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Review Article

Transient Receptor Potential Canonical Channels and Sphingosine-1-Phosphate Signaling in Lung Vascular Endothelial Barrier Regulation: From Molecular Mechanisms to Therapeutic Opportunities

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Abstract

The pulmonary vascular endothelium serves as a dynamic, semi-permeable barrier that precisely governs the passage of fluid, solutes, and immune cells between the intravascular compartment and the surrounding alveolar tissue. Dysregulation of this barrier is the cardinal pathophysiological event in acute lung injury (ALI) and acute respiratory distress syndrome (ARDS), life-threatening conditions characterized by non-cardiogenic pulmonary edema, refractory hypoxemia, and respiratory failure. Despite decades of intensive investigation, ARDS mortality remains unacceptably high - ranging from 30% to over 40% in severe cases - and no pharmacotherapy targeting endothelial barrier restoration has achieved regulatory approval. Central to endothelial barrier disruption is the elevation of intracellular calcium (Ca^{2+}), which activates myosin light chain kinase (MLCK), drives actomyosin contraction, and ultimately disrupts intercellular adherens junctions (AJs). The transient receptor potential canonical (TRPC) family of non-selective cation channels - particularly TRPC1 and TRPC6 - mediates the primary Ca^{2+} entry events downstream of inflammatory stimuli including lipopolysaccharide (LPS)-Toll-like receptor 4 (TLR4) activation and thrombin-protease activated receptor-1 (PAR-1) signaling. TRPC6-dependent Ca^{2+} influx directly couples innate immune activation to lung vascular hyperpermeability and amplifies NF-κB-driven inflammatory gene transcription through a novel MLCK-MyD88IRAK4 signaling nexus. TRPC1, by contrast, operates as a constitutive suppressor of sphingosine kinase 1 (SPHK1), thereby setting the basal threshold for AJ stability. Counterbalancing this barrier-disruptive axis is the sphingosine-1-phosphate (S1P) signaling pathway. SPHK1-catalyzed generation of S1P, acting through its cognate receptor $S1P_1$, potently activates Rac1 GTPase, promotes cortical actin assembly, stabilizes VE cadherin at AJs, and restores endothelial barrier integrity. SPHK1 is dynamically induced during the resolution phase of inflammatory barrier disruption, representing an endogenous vascular repair signal. Failure of this compensatory response - as observed in SPHK1 deficient animals - results in sustained, unresolved permeability increases and increased mortality. This comprehensive review synthesizes mechanistic, preclinical, and emerging clinical evidence for the functional interplay between TRPC channels and S1P signaling in the lung endothelium. We discuss the molecular architecture of the endothelial barrier, the upstream inflammatory triggers, the downstream effectors of barrier disruption and recovery, and the translational implications for therapeutic targeting in ARDS, sepsis, and COVID-19-associated endotheliopathy. We propose that pharmacological strategies directed at inhibiting TRPC6, amplifying SPHK1-S1P-$S1P_1$ signaling, or simultaneously modulating both axes represent the most promising approaches to barrier-targeted therapy in inflammatory lung disease.

Keywords: *Endothelial Permeability; Acute Lung Injury; ARDS; Transient Receptor Potential Channel; Sphingosine-1-Phosphate; Adherens Junctions; Vascular Barrier; Sepsis*

Abbreviations

ARDS: Acute Respiratory Distress Syndrome; ALI: Acute Lung Injury; AJ: Adherens Junction; Ca^{2+}: Calcium; DAG: Diacylglycerol; FAK: Focal Adhesion Kinase; HDL: High-Density Lipoprotein; IP_3: Inositol Trisphosphate; IRAK4: Interleukin-1 Receptor-Associated Kinase 4; LPS: Lipopolysaccharide; MLCK: Myosin Light Chain Kinase; MLC: Myosin Light Chain; MyD88: Myeloid Differentiation Primary Response Gene 88; NF-κB: Nuclear Factor Kappa Light-Chain-Enhancer of Activated B Cells; p120: p120-Catenin; PAR-1: Protease-Activated Receptor-1; PI3K: Phosphoinositide 3-Kinase; PKC: Protein Kinase C; ROCK: Rho-Associated Protein Kinase; ROCE: Receptor-Operated Ca^{2+} Entry; S1P: Sphingosine-1-Phosphate; SOCE: Store-Operated Ca^{2+} Entry; SPHK1: Sphingosine Kinase 1; TLR4: Toll-Like Receptor 4; TRP: Transient Receptor Potential; TRPC: Transient Receptor Potential Canonical; VE-Cadherin: Vascular Endothelial Cadherin

Introduction

The endothelium is one of the most metabolically active and functionally diverse tissues in the human body. Comprising a continuous monolayer of approximately 10^{13} cells lining the entire vasculature, endothelial cells perform tasks ranging from regulation of vascular tone and coagulation to modulation of leukocyte trafficking and control of macromolecular permeability [1,2]. Of all endothelial functions, the maintenance of a selective semipermeable barrier is perhaps the most critical in the context of organ homeostasis. In the lung, this function acquires particular urgency: microvascular endothelial barrier integrity directly determines the degree of alveolar loading in response to inflammatory insult. Even a modest increase in pulmonary microvascular permeability allows protein-rich fluid to accumulate in the alveolar spaces, impairing gas exchange and driving the pathophysiology of ARDS [3,4].

ARDS is defined by the Berlin criteria as an acute, diffuse, inflammatory lung injury leading to impaired gas exchange, with the hallmarks of bilateral opacities on chest imaging, absence of cardiogenic edema, and a PaO_2/FiO_2 ratio below 300 mmHg [4]. The condition affects approximately 190,000 patients annually in the United States alone, with in-hospital mortality ranging from 27% in mild disease to over 45% in severe ARDS [4,5]. Despite intensive research into its pathogenesis, the only interventions that have consistently reduced ARDS mortality are low-tidal-volume lung-protective ventilation and prone positioning - supportive measures that mitigate secondary injury but do not target the primary vascular pathology [6]. There are currently no approved pharmacological therapies directed at restoring the disrupted endothelial barrier.

The molecular underpinnings of endothelial barrier disruption in ARDS are now understood in considerable mechanistic detail. Inflammatory mediators - including bacterial LPS, cytokines such as TNF-α and IL-1β, thrombin generated by the coagulation cascade, and hypoxia - converge on the endothelial cell to elevate intracellular Ca^{2+}, activate contractile machinery, and disrupt the adhesive protein complexes that maintain interendothelial seals [2,7]. Ca^{2+} influx through plasma membrane ion channels is the principal mechanism for this elevation, and members of the transient receptor potential canonical (TRPC) subfamily have emerged as the most clinically relevant such channels in the pulmonary endothelium [8,9].

In parallel, the past two decades have produced a compelling body of evidence implicating bioactive sphingolipid signaling - specifically the sphingosine kinase 1 (SPHK1)/sphingosine-1-phosphate (S1P)/S1P receptor 1 ($S1P_1$) axis - as the endothelium's primary endogenous barrier-repair pathway [10,11]. S1P, generated by the phosphorylation of sphingosine, acts in a paracrine and autocrine manner to activate the small GTPase Rac1, drive cortical actin polymerization, and stabilize vascular endothelial (VE)-cadherin at adherens junctions [10]. The dynamic antagonism between Ca^{2+}-driven TRPC channel activation and SPHK1-S1P barrier-restorative signaling determines the net permeability state of the pulmonary endothelium at any given moment [12].

This review provides a comprehensive, mechanistically detailed account of both pathways, their molecular interactions, their roles in the clinical syndromes of ALI and ARDS, and the therapeutic opportunities they present. We draw on seminal contributions from our laboratory and others in the field, integrating *in vitro* mechanistic data, rodent *in vivo* experiments, and emerging clinical observations to construct a unified model of lung vascular barrier regulation with direct translational implications.

Endothelial barrier architecture and regulatory principles

Structural organization of the endothelial barrier

The endothelial barrier is a complex structure whose integrity depends on three interlocking systems: (1) intercellular junctional complexes that physically link adjacent endothelial cells, (2) the cortical actin cytoskeleton that provides structural tension and mechanical resistance, and (3) cell-matrix adhesion via focal adhesion complexes that anchor the monolayer to the underlying basement membrane [13]. In lung microvessels, adherens junctions (AJs) constitute the primary structural determinant of paracellular permeability, while tight junctions (TJs) contribute a secondary seal that is more prominent in the blood brain barrier [14].

AJs are formed by the homophilic, calcium-dependent adhesion of vascular endothelial (VE) cadherin, a type II classical cadherin whose extracellular domain mediates trans-interaction with VE-cadherin on the opposing cell surface. The cytoplasmic tail of VE-cadherin engages a complex of catenin proteins: p120-catenin (p120) binds the juxtamembrane domain and controls VE-cadherin surface expression and endocytic trafficking; β-catenin and plakoglobin bind the distal cytoplasmic domain and link VE-cadherin to α-catenin, which serves as the mechanical link to the actin cytoskeleton (Figure 1) [14,15]. The stability of VE-cadherin at the AJ is regulated by the phosphorylation state of its cytoplasmic partners, by Rac1/RhoA GTPase balance, and by the local architecture of the actin cytoskeleton [14,16].

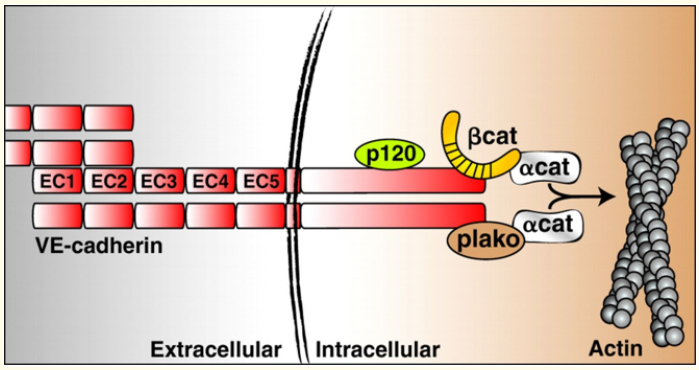

Figure 1: *Molecular organization of endothelial adherens junctions. Vascular endothelial (VE)-cadherin is represented as a dimer, which is the minimal functional unit of cadherins. EC1-EC5 denote the five homologous extracellular cadherin domains. Clustering of VE-cadherin at cell-cell contacts promotes the formation of multimolecular complexes comprising signaling, regulatory, and scaffold proteins. Key interacting partners include p120-catenin (p120), β-catenin (βcat), and plakoglobin (plako), which bind directly to the VE-cadherin cytoplasmic tail. β-catenin and plakoglobin in turn associate with α-catenin (αcat), which links the complex to the actin cytoskeleton. Some VE-cadherin-interacting proteins possess enzymatic activity (tyrosine or serine kinases, tyrosine phosphatases, and GTPases), while others serve scaffolding functions. The resulting multimeric adherens junction complex modulates endothelial barrier function by regulating VE-cadherin activity and transducing intracellular signals. Reproduced with permission from Dejana E, Orsenigo F, and Lampugnani MG (2008). The role of adherens junctions and VE-cadherin in the control of vascular permeability. Journal of Cell Science, 121(13), 2115-2122. https://pubmed.ncbi.nlm.nih.gov/18565824/*

Abbreviations: AJ: Adherens Junction; EC1-EC5: Extracellular Cadherin Domains 1 through 5; p120: p120-Catenin; βcat: β-Catenin; Plako: Plakoglobin; αcat: α-Catenin; VE-Cadherin: Vascular Endothelial Cadherin.

VE-cadherin and p120-catenin dynamics

A critical molecular switch governing AJ stability is the binding of p120 to VE-cadherin. When p120 is bound, it sterically masks an endocytic targeting motif in the VE-cadherin juxtamembrane domain, preventing clathrin-mediated internalization and maintaining the junction [17]. Conversely, dissociation of p120 from VE-cadherin exposes this motif, triggering VE-cadherin endocytosis, AJ disassembly, and a consequent increase in paracellular permeability. We demonstrated that protein kinase C alpha (PKCα), activated downstream of Ca^{2+}-dependent signaling, phosphorylates p120 at serine 879, causing its dissociation from VE-cadherin and driving AJ disassembly (Figure 2) [18]. Pharmacological inhibition of PKCα or mutation of the Ser879 phosphorylation site to alanine prevented LPS- and thrombin-induced barrier disruption *in vitro* and *in vivo* [18]. This positioned PKCαSer879-p120 as a critical downstream effector of Ca^{2+}-dependent barrier disruption, directly connecting channel-mediated Ca^{2+} influx to junction disassembly at the molecular level.

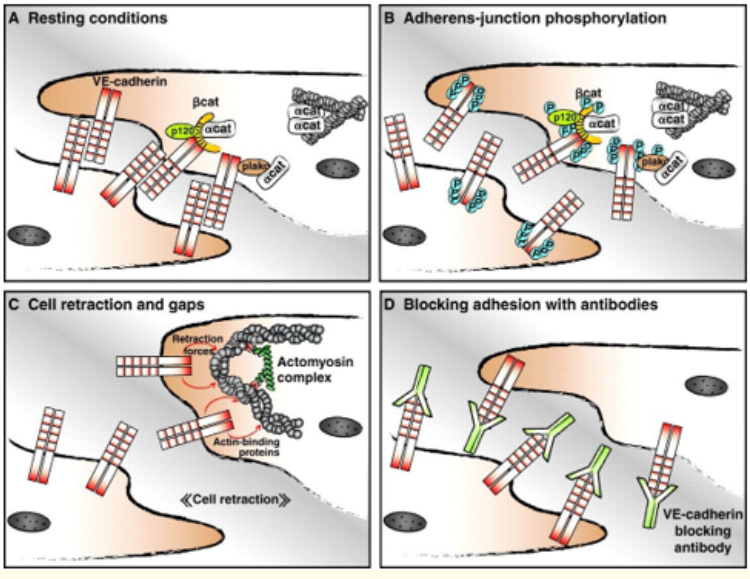

Figure 2: *Functional modifications of endothelial adherens junctions under conditions that increase vascular permeability. (A) Resting conditions: (VE)-cadherin clusters at cell-cell junctions in zipper-like structures. p120-catenin (p120), β-catenin (βcat), and plakoglobin (plako) bind directly to the VE-cadherin cytoplasmic tail, while αcatenin (αcat) associates indirectly via β-catenin or plakoglobin, linking the complex to the actin cytoskeleton. (B) Adherens junction phosphorylation: Phosphorylation (P) of tyrosine residues on VE-cadherin, β-catenin, plakoglobin, and p120 reduces adherens junction strength. The VE-cadherin complex may become partially disorganized in the absence of overt cell retraction. Phosphorylation of VE-cadherin at Ser665 has also been reported, which mediates VE-cadherin internalization and increases vascular permeability. (C) Cell retraction and gap formation: Permeability-increasing agents, inflammatory stimuli, or lytic enzymes can cause intercellular gap formation. This process is mediated by actomyosin contraction linked to the cadherin complex via actin-binding proteins, generating centripetal retraction forces that open paracellular clefts. (D) Blockade of adhesion with antibodies: Anti-VE-cadherin blocking antibodies bind to the extracellular adhesive domain of VE-cadherin and inhibit homophilic clustering. The effect of such antibodies can persist for several hours in vivo, resulting in a dramatic and sustained increase in vascular permeability. Reproduced with permission from Dejana E, Orsenigo F, and Lampugnani MG (2008). The role of adherens junctions and VE-cadherin in the control of vascular permeability. Journal of Cell Science, 121(13), 2115-2122. https://pubmed.ncbi.nlm.nih.gov/18565824/ Abbreviations: AJ: Adherens Junction; P: Phosphorylation; p120: p120-Catenin; βcat: β-Catenin; Plako: Plakoglobin; αcat: α-Catenin; VE-Cadherin: Vascular Endothelial Cadherin.*

In addition to p120, the phosphorylation of VE-cadherin itself - particularly at tyrosine residues Y658 and Y731 - has been implicated in barrier disruption. Tyrosine phosphorylation of VE-cadherin weakens its interaction with p120 and β-catenin, respectively, providing an additional mechanism by which kinases activated downstream of inflammatory Ca^{2+} signals can destabilize AJs [19]. Src kinase and Pyk2, both activated in response to Ca^{2+} elevation, are the primary kinases responsible for this phosphorylation. The interplay between serine and tyrosine phosphorylation events in VE-cadherin and its binding partners thus constitutes a phosphorylation code that ultimately determines the open or closed state of the paracellular cleft [20].

The actomyosin contractile apparatus

The actomyosin cytoskeleton provides the mechanical force that drives AJ opening during barrier disruption. Under basal conditions, the endothelial cell maintains a balance between peripheral (cortical) actin filaments that support AJ integrity and transendothelial actin stress fibers that exert contractile tension [21]. This balance is controlled by two opposing regulatory pathways: (1) the RhoA/ROCK pathway, which promotes MLC phosphorylation and stress fiber formation, and (2) the Rac1/PAK pathway, which promotes cortical actin assembly and peripheral VE-cadherin enrichment [22,23].

Elevated intracellular Ca^{2+} activates MLCK directly via calmodulin, phosphorylating MLC at Thr18/Ser19 and driving actin-myosin crossbridge cycling. The resulting centripetal contractile force pulls cell bodies apart, generating the paracellular gaps through which fluid and plasma proteins extravasate into the alveolar interstitium and airspace (Figure 3) [24]. Simultaneously, Ca^{2+}-activated PKC isoforms, including PKCα, inhibit myosin phosphatase (MYPT1), further elevating MLC phosphorylation. This dual mechanism - direct MLCK activation and myosin phosphatase inhibition - ensures a robust and sustained contractile response to Ca^{2+} elevation [2,21]. Pharmacological inhibition of MLCK using membrane permeant inhibitory peptides (PIK or staurosporine analogues) attenuates LPS- and thrombin-induced barrier disruption in animal models, validating the centrality of this pathway [14,25].

Tight junctions in pulmonary endothelial permeability

While AJs are the dominant junctional complex governing pulmonary endothelial permeability, tight junctions (TJs) composed of claudins, occludin, and ZO-1/ZO-2 scaffolding proteins contribute to the barrier, particularly in larger pulmonary vessels [26]. Claudin-5 is the most abundant claudin in lung endothelial cells and is a direct transcriptional target of the β-catenin/TCF pathway downstream of VE-cadherin [27]. When VE-cadherin is internalized during barrier disruption, β-catenin is released and can undergo nuclear translocation, where it paradoxically suppresses claudin-5 transcription, further compromising barrier integrity. This molecular crosstalk between AJs and TJs creates a coordinated and self-amplifying barrier disruption response [23,27].

TRPC channels as mediators of Ca^{2+} entry and endothelial barrier disruption

The TRP superfamily: An overview

The transient receptor potential (TRP) superfamily comprises 28 mammalian channels organized into six subfamilies: TRPC (canonical), TRPV (vanilloid), TRPM (melastatin), TRPML (mucolipin), TRPP (polycystin), and TRPA (ankyrin) [28]. All TRP channels share a common topology - six transmembrane segments with a pore-forming loop between segments 5 and 6, cytoplasmic N- and C-termini, and homo- or heterotetrameric assembly - but diverge considerably in their activation mechanisms, ion selectivity, and tissue distribution [8]. TRP channels are generally non-selective cation channels, permeable to Na^+, K^+, and Ca^{2+}, with varying Ca^{2+}-to-Na^+ selectivity ratios. In the vasculature, TRP channels in endothelial and smooth muscle cells regulate Ca^{2+} homeostasis and thereby govern vasomotor tone, permeability, and inflammatory responses [29,30].

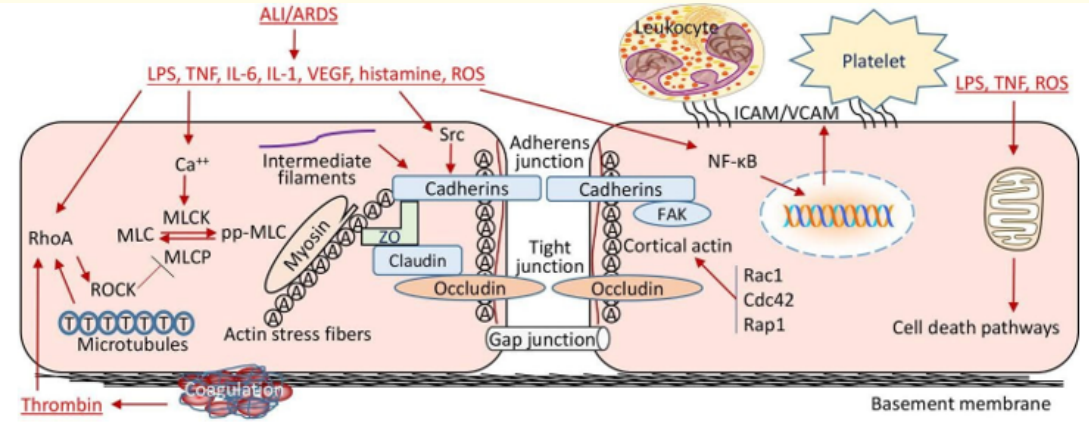

Figure 3: *Mechanisms of pulmonary endothelial barrier disruption in acute lung injury (ALI) and acute respiratory distress syndrome (ARDS). The schematic summarizes the major cellular and molecular pathways driving lung endothelial barrier failure in ALI/ARDS. Pro-inflammatory stimuli - including LPS, TNF, IL-6, IL-1, VEGF, histamine, and ROS - activate RhoA, which potentiates MLC phosphorylation by inhibiting MLC phosphatase (MLCP) activity through ROCK, promoting actin stress fiber formation and dispersal of cortical actin bundles. Actomyosin-driven contractile tension pulls VE-cadherin inward and forces its dissociation from adjacent partners, opening interendothelial gaps. Src activation by pro-inflammatory mediators induces VE-cadherin phosphorylation, disorganizing adherens junction (AJ) proteins and detaching VE-cadherin from the actin cytoskeleton. In contrast, Rac1, Cdc42, and Rap1 promote barrier integrity by supporting cortical F-actin formation. Focal adhesion kinase (FAK) stabilizes AJ formation and focal adhesion turnover. NF-κB activation upregulates ICAM-1, VCAM-1, and selectins on the endothelial surface, facilitating leukocyte and platelet adhesion. Platelet aggregation and coagulation activation lead to thrombin generation and prothrombin cleavage. Bacterial infection, inflammatory infiltration, and cytokine release induce EC death, further compromising the structural integrity of the lung endothelial barrier.*

Abbreviations: AJ: Adherens Junction; ALI: Acute Lung Injury; ARDS: Acute Respiratory Distress Syndrome; Cdc42: Cell Division Cycle 42; EC: Endothelial Cell; FAK: Focal Adhesion Kinase; ICAM: Intercellular Adhesion Molecule 1; IL: Interleukin; LPS: Lipopolysaccharide; MLC: Myosin Light Chain; MLCK: MLC Kinase; MLCP: MLC Phosphatase; NF-κB: Nuclear Factor-κB; Rac1: Ras-Related C3 Botulinum Toxin Substrate 1; Rap1: Ras-Related Protein 1; RhoA: Ras Homolog Family Member A; ROCK: Rho Kinase; ROS: Reactive Oxygen Species; TNF: Tumor Necrosis Factor; VCAM: Vascular Cell Adhesion Molecule 1; VE-Cadherin: Vascular Endothelial Cadherin; VEGF: Vascular Endothelial Growth Factor; ZO: Zonula Occludens.

TRPC subfamily: Structure and gating mechanisms

The canonical TRPC subfamily consists of seven members (TRPC1-7) that are grouped into two functional clusters based on sequence homology and activation mechanisms: TRPC1/4/5, which are activated primarily by store depletion and Gq-coupled receptor stimulation, and TRPC3/6/7, which are gated directly by diacylglycerol (DAG) in a store independent manner [30,31]. All TRPC proteins share conserved structural features including ankyrin repeat domains in the N-terminus, a highly conserved TRP box sequence in the C-terminus (EWKFAR), and a calmodulin/IP_3 receptor-binding domain (CIRB) in the C-terminal region [32].

TRPC channels are regulated by numerous post-translational modifications including phosphorylation, ubiquitination, and palmitoylation. Importantly, their membrane trafficking and channel activity are regulated by interaction with caveolae-associated scaffolding proteins including caveolin-1 and the scaffolding protein STIM1 (stromal interaction molecule 1), which acts as a sensor of ER Ca^{2+} store depletion and subsequently gates TRPC1 and TRPC4 channels [33]. The interaction of STIM1 with TRPC channels is direct and Ca^{2+}-dependent, providing a molecular mechanism for store-operated Ca^{2+} entry (SOCE) in endothelial cells [30,34].

TRPC expression in lung endothelial cells

Pulmonary microvascular endothelial cells (PMVECs) express multiple TRPC isoforms, with TRPC1, TRPC4, and TRPC6 being the most abundant [32]. The expression levels and subcellular distribution of these channels are dynamically regulated by inflammatory stimuli. LPS and TNF-α have been shown to upregulate TRPC6 expression in human pulmonary arterial endothelial cells within hours of exposure, creating a feed-forward mechanism that amplifies Ca^{2+} entry and perpetuates barrier disruption during sustained inflammation [35]. Conversely, hypoxia has been reported to increase TRPC1 and TRPC6 expression in pulmonary arterial smooth muscle cells, contributing to hypoxic pulmonary vasoconstriction - a distinct but potentially interacting pathophysiology in ARDS [36].

Single-channel electrophysiology studies have demonstrated that TRPC1, when activated by store depletion, mediates a low-conductance (approximately 15-16 pS) non-selective cation current in pulmonary endothelial cells, while TRPC6 mediates a higher-conductance (approximately 25-35 pS) DAG-activated current (Figure 4) [37]. These channels are preferentially localized to membrane microdomains enriched in cholesterol and sphingolipids (lipid rafts), where they co-localize with relevant signaling partners including PLCβ, Gq/11 proteins, and the IP_3 receptor [37]. Disruption of lipid raft integrity using methyl-β-cyclodextrin prevents TRPC-mediated Ca^{2+} entry, emphasizing the structural importance of the membrane microenvironment for channel function [37].

TRPC6 and TLR4-mediated lung injury: The LPS-DAG-Ca^{2+} axis

One of the most significant advances in understanding the mechanistic link between innate immune activation and vascular barrier disruption was the elucidation of the TRPC6 dependent signaling pathway downstream of TLR4. LPS, the major structural component of gram-negative bacterial cell walls and the canonical TLR4 ligand, is the proximate driver of endothelial injury in bacterial sepsis - the most common clinical cause of ARDS [38].

We established that LPS-TLR4 engagement in pulmonary endothelial cells activates phospholipase Cβ (PLCβ) through a MyD88-independent, TRIF-dependent mechanism that ultimately couples to Gq/11 signaling, generating inositol trisphosphate (IP_3) and DAG from membrane phosphatidylinositol 4,5-bisphosphate (PIP_2) [39]. While IP_3 triggers release of Ca^{2+} from ER stores, the resulting DAG directly gates TRPC6 at the plasma membrane, mediating a sustained Ca^{2+} influx that outlasts the initial IP_3-induced Ca^{2+} transient [39]. This TRPC6-dependent Ca^{2+} entry activates MLCK, driving MLC phosphorylation, acto-myosin contraction, and paracellular gap formation. The result is the characteristic lung vascular hyperpermeability that defines early ALI.

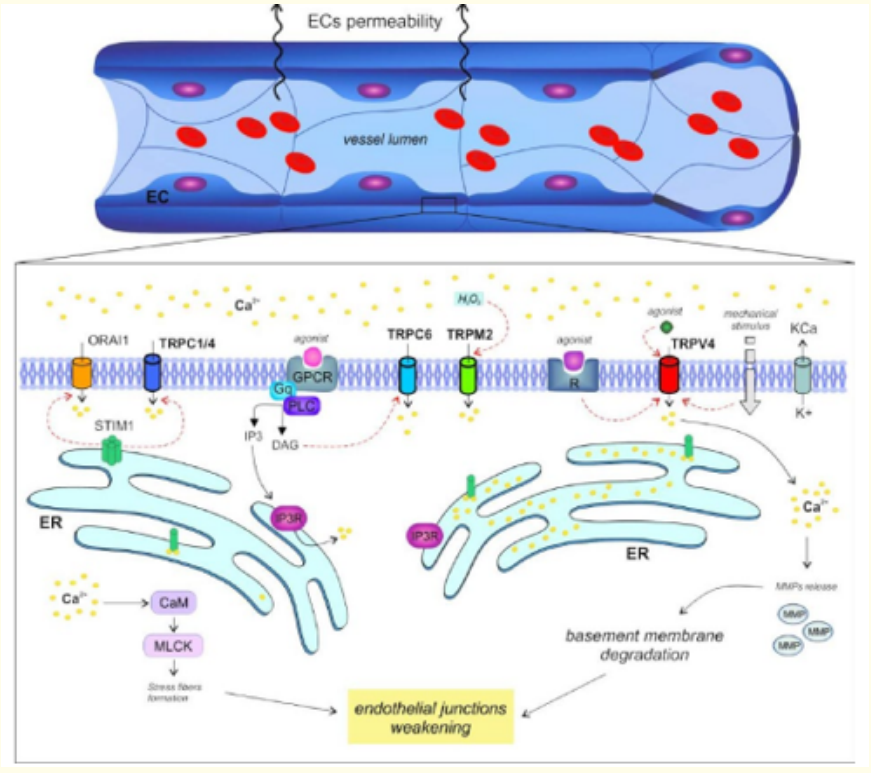

Figure 4: *Regulation of endothelial permeability by TRP channels. The diagram illustrates how endothelial calcium signaling regulates vascular permeability in both large vessels and capillaries through distinct mechanisms. In large vessels (left panel), store operated calcium entry is mediated through ORAI1 and TRPC1/4, regulated by STIM1 at the ER. Agonist-mediated GPCR activation generates IP3 and DAG via PLC, triggering ER calcium release through IP3 receptors (IP3R). The resulting rise in cytosolic Ca^{2+} activates calmodulin (CaM) and MLCK, driving actin stress fiber formation. In capillaries (right panel), TRPC6 responds to DAG and TRPM2 to oxidative stress, triggering ER Ca^{2+} release through IP3R. TRPV4, activated by agonists and mechanical stimuli, promotes matrix metalloproteinase (MMP) release, leading to basement membrane degradation. Both pathways converge on endothelial junction weakening and increased permeability.*

Abbreviations: Ca^{2+}: Calcium; CaM: Calmodulin; DAG: Diacylglycerol; ER: Endoplasmic Reticulum; GPCR: G Protein-Coupled Receptor; IP3: Inositol 1,4,5-Trisphosphate; IP3R: IP3 Receptor; Kca: Calcium-Activated Potassium Channel; MLCK: Myosin Light Chain Kinase; MMP: Matrix Metalloproteinase; ORAI1: Calcium Release-Activated Calcium Channel Protein 1; PLC: Phospholipase C; STIM1: Stromal Interaction Molecule 1; TRP: Transient Receptor Potential; TRPC1/4/6: Transient Receptor Potential Canonical 1/4/6; TRPM2: Transient Receptor Potential Melastatin 2; TRPV4: Transient Receptor Potential Vanilloid 4.

Adapted from Genova T, Gaglioti D, and Munaron L (2020). Regulation of Vessel Permeability by TRP Channels. Frontiers in Physiology, 11:421.

Using Trpc6 knockout mice (*Trpc6*$^{-/-}$), we demonstrated that genetic ablation of TRPC6 substantially protected against LPS-induced lung vascular permeability, as measured by Evans Blue dye extravasation and bronchoalveolar lavage protein concentration [39]. Crucially, *Trpc6*$^{-/-}$ mice also exhibited markedly reduced lung inflammation, including significantly lower levels of TNF-α, IL-6, IL-1β, and CXCL2 in bronchoalveolar lavage fluid and lung homogenates. This observation suggested that TRPC6-mediated Ca^{2+} entry not only drives barrier disruption but also amplifies inflammatory gene transcription - an unexpected coupling between ion channel activity and innate immune signaling [39].

Mechanistically, we discovered that MLCK activated downstream of TRPC6-Ca^{2+} entry forms a protein complex with MyD88 and IRAK4, two critical proximal signaling components of the TLR4-NF-κB pathway. This interaction promotes IRAK4 kinase activity and downstream NFκB activation, linking Ca^{2+} entry directly to inflammatory gene transcription [39]. This finding revealed MLCK as a bi-functional effector molecule: in addition to its canonical role in actomyosin contraction, it serves as an adaptor protein amplifying innate immune signaling. This paradigm-shifting observation provides a molecular explanation for the well recognized clinical association between vascular leak and the systemic inflammatory response in sepsis-associated ARDS and identifies TRPC6-MLCK as a convergence point where barrier disruption and inflammation are jointly amplified.

TRPC6 in non-septic models of ALI

The role of TRPC6 extends beyond LPS-TLR4-mediated injury. TRPC6-dependent barrier disruption has been demonstrated in models of ventilator-induced lung injury (VILI), ischemia-reperfusion injury, and acid aspiration injury - all clinically relevant ARDS triggers [40]. In VILI, cyclic mechanical stretch activates TRPC6 via a mechanism involving RhoA dependent membrane tension increases, leading to Ca^{2+} entry and downstream MLC phosphorylation [41]. This stretch-activated TRPC6 activity was shown to contribute to the volutrauma that defines high-tidal-volume ventilation-associated lung injury, providing a mechanistic basis for why low-tidal-volume protective ventilation strategies reduce endothelial injury [42].

In ischemia-reperfusion injury - relevant to lung transplantation and pulmonary embolism with reperfusion - reactive oxygen species (ROS) generated during reperfusion activate TRPC6 through direct cysteine oxidation of the channel pore, as well as through ROS dependent activation of phospholipase A2 and generation of endogenous DAG analogues [43]. The resulting Ca^{2+} entry drives the rapid permeability increase that characterizes reperfusion lung injury. Importantly, pretreatment with the selective TRPC6 inhibitor SAR7334 attenuated reperfusion-induced permeability in isolated perfused lung preparations, underscoring the therapeutic relevance of this target [43].

TRPC1 and store-operated barrier disruption

While TRPC6 is the dominant Ca^{2+} entry channel in receptor-operated, DAG-driven barrier disruption, TRPC1 plays a distinct and mechanistically important role in store-operated Ca^{2+} entry (SOCE) downstream of PAR-1 activation by thrombin. Thrombin, generated in abundance during the coagulopathy of sepsis, cleaves and activates PAR-1 on the endothelial surface, leading to Gq/11-mediated PLCβ activation, IP_3-dependent ER Ca^{2+} release, and STIM1-mediated activation of TRPC1 at the plasma membrane [44].

We made the unexpected discovery that TRPC1 exerts its primary barrier-regulatory function not through acute Ca^{2+} entry alone, but through a tonic suppression of SPHK1 expression and activity under basal conditions. Trpc1 knockout (*Trpc1*$^{-/-}$) endothelial cells exhibited a 1.5-fold increase in SPHK1 mRNA and protein compared to wild-type cells, accompanied by a 2-fold elevation in intracellular and secreted S1P levels [12]. The elevated S1P in *Trpc1*$^{-/-}$ cells were associated with greater VE-cadherin surface expression, reduced VE-cadherin endocytosis, and a more stable AJ phenotype at baseline [12]. When challenged with thrombin or LPS, *Trpc1*$^{-/-}$ mice were significantly protected against lung vascular hyperpermeability and showed improved survival compared to wild-type controls [12].

These data establish TRPC1 as a constitutive suppressor of SPHK1, dynamically balancing barrier stability against plasticity. By keeping basal SPHK1 and S1P levels low, TRPC1 maintains AJs in a dynamically responsive state that can rapidly disassemble in response to acute inflammatory signals - a physiologically essential property for immune surveillance. This molecular interaction places TRPC1 upstream of the SPHK1-S1P axis as a regulator of the set point for AJ stability, providing a previously unrecognized link between Ca^{2+} channel biology and sphingolipid signaling in the endothelium.

Sphingosine-1-phosphate signaling in endothelial barrier protection

Sphingolipid metabolism: From ceramide to S1P

The sphingolipid metabolic network is a complex, highly regulated system with diverse biological outputs depending on the specific metabolite predominating at any given time and cellular location [45]. Ceramide, generated by *de novo* synthesis in the ER or by sphingomyelinase-mediated hydrolysis of sphingomyelin in the plasma membrane, is a proapoptotic, pro-inflammatory mediator that promotes endothelial barrier disruption [46]. Ceramide can be deacylated to sphingosine by ceramidase, and sphingosine is then phosphorylated to S1P by sphingosine kinases 1 and 2 (SPHK1 and SPHK2). The balance between ceramide/sphingosine (barrier disruptive, pro-apoptotic) and S1P (barrier protective, pro-survival) has been termed the 'sphingolipid rheostat,' and is a fundamental determinant of endothelial fate during inflammatory stress [47].

Sphingosine kinase 1: Regulation and activation

SPHK1 is a cytosolic enzyme that translocates to the plasma membrane upon activation, where it has access to its substrate sphingosine and generates S1P for intracellular signaling and extracellular secretion [48]. SPHK1 is activated by a diverse array of stimuli including growth factors (VEGF, FGF, PDGF), cytokines (TNF-α, IL-1β), phorbol esters, and Ca^{2+} [49]. Importantly, SPHK1 is activated downstream of PKCδ and ERK1/2, both of which can be stimulated by Ca^{2+}-dependent mechanisms - creating a negative feedback loop whereby initial Ca^{2+}-driven barrier disruption eventually leads to SPHK1 activation and barrier restoration [50].

SPHK1 translocation to the plasma membrane is mediated by its interaction with Ca^{2+}/calmodulin and by phosphorylation at Ser225 by ERK1/2 [51]. Once at the membrane, SPHK1 co-localizes with caveolin-1 in lipid rafts, placing it in close proximity to sphingosine generated by ceramidase activity on ceramide at the cell surface. The resulting S1P is then secreted into the extracellular space by the S1P transporter spinster homolog 2 (SPNS2) and to a lesser extent by ABC transporters, where it engages S1P receptors in an autocrine and paracrine fashion [52].

S1P receptors and endothelial signaling

S1P signals through five G-protein-coupled receptors, $S1P_1$ through $S1P_5$, which couple to distinct G-proteins and activate divergent downstream pathways [53]. In endothelial cells, $S1P_1$ is the most abundantly expressed receptor and mediates the canonical barrier protective response. $S1P_1$ couples primarily to Gi, activating the Gβγ dimer to stimulate PI3K, Rac1 GEF (Tiam1, Vav2), and downstream Rac1 activation [54]. Rac1-GTP promotes lamellipodia formation, peripheral actin polymerization, and stabilization of VE-cadherin containing AJs. $S1P_1$ also activates endothelial nitric oxide synthase (eNOS) via a PI3K/Akt dependent mechanism, generating NO which further promotes barrier integrity through cGMP-mediated cytoskeletal relaxation [55].

$S1P_2$ and $S1P_3$, in contrast, couple to $G\alpha_{12/13}$ and Gαq, respectively, and activate RhoA, leading to actomyosin contraction and barrier disruption [56]. The relative expression levels of $S1P_1$ versus $S1P_{2/3}$, and the availability of their respective downstream effectors, therefore, determine whether S1P exerts a net barrier-protective or barrier-disruptive effect in a given cell type and context. In pulmonary microvascular endothelial cells, $S1P_1$ predominates and the net effect of S1P is strongly barrier-protective, in contrast to larger pulmonary arterial cells where $S1P_2$ expression is relatively higher [57].

SPHK1 as an endogenous barrier repair signal

We established that SPHK1 is not merely a constitutive barrier maintainer but is dynamically and specifically induced during the resolution phase of inflammatory barrier disruption, representing a controlled endogenous repair response rather than a passive constitutive function [11]. In time-course experiments following PAR-1 activation by thrombin, we demonstrated a characteristic biphasic pattern: an immediate (0 - 30 minutes) permeability increase associated with Ca^{2+} influx and MLCK activation, followed by a delayed (2 - 4 hours) compensatory phase in which SPHK1 activity increased, S1P secretion rose, and barrier function recovered toward baseline [11].

The functional indispensability of SPHK1 for barrier repair was established using Sphk1 knockout (*Sphk1*$^{-/-}$) mice [11]. These animals exhibited a grossly amplified and sustained permeability response to both thrombin and LPS, with markedly greater lung edema formation and significantly higher mortality compared to wild-type littermates. Reconstitution with exogenous S1P or intravenous administration of recombinant human SPHK1 partially rescued barrier function in *Sphk1*$^{-/-}$ mice, confirming the specificity of the SPHK1-S1P pathway in barrier recovery [11].

Mechanistically, barrier recovery by SPHK1/S1P requires secretion of S1P into the extracellular space, where it acts on $S1P_1$ to activate Rac1 and simultaneously suppress RhoA through Rac1-mediated inactivation of Rho GEFs [58]. This shift in GTPase balance - from RhoA dominance during the disruptive phase to Rac1 dominance during recovery - drives a reorganization of the actin cytoskeleton from central stress fibers to peripheral cortical actin, reestablishes VE-cadherin at junctions, and closes paracellular gaps [58]. The temporal regulation of this GTPase switch is exquisitely sensitive to the local concentration of S1P, which is itself determined by the balance between SPHK1-driven synthesis and S1P lyase driven catabolism [59].

Gβγ-Fyn-FAK signaling in AJ reassembly

Beyond the Rac1/RhoA GTPase switch, AJ reassembly following barrier disruption requires additional scaffolding events coordinated by Gβγ subunits released from activated $S1P_1$ [60]. We demonstrated that Gβγ subunits downstream of $S1P_1$ engage Fyn kinase, a Src-family tyrosine kinase that phosphorylates and activates focal adhesion kinase (FAK). FAK then translocates to nascent AJ sites, where it associates with VE-cadherin-β-catenin complexes and stabilizes them through phosphorylation-dependent interactions [60]. This Gβγ-Fyn FAK axis operates in parallel with Rac1 activation and is required for the structural reassembly of AJs rather than merely their biochemical stabilization.

Receptor for activated C kinase 1 (RACK1), a scaffolding protein that we found to negatively regulate Gβγ-Fyn interaction, provides an additional layer of regulation [60]. Under basal conditions, RACK1 sequesters Gβγ and prevents constitutive Fyn activation. During barrier recovery, the $S1P_1$-stimulated release of Gβγ overcomes this RACK1-dependent inhibition, enabling Fyn activation and AJ reassembly [60]. Disruption of RACK1 by siRNA knockdown potentiated Fyn-FAK activation and accelerated barrier recovery following PAR-1-induced disruption, suggesting that pharmacological targeting of the RACK1-Gβγ interaction may represent a strategy to accelerate barrier repair [60].

Functional interplay between TRPC channels and S1P signaling: A molecular rheostat

Opposing regulatory arms of the endothelial permeability rheostat

The concept of a 'permeability rheostat' governed by the opposing actions of TRPC channels and S1P signaling provides a unifying framework for understanding how the endothelium maintains homeostatic barrier function while retaining the capacity for rapid, appropriate permeability responses to inflammatory signals [61]. Under basal conditions, the rheostat is set at a moderate state of AJ stability: TRPC1 activity maintains SPHK1 at low-to-moderate activity, generating enough basal S1P to stabilize AJs but not so much that the barrier becomes completely resistant to physiological permeability signals [12].

During acute inflammatory challenge, TRPC6-mediated Ca^{2+} entry drives the rheostat toward barrier disruption: MLCK is activated, MLC is phosphorylated, RhoA is activated, AJs disassemble, and paracellular gaps open. The initial magnitude of this disruptive phase is determined in part by the basal set point of S1P-mediated barrier protection - a lower basal S1P (as maintained by tonic TRPC1 activity) means less initial resistance to barrier disruption [12,39]. This design principle ensures that the barrier can respond rapidly to acute inflammatory stimuli without requiring an overwhelming signal.

As the acute phase subsides and inflammatory signals wane, the compensatory SPHK1 activation response is initiated. SPHK1 is induced by the same Ca^{2+} transient that initially drove barrier disruption - acting through PKCδ and ERK1/2 - generating a delayed wave of S1P that drives Rac1 activation and barrier recovery [50,62]. The temporal delay between the disruptive and restorative phases ensures that the barrier remains open long enough to allow immune cell transmigration and pathogen clearance before closing. This timed resolution is an essential feature of normal inflammatory physiology, not merely a failure to prevent permeability [62,63].

Transcriptional regulation of TRPC-S1P axis components

The long-term adaptation of the endothelial barrier to chronic inflammatory conditions involves transcriptional regulation of both TRPC channels and S1P pathway components. NFATc1, activated by calcineurin downstream of sustained Ca^{2+} elevation, promotes TRPC6 transcription, creating a Ca^{2+}-dependent positive feedback loop [2,64]. Conversely, KLF2 (Krüppel-like factor 2), a flow-responsive transcription factor that is a master regulator of endothelial quiescence, transcriptionally induces $S1P_1$ and suppresses inflammatory signaling genes including TRPC6 [65]. This KLF2-mediated transcriptional program is part of the laminar shear stress response that maintains endothelial barrier integrity in regions of high flow and is disrupted in areas of low or oscillatory shear—conditions associated with atherosclerosis and vascular inflammation [65].

MicroRNA regulation provides an additional layer of post-transcriptional control. miR-181b, an endothelial-enriched microRNA, has been shown to suppress multiple components of the NF-κB/inflammatory signaling pathway, including TRPC6 indirectly through suppression of upstream activators [65]. miR-193a-3p directly targets TRPC6 mRNA in lung endothelial cells and is downregulated by LPS, contributing to LPS-induced TRPC6 upregulation and barrier disruption [66]. These microRNA-mediated regulatory mechanisms represent potential therapeutic targets for modulating TRPC-S1P axis activity in inflammatory lung disease.

Crosstalk with oxidative stress and mitochondrial signaling

Reactive oxygen species (ROS), generated in abundance during inflammatory lung injury by NADPH oxidase (NOX2) and mitochondrial electron transport chain dysfunction, modulate both TRPC channel activity and S1P signaling in ways that amplify barrier disruption [67]. Hydrogen peroxide (H_2O_2) and superoxide directly oxidize TRPC6 at cysteine residues C553 and C558, potentiating channel activity and Ca^{2+} entry [43]. Simultaneously, ROS inactivate SPHK1 through oxidation of critical cysteine residues in its active site, reducing S1P generation and thereby compromising the barrier repair response [68]. This dual action of ROS - activating TRPC6 while inactivating SPHK1 - creates a pro-permeability redox shift that amplifies the initial inflammatory signal. Antioxidant strategies, including N-acetylcysteine and MitoTEMPO (a mitochondria-targeted antioxidant), partially prevent this ROS-mediated shift *in vitro* and *in vivo*, suggesting that oxidative stress management may synergize with TRPC or S1P-targeted therapies [68].

Clinical relevance: ARDS, sepsis, and COVID-19-associated endotheliopathy

ARDS and the endothelial basis of non-cardiogenic pulmonary edema

ARDS is the prototypical clinical manifestation of lung vascular endothelial barrier failure.

The Berlin Definition classifies ARDS by severity based on the PaO_2/FiO_2 ratio: mild (200 - 300 mmHg), moderate (100 - 200 mmHg), and severe (< 100 mmHg), with corresponding 28 days mortalities of approximately 27%, 32%, and 45% [4]. Histopathologically, early ARDS (within the first 7 days) is characterized by diffuse alveolar damage (DAD), with deposition of protein-rich hyaline membranes in the alveolar spaces, interstitial edema, loss of type I alveolar epithelial cells, and activation of alveolar macrophages - all consistent with failure of the alveolocapillary barrier [3].

The pivotal role of endothelial permeability in ARDS pathogenesis is supported by biomarker studies in critically ill patients. Plasma levels of angiopoietin-2 (Ang-2), a destabilizer of the endothelial Tie-2 receptor that promotes barrier disruption, are elevated in ARDS and independently predict mortality [69]. Similarly, soluble VE-cadherin, shed from AJs during barrier disruption, circulates at elevated levels in sepsis-associated ARDS patients and correlates with both permeability indices and clinical outcomes [70]. Plasma S1P levels are significantly reduced in critically ill patients with ARDS compared to healthy controls, consistent with a failure of endogenous barrier-repair mechanisms [71]. These biomarker data provide direct clinical evidence that the TRPC-S1P regulatory axis described in experimental models operates and is disrupted in human ARDS.

Sepsis-induced ARDS: The LPS-TLR4-TRPC6 pathway in clinical context

Sepsis is the most common cause of ARDS, accounting for approximately 35 - 40% of all ARDS cases [72]. The pathogenesis of sepsis-associated ARDS begins with dissemination of bacterial products (primarily LPS from gram-negative bacteria) into the systemic circulation, where TLR4 activation on circulating monocytes and vascular endothelial cells drives the systemic inflammatory response. In the lung, TRPC6-mediated Ca^{2+} entry downstream of LPS-TLR4 activation is a critical early event in the vascular leak response, as demonstrated in our murine model [39]. The clinical implication is that patients with higher TRPC6 expression or activity at the time of sepsis onset may be at greater risk of developing ARDS - a hypothesis that could be tested through TRPC6 expression profiling of circulating endothelial cells or platelets, which also express TRPC6 [73].

In clinical practice, the management of sepsis-associated ARDS remains predominantly supportive. Fluid resuscitation, guided by dynamic hemodynamic parameters, must balance the need for cardiac output against the risk of worsening pulmonary edema through increased hydrostatic pressure acting on an already-compromised endothelial barrier [74]. The recognition that TRPC6-Ca^{2+}-MLCK signaling amplifies both permeability and NF-κB driven inflammation [39] suggests that anti-inflammatory interventions downstream of TLR4 may inadvertently also reduce endothelial barrier disruption, potentially explaining the modest barrier-protective effects of corticosteroids and statin therapy that have been observed in some ARDS trials [75].

COVID-19-associated endotheliopathy: TRPC and S1P in SARS-CoV-2 infection

The COVID-19 pandemic dramatically highlighted the clinical importance of endothelial barrier regulation, as SARS-CoV-2 infection was found to produce a distinctive pattern of endotheliopathy characterized by widespread vascular inflammation, microthrombus formation, endothelial cell apoptosis, and - in severe cases - frank ARDS [76]. Histopathological studies of COVID-19 lungs revealed extensive endothelial disruption with intracapillary neutrophil infiltration, complement deposition, and a pattern of angiogenesis distinct from classical ARDS, suggesting that SARS-CoV-2 directly injures the endothelium in addition to causing secondary injury through immune-mediated mechanisms [77].

Emerging evidence implicates both TRPC channels and S1P signaling in COVID-19-associated endotheliopathy. The SARS-CoV-2 spike protein, through its interaction with ACE2 and subsequent activation of membrane-bound and shed ACE2 signaling, can activate phospholipase C and generate IP_3 and DAG, which could in principle activate TRPC-mediated Ca^{2+} entry in endothelial cells [78]. Spike protein fragments that engage AT1R (angiotensin receptor type 1) - an established activator of TRPC6 in cardiovascular cells - may

constitute an additional activation mechanism [78]. Critically ill COVID-19 patients have been found to have significantly reduced circulating S1P levels, consistent with impaired SPHK1 activity [79]. The mechanism of SPHK1 suppression in COVID-19 may involve viral driven oxidative stress, cytokine-mediated suppression of SPHK1 expression, or direct interactions between viral proteins and SPHK1 [79,80]. These observations suggest that therapeutic augmentation of S1P signaling may have specific application in COVID-19 associated vascular injury.

Pulmonary hypertension and chronic vascular remodeling

Beyond acute permeability regulation, TRPC and S1P signaling pathways are implicated in the chronic vascular remodeling that underlies pulmonary arterial hypertension (PAH). PAH is characterized by progressive remodeling of pulmonary arteries, including endothelial dysfunction, smooth muscle hypertrophy, and formation of plexiform lesions [81]. TRPC6 expression is upregulated in pulmonary arterial smooth muscle cells from PAH patients compared to controls, and contributes to the enhanced Ca^{2+} entry and proliferative phenotype of PAH-SMCs [82]. In the endothelium, loss of $S1P_1$ signaling - as occurs with chronic receptor internalization due to high S1P levels in inflammatory states - paradoxically promotes endothelial-to-mesenchymal transition (EndMT), a process that contributes to the fibrotic vascular remodeling of PAH [83]. These observations suggest that the TRPC-S1P axis is relevant not only to acute lung injury but also to the chronic vascular pathology of PAH, broadening its potential therapeutic significance.

Therapeutic targeting of the TRPC-S1P axis in lung vascular disease

TRPC6 inhibitors

The identification of TRPC6 as a critical mediator of LPS-induced lung vascular hyperpermeability and inflammation has generated considerable interest in TRPC6 inhibitors as potential therapeutics for ARDS and sepsis. Several small-molecule TRPC6 inhibitors have been developed and characterized in preclinical settings. SAR7334 inhibits TRPC6-mediated Ca^{2+} influx with an IC_{50} of 9.5 nM (confirmed at 7.9 nM by patch-clamp), with significantly lower potency against TRPC3 (IC_{50} = 282 nM) and TRPC7 (IC_{50} = 226 nM), and no significant activity at TRPC4 or TRPC5, and has demonstrated efficacy in reducing pulmonary edema in rodent models of ALI [84]. Larixyl acetate selectively inhibits TRPC6 with an IC_{50} of approximately 0.1-0.6 μM, exhibiting ~12-fold and ~5-fold selectivity over TRPC3 and TRPC7, respectively, and has shown anti-permeability effects in isolated perfused lung preparations [85]. GSK2833503A is a highly selective TRPC6 inhibitor (>100-fold selectivity over TRPC3 and TRPC7) that has been used as a tool compound to dissect TRPC6 specific contributions to Ca^{2+} signaling in endothelial cells [85,86].

A key challenge in translating TRPC6 inhibition to clinical practice is selectivity across vascular beds. TRPC6 is expressed in pulmonary, systemic, and renal vasculature, where it plays distinct physiological roles. In the kidney, TRPC6 is expressed in podocytes and contributes to glomerular filtration barrier integrity; loss-of-function TRPC6 mutations have been associated with familial focal segmental glomerulosclerosis (FSGS) in humans, while gain-of-function mutations cause nephrotic syndrome [87]. Systemic TRPC6 inhibition could therefore impair podocyte function, a concern that underscores the need for endothelial targeted delivery strategies. Nanoparticle-based delivery platforms functionalized with endothelial-targeting ligands (e.g. anti-ICAM-1 antibodies, RGD peptides) have demonstrated the ability to achieve selective endothelial delivery of pharmacological payloads in rodent ALI models and represent a promising strategy for TRPC6-targeted therapy [88].

Targeting TRPC1 to amplify basal S1P signaling

The discovery that TRPC1 tonically suppresses SPHK1 [12] opens an alternative therapeutic strategy: rather than directly supplementing S1P, modulating TRPC1 activity to elevate endogenous SPHK1 and S1P could achieve sustained barrier protection without the limitations of exogenous S1P administration. Pharmacological inhibitors of TRPC1, including SKF-96365 (which inhibits TRPC1 in addition to other TRPC channels) and the more selective TRPC1-blocking antibody 1E6, have been used experimentally to increase basal SPHK1 activity

and enhance VE-cadherin surface expression in cultured endothelial cells [12]. Whether chronic TRPC1 inhibition would be tolerated systemically remains to be evaluated, given TRPC1's roles in vascular smooth muscle tone and salivary gland secretion.

Exogenous S1P and S1P receptor agonists

Exogenous S1P administration has demonstrated consistent barrier-protective efficacy in rodent models of ALI, including LPS-induced, mechanical stretch-induced, and ischemia reperfusion-induced models [89]. Recombinant S1P administered intratracheally or intravenously reduces lung vascular leak, improves oxygenation, and decreases inflammatory cytokines in murine ARDS models. The challenges of clinical translation include the short plasma half-life of S1P (minutes), its rapid metabolism by S1P lyase, its pleiotropic effects through multiple receptor subtypes, and the potential for paradoxical barrier disruption through $S1P_{2/3}$ at high concentrations [90].

These limitations have driven the development of S1P receptor subtype-selective agonists. FTY720-phosphate is a full agonist at $S1P_1$, $S1P_3$, $S1P_4$, and $S1P_5$, but causes prolonged, irreversible internalization and downregulation of $S1P_1$ specifically - producing functional antagonism of this subtype. This distinguishes FTY720's mechanism from classical agonism, where receptor recycling normally occurs [91]. FTY720 initially enhances barrier function in endothelial cells but causes receptor internalization with prolonged exposure, paradoxically disrupting the barrier at later time points - a significant concern for ARDS therapy [91]. More recently, biased $S1P_1$ agonists that activate Gi-Rac1 signaling without triggering receptor internalization have been developed. AUY954 represent this class and have shown sustained barrier-protective activity without the rebound permeability seen with FTY720 [92,93]. These biased agonists represent the most clinically promising $S1P_1$-targeted agents for ARDS therapy.

HDL-S1P as a therapeutic strategy

Approximately 65% of circulating S1P is bound to HDL particles, primarily via interaction with apolipoprotein M (ApoM), while 30% is bound to albumin [94]. ApoM-bound S1P on HDL engages $S1P_1$ with different kinetics and downstream signaling characteristics compared to albumin-bound S1P; ApoM-S1P preferentially activates β-arrestin-biased signaling that promotes sustained $S1P_1$ surface expression and more durable barrier protection [94]. Patients with low HDL-S1P levels - including those with sepsis, critical illness, and COVID-19 - may be particularly susceptible to barrier failure due to loss of this endogenous barrier-protective carrier system [79,94].

Reconstituted HDL (rHDL) infusion, originally developed to enhance reverse cholesterol transport in cardiovascular disease, can increase circulating S1P particularly when enriched with apolipoprotein M. HDL-associated S1P has been shown to promote endothelial barrier integrity via $S1P_1$ receptor signaling, and preclinical studies suggest that modulation of the HDL-S1P axis may attenuate vascular leak and inflammation in models of sepsis and acute lung injury [95]. Clinical translation of vascular-targeted therapies in sepsis has proven challenging. For example, the EUPHRATES trial of polymyxin B hemoperfusion targeting endotoxemia did not demonstrate a mortality benefit in the overall population, highlighting the difficulty of translating mechanistic insights into effective therapies [96]. ApoM overexpression using adeno-associated viral vectors or ApoM-Fc fusion proteins that deliver S1P to $S1P_1$ in a controlled manner represent next-generation strategies for HDL-S1P-based barrier therapy [97].

SPHK1 activators and gene therapy approaches

Direct activation of SPHK1 as a therapeutic strategy has been explored using small-molecule activators. K145 and Compound 31 are selective SPHK1 activators that have demonstrated the ability to increase cellular S1P, activate Rac1, and enhance barrier function in endothelial monolayer assays [98]. However, SPHK1 activation must be carefully controlled, as chronic overactivation can paradoxically promote barrier disruption through $S1P_{2/3}$ engagement at high S1P concentrations, and excessive S1P generation has been linked to NLRP3 inflammasome activation in macrophages [98].

Gene therapy approaches using adeno-associated virus (AAV) serotype 2 or lipid nanoparticle-delivered mRNA encoding SPHK1 have shown promise in restoring barrier function in *Sphk1*$^{-/-}$ mice and in models of aging-associated endothelial dysfunction where SPHK1 expression is constitutively reduced [99]. The selectivity of endothelial promoters (ICAM2, TIE2, VE-cadherin promoters) for driving SPHK1 expression specifically in the vascular endothelium addresses the concern of off-target effects in inflammatory cells where S1P promotes different biological outcomes [99].

Combination strategies

Given the functional opposition between TRPC6-mediated Ca^{2+} influx, which promotes endothelial barrier disruption, and $S1P_1$ receptor signaling, which enhances barrier integrity, a combinatorial therapeutic strategy targeting both pathways is conceptually attractive. TRPC6 contributes to pulmonary vascular dysfunction, and its pharmacologic inhibition with compounds such as SAR7334 suppresses Ca^{2+} influx and attenuates lung inflammatory responses [84,100]. In contrast, activation of $S1P_1$ signaling with agonists such as FTY720 enhances endothelial barrier function and reduces vascular leak in models of acute lung injury [101]. Although these findings suggest that simultaneous TRPC6 inhibition and $S1P_1$ activation may provide complementary protective effects, direct experimental evidence demonstrating additive or synergistic benefit of this combination in lung injury models remains limited, and further studies are required to define optimal therapeutic strategies.

Precision medicine approaches to TRPC-S1P axis targeting may be facilitated by emerging biomarker panels. Plasma S1P levels, circulating SPHK1 activity (measurable by enzymatic assay in peripheral blood mononuclear cells), and soluble VE-cadherin as a marker of ongoing AJ disruption could be combined into a 'barrier function index' to guide targeted therapy [70,101]. Patients identified as having both low SPHK1 activity (impaired repair) and high TRPC6 expression (exaggerated disruptive signaling) may represent the subgroup most likely to benefit from combination TRPC6 inhibitor plus $S1P_1$ agonist therapy.

Conclusion

The endothelial barrier of pulmonary microcirculation is the central determinant of lung fluid homeostasis, and its failure underlies the pathogenesis of ARDS and sepsis-associated organ dysfunction. Over the past two decades, the molecular mechanisms governing barrier disruption and restoration have been elucidated to a remarkable degree of precision, revealing a central regulatory axis defined by the functional antagonism between Ca^{2+}conducting TRPC channels and the SPHK1-S1P-$S1P_1$ barrier-repair pathway.

TRPC6, gated by DAG downstream of TLR4 activation, is a critical transducer of innate immune signals into vascular permeability, and its activity additionally amplifies NF-κB driven inflammation through a novel MLCK-MyD88-IRAK4 signaling nexus. TRPC1, through tonic suppression of SPHK1, sets the basal threshold for AJ stability and determines the magnitude of the permeability response to acute inflammatory signals. Counterbalancing these disruption signals, SPHK1-derived S1P - acting through $S1P_1$-Rac1 signaling and the Gβγ-Fyn-FAK scaffolding axis - drives AJ reassembly and barrier recovery during the resolution phase of inflammation. The failure of this compensatory response, as occurs in genetic SPHK1 deficiency, sepsis-associated ARDS, and COVID-19, results in sustained, unresolved barrier disruption and worsened outcomes.

The clinical landscape of ARDS management remains devoid of approved barrier-targeted pharmacotherapies, representing a critical unmet need given the persistently high mortality of this syndrome. The mechanistic insights reviewed here identify at least five distinct therapeutic intervention points: TRPC6 inhibition, TRPC1 modulation to amplify SPHK1, exogenous S1P or biased $S1P_1$ agonists, HDL-S1P reconstitution, and SPHK1 activators or gene augmentation. Each strategy has demonstrated efficacy in preclinical models, and the most advanced clinical data come from rHDL infusion trials and FTY720-based approaches.

Looking forward, three areas of investigation are particularly promising. First, the development of endothelial-targeted drug delivery systems using nanoparticles conjugated to ICAM-1-targeting ligands or other endothelial-selective moieties will be essential for translating TRPC6 inhibitors and SPHK1 activators into clinical practice without off-target effects on podocytes, smooth muscle cells, and inflammatory cells. Second, the identification of patient subpopulations most likely to benefit from specific interventions - using biomarkers such as plasma S1P levels, circulating SPHK1 activity, and soluble VE-cadherin - will be critical for demonstrating efficacy in clinical trials, given the heterogeneity of ARDS. Third, the emerging appreciation of COVID-19-associated endotheliopathy as a distinct mechanistic entity that engages the TRPC-S1P axis through unique viral mechanisms provides both a new model system for studying endothelial barrier biology and an urgent new clinical context in which barrier-targeted therapeutics are needed.

In conclusion, the TRPC-S1P signaling axis represents one of the most thoroughly validated and mechanistically complete therapeutic targets in lung vascular biology. With continued progress in targeted drug delivery, biased receptor pharmacology, and precision biomarker guided patient selection, the translation of these mechanistic insights into life-saving therapies for ARDS, sepsis, and COVID-19 represents both an achievable and urgently needed clinical goal.

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Conflict of Interest

The authors declare no financial or personal conflicts of interest with respect to the content of this article.

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