Transient receptor potential channels and regulation of lung endothelial permeability

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Abstract: This review highlights our current knowledge regarding expression of transient receptor potential (TRP) cation channels in lung endothelium and evidence for their involvement in regulation of lung endothelial permeability. Six mammalian TRP families have been identified and organized on the basis of sequence homology: TRPC (canonical), TRPV (vanilloid), TRPM (melastatin), TRPML (mucolipin), TRPP (polycystin), and TRPA (ankyrin). To date, only TRPC1/4, TRPC6, TRPV4, and TRPM2 have been extensively studied in lung endothelium. Calcium influx through each of these channels has been documented to increase lung endothelial permeability, although their channel-gating mechanisms, downstream signaling mechanisms, and impact on endothelial structure and barrier integrity differ. While other members of the TRPC, TRPV, and TRPM families may be expressed in lung endothelium, we have little or no evidence linking these to regulation of lung endothelial permeability. Further, neither the expression nor functional role(s) of any TRPML, TRPP, and TRPA family members has been studied in lung endothelium. In addition to this assessment organized by TRP channel family, we also discuss TRP channels and lung endothelial permeability from the perspective of lung endothelial heterogeneity, using outcomes of studies focused on TRPC1/4 and TRPV4 channels. The diversity within the TRP channel family and the relative paucity of information regarding roles of a number of these channels in lung endothelium make this field ripe for continued investigation.

Keywords: TRP channels, calcium, lung endothelial permeability, lung endothelial heterogeneity.

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INTRODUCTION

The transient receptor potential (TRP) channel superfamily is perhaps the largest known cation channel family contributing to calcium entry in lung endothelium. Since calcium influx through plasmalemmal calcium channels can impair the integrity of the lung endothelial barrier, and thus its permeability to fluid and protein, TRP channels have the potential to be both critical participants in and therapeutic targets for acute lung injury. This review focuses on our current knowledge regarding TRP channel expression in the lung and evidence supporting a role for TRP channels in regulation of lung endothelial barrier integrity and permeability. Changes in lung endothelial permeability due to calcium influx can certainly be modulated by the availability of cyclic nucleotide pools and mediated by myriad downstream signaling pathways. However, these issues have been reviewed at length.¹⁻⁶ Thus,

we focus on TRP channel-mediated calcium entry and loss of endothelial barrier integrity. Given the growing recognition of structural and functional heterogeneity of lung endothelium,⁷⁻¹⁰ we also discuss the evidence for TRP channels' involvement in regulation of lung endothelial permeability from that perspective as well.

Currently, 27 human TRP isoforms have been identified (28 in mouse), grouped into 6 families based on amino acid homology: TRPC (canonical), TRPV (vanilloid), TRPM (melastatin), TRPML (mucolipin), TRPP (polycystin), and TRPA (ankyrin). A seventh family—TRPN (NO-mechano potential C)—has not been identified in any vertebrate species other than zebra fish.¹¹ TRP channel proteins contain 6 transmembrane spanning domains, with a pore loop region between transmembrane domains 5 and 6. Domains within the intracellular N- and C-termini contribute to

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channel assembly and regulation. Individual TRP proteins assemble into homo- or heterotetramers to form functioning cation channels. While Nilius and Owsianik¹¹ recently suggested that most TRP channels exist as homotetramers, heteromultimeric assembly of several TRP proteins from within or between subfamilies into functional cation channels has been described.¹²⁻¹⁵ Nonetheless, aside from a recent report using Förster resonance energy transfer (FRET) to document assembly of TRPC1 and TRPC4 into heteromeric channels in pulmonary artery endothelial cells and endothelial caveolar fractions isolated from intact lung,¹⁶ there is very little direct evidence for TRP channel composition and stoichiometry in lung endothelium. To further complicate this issue, protein expression, channel assembly, and trafficking to the plasma membrane may potentially be dictated by developmental stage and/or disease in vivo,^{17,18} passage of endothelial cells in culture,¹⁹ or the degree of confluency of endothelial monolayers in culture.²⁰

TRP CHANNELS, CALCIUM ENTRY, AND LUNG ENDOTHELIAL PERMEABILITY

TRPC (canonical)

TRPC family members 1–7 share an invariant amino acid sequence (EWKFAR) in the C-terminus called the TRP box, as well as 3–4 NH₂-terminal ankyrin repeat domains. Generally, TRPCs form nonselective cation channels, with calcium-to-sodium selectivity ratios ranging from 1.1 to 9.²¹ Protein expression for TRPC1, 3, 4, 6, and 7 has been variously identified in pulmonary vascular endothelium in the intact lung by Western blot or immunohistochemical approaches.^{18,22-24} In addition, expression of TRPC1, 2, 4, and 6 messenger RNA (mRNA) and protein has been identified in human, mouse, and rat pulmonary artery endothelial cells.^{7,23,25-30}

In terms of activation mechanisms, TRPC channels can be distinguished as those activated by depletion of endoplasmic reticulum (ER) calcium stores (store-operated channels)—TRPC1/4—and those activated by diacylglycerol (DAG)—TRPC3/6/7. While the latter are often termed receptor-operated channels, this is somewhat of a misnomer, as synthesis of both inositol-1, 4, 5-trisphosphate (IP₃, an endogenous initiator of store depletion) and DAG can result from receptor-ligand interactions in lung endothelial cells. However, this functional distinction may not clearly delineated between TRPC subfamily groups because of potential heterologous channel assembly, i.e., interaction between TRPCs typically associated with store-operated channels (TRPC1 and 4) and those associated with receptor-operated channels (TRPC3, 6, and 7). Reso-

lution of this issue is complicated by the relative lack of information regarding stoichiometry for TRPC channel assembly in lung endothelium. The only available information has been provided by Cioffi and colleagues,¹⁶ who used a FRET-based reporter system to identify the composition of the channel responsible for the calcium-selective store-operated current (I_{SOC}) in cultured pulmonary artery endothelium and in caveolar fractions harvested from intact lung endothelium. They found that the I_{SOC} channel consists of at least one TRPC1 and 2 TRPC4 proteins and that interaction of TRPC4 with Orai1 is responsible for the channel's calcium selectivity. While this study resolves stoichiometry for one store-operated channel, lung endothelium also expresses nonselective cation channels that are activated by store depletion.²²

Agents that evoke depletion of ER calcium stores, resulting in activation of store-operated channels, include IP₃ and ER calcium ATPase (SERCA) inhibitors such as the plant alkaloid thapsigargin and cyclopiazonic acid, as well as the calcium chelators 1,2-bis(o-aminophenoxy) ethane-*N,N,N',N'*-tetraacetic acid (BAPTA) and *N,N,N',N'*-tetrakis(2-pyridylmethyl)ethylene diamine (TPEN). The mechanisms by which these agents act vary: IP₃ binds IP₃ receptors, leading to efflux of calcium from ER stores; SERCA inhibitors prevent calcium reuptake into ER stores and thus cause depletion; and finally, BAPTA and TPEN chelate either cytosolic or ER calcium, thus preventing refilling or store depletion, respectively. Endogenous agonists, such as thrombin and angiotensin II, act through plasma membrane receptors to evoke store depletion in lung endothelium.^{29,31} Store depletion, rather than the subsequent rise in cytosolic calcium, is responsible for activation of store-operated channels per se.³²⁻³⁴

Regardless of the tools used to elicit store depletion, it is clear that calcium entry into lung endothelium following store depletion increases lung endothelial permeability, both in vitro and in vivo.^{18,29,35-37} The mechanisms linking store depletion and activation of store-operated channels are actively debated.³⁸⁻⁴⁰ However, the increased permeability response to I_{SOC} activation is due to inter-endothelial gap formation, classically attributed to myosin light-chain kinase-dependent retraction of endothelial cell borders.^{5,6,41,42} For example, thapsigargin induces gap formation in cultured pulmonary artery endothelial cells (visualized via light microscopy) and in extra-alveolar vessels from isolated rat lung (visualized via scanning electron microscopy), structural changes that correlate with increased transendothelial diffusion of macromolecules and increases in the filtration coefficient (K_f), respectively.^{24,35,37,42} At the ultrastructural level, interen-

dothelial gap formation was observed in extra-alveolar vessels at least 100 μm in diameter, signifying that the increased permeability effect induced by store-operated calcium entry activation is due to weakening of endothelial barrier integrity in the extra-alveolar compartment (Fig. 1).³⁵ Notably, despite a 3-fold or more increase in K_f , thapsigargin-induced store depletion does not evoke alveolar flooding.⁷ Tiruppathi et al.²⁹ found that the calcium entry response to thrombin-induced PAR-1 activation in lung endothelium, which initiates store depletion, was significantly reduced in endothelial cells isolated from TRPC4^{-/-} mice. Further, in TRPC4^{-/-} lung endothelial cells, the decrement in thrombin-induced calcium entry correlated with loss of actin stress-fiber formation and less cell retraction. In parallel studies, they found that the thrombin-induced increase in K_f was attenuated in lungs from TRPC4^{-/-} mice. In agreement with these observations, the lung endothelial permeability response to thapsigargin-induced store depletion is ablated in animals with congestive heart failure,^{18,31,36} coincident with downregulated expression of TRPC1, 3, and 4 in extra-alveolar vessels.¹⁸

Unlike store-operated calcium entry, activation of TRPC3, 6, and 7 is triggered by DAG and occurs independent of store depletion.⁴³ Alvarez et al.¹⁸ found that K_f did not increase in isolated rat lungs treated with the DAG mimic 1-oleoyl-2-acetyl-*sn*-glycerol (OAG), despite confirmed protein expression for TRPC 3 and 6/7 in extra-alveolar vessels. They concluded that calcium entry through a putative TRPC 3-, 6-, and/or 7-containing chan-

nel does not play a role in mediating increases in lung permeability. However, Singh et al.²⁸ reported that activation of calcium entry by OAG in human pulmonary artery endothelial cells led to protein kinase C- α (PKC α)-dependent activation of RhoA, which in turn induced interendothelial cell gap formation. In their study, both OAG- and thrombin-induced calcium entry was significantly attenuated in cells treated with small interfering RNA (siRNA) against TRPC6. In concert with decreased calcium entry, PKC α activity, RhoA activity, myosin light-chain phosphorylation, actin stress-fiber formation, and monolayer permeability were all decreased. In an extension of this study, Kini et al.²⁶ found that phosphatase and tensin homolog (PTEN) interacts with TRPC6 but not with TRPC1, 3, or 4 in human pulmonary artery endothelial cells. The PTEN interaction with TRPC6 was required for OAG-induced calcium entry and subsequent increases in monolayer permeability. Consistent with the latter findings, ischemia and OAG-induced rises in intracellular calcium levels and subsequent increases in monolayer permeability were attenuated in endothelial cells isolated from TRPC6^{-/-} mouse lungs.²⁶ Similarly, TRPC6^{-/-} mouse lungs are protected from ischemia/reperfusion-induced increases in lung permeability and edema.²⁶ Collectively, these results support the notion that calcium entry via TRPC6 can mediate increases in lung permeability. Nonetheless, the disparate findings with respect to the permeability response to OAG-induced calcium entry in the intact rat lung versus those in mouse lung and human pulmonary artery endothelial monolayers have yet to be resolved.

Figure 1. Scanning electron microscopy in thapsigargin-treated rat lung. Thapsigargin-mediated activation of store-operated calcium entry promoted interendothelial gap formation in pulmonary arteries (A) but not in alveolar capillary endothelium (B). These findings are consistent with the notion that extra-alveolar and septal capillary endothelium have distinctive phenotypes, such that store-operated calcium entry via transient receptor potential channels increases lung endothelial permeability only in the extra-alveolar compartment. alv: alveolar space; c: alveolar capillary; arrowheads indicate interendothelial junctions. Scale bars: 10 μm . Adapted from Chetham et al.³⁵

TRPV (vanilloid)

The TRPV family members (1–6) are broadly classified into 4 groups based on their structure and function: TRPV1/2, TRPV3, TRPV4, and TRPV5/6. The TRPV proteins contain versions of the TRP box, the TRP domain, and 3–6 N-terminal ankyrin repeat domains. TRPV1 has been described as both nonselective and calcium selective.^{21,44} TRPV2–4 form nonselective channels, with calcium-to-sodium selectivity ratios ranging from ~1 to 10. In contrast, TRPV5 and 6 have been identified as selective calcium channels, with calcium-to-sodium ratios greater than 100.²¹ TRPV proteins tend to form homomeric channels,^{11–13} although interactions between TRPV1 and 2 and between TRPV5 and 6 have been observed by FRET, co-immunoprecipitation, and colocalization studies in heterologous expression systems.¹³

Expression of TRPV1 has been identified in human pulmonary artery and human lung microvascular endothelial cells by reverse-transcription polymerase chain reaction (RT-PCR).^{23,45} TRPV1 is activated by the vanilloid capsaicin, capsaicin analogs (e.g., nonivamide, resiniferatoxin, and olvanil), moderate heat ($\geq 43^{\circ}\text{C}$), and low pH (≤ 5.9).^{21,44,46} TRPV1 can also be activated by endogenous lipid compounds referred to as “endovanilloids,” such as anandamide, 2-arachidonoyl glycerol, and *N*-arachidonoyl amino acids.^{47–51} There is little evidence supporting a role for TRPV1 in regulation of lung endothelial permeability. Neither pharmacological silencing nor genetic deletion of TRPV1 affected the edema response to lipopolysaccharide, or LPS.⁴⁵ In support of these findings, treatment of isolated rat lungs with the TRPV1 agonist 4 α -phorbol-12,13-didecanoate-20 homovanillate (4 α PDDHV) had no impact on K_f in rat lung.⁷ Finally, in a model of lung ischemia/reperfusion injury in rabbits,⁵² activation of TRPV1 by capsaicin actually attenuated the increased lung wet-to-dry weight ratio and protein levels in the bronchoalveolar lavage fluid. Although TRPV1 mRNA is highly expressed in the alveolar region of the mouse lung, expression is minimal in human lung microvascular endothelial cells.⁴⁵ Thus, any role of TRPV1 in regulation of lung endothelial permeability in the intact lung may be indirect.

TRPV4 protein is expressed in cultured rat pulmonary arterial and microvascular endothelial cells and in endothelial cells from rat lung.^{7,25} Further, immunohistochemistry reveals prominent expression of TRPV4 in the alveolar septal wall of human, rat, and mouse lung, while expression in extra-alveolar vessels is more variable.⁷ TRPV4 can be activated by heat,⁵³ cell swelling,^{54,55} mechanical and shear stress,^{53,55,56} epoxyeicosatrienoic acids (EETs),^{57,58} and synthetic phorbol esters such as 4 α -phorbol-12,13-

didecanoate (4 α PDD).⁵⁹ The impact of TRPV4 activation on K_f was clearly dependent on calcium entry, because the response was mitigated by either perfusion with a low calcium buffer or pretreatment of lungs with the TRPV antagonist ruthenium red.⁷ Further, the 4 α PDD-induced increase in K_f in isolated mouse lungs, but not that evoked by thapsigargin, was absent in lungs from TRPV4^{-/-} mice (Fig. 2). Despite similar increases in K_f due to activation of store-operated channels or TRPV4, only the latter is associated with alveolar flooding (Fig. 3). Transmission electron microscopy revealed that TRPV4 activation, but not activation of store-operated channels, elicits disruption of the alveolar septal endothelial barrier leading to alveolar flooding in isolated rat and mouse lungs.⁷ Notably, this disruption does not appear to involve interendothelial cell gap formation but rather a complex array of perturbations in cell morphology (Fig. 4). More recently, the advent of specific small-molecule agonists and antagonists for TRPV4 has provided new tools. Intravenous administration of the TRPV4 agonist GSK1016790A in rats significantly increases lung wet-to-dry weight ratio, resulting in perivascular edema as well as multifocal alveolar flooding.⁶⁰ Similarly, we have found increases in lung wet-to-dry weight ratio and albumin levels in the bronchoalveolar lavage fluid after GSK1016790A administration to perfused mouse

Figure 2. Activation of either TRPV4 or store-operated channels increases endothelial permeability in isolated, perfused mouse lung. Challenge of lungs with either 4 α -phorbol-12,13-didecanoate-20 (4 α PDD) or thapsigargin (TG) to activate TRPV4 or store-operated transient receptor potential (TRP) channels, respectively, similarly increased endothelial permeability, as measured by the filtration coefficient K_f . Only the response to 4 α PDD was abrogated in lungs from TRPV4^{-/-} mice. Asterisk indicates $P < 0.05$ versus baseline. DMSO: dimethyl sulfoxide. From Alvarez et al.⁷

Figure 3. Activation of calcium entry via TRPV4, but not that via store-operated channels, elicits lung endothelial barrier disruption via distinct targeting of the alveolar septal barrier. Activation of TRPV4 by 4 α -phorbol-12,13-didecanoate-20 (4 α PDD) significantly increased the frequency of blebs and breaks in the alveolar septal wall in both rat and mouse lung (not shown), which led to significant increases in the alveolar fluid volume fraction (V_{af}/V_{as}) in mouse lung. A similar trend was observed in rat lung. Despite 2–3-fold elevation in K_f , thapsigargin had no impact on the integrity of the alveolar septal barrier or alveolar fluid accumulation in either group. Asterisk indicates $P < 0.05$ versus control; pound sign indicates $P < 0.05$ versus thapsigargin. Data from Alvarez et al.⁷

lungs (P. C. Villalta and M. I. Townsley, unpublished observations), effects mitigated in lungs from TRPV4^{-/-} mice.

TRPV4 activation by mechanical stress induces lung injury as well. Jian et al.⁶¹ found that high venous pressure-induced increases in lung permeability were mediated by cytochrome P450 epoxygenase-dependent activation of TRPV4 in the mouse lung. P450 epoxygenases are enzymes that synthesize EETs from arachidonic acid.⁶² Thus, these data are consistent with EET-induced activation of TRPV4 noted in vitro.^{57,58,63} In a model of ventilator-induced lung injury, calcium-dependent increases in lung permeability were exacerbated by heat in TRPV4^{+/+} mice but were absent in TRPV4^{-/-} mice. This mechanical stress-induced lung injury was also attenuated by use of cytochrome P450 epoxygenase inhibitors.⁶⁴ However, the permeability response to ventilator-induced injury in mouse lung appears to specifically require TRPV4 expression in alveolar macrophages, because repletion of wild-type macrophages to lungs of TRPV4^{-/-} mice restored the injury response.⁶⁵ Collectively, these studies support a role for EETs in initiating TRPV4 activation and the

permeability response to mechanical stress in the intact lung, but they also suggest differences in the cellular repertoire recruited to elicit lung injury. The role of TRPV4 in the permeability response to high airway and vascular pressure in mouse lung has been well documented by us and others.^{61,64–66} In both experimental paradigms, the permeability response is significantly attenuated by pretreatment with the nonspecific TRPV antagonist ruthenium red and by use of TRPV4^{-/-} mice. However, until recently, extension of these observations to rats and larger mammals has been hampered by the lack of pharmacologic and genetic tools to specifically target TRPV4 in these species. New studies using small-molecule antagonists—GSK2193874 and GSK2263095—that are selective for TRPV4 have confirmed a TRPV4-mediated permeability response to high vascular pressure in rat and canine lung.⁶⁷ Further, use of these specific TRPV4 antagonists has provided support for involvement of TRPV4 in the development of pulmonary edema in acute pulmonary venous hypertension induced by aortic banding in rats, as well as the development and resolution of pulmonary edema in a murine model of chronic myocardial infarction.⁶⁷

The downstream targets of TRPV4-mediated calcium entry that mediate permeability increases in the lung are unknown. Our observation that interendothelial junctions of capillary endothelial cells in mouse or rat lung appear to be intact after TRPV4 activation (Fig. 4)⁷ challenges the established paradigm that increases in lung endothelial permeability are invariably due to endothelial cell retraction and subsequent gap formation. In cultured human umbilical vein endothelial cells, TRPV4 activation leads to dose-dependent cell detachment,⁶⁰ leading us to consider activation of proteases to mediate basement membrane degradation, loss of cell-matrix tethering, and/or initiation of cell death processes as potential mechanisms for injury. In preliminary work, we have identified increased active matrix metalloproteinase (MMP) 2 and 9 expression in mouse lungs in concert with TRPV4-induced lung injury,⁶⁸ although whether these MMPs actually cause the TRPV4-induced increase in lung permeability remains to be determined. Increases in intracellular calcium, if sufficient, can orchestrate cell injury and death,^{69,70} and TRPV4 activation is capable of initiating cell death in several tissues.^{71–73} However, we do not observe activated caspase-3 or positive TUNEL (terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling) staining in mouse lungs where TRPV4-mediated lung injury occurred (P. C. Villalta and M. I. Townsley, unpublished observations). As an alternative, the cell swelling, blebbing, and

Figure 4. TRPV4 activation preferentially targets the alveolar septal network. Isolated rat and mouse lungs treated with 4 α -phorbol-12,13-didecanoate-20 to activate TRPV4 were visualized by transmission electron microscopy. The increase in V_{af}/V_{as} noted in Figure 3 was not associated with development of interendothelial gaps (gray arrowheads). Rather, TRPV4 activation resulted in endothelial cell detachment from the basement membrane (A, arrow) and microparticle formation (B, arrow), as well as endothelial cell vacuolization (C) and swelling (D). A color version of this figure is available online.

vacuolation induced by TRPV4 activation resemble structural defects induced by another form of programmed cell death, oncosis.⁷⁴ Oncosis, implicated as a mode of cell death in a few models of acute lung injury,⁷⁵⁻⁷⁷ can be initiated by calcium-dependent activation of calpains, a family of calcium-dependent cysteine proteases.⁷⁸ Although there are no reports linking calpains and oncosis in TRPV4-induced lung injury, calpain inhibition does attenuate lung interstitial and air space edema during inflammation,⁷⁹ suggesting a potential role for deformation of the septal barrier. In vitro, separate reports have demonstrated that cyclic stretch both activates TRPV4⁸⁰ and induces calpain-mediated alveolar epithelial barrier disruption.⁸¹ Mechanisms by which TRPV4 activation leads to endothelial barrier disruption in the lung should be further resolved.

Involvement of other TRPV channels in regulation of lung endothelium permeability is uncertain. Although TRPV2 mRNA and protein are indeed expressed in rat lung endothelium,^{23,25} there is no evidence to date linking this TRP channel to regulation of endothelial permeability. In human pulmonary artery endothelium, mRNA for TRPV3, 5, and 6 was not identified with RT-PCR.²³ No studies to date have assessed expression of these TRPV channels in lung microvascular endothelium.

TRPM (melastatin)

The TRPM (1–8) family members, divided into the TRPM1/3, 2/8, 4/5, and 6/7 groups, lack N-terminal ankyrin repeat domains.²¹ TRPM channels vary greatly in calcium selectivity ratio, ranging from those that are not calcium permeable (TRPM4/5) to those with selectivity ratios greater than 3.

Expression of all TRPMs, with the exception of TRPM5, has been verified by RT-PCR in human pulmonary artery endothelial cells.^{23,82} In addition, protein expression of the alternate splice form TRPM2-s and the full-length protein TRPM2-l, as well as TRPM4, has been confirmed by Western blot in human pulmonary artery endothelial cells.^{23,82} TRPM2 can be activated in the setting of oxidant stress, although this effect is now recognized to be indirect. Direct activation of TRPM2 is elicited by intracellular adenosine diphosphate ribose (ADPR),^{83,84} cyclic ADPR, H₂O₂,⁸⁵ nicotinic acid adenine dinucleotide phosphate,⁸⁶ and arachidonic acid.⁸⁷ Nonetheless, the role of TRPMs in lung endothelial permeability is unclear. Hecquet et al.⁸² provided evidence for involvement of TRPM2 in oxidative stress-induced damage to human pulmonary artery endothelium. They determined that treatment of human pulmonary artery endothelial monolayers with sublytic concentrations of H₂O₂ led to calcium influx and decreased

monolayer transendothelial resistance in a dose-dependent fashion. Inhibition of calcium entry by blockade of intracellular ADPR generation or siRNA-mediated suppression of TRPM2-l expression diminished the decrease in monolayer barrier integrity. The authors also confirmed a regulatory mechanism—noted in other cell lines^{88,89}—by which TRPM2-s isoform overexpression modulates the activity of the TRPM2 channel and the increased monolayer permeability response to H₂O₂. The involvement of TRPM2 in the intact lung is more complex. In a model of endotoxin-induced lung injury, where oxidative stress is prevalent, Di and colleagues⁹⁰ found that lung edema increased and survival decreased in TRPM2^{-/-} mice bred into a C57BL/6 background, compared to wild-type controls. However, Hardaker et al.⁹¹ reported that TRPM2 deficiency in a BALB/c mouse strain had no effect on bronchoalveolar-lavage albumin levels following LPS challenge, compared to that in wild-type controls. These discrepancies between the permeability effects of TRPM2 activation in human pulmonary artery endothelial cells and those in mouse lung could be due a number of factors. However, the varied sensitivity to lung injury among different mouse strains may be an important consideration. Notably, BALB/c mice display a lower sensitivity to lung injury induced by LPS or ischemia/reperfusion than do C57BL/6 mice.^{92,93} Thus, no definitive conclusions can be reached yet regarding the role of TRPM2 in mediating increases in lung endothelial permeability.

TRPML (mucolipin)

The mammalian members of the TRPML family, TRPML1–3, have been identified largely as endolysosomal nonselective cation channels that mediate intralysosomal calcium release in the endocytic pathway.^{94–96} There is no evidence of TRPML expression or activity in the plasma membrane, and only recently have technological advances allowed for assessment of the channel in lysosomal membranes.⁹⁷ TRPML1 mRNA is ubiquitously expressed in all major tissues, and TRPML3 mRNA has been detected in mouse lung.⁹⁸ Neither the expression nor the functional role of TRPML channels in lung endothelium is known. Studies are needed to determine whether these channels influence endothelial barrier properties.

TRPP (polycystin)

The TRPP family members TRPP2, 3, and 5 are more homologous to the TRPML family than to any other TRP family.⁹⁹ These calcium-regulated, nonselective channels have been extensively studied in renal epithelium, where

their role in mechanosensation and involvement in polycystic kidney disease have been reported.^{99–102} Berrout et al.¹⁰³ implicated TRPP2 in a model of traumatic brain injury, where mechanical stretch of brain microvascular endothelial cells promoted calcium influx and actin stress-fiber formation independent of store depletion. These investigators reported protein expression of TRPP2, along with TRPC1, TRPV4, and other TRPs, using immunocytochemistry and Western blot in mouse brain microvascular endothelial cells. Knockdown of TRPP2, TRPC1, or both by siRNA, as well as nonselective inhibition of TRPPs and TRPCs by amiloride and LOE908, respectively, resulted in a decreased calcium response and stress-fiber formation, suggesting that at least TRPP2 and TRPC1 may mediate calcium-dependent rearrangement of the cytoskeleton that could promote increases in endothelial permeability. Indeed, inhibition of calcium-permeable channels in the plasma membrane of brain microvascular endothelial cells, which prevents rises in cytosolic calcium, results in protection against increases in blood-brain barrier permeability.^{104,105}

The channels that may influence the barrier properties of the microvascular component of the blood-brain barrier are highlighted here because there are many parallels between brain and lung microvasculature. In both brain and lung, the microvascular barrier segments are very tight, compared to their arterial counterparts.^{106–108} Further, cytosolic calcium influx via TRPC 1/4 and TRPV4 in the lung^{5,7,22,24,35,37,109} and via TRPC and TRPV in brain^{110–113} leads to a compromise of barrier integrity and increases in endothelial permeability. This may be particularly relevant because TRPP2 has been described as part of a functional, mechanosensitive complex with TRPC1 and TRPV4.^{114–116} Thus, studies are needed to determine whether TRPP2 is expressed in lung endothelium, whether it interacts with other members of the TRPC and TRPV families known to mediate increases in lung endothelial permeability, and whether its activation per se compromises lung endothelial barrier integrity.

TRPA (ankyrin)

The lone member of the TRPA family, TRPA1, is unique in that it possesses 14–19 ankyrin repeats on its amino terminus.¹¹⁷ In the lung, TRPA1 mRNA and protein expression has been verified in human A549 cells and human lung fibroblasts by RT-PCR and Western blot, respectively,¹¹⁸ but an expression profile in lung endothelium is lacking. In cerebral arteries, TRPA1 activation evokes dynamic cytosolic calcium transients and vasodilation.^{129,120} While TRPA1 appears to play a role in mediat-

ing cough and airway inflammation,¹²¹⁻¹²⁵ no studies to date have explored a functional role for TRPA1 in lung endothelium.

IMPACT OF LUNG ENDOTHELIAL-HETEROGENEITY

Our understanding of the molecular players and mechanisms that mediate lung endothelial permeability responses to TRP channel activation is emerging with a key message: not all endothelial cells, endothelial calcium pools, and functional endothelial responses to calcium entry are cut from the same cloth.

First, the notion that calcium-mediated loss of barrier integrity is solely a consequence of endothelial cell retraction is now being challenged. As discussed above, we have documented increases in lung endothelial permeability in the alveolar septal compartment without the formation of interendothelial gaps,⁷ suggesting that other mechanisms are responsible for the loss in barrier integrity.

Second, we have determined that TRP channels and other calcium channels may be coexpressed in the same lung endothelial compartment but still elicit unique downstream signals. Specifically, calcium entry via TRPV4 and that via the voltage-gated T-type channel, both expressed in lung septal capillary endothelium, have divergent effects. Only calcium transients resulting from TRPV4 activation increase lung endothelial permeability, and only those resulting from activation of the T-type channel lead to surface expression of P-selectin.¹⁰

Third, work from our group and others suggests that calcium entry-dependent alterations in lung endothelial barrier integrity can have distinct outcomes for overall lung function based on the targeted endothelial compartment (Fig. 5). Specifically, activation of store-operated TRP channels in extra-alveolar vessels leads to perivascular/peribronchial cuffing and decreased lung compliance.¹²⁶ In contrast, activation of TRPV4 leads to alveolar flooding and depressed gas exchange.^{7,126}

Finally, heterogeneity should be interpreted with recognition of potential signaling- or remodeling-induced endothelial plasticity. This concept can be evidenced acutely with manipulation of signaling pathways and/or chronically with modulation of TRP channel expression. For example, Wu et al.²² found that acute rolipram pretreatment of rat pulmonary artery and microvascular endothelial cells abolished or unmasked thapsigargin-induced I_{SOC} activation in these cells, respectively. In the intact rat lung, rolipram pretreatment shifted the thapsigargin-induced leak site from the extra-alveolar endothelial compartment to the alveolar septal compartment. Close association of the ER and the plasma membrane has been proposed as a requirement for activation of store-operated TRP channels,⁴⁰ and the distance between the ER and the plasma membrane is more than 2-fold greater in pulmonary microvascular endothelial cells than in pulmonary artery endothelium.⁹ Interestingly, Wu et al.¹⁰⁹ also found that rolipram treatment leads to translocation of the ER closer to the plasma membrane in pulmonary microvascular en-

Figure 5. Activation of store-operated channels and TRPV4 leads to distinctive leak sites in the extra-alveolar and alveolar septal networks, respectively. Corrosion casting revealed no leak sites in the normal rat lung (A) but extra-alveolar leak sites in rat lungs treated with thapsigargin to activate store-operated calcium entry (B). In contrast, in rat lungs treated with 14,15-epoxyeicosatrienoic acid (14,15-EET) to activate TRPV4, microvascular leak resulted in alveolar flooding evident just subjacent to the pleural surface (C) and blebbing across the alveolar surface (D). From Townsley et al.¹²⁷

dothelial cells. They further demonstrated that dynein, a microtubule motor protein that directs organelle movement away from the plasma membrane, predominates in microvascular endothelial cells, while kinesin, a motor protein that directs organelle movement toward the plasma membrane, predominates in pulmonary artery endothelial cells. Disruption of dynein expression in pulmonary microvascular endothelial cells unmasked thapsigargin-induced I_{SOC} activation, while disruption of kinesin in pulmonary artery endothelial cells ablated thapsigargin-induced I_{SOC} activation. The authors concluded that the ER distribution, influenced by rolipram-exposed cyclic adenosine monophosphate (cAMP) pools and microtubule motor function, plays a crucial and functional role in I_{SOC} entry-mediated increases in lung endothelial permeability. In addition to such acute mediator-induced remodeling, chronic remodeling in lung with disease can evoke different alterations. For example, we found that in a model of heart failure, downregulated expression of TRPC1, 3, and 4 in extra-alveolar vessels led to loss of thapsigargin-mediated permeability responses unrelated to alteration in the ER-plasma membrane distance, yet also led to retention of TRPV4-mediated increases in permeability.¹⁸

Collectively, we interpret these observations as reflecting endothelial heterogeneity, not only between extra-alveolar and microvascular compartments in lung but also between unique calcium microdomains in any one lung endothelial compartment. Calcium channels expressed in lung endothelium may be coupled to distinct downstream signaling pathways and thus linked to distinct functional outcomes. As a corollary, some TRP channels expressed in lung endothelium are likely not coupled to regulation of lung endothelial permeability.

SUMMARY AND FUTURE DIRECTIONS

From the large library of mammalian TRP channels now identified—28 members to be exact—evidence links a number of TRP channels to regulation of lung endothelial barrier integrity. We are just beginning to appreciate the complex nature by which specific endothelial responses to calcium entry via TRP channels are orchestrated. Thus far, the TRPC1/4 (I_{SOC}) and TRPV4 channels provide models for overall concepts as we begin to unravel the role of other TRP channels in lung endothelial barrier function. One very important concept is that calcium entry through TRP channels can mediate divergent responses that hinge on lung endothelial cell phenotype (Fig. 6). That is, phenotypic heterogeneity in lung endothelium dictates segmental ex-

Figure 6. Heterogeneity of injury model. The sequential-filling model of pulmonary edema accumulation initially described by Staub and others,¹²⁸⁻¹³¹ shown at left, is applicable when lungs are challenged with nonspecific agents to increase endothelial permeability. However, given newer evidence on endothelial heterogeneity, we should appreciate that extra-alveolar and alveolar septal endothelium can be differentially targeted. We, and others, have provided evidence for such a differential distribution of effects following activation of store-operated channels and TRPV4.^{7,35,127} If lung endothelial phenotypic heterogeneity extends to differential, compartment-specific expression of other transient receptor potential (TRP) channels, then their activation may similarly elicit direct edema formation in the extra-alveolar or septal compartments of the lung, thus bypassing the sequential filling model. A: artery; B: bronchiole. Adapted from Townsley et al.¹²⁷ A color version of this figure is available online.

pression of TRP channels. For example, activation of the I_{SOC} channel (TRPC1/4) can increase lung endothelial permeability by compromising the barrier properties of the extra-alveolar vasculature, thus promoting perivascular cuff formation. Conversely, TRPV4 activation increases lung endothelial permeability by affecting the alveolar septal compartment, thus promoting alveolar flooding. These differences in regional changes in permeability are due in part to innate phenotypic differences between the pulmonary artery and microvascular endothelium. Another important concept is that permeability increases are not always reflected simply by interendothelial gap formation. TRPV4 activation leads to barrier disruption with no apparent alteration in interendothelial junctional integrity. Thus, different signaling pathways must mediate the impact of TRP channel activation in different vascular segments to orchestrate changes in endothelial barrier properties.

Increasing evidence for TRP channel involvement in lung endothelial permeability makes this an exciting opportunity for discovery, and much research is still needed. The set of TRP channels that mediate increases in lung endothelial permeability must be completely identified. Once identified, the physiological and signaling mechanisms involved in mediating the permeability increases should be resolved. In addition, we have yet to fully understand whether permeability responses are modulated by the influx of other ions, such as magnesium and sodium, through activated but nonselective TRP channels. In the context of therapeutic value, a global understanding of the way these channels and their downstream effectors influence alterations in endothelial barrier properties could open up a new array of targets for drug development.

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