TRP Channels and the Regulation of Vascular Permeability
New Insights From the Lung Microvasculature

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One of the most widely recognized mechanisms to initiate an inflammatory response is calcium entry into endothelial cells. Recent investigations have demonstrated that there are multiple mechanisms which determine calcium flux into endothelial cells (including ligand gated calcium channels, store operated calcium channels and mechanosensitive channels), and that these different mechanisms are preferentially distributed between different endothelial cells. The functional consequences of these observations for regulation of vascular tone and remodeling are beginning to be understood, but this is not the case for the regulation of endothelial barrier function in intact organs. In this issue Alvarez et al describe an important example of segmental vascular permeability regulation in lung. They demonstrate that activation of the vanilloid subset of the family of calcium channels known as transient receptor potential channels (in this case the TRPV4 channels) preferentially increased the permeability of the endothelial and epithelial layers of the primary gas exchanging septal regions of the lung microvasculature whereas other calcium channels, such as the store operated calcium channels, increased permeability outside the primary gas exchange regions (so called extra-alveolar regions). These are important observations because disruption of the endothelium at the septal barrier is more likely to cause alveolar flooding and impair gas exchange than disruption in extra-alveolar vessels.

So far at least 28 mammalian TRP isoforms have been discovered. TRP channels have been subdivided into 3 main classes, TRPC (canonical), TRPM (melastatin), and TRPV (vanilloid) although more recently other classes have also been proposed (TRPP [polycystin], TRPML [mucolipin], and TRPA [ankyrin]). A general feature of the channels is that they pass only very small currents (calcium ion flux) and act as passive conductance pathways for calcium entry, i.e., they are not voltage gated. Thus the calcium flux depends on the local density of channel expression and the electrochemical driving force driving calcium into the cell. In particular hyperpolarization of the endothelial cell membrane potentiates calcium entry by increasing the electrical driving force for positively charged ions such as calcium. When TRP channels are activated close to calcium regulated potassium channels, they may also hyperpolarize the membrane and amplify calcium influx through all open calcium channels. As described in recent reviews, TRP channels have many functions in the vasculature including the regulation of vascular tone (TRPC4, TRPV1, TRPV4), angiogenesis (TRPM6 and TRPM7), vascular remodeling (TRPC4), oxidative stress-induced responses (TRPC3, TRPC4, TRPM2) and mechanosensing (TRPV4). TRP channels have also been implicated in the regulation of vascular permeability including TRPC1, TRPC4, TRPC6, and TRPV1. With relatively few specific antagonists for the TRP channels available at this time, it can be difficult to identify which TRP channel is involved in a given signaling event. Alvarez et al have used several strategies to distinguish the TRP channels in their lung preparation. As discussed below there are limitations to the confidence that can be placed in any one of these strategies, but a strength of the present article is that it focuses attention of several converging themes that are important for further investigations of calcium dependent regulation of fluid accumulation in the lung.

The strategies used by Alvarez et al include immunohistochemical localization of TRPV4 expression, light and electron microscopy of localized breaks in the endothelial barrier, selective activation of the channels, with particular attention of the role the arachidonic acid metabolites (EETs, epoxyeicosatrienoic acids), use of putative selective blocking of calcium entry, and the use of TRPV4−/− mice. The importance of using a multiple approach is well illustrated by the following example. The authors measure changes in the lung filtration coefficient to report modulation of lung vascular permeability. The filtration coefficient is determined from the rate of fluid accumulation (lung weight) after a step increase in microvessel pressure. They demonstrate that the increase in filtration coefficient after exposure to a putative activator of TRPV4 channels is attenuated in TRPV4−/− mice. These results support a role for TRPV4 in the regulation of permeability only if the known role of TRPV4 as a modulator of vascular tone is not a dominant mechanism. For example, at least part of the increase in lung weight after activating TRPV4 channels could involve recruitment of more blood vessels resulting in an increase in vascular volume and surface area available for fluid filtration. As appropriate, the authors attempt to distinguish such changes in vascular volume from true changes in hydraulic conductivity (a measure of vascular permeability) by only measuring fluid accumulation after a period when changes in vascular tone are assumed to have ceased. Nevertheless the loss of TRPV4 dependent mechanisms of vascular recruitment in knockout mice could be misinterpreted as a reduced permeability. In this regard the
Expression patterns of the TRP channels in rat lung. A heterogeneous staining for TRPV4 was detected in the endothelial cells of the alveolar-septal barrier (A). TRPV4 staining was also detected in the vascular smooth muscle cells of the extraalveolar vessels. In these vessels the endothelial cells were shown to express TRPC1,3, and 4 (B). TRPV4+ cells are shown in brown, TRPC1,3,4+ cells are shown in orange. EC=endothelial cell, EP=epithelium, BM=basement membrane, VSMC=vascular smooth muscle cell, AS=alveolar space, C=capillary, PA=pulmonary arteriole, RBC=red blood cell.

direct light and electron microscopy results described in the article are a strength. Although the authors do not show direct evidence for protection of the endothelial barrier from their knockout mice, they do show that the putative activator of TRPV4 channels resulted in blebs or breaks in the septal endothelium and epithelium in mice and rats. They also found evidence to support their argument that the main TRPV4 action is in the septal region by showing that in control mice TRPV4 activation caused no increase in extraalveolar cuffs. The Figure illustrates the segmental expression patterns of TRP channels in the alveolar-septal barrier and in the extraalveolar vessels as described by Alvarez et al in this issue8 and previously.9

Although knockout models are very informative there are limitations with their use. If TRPV channels form heterotetramers with other TRP channels then the properties of these channels would likely be altered in the absence of TRPV4. There is some evidence that TRPV channels can exist as heterotetramers but TRPV4 appears to preferentially form homotetramers.9 The absence of TRPV4 in knockout mice may also be compensated for by the upregulation of other TPR channels whose function is likely to depend on their expression levels.10 Whereas immunohistochemical localization provides qualitative information about the channel distribution, the definitive evidence for a functional contribution of the channels will always require additional information about endogenous channel activity. In summary, these investigations are a good example in the area of vascular permeability regulation of the growing need for investigations by teams that not only recognize the strengths and weakness of investigation at the molecular level, but can also evaluate the physiological and pathophysiological importance of new molecular insights taking into account the well known limitations of separating real change in the endothelial barrier function from change in vascular hemodynamics.

This leads us to briefly discuss other strategies used by Alvarez et al, including the attempts at selective activation of the channels. The difficulty is that far less is known about the TRPV family of channels than the canonical TRPC channels. TRPV4 is activated by a range of stimuli including fluid shear stress, extracellular hypo-osmolarity, phorbol esters such as 4α-phorbol-12,13-didecanoate (4aPDD),11 and arachidonic acid metabolites, EETs. There is also considerably less known about the gating mechanisms for the TRPV family as compared with the TRPC family. Although some members (eg, TRPV1) have been shown to translocate to the plasma membrane in response ligand binding to receptors (implying the conformational coupling mechanism), TPRV4 is activated by endogenous second messengers (EETs) and appears to be ligand gated. The identification of TRPV4 as a key calcium channel in the experiments of Alvarez et al is dependent of the specificity of the agonists 4aPDD for TRPV411 and of exogenous EETs to activate TRPV4. To further distinguish ligand gated from store operated calcium channels Alvarez et al used extracellular ruthenium red to block the actions of the TRPV channels.

The role of EET’s in pulmonary disease has previously focused mainly on their action to regulate vascular tone, and recent investigations demonstrate important roles of these cytochrome P450 epoxigenase metabolites of arachidonic acid in acute pulmonary hypertension and chronic hypoxic states.13 There is growing evidence for a role for both TRPC and TRPV channels as regulators of permeability vascular permeability in the lung. Of particular interest with respect to the present findings are the previous observations in the author’s laboratory of down regulation of TRPC1 and TRPC4 channels in rat lung in a model of heart failure.8 This was accompanied by a loss of a calcium-dependent increase in permeability in response to calcium store depletion by thapsigargin. In this same heart failure model, permeability was still increased by 14,15 EET. Alvarez et al can now account for these observations because the TRPC1 and TRPC4 channels in rat lung in a model of heart failure.8 The Figure provides qualitative information about the channel distribution, the definitive evidence for a functional contribution of the channels will always require additional information about endogenous channel activity. In summary, these investigations are a good example in the area of vascular permeability regulation of the growing need for investigations by teams that not only recognize the strengths and weakness of investigation at the molecular level, but can also evaluate the physiological and pathophysiological importance of new molecular insights taking into account the well known limitations of separating real change in the endothelial barrier function from change in vascular hemodynamics.

Finally it is noted that the TRP channels, as the newest family of ions channels to be discovered, offer important new targets for therapeutic interventions. In relation to strategies to reduce inflammatory injury, it is important to remember that much of the effort over the past 2 decades has focused on...
inhibition of the interaction of leukocytes with endothelium using antibodies or small molecule antagonists to the block leukocyte/endothelial cell adhesion molecules, and attempts to block the binding of inflammatory mediators (TNFα, VEGF, histamine) to their receptors on endothelial cells using receptor antagonists. Although some of these approaches have achieved limited success, the disappointing outcome of several key clinical trials14,15 has refocused attention on alternative antiinflammatory strategies which modulate the signal transduction pathways within the endothelial cells and their down stream targets that increase permeability. This is an active field of investigation. In the lung the striking results of investigations using modulators of the sphingosine-1-phosphate to attenuate acute lung inflammation is a current example.16 In the longer term, strategies to attenuate permeability responses based on more detailed understanding of calcium channel such as the TRPV4 described by Alvarez et al may be important in the treatment of acute lung injury and respiratory distress syndromes in humans.

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References


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