Mechanisms regulating endothelial cell barrier function

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Stevens, Troy, Joe G. N. Garcia, D. Michael Shasby, Jahar Bhattacharya, and Asrar B. Malik. Mechanisms regulating endothelial cell barrier function. Am J Physiol Lung Cell Mol Physiol 279: L419–L422, 2000.—Endothelium forms a physical barrier that separates blood from tissue. Communication between blood and tissue occurs through the delivery of molecules and circulating substances across the endothelial barrier by directed transport either through or between cells. Inflammation promotes macromolecular transport by decreasing cell-cell and cell-matrix adhesion and increasing centripetally directed tension, resulting in the formation of intercellular gaps. Inflammation may also increase the selected transport of macromolecules through cells. Significant progress has been made in understanding the molecular and cellular mechanisms that account for constitutive endothelial cell barrier function and also the mechanisms activated during inflammation that reduce barrier function. Current concepts of mechanisms regulating endothelial cell barrier function were presented in a symposium at the 2000 Experimental Biology Conference and are reviewed here.

gp60; myosin light chain kinase; vascular endothelial-cadherin; adenyl cyclase; calcium

IN THE INTACT BLOOD VESSEL, the endothelium forms a continuous, semipermeable barrier. Barrier integrity differs between organs, and it has also become apparent that barrier integrity even differs within vascular segments of the same organ (1, 5, 14, 22, 27). For example, the lung endothelium in vessels < 30 μm in diameter forms a more restrictive barrier than either arterial or venular endothelium (1, 5, 22). In response to inflammatory stimuli, the endothelial barrier becomes less restrictive, resulting in increased water and protein permeability. Two mechanisms can account for such an increase in permeability: paracellular (i.e., between cell) pathway and transcytotic (i.e., through cell) transport. Paracellular transport of molecules was first reported in 1961 by Majno and Palade (17), who suggested that histamine induced the formation of inter-endothelial cell gaps at inflammatory sites. The concept that intercellular gaps represent the sites of small- and large-“pore” pathways has been the general model of endothelial permeability. Recently, it has become apparent that the endothelial barrier does not simply behave as a physical sieve (23–25, 28, 38). Transcellular albumin transport may also occur secondary to albumin binding to a specific docking protein (gp60) that induces vesicular transport across the endothelium (9, 28–30, 33, 37). This discovery prompted speculation that the transcytotic pathway may also account for the large-pore pathways (24), leading to a flurry of experiments to determine whether gp60-activated signaling represents a mechanism of site-directed protein delivery and increased transendothelial

1 Presented by Asrar B. Malik.

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permeability. These advances were reviewed at the 2000 Experimental Biology Conference held in San Diego, CA.

**TRANSCELLULAR PATHWAYS IN ENDOTHELIAL CELLS**

In the past five years, the presence of a gp60-mediated albumin transport pathway has been well established (9, 23–26, 28–30, 33, 37, 38), although physiological function and modes of regulation by specific signal transduction pathways are still poorly understood. New data indicate that gp60 activation induces the transport of albumin without a concurrent increase in hydraulic conductivity across either endothelial cell monolayers in vitro or isolated perfused rat lungs. These data suggest that one mechanism of uncoupling protein permeability from water permeability involves the gp60-mediated transport pathway. Whether inflammatory mediators induce albumin transport and whether the gp60-mediated pathway is capable of bulk albumin transport in physiological and pathophysiological conditions remain important future challenges. Localization of gp60 within caveolae suggests that its presence is associated with endocytotic vesicles (10, 11, 34). Identification that albumin binding to gp60 induces tyrosine phosphorylation, activation of Src tyrosine kinases, pp60−src, and Fyn implicates receptor tyrosine kinase activity in albumin transcytosis (38). Interestingly, new studies demonstrate that selective inhibition of Gα prevented albumin from activating tyrosine kinase activity and from increasing albumin transport, suggesting that gp60 is linked to activation of tyrosine kinase through heterotrimeric G proteins (18). These observations generally support the findings that genistein and herbimycin A prevent gp60-mediated albumin transport (38). Thus while considerable progress has been made in identifying certain components of the signaling pathways activated on albumin binding to the endothelium, key molecular events remain to be described. Similarly, how these signaling events initiate transcytosis and the molecular sequelae required for directed apical to basal vesicular movement remain important future challenges.

**KINASE REGULATION OF BARRIER FUNCTION**

Considerable progress has been made in understanding how inflammatory agonists act to promote formation of intercellular gaps. The discovery that endothelial cells possess the molecular machinery needed to initiate and sustain a "contraction" had lead to the idea that inflammatory agonists increase constitutive actomyosin interaction sufficient to increase inwardly directed tension and pull cells apart at their sites of adhesion (16, 42). Several reports (7, 20, 31) showing that inhibition of nonmuscle myosin light chain kinase (MLCK), an enzyme required to initiate actomyosin interaction, prevented Gq-linked agonists from decreasing both cell-cell adhesion and endothelial barrier integrity are consistent with this idea. However, reports that inhibition of MLCK does not prevent direct intracellular Ca2+ concentration ([Ca2+]i) elevating agents from increasing permeability do not support this concept (16, 19). This evidence suggests that disruption of cell-cell adhesion is sufficient to increase endothelial cell permeability without an increase in tension per se (2). Cloning and expression of multiple endothelial cell-specific MLCK isoforms (and splice variants) by Garcia and colleagues (6, 8, 15, 32, 39, 40) provide an exciting advancement that may underlie these disparate findings. MLCK isoforms each possess unique regulatory sites. MLCK1 possesses a 922-amino acid NH2-terminal sequence that is not present in smooth muscle MLCK. Of particular interest is the presence of both SH2 and SH3 binding domains and a tyrosine residue (Tyr485) that is sufficient to activate kinase activity on phosphorylation without involvement of Ca2+/calmodulin. Indeed, Garcia has shown that stimulation of tyrosine kinase activity results in activation of the "contractile complex" that includes MLCK, Src, and cortakin. Completion of future key studies will require that these observations be placed in the context of endothelial cell barrier function in vivo. In addition, site-specific localization of MLCK isoforms and control of their discrete regulatory sites will also need to be considered. Finally, it will be important to determine whether MLCK isoforms control both intercellular gap formation and the molecular motor important for gp60-mediated vesicular trafficking.

**TENSION, ADHESION, AND CONTROL OF ENDOTHELIAL PERMEABILITY**

That disruption of cell-cell adhesion mediated by vascular endothelial (VE)-cadherin is sufficient to induce inter-endothelial cell gap formation provides compelling evidence that tethering forces regulate barrier function (2). Although cell-cell adhesion in endothelial cells may be due to both tight and adherens junctions, VE-cadherins play a prominent role in cell tethering (2, 4). Disruption of VE-cadherin function resulted in interstitial edema and accumulation of inflammatory cells in the heart and lung microcirculation (2). Shasby and colleagues (21, 41) have recently shown that inhibition of VE-cadherin function decreases cell-cell but not cell-matrix resistance, suggesting that cadherins mediate cell-cell adhesion important for the control of barrier integrity. Moreover, they demonstrated that agonists such as histamine that raise [Ca2+]i, activate signal transduction cascades that decrease the VE-cadherin-dependent sites of adhesion without increasing cell tension. These findings have begun to uncouple the Gq signaling events regulating cell tension from the Gq-activated signaling events that control cell adhesion. Future efforts will be required to establish the

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2 Presented by Joe G. N. Garcia.

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key signal transduction pathways that link inflammatory agonists to the sites of cell adhesion.

**CALCIUM REGULATION OF ADENOSINE 3',5'-CYCLIC MONOPHOSPHATE: CONTROL OF ENDOTHELIAL PERMEABILITY**

Endothelial cell biologists have recognized that a rise in \([\text{Ca}^{2+}]_i\) is sufficient to induce inter-endothelial cell gap formation and increase permeability to macromolecules. Generally, however, agonists that elevate \([\text{Ca}^{2+}]_i\), only increase permeability in the absence of a rise in cAMP (19). Stevens presented his work addressing the link between \([\text{Ca}^{2+}]_i\) and cAMP. The observation that endothelial cells express the \(\text{Ca}^{2+}\)-inhibited isoform of adenyl cyclase, the enzyme responsible for cAMP synthesis, provides a compelling mechanism through which physiological increases in \([\text{Ca}^{2+}]_i\) decrease cAMP and thereby control endothelial cell barrier function (36). However, direct \([\text{Ca}^{2+}]_i\) elevating agents (i.e., agonists that increase \([\text{Ca}^{2+}]_i\), without concurrent activation of heterotrimeric G proteins) only increase lung microvascular endothelial cell permeability and not microvascular permeability. Although microvascular endothelial cells express the \(\text{Ca}^{2+}\)-inhibited isoform of adenyl cyclase, agonists elevating \([\text{Ca}^{2+}]_i\) do not decrease cAMP, suggesting that maintenance of cAMP is an important homeostatic mechanism contributing to enhanced barrier properties of the microvascular endothelium (35). Further support for this idea has recently come from a study (3) showing that the physiological rise in \([\text{Ca}^{2+}]_i\) induced microvascular endothelial cell gap formation only under experimental conditions in which \(\text{Ca}^{2+}\) inhibited adenyl cyclase. These findings strongly support the involvement of this enzyme in control of endothelial cell barrier function through the integration of \([\text{Ca}^{2+}]_i\) and cAMP signaling events. Perhaps most importantly, the findings support the developing idea that endothelial cells derived from conduit vessels and microvessels are phenotypically distinct. Thus unique signaling circuits in these cell populations may underlie site-specific vascular responses to inflammation. Systematic exploration of cell-specific signaling cascades and their link to segment-specific function represent an important future challenge.

**CELL SIGNALING IN THE PULMONARY MICROCIRCULATION**

The proinflammatory effects of increased endothelial cell \([\text{Ca}^{2+}]_i\) in situ have been identified by Bhattacharya and co-workers (12, 13), providing critical integration between studies performed in culture and in the intact organ. An increase in vascular pressure was shown to activate mechanogated cation channels, resulting in \(\text{Ca}^{2+}\) entry that stimulated the expression of endothelial cell P-selectin. More recently, this group has demonstrated that direct instillation of tumor ne-

crosis factor-\(\alpha\) into alveoli increased \([\text{Ca}^{2+}]_i\) not only in alveolar epithelium but also, remarkably, in adjacent microvascular endothelium (12). This rise in endothelial cell \([\text{Ca}^{2+}]_i\) also resulted in an increase in P-selectin expression. These data are provocative because they indicate first that increased \([\text{Ca}^{2+}]_i\) may direct an inflammatory response to appropriate vascular segments through the upregulation of endothelial adhesion molecules, perhaps without inter-endothelial cell gap formation, and second that communications between alveolar and vascular microcirculatory compartments may direct leukocyte recruitment to sites of alveolitis. Future studies will be critical to assess how these signaling events proceed and to determine how a rise in microvascular versus macrovascular endothelial cell \([\text{Ca}^{2+}]_i\) coordinates with other signaling events to induce a site-specific inflammatory response.

In conclusion, considerable progress has been made in the understanding of molecular events that regulate endothelial cell shape and signaling processes that initiate transcytotic and paracellular transport of macromolecules. Discriminating between the key events that regulate the site-specific endothelial cell response to inflammation and the unique signaling events activated to coordinate these processes represent important future challenges in microvascular biology.

**REFERENCES**


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