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**“ENDOTHELIAL BARRIER RESTORATION AFTER INFLAMMATION-  
INDUCED HYPERPERMEABILITY”**

by

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## **ABSTRACT**

Inflammation is characterized by an increase in endothelial barrier permeability (hyperpermeability) to macromolecules. Major efforts focus on understanding the mechanisms involved in onset of hyperpermeability in endothelial cells (EC); however, much less is known about the restoration of vascular barrier integrity following hyperpermeability. We investigated mechanisms that terminate hyperpermeability and thereby restore microvascular barrier properties. Understanding the mechanisms involved in the restoration of endothelial barrier integrity will advance clinical knowledge and assist vascular surgeons in treating diseases associated with trauma and ischemia-reperfusion. Translocation of endothelial nitric oxide synthase (eNOS) from plasma membrane to cytosol is required for the onset of hyperpermeability. Increased production of cyclic adenosine monophosphate (cAMP), stimulates the exchange protein activated by cAMP (Epac1)-Rap1 axis and contributes to restoration of basal permeability after ischemia-reperfusion. Based on our publications and preliminary data, we tested the central hypothesis that ***Inflammatory agonist signaling leads to hyperpermeability and initiates a delayed cascade of second messenger dependent pathways, which causes inactivation of hyperpermeability.*** We used human microvascular (HMVEC) and mouse myocardial endothelial cells (MyEnd) to study the inactivation of endothelial barrier after hyperpermeability induced by platelet-activating factor (PAF). Importantly for our hypothesis, PAF caused a delayed increase in cAMP concentration. Stimulation of Epac1 in HMVEC inactivated PAF-induced hyperpermeability. Interestingly, Epac1 stimulation also failed to inactivate PAF-induced hyperpermeability in VASP-KO MyEnd EC. PAF activates VASP (vasodilator-stimulated phosphoprotein) by phosphorylation at Serine 157 and Serine 239. This phosphorylation is sensitive to eNOS inhibition indicating that PAF stimulates production of eNOS-derived NO and VASP phosphorylation in a sequential manner. In addition, stimulation of Epac1 after the onset of PAF-induced hyperpermeability translocates eNOS from the cytosol back to the plasma membrane in HMVEC and in control MyEnd cells. eNOS is preferentially located in the cytosol in MyEnd VASP KO cells. Knockout of VASP prevents translocation of eNOS associated with stimulation of Epac1. We conclude that PAF triggers a series of events that lead to an increase in permeability and to delayed stimulation of the cAMP-Epac1 axis. This stimulation is accompanied with VASP-modulated return of eNOS to the plasma membrane, thus switching off the signal for endothelial hyperpermeability.