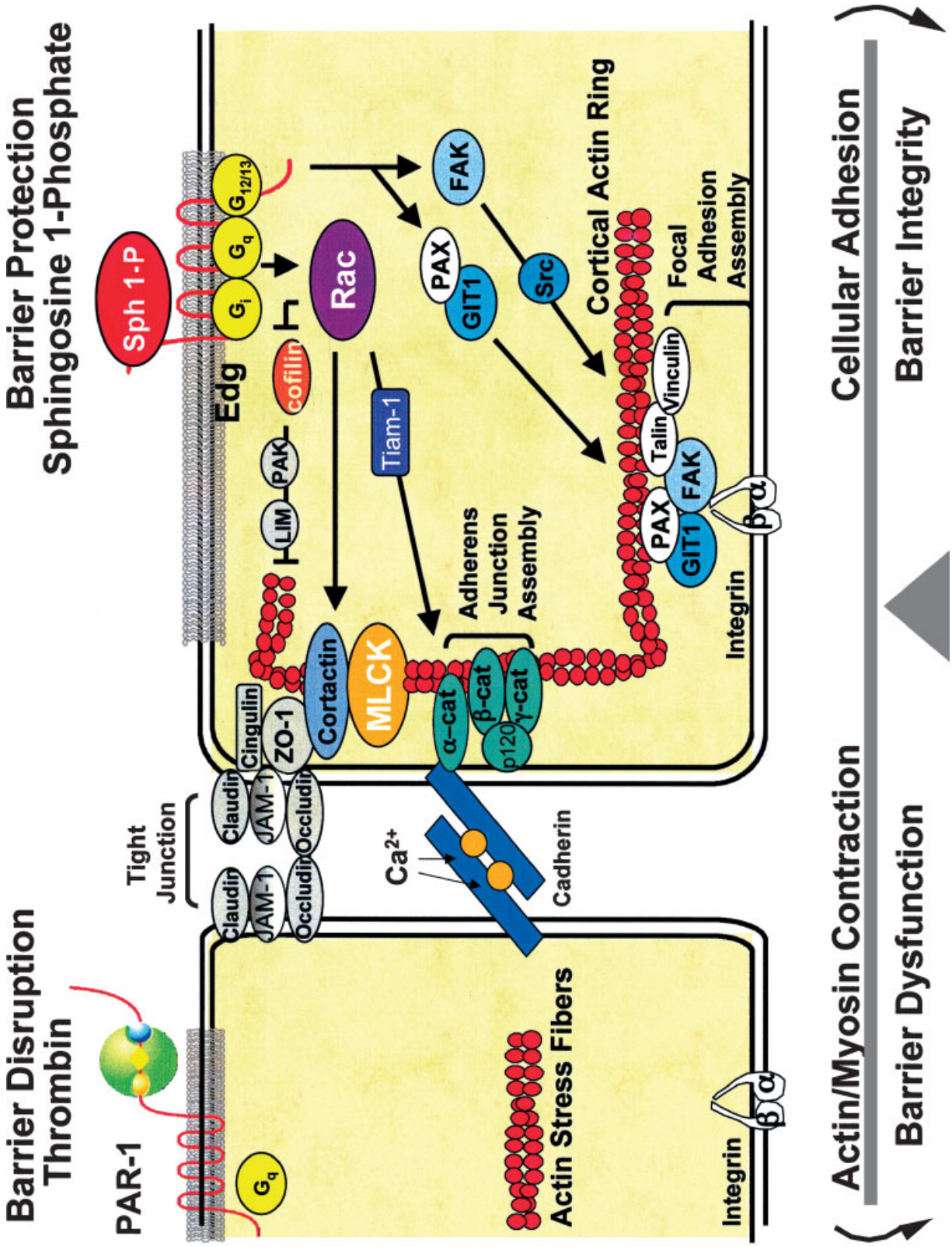
Barrier Protection
Sphingosine 1-Phosphate
Sph 1-P
Edg
G_i
G_q
$G_{12/13}$
Rac
cofilin
LIM
PAK
Tiam-1
FAK
PAX
GIT1
Src
Cortical Actin Ring
Focal Adhesion Assembly
Talin
Vinculin
Adherens Junction Assembly
Cortactin
MLCK
α–cat
β-cat
p120
γ-cat
Integrin
Tight Junction
Claudin
JAM-1
Occludin
Cingulin
ZO-1
Ca^{2+}
Cadherin
Actin Stress Fibers
Barrier Disruption
Thrombin
PAR-1
G_q
Actin/Myosin Contraction
Barrier Dysfunction
Cellular Adhesion
Barrier Integrity

Fig. 1.

may promote the interaction of myosin with filamentous actin stabilizing the actin cytoskeleton and thereby stabilizing cortical interactions with adhesive elements. However, inhibitors of MLCK, ML-7, and the intracellular calcium chelator BAPTA only weakly alter barrier function in S1P-treated EC monolayers [Garcia et al., 2001] suggesting a role for MLCK-independent cytoskeletal rearrangement in S1P modulated barrier function. Inhibition of Rho-kinase, leading to increased MLC phosphatase activity, results in a 30% decrease in TER measured across EC monolayers but does not appear to interrupt the formation of the cortical actin ring [Garcia et al., 2001]. This implies an important role for MLC phosphorylation in endothelial barrier enhancement whether fostered by MLCK-dependent or independent pathways or a combination of both. Further elucidation of the role of MLCK in endothelial barrier function remains a focus of investigation in our laboratory.

The Rho family of small GTPases plays a pivotal role in the regulation of endothelial barrier function in that they are intimately involved in cytoskeletal rearrangement and the cytoskeletal interactions that distribute and assemble intercellular junctional complexes and focal adhesions (FAs) (Fig. 1). Sequential activation of Rho and Rho kinase, leads to actin stress fiber formation in endothelial cells partially mediated by the increases in phosphorylation of MLC (via myosin phosphatase inhibition) and resultant actomyosin contraction [Garcia et al., 1999; Wojciak-Stothard et al., 2001; Vouret-Craviari et al., 2002]. At physiologic concentrations (less than 1–2 μM), S1P preferentially activates the small GTPase Rac, in a pertussis toxin-sensitive fashion [Garcia et al., 2001; Shikata et al., 2003a], relative to Rho, which is preferentially stimulated by thrombin or by supraphysiologic concentrations of S1P [Vouret-Craviari et al., 2002; Shikata et al., 2003a]. Rac activation appears to be central to the cellular locomotion and barrier enhancing effects of S1P having been associated with lamellipodia formation, membrane ruffling, the formation of cortical actin filaments, and the spreading of EC [Vouret-Craviari et al., 2002]. Rac activation likely plays a key role in endothelial barrier function as inhibition of Rac leads to increased monolayer permeability and enhances the thrombin-mediated barrier dysfunction response [Wojciak-Stothard et al., 2001] and may proceed through multiple signaling mechanisms in doing so. Rac inactivates cofilin, an actin severing protein, in the cell periphery through a signaling cascade involving PAK-1 and LIM kinase [Garcia et al., 2001]. Inhibition of cofilin may play a key role in EC barrier function as over-expression of dominant-negative PAK-1 significantly reduces cortical actin assembly induced by S1P [Garcia et al., 2001]. Rac activation has been linked to the translocation of cortactin, an F-actin binding protein that stimulates actin polymerization and stabilizes the filamentous actin network, to the cell periphery following EC monolayer stimulation by S1P of exposure to shear stress [Birukov et al., 2002; Dudek et al., 2004]. Normal expression of cortactin is, in fact, necessary for the peak barrier enhancing response to S1P in vitro [Dudek et al., 2004]. Inhibition of Rac activity with a dominant negative construct prevents the translocation of cortactin and subsequent actin polymerization in the cell periphery [Vouret-Craviari et al., 2002]. S1P-mediated cortactin translocation does not appear to involve PAK-1 or ERK activation [Dudek et al., 2004]. In addition, Src activation does not appear to be necessary for the translocation of cortactin to the cell periphery as this effect is not altered in the presence of PP-2, a selective Src inhibitor, or in HUVEC expressing tyrosine deficient mutant cortactin [Dudek et al., 2004]. However, Src-mediated phosphorylation of cortactin residues Y^{421}, Y^{466}, and Y^{482} may be necessary for the peak barrier enhancement induced by S1P as it alters the binding of cortactin, via its SH-3 domain, to MLCK, a molecule potentially important for the organization of filamentous actin in the cortical

Fig. 1. Sphingosine 1-phophate (S1P) modulates endothelial barrier function through combined effects on cytoskeletal arrangement and regulation of intercellular and cell–cell matrix junctional complexes. Thrombin cleavage of the protease activated receptor (PAR)-1 results in actin stress fiber formation via Rho GTPase activation, disrupts adherens complex formation via Rac GTPase inhibition, and interferes with the assembly of focal adhesion plaques. S1P ligation of the endothelial differentiation gene (Edg)-1 receptor activates Rac GTPase resulting downstream in cortical actin rearrangement and the assembly and stabilization of adherens junctions, tight junctions, and focal adhesions. G, G-protein; LIM, LIM kinase; PAK, p21 activated kinase-1; ZO, zona occludins; JAM, junctional adhesion molecule; MLCK, myosin light chain kinase; Src, Src kinase; GIT, G-protein coupled receptor kinase-interacting protein; PAX, paxillin; FAK, focal adhesion kinase; cat, catenin.

ring [Dudek et al., 2004]. We speculate that cortactin binds MLCK at the site of cortical actin polymerization in EC treated with S1P [Dudek et al., 2004], thus localizing the actomyosin interaction at an optimal location to enhance barrier function. This speculation is supported by the observation that the introduction of a blocking peptide that interrupts the binding of cortactin to MLCK reduces both peripheral MLC phosphorylation and the barrier protection induced by S1P [Dudek et al., 2004]. The blocking peptide, however, may nonspecifically block protein binding at the SH-3 domain disturbing cortactin interaction with junctional proteins such as ZO-1, and therefore influence the barrier-enhancing effect of S1P.

Thus, ligation of cell surface S1P receptors triggers a signaling cascade mediated by intracellular G-proteins ultimately resulting in enhancement of endothelial barrier function. Cytoskeletal rearrangement and the formation of a strong cortical actin ring, a necessary process for endothelial barrier enhancement, proceeds in response to S1P receptor ligation via activation of Rac GTPase and the modulation of molecular trafficking to and enzymatic activity at the cell periphery. We speculate that the cortical actin ring stabilizes the cell and integrates with intercellular adhesion complexes thus tightening the barrier to paracellular fluid and solute translocation.

MECHANISMS OF INCREASED CELLULAR ADHESION

As fluid and solute transport across the endothelial barrier primarily occurs via the paracellular route [Dudek and Garcia, 2001], endothelial barrier function is intimately dependent upon adherence of neighboring cells to one another and to the underlying matrix. Two molecular complexes, adherens junctions (AJ) and tight junctions (TJ) primarily account for intercellular adhesion, although the contribution of TJ appears to be minimal in endothelium compared to epithelium. AJ consist of cadherin proteins (specifically vascular endothelial (VE) cadherin) associated with the actin cytoskeleton via complexes of proteins including α-, β-, and γ-catenin and p120. Calcium-dependent homotypic linkage of neighboring AJ is necessary for the stability of the AJ complex and for the control of vascular barrier function. Corada et al. [1999] describe increased cardiac and pulmonary vascular permeability in mice treated with intravenous BV-13, a monoclonal antibody to mouse VE-cadherin. Furthermore, increased neutrophil diapedesis is noted across lung and cardiac microvessels in mice treated with BV-13 compared to control. Interestingly, brain, adrenal gland, liver, and lymph nodes of treated animals do not exhibit similar changes [Corada et al., 1999]. Rabiet et al. [1996] demonstrated disruption of VE-cadherin/catenin complexes and the disappearance of cadherin junctional complexes at sites of intercellular gap formation in HUVEC monolayers exposed to thrombin. Interestingly, these effects occurred along the same time-course as cytoskeletal rearrangement and resultant macromolecular permeability [Rabiet et al., 1996] suggesting a key role for the function of AJ and its interaction with the cytoskeleton in the maintenance and enhancement of the endothelial barrier. In confluent human umbilical vein endothelial cells (HUVEC), S1P stimulates the localization of VE-cadherin, α-, β-, and γ-catenin to sites of cell–cell junctions within 1h [Lee et al., 1999]. Furthermore, VE-cadherin localizes to discontinuous structures at these contact regions and partitions into the Triton insoluble fraction suggesting the assembly of functional AJ. Interestingly, related phospholipids that do not interact with S1P receptors have no effect [Lee et al., 1999], though FTY720-P, a molecule structurally similar to S1P and a potent ligand for S1PR1, has been demonstrated to stimulate the assembly of AJ in HUVEC [Sanchez et al., 2003].

AJ assembly appears to be mechanistically related to S1P induced cytoskeletal rearrangement (Fig. 1). As previously discussed, Rac activation leads to the cortical redistribution of multiple proteins involved in the formation of the cortical actin ring. Similarly, Rac, and an associated guanine nucleotide exchange factor Tiam1, redistributes to areas of cell–cell contact within 30 min of exposure to S1P [Lee et al., 1999]. Dominant-negative Rac polypeptide attenuates S1P-induced VE-cadherin and β-catenin localization to areas of cell–cell contact [Lee et al., 1999]. Furthermore, thrombin, which is known to inhibit Rac weakens both AJ and TJ [Vouret-Craviari et al., 2002]. Microinjection of HUVEC with oligonucleotides designed to interrupt the translation of Edg-1 and Edg-3 receptor mRNA resulted in decreased translocation of VE-cadherin to cell–cell

contact regions after treatment with S1P, suggesting the necessity of Edg receptor expression for AJ assembly [Lee et al., 1999]. Clearly, AJ assembly is important for the maintenance of endothelial barrier function. S1P, through its action on G-protein coupled Edg receptors and activation of Rac, induces the formation and stabilization of AJ in EC thus decreasing permeability both in vitro and in vivo.

TJ consist of a complex of proteins including the claudins, occludin, and junctional adhesion molecule (JAM-1). These protein complexes interact with EC cytoskeletal actin via the zona occludins family of proteins and cingulin. The SH-3 domain of cortactin interacts with ZO-1 providing one potential link between the tight junction and the cytoskeletal redistribution invoked by S1P [Katsube et al., 1998]. A recent report suggests a potential role for TJ in rat brain endothelial barrier modulation, however, their specific contribution to endothelial barrier function remains unclear and likely modest.

Focal adhesion (FA) complexes tether the cell to the underlying matrix, an activity under constant rearrangement. Reports suggest the assembly of FA promotes stress fiber formation, and their disassembly leads to break down of stress fibers, although this is clearly an oversimplification of a dynamic and highly orchestrated response. FA consist of transmembrane α- and β-integrin molecules linked to the actin cytoskeleton by a complex of proteins including vinculin, talin, paxillin, α-actinin, and focal adhesion kinase (FAK). Recent evidence suggests a role for G-protein coupled receptor kinase interacting proteins, GIT-1 and GIT-2, in FA remodeling and for FAs in EC barrier regulation in response to S1P (Fig. 1) [Shikata et al., 2003a]. In cultured EC, S1P at physiologic concentrations induces tyrosine phosphorylation of FAK, disassembly of the FA complex, association of GIT-1 with paxillin, and redistribution of paxillin and FAK to the cell periphery concomitant with the associated cytoskeletal changes, specifically the formation of the cortical actin ring [Shikata et al., 2003a]. In contrast, FAK redistributes to the ends of stress fibers when EC were stimulated with thrombin [Shikata et al., 2003b]. Further distinguishing the S1P response from the thrombin response, the FAK tyrosine phosphorylation sites differ between cells stimulated with S1P and those induced by thrombin. S1P induces phosphorylation only at the Y^{576} site corresponding with increased catalytic activity, whereas thrombin phosphorylates FAK at Y^{397}, Y^{576}, and Y^{925} sites correlating with the major autophosphorylation site, the catalytic domain activation loop, and the docking site for the Grb2 SH2 domain respectively [Shikata et al., 2003a,b]. Src activation appears to be involved in the S1P induced redistribution of FAK and the formation of the cortical actin ring as PP2 abolished the response [Shikata et al., 2003b]. In contrast, PP2 failed to alter thrombin-induced FAK and cytoskeletal alterations. Clearly, FA complexes play an integral role in the maintenance and disruption of the endothelial barrier. The mechanisms by which S1P affects the distribution of FA complexes are intricate, but likely depend upon the activation of Src and the involvement of small GTPases and associated factors.

ENDOTHELIAL HETEROGENEITY

Structural and functional differences exist between endothelial cells resident in different organ systems, and recent observations suggest distinct differences between those present in the macrovasculature and microvasculature of the same organ. The albumin permeability value in cultured pulmonary microvessel endothelial cells was about one-half to one-fifth of the values of similarly cultured cells from the main stem pulmonary artery, with particularly more rigorous regulation of the paracellular space compared to large vessel endothelium, a feature also noted in vivo [Parker, 2000]. Finally, microvascular cells proliferate at a higher level than macrovascular cells and retain phenotypically distinct calcium and cyclic nucleotide signaling responses as well as oxidant-mediated signaling transduction.

Little is known about the permeability effects of S1P in different organ systems such as kidney or brain despite the wide distribution of S1P receptors in these organs. Primarily, in vitro examinations of endothelial permeability have been conducted on human or bovine conduit vessel endothelium or human umbilical vein endothelial cells. Phenotypic differences exist between conduit endothelial cells and those found in the microvasculature with pulmonary microvascular endothelial cells (PMVEC) forming a tighter, less permeable barrier to fluid and solute than pulmonary artery endothelial cells (PAEC) [Schnitzer et al., 1994]. This

differential barrier function has been attributed to a significantly greater population of FA complexes in PMVEC [Schnitzer et al., 1994]. Scanning electron micrographs following permeability studies have disclosed visible junctional gaps between conduit vessel endothelial cells whereas microvascular cells form tight intercellular junctions without visible gap formation [Kelly et al., 1998]. We reported slightly attenuated increases in transmonolayer endothelial resistance in bovine PMVEC compared to bovine PAEC in response to escalating doses of S1P [Garcia et al., 2001]. Similar barrier protective mechanisms may be operative in capillaries as in conduit vessels, however, the nuances of signal transduction in the microvasculature have yet to be completely elucidated.

EFFECTS OF S1P IN VIVO

Endothelial dysfunction has recently been recognized as a central pathophysiologic mechanism underlying primary vascular diseases including atherosclerosis, hypertension, pulmonary hypertension, and cerebrovascular disease. Indeed, endothelial dysfunction is central to the pathogenesis of inflammatory disease states such as the systemic inflammatory response underlying severe sepsis and ALI. Lysophospholipid and sphingolipid mediators including S1P are emerging as important and potent mediators of endothelial function. A recent report correlates serum levels of S1P with the development of obstructive coronary artery diseases and implicates S1P as a more sensitive predictor of coronary risk than any of the more conventional risk factors including tobacco use, family history or hypertension [Deutschman et al., 2003].

The S1P signaling cascade may play a key role in the pathogenesis of inflammatory processes and potential therapeutic or preventive measures directed against these maladies. The phosphorylated form of FTY720, a structural analogue to sphingosine and an in vivo substrate for sphingosine kinase, is a potent agonist of the S1P family of receptors. It has been shown to alter endothelial permeability both in vitro and in vivo [Sanchez et al., 2003; Peng et al., 2004]. Furthermore, it is a potent immunosuppressive agent currently under investigation for application in solid-organ transplantation. The immunosuppressive effect appears to be related to the sequestration of lymphocytes in the secondary lymphoid organs, unable to enter the circulation through a tight endothelial barrier [Brinkmann et al., 2002; Mandala et al., 2002].

Our group has been intrigued by the potential role for S1P and FTY720 in treatment of ALI (Fig. 2), a devastating inflammatory lung disease characterized by increases in vascular permeability. Cyclic stretch, greatly accentuated by mechanical ventilation, exacerbates this endothelial barrier dysfunction. Studies in isolated, perfused rodent lungs have demonstrated that ventilator-induced mechanical stresses increase fluid flux across capillaries, primarily through an active endothelial response involving the cytoskeleton [Parker, 2000; Birukov et al., 2003]. Our recent data in a mouse model of ALI suggests that S1P attenuates the formation of lung edema and modulates neutrophil chemotaxis and function in response to intratracheal administration of LPS [Peng et al., 2004]. Further investigation demonstrates that intravenous S1P attenuates protein extravasation into the alveolar space and shunt formation in a clinically and mechanically relevant canine model of ALI induced by intrabronchial LPS and high tidal volume mechanical ventilation [McVerry et al., 2003]. We believe that this effect will translate clinically into a shorter duration of mechanical ventilation and subsequently decreased morbidity associated with mechanical ventilation in patients with ALI or ARDS.

Multiple mechanisms may be operative underlying potential clinical benefits of S1P (Fig. 2). These potential mechanisms highlight various controversies and peculiarities regarding S1P signaling. We have shown S1P to impair leukocyte migration into the lungs and kidneys of rodents exposed to LPS [Peng et al., 2004]. As our data convincingly demonstrate, S1P enhances endothelial barrier integrity. S1P-induced prevention of edema fluid accumulation in the lung in response to LPS may be the result neutrophil chemotaxis modulation, the tightening of the microvascular endothelium, or both. Similarly, reduction of protein extravasation into alveolar spaces has been demonstrated in both rodent and canine models of LPS-induced lung injury [McVerry et al., 2003; Peng et al., 2004]. Whether this phenomenon is due solely to overt enhancement of endothelial barrier integrity, or if it involves an epithelial response to S1P remains unknown. Finally, given recent

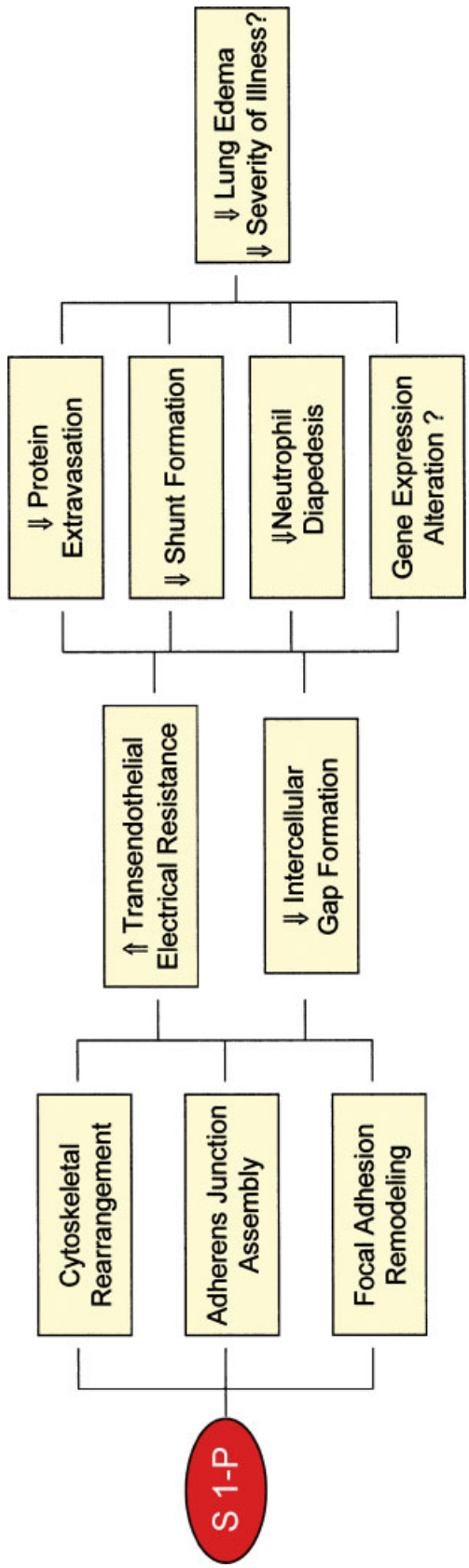

Fig. 2. S1P modulates endothelial cellular biology and physiology to prevent acute lung injury (ALI). Schematic diagram how S1P effects on endothelial cell biology, endothelial barrier function, and tissue physiology interact to provide clinical benefit in patients with ALI.

evidence supporting the involvement of gene expression modulation in the development of ALI, S1P-mediated lung protection may be related to reduction in inflammatory and/or innate immunity gene expression. The expression of pre B-cell enhancing factor (PBEF) has been linked to the development of lung injury in specific populations [Ye et al., 2004]. In addition, inflammatory cytokines such as IL-6 and various coagulation cascade components appear to be upregulated in models of ALI. Modulation of candidate gene expression represents another potential mechanism of action of S1P in vivo currently under investigation.

SUMMARY

Sphingolipids are now recognized as important mediators of cellular function. Clearly, modulation of barrier integrity is a key aspect of S1P signaling. The mechanisms of barrier enhancement afforded by S1P are undoubtedly complex and continue to be the focus of intense ongoing basic research. It is clear that through external signaling via GPCR and the activation of the small GTPase Rac, S1P induces the reorganization of the endothelial cytoskeleton into a strong cortical actin ring and the distribution, assembly, and stabilization of adherens junction and FA complexes on the cell membrane. The combined effect of these events is the reduction in endothelial permeability to fluid and solute translating into decreased edema fluid accumulation in animal models of ALI. Modulation of inflammatory cell function, epithelial cell biology, and gene expression profiles may also contribute to the anti-inflammatory function of S1P, though further investigation to elucidate these mechanisms is required.

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