Neutrophil infiltration into tissues, a hallmark of acute inflammation, is a complex process requiring an intricate orchestration of signals between microvascular endothelial cells and both circulating and adherent neutrophils. Cytokines and inflammatory mediators elaborated during injury or infection activate both endothelial cells and circulating neutrophils (primary paracrine mechanism), causing neutrophil rolling to slow, formation of endothelial-neutrophil tethering, and ultimately, firm neutrophil adhesion. Neutrophil adhesion to the endothelial surface, via E- and P-selectins and vascular cell adhesion molecule-1 (VCAM-1), contributes to further activation of both cell types (adhesion-dependent activation). This adhesion-dependent activation of the neutrophil through ligation of the CD11/18 complex promotes the release of neutrophil granules that propagate the inflammatory process (secondary paracrine). One important consequence of this inflammatory process is an increase in endothelial permeability, which leads to excessive tissue edema with attendant changes in microvascular hemodynamics, tissue oxygenation, and organ failure. Although the molecular determinants that regulate the intimate cell-cell interaction between the adherent leukocyte and the vascular endothelium have been extensively evaluated, relatively little information exists on the mechanisms by which polymorphonuclear leukocytes (PMNs) alter paracellular junctional integrity to allow PMNs to traverse the endothelium and increase leakage of circulating proteins and fluid. Historically, the concepts of leukocyte-mediated vascular dysfunction viewed the endothelial cell as a passive target for PMN-derived reactive oxygen species and granule products with endothelial toxicity the valued endpoint for these studies. The notion that endothelium can be an active participant in leukocyte-mediated vascular dysregulation began to emerge when a rise in endothelial cell cytosolic Ca\(^{2+}\) was observed in response to N-formylmethionyl-leucyl-phenylalanine (fMLP)-mediated PMN migration. The mechanism by which PMN interaction with the surface of the endothelium elicits this increase in endothelial cell cytosolic Ca\(^{2+}\) was unclear, and the exact downstream signaling pathways evoked by PMN-mediated increases in endothelial cell Ca\(^{2+}\) were not defined. However, it was reasoned that PMN-endothelial cell interaction in the presence of a chemotactic gradient could produce a Ca\(^{2+}\)-dependent opening of the intercellular junction, a phenomenon first reported by Majno and later Simionescu in response to bioactive agents.

The regulation of endothelial-specific paracellular gap formation and barrier responses is now recognized as dependent on Ca\(^{2+}\)-related signal transduction pathways that modulate the activity of the endothelial cell cytoskeleton, a complex array of structural filaments. The endothelial contractile apparatus is composed of proteins such as actin, myosin heavy chain, myosin light chain (MLC), and associated cytoskeletal regulatory proteins including myosin light chain kinase (MLCK), calmodulin, MLC phosphatase, Rho kinase, and p60\(^{SH_2\text{ and }SH_3}\) (Figure). A key determinant of centripetal tension development and endothelial cell contraction is the activity of the endothelial MLCK, which phosphorylates MLC on Ser\(^{19}\) and Thr\(^{18}\). Endothelial cells express a unique form of MLCK that differs both structurally and functionally when compared with smooth muscle MLCK. Although the Ca\(^{2+}\)-calmodulin regulatory regions are conserved, endothelial MLCK possesses a unique NH\(_2\)-terminus with multiple p60\(^{SH_2\text{ and }SH_3}\) phosphorylation sites, as well as two SH\(_2\) and two SH\(_3\) binding regions. Because an initial event common to both PMN paracrine- and adhesion-dependent barrier dysfunction is an increase in endothelial cell Ca\(^{2+}\) with subsequent activation of endothelial contractile apparatus, we and others speculated that PMN-stimulated activation of the endothelial cell MLCK by Ca\(^{2+}\)-dependent pathways would be a primary determinant of paracellular gap formation, diapedesis, and barrier dysfunction.

In this issue of Circulation Research, Yuan and colleagues provide in situ experimental evidence supporting the contribution of the endothelial cytoskeleton to the pathological regulation of neutrophil-induced corneal venular barrier dysfunction. Their convincing data indicate that MLCK-mediated MLC phosphorylation and actomyosin reorganization play an important role in the development of microvascular leakage during neutrophil stimulation. Activated neutrophils added to the vessel-bathing chamber (rather than infused intraluminally) produced consistent paracrine-dependent (not adhesion-dependent) vascular permeability. These results were validated by both pharmacological and molecular approaches, and the time course of endothelial MLC phosphorylation correlated with alterations in neutrophil-induced permeability. These well-designed in vivo studies extend previous work in primarily in vitro models to confirm that the level of MLC phosphorylation as determined by PMN-mediated endothelial cell MLCK activation served as a key determinant of junctional integrity. Importantly, there was good correlation between the effects observed in the whole vessel preparation and responses of cultured endothelial cells derived from the same type of vessels. These data confirm the importance of MLCK activity in regulating neutrophil-induced barrier dysfunction (both adhesion and paracrine) via focally produced increases in contractile forces that...
likely disrupt adhesive forces maintained by adherens junctions and focal adhesion interactions with the cytoskeleton (Figure). The exact mechanisms by which PMN-adhesion and PMN-released factors increase Ca\textsuperscript{2+} to activate the MLCK-driven endothelial cell cytoskeleton and increase vascular permeability are unclear. Recently, Gautam et al\textsuperscript{4} demonstrated that vascular permeability-enhancing activity from activated neutrophils could be attributed to the release of heparin-binding protein known as CAP37/azurocidin. This highly cationic peptide increases endothelial cell Ca\textsuperscript{2+}, promotes actin stress fiber formation, and increases permeability, suggesting, but not directly tested, that CAP37 may act through MLCK-mediated pathways. Our preliminary studies suggest that the syndecan family of endothelial heparan sulfates (proteoglycans) within the glyocalyx may act as “receptor-like” molecules for cationic peptides such CAP37 and are capable of regulating cytoskeletal reorganization and endothelial barrier function.\textsuperscript{19} Given the multifaceted role of heparan sulfates and syndecans in cytoskeletal regulation, cell-cell adhesion, focal adhesion kinase activity, and integrin-mediated signaling,\textsuperscript{20} these molecules are excellent candidates to strongly influence PMN-mediated barrier regulation.

In summary, it is now obvious that the coordinated events leading to PMN recruitment, attachment, activation, and extravasation rely heavily on the signaling pathways of the endothelium. Studies from the Yuan laboratory are consistent with the notion that the activation of the actomyosin contractile apparatus via MLCK is an essential mechanism by which neutrophils produce paracellular gap formation and vascular permeability. While excellent molecular targets are continually being explored, further studies examining the regulation of the PMN-stimulated endothelial cell contractile apparatus will undoubtedly lead to a better understanding as to how endothelial cell cytoskeletal “tweaks” participate in regulating vascular leak.

References


Key Words: leukocytes ■ endothelium ■ permeability ■ microvascular ■ myosin light chain kinase
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Randal O. Dull and Joe G.N. Garcia

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