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Neutrophil transmigration, focal adhesion kinase and endothelial barrier function

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Abstract

Neutrophil activation is an essential component of innate immune defense against infection and injury. In response to inflammatory stimulation, circulating neutrophils undergo a series of dynamic and metabolic changes characterized by β 2-intergrin mediated adhesion to microvascular endothelium and subsequent transendothelial migration. During this process, neutrophils release granular contents containing digestive enzymes and produce cytotoxic agents such as reactive oxygen species and cytokines. These products target endothelial barriers inducing phosphorylation-triggered junction dissociation, actin stress fiber formation, and actomyosin contraction, manifest as paracellular hyperpermeability. Endothelial cell-matrix focal adhesions play an integral role in this process by providing structural support for endothelial conformational changes that facilitate neutrophil transmigration, as well as by recruiting intracellular molecules that constitute the hyperpermeability signaling cascades. As a central connector of the complex signaling network, focal adhesion kinase (FAK) is activated following neutrophil adhesion, and further mediates the reorganization of endothelial integrin-matrix attachments in a pattern coordinating with cytoskeleton contraction and junction opening. In this review, we present recent experimental evidence supporting the importance of FAK in neutrophil-dependent regulation of endothelial permeability. The discussion focuses on the mechanisms by which neutrophils activate FAK and its downstream effects on endothelial barriers.

Keywords

neutrophil; FAK; endothelial barrier; vascular permeability

The inner surface of exchange microvessels (capillaries and postcapillary venules) is formed of a monolayer of juxtaposed endothelial cells that are joined together at cell-cell junctions. The basolateral side of the endothelium is attached to a basement membrane composed of collagen fibrils, laminin, fibronectin, and glycosaminoglycans. Endothelial cells are anchored to the extracellular matrix via cell surface-expressing integrins localized at focal adhesions. This cell-cell/matrix adhesion-based structure is semi-permeable to water and non-lipophilic molecules, providing size- and charge-selectivity for solute transport across the microvessel wall. The permeability properties of the endothelial barrier are critical in the maintenance of fluid homeostasis and in the regulation of physiological functions of tissues and organs. Endothelial barrier dysfunction, often described as increased permeability,

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occurs during inflammation, a pathophysiological process associated with various disease states or injurious conditions, including trauma, ischemia-reperfusion injury, infection, sepsis, diabetes, and other types of disorders (Groeneveld, 2002; Kumar et al., 2009; Lush and Kvietys, 2000; van Nieuw Amerongen and van Hinsbergh, 2002; Yuan et al., 2007). A hallmark of inflammation is extravasation of white blood cells from the blood to the tissue across the microvascular endothelium (diapedesis) (Cavanagh et al., 1998; Lewis and Granger, 1986; Nathan, 2006; Rodrigues and Granger, 2010).

As a major component of the innate immune system, polymorphonuclear neutrophils (PMNs) provide the most important mechanism in host defense against infection or invasion of sterile tissues or fluids during traumatic injury. Circulating neutrophils are capable of sensing the danger signals and migrating toward compromised tissues to eradicate harmful materials (Heissig et al., 2010; Nathan, 2006). Endothelial barrier dysfunction, due to opening of cell-cell/matrix junctions and/or increased vesicle-mediated transcytosis, facilitates the movement of neutrophils across the endothelium. In addition, vasoactive factors and cytotoxic agents (reactive oxygen species and digestive proteases) released by activated neutrophils can increase or prolong endothelial hyperpermeability during inflammation (Boueiz and Hassoun, 2009; Pun et al., 2009). Thus, microvascular barrier injury is both a cause and consequence of neutrophil transmigration.

Neutrophil Adhesion and Transendothelial Migration

Neutrophil diapedesis is a multi-step process involving rolling, activation, adhesion and transmigration (Granger and Schmid-Schönbein, 1995; Engelhardt and Vestweber, 2006) (Figure 1). Normal blood flow velocity is extremely fast in microvascular beds, preventing sustained interactions of blood cells with the microvessel wall. Temporary attachment of neutrophils to the endothelium occurs through interactions of membrane surface receptors (selectins) and sialylated glycoproteins present on both neutrophil and endothelial surfaces. This interaction induces leukocyte rolling along the microvessel wall (Yuan et al., 1995). Rolling is seen both in the presence and absence of inflammation. In response to pathogen invasion or inflammatory stimulation, neutrophils become immobilized by firm adhesion to the endothelial surface followed by transmigration across the microvascular (especially venular) wall (Allport et al., 1997; Lawrence and Springer, 1991). This process can be initiated with increased expression of adhesion molecules (Granger and Schmid-Schönbein, 1995; Kuby, 1997; Lodish, 2000; Engelhardt and Vestweber, 2006; Wittchen, 2009), including endothelial (E)-selectin and platelet (P)-selectin expressed on the surface of endothelium that binds to leukocyte cell surface glycoproteins (P-selectin glycoprotein ligand 1, PSGL-1). Firm adhesion is mediated by binding between intercellular adhesion molecule 1 (ICAM-1) expressed on endothelial cells and leukocyte β_2 integrins, including LFA-1 (CD11a/CD18; $\alpha_L\beta_2$), Mac-1 (CD 11b/CD18, $\alpha_M\beta_2$) and p150,95 ($\alpha_X\beta_2$). In addition, leukocyte $\alpha_D\beta_2$ binds to vascular cell adhesion molecule 1 (VCAM-1) (Clayton et al., 1998; Fabbri et al., 1999; Granger and Schmid-Schönbein, 1995; Kuby, 1997; Lodish, 2000; Engelhardt and Vestweber, 2006). During firm adhesion, neutrophils are preferentially arrested at the tri-endothelial cellular junctions, followed by spreading, invadopodia formation, and crawling through the intercellular space (Bianchi et al., 1997; Burns et al., 1997; Fabbri et al., 1999). In addition to the paracellular transmigration, neutrophils are capable of crossing the endothelial cell body via a pathway similar to transcytosis (Phillipson et al., 2008; Wittchen, 2009; Wolburg et al., 2005). A recent study by Millan et al demonstrates that leukocytes induce ICAM-1 clustering and translocating to caveolin-rich domains on the cell membrane in close proximity to the ends of stress fibers. ICAM-1 undergoes further internalization and transcytosis, forming actin-rich transcellular pores allowing leukocyte TEM (Millan 2005, Rahman 2009).

Neutrophil Migration and Endothelial Hyperpermeability

Neutrophil transendothelial migration (TEM) via the paracellular pathway requires sequential opening and re-annealing of endothelial cell-cell junctions. Initially it was believed that mechanical disruption or proteolytic breakdown of the endothelial barrier was necessary for neutrophils to move through, hence the coupling between TEM and hyperpermeability. Recently, this conventional concept has been challenged by experimental evidence suggesting these two processes as uncoupled events or without cause-effect relationship (Carman, 2009; Carman et al., 2007; Carman and Springer, 2004; Carman and Springer, 2008; He, 2010; Huber and Weiss, 1989; Lewis and Granger, 1986; Lewis and Granger, 1988). An elegant study by Granger and colleagues clearly demonstrates the process of a neutrophil moving through two adjacent endothelial cells without tracer protein leakage (Granger et al., 1994; Lewis and Granger, 1988). More recently, fluorescence microscopic imaging of neutrophil-endothelial cell interactions confirms that the both neutrophil and endothelial cell membranes remain in close contact during transmigration (Carman et al., 2007; Carman and Springer, 2004). Another study in aseptic cutaneous wounds shows that neutrophil transmigration and endothelial hyperpermeability are temporally separated, with hyperpermeability preceding TEM by several hours. Vascular permeability increases approximately three times faster than neutrophil influx (Kim et al., 2009).

Endothelial Barrier Structure

The barrier function of exchange microvascular endothelium derives from the integrity of the endothelial cell-cell junctions and cell-matrix focal adhesions. Traditionally, compromised endothelial junction integrity is considered to account for plasma leakage in response to inflammatory injury. However, recent evidence demonstrates that plasma proteins and even neutrophils can pass through the endothelium through the cell body via the transcellular pathway (Carman et al., 2007; Carman and Springer, 2004; Dvorak et al., 1996; Mehta and Malik, 2006; Hu 2008, Sun 2009).

With respect to the paracellular permeability, two types of junctions have been characterized as the major cell-cell adhesive barrier structures in the microvascular endothelium: the adherens junction (AJ) and tight junction (TJ). While TJs are mainly expressed in the blood-brain or blood-retinal barriers (Hawkins and Davis, 2005; Pardridge, 1998), AJs have been identified in nearly all types of vascular beds, especially in the peripheral microvasculature. An important molecular structure of AJs is vascular endothelial (VE)-cadherin, a transmembrane receptor with its extracellular domain binding to the extracellular domain of another VE-cadherin expressed on the membrane of an adjacent endothelial cell. This homotypic binding interaction is dependent upon extracellular calcium (Rudini and Dejana, 2008). Intracellularly, VE-cadherin is connected to the actin cytoskeleton via a family of catenins (α -, β -, γ -catenins) (Burridge et al., 2006). Connection to the actin cytoskeleton is further stabilized by p120-catenin which binds to protein kinases (Src family kinases) and phosphatases, serving as a scaffold to bring these signaling molecules into proximity with adherens junction proteins for further interactions (Piedra et al., 2003; Reynolds and Roczniak-Ferguson, 2004). Thus, the catenins not only serve as a structural linkage between VE-cadherin and the cytoskeleton, they also transduce biochemical signals for cell-cell communications. Phosphorylation of VE-cadherin and catenins is considered a triggering mechanism in the conformational changes or disassembly of intercellular junctions (Esser et al., 1998; Lampugnani et al., 1997; Young et al., 2003).

Under normal conditions, the stability of the junction-cytoskeleton complex is maintained through balanced cell-cell/matrix adhesive forces and cytoskeleton contractile tension (Shen

et al., 2009). In endothelial cells during stimulation by inflammatory agents, actin polymerization and stress fiber formation occur concomitantly with myosin light chain phosphorylation (Yuan, 2000). These cytoskeletal responses act in concert producing a centripetal contractile force that competes cell-cell adhesions rendering widened intercellular space (Guo et al., 2009; Huang et al., 2003; Reynoso et al., 2007; Sun et al., 2006; Tinsley et al., 2004; Yuan et al., 2002).

The cytoskeletal contractile response is supported by integrin-matrix binding within focal adhesions. Integrins provide an anchoring structure for maintaining endothelial barriers, and also coordinate the moment-to-moment changes in the cytoskeleton and junctions. This interaction is multidirectional and may not be limited to the basal lateral site of endothelial cells. Indeed, some members of the integrins family have been identified to be located at endothelial cell-cell borders (Lampugnani et al., 1991). It is suggested that these integrins collaborate with other intercellular molecules to form lateral junctions. Thus, blocking integrin function could alter the junctional connection allowing cells or macromolecules to move across endothelial monolayers. Synthetic peptides that compete the RGD-binding sequence, or antibodies directed against the $\beta 1$ subunit of integrins produce a dramatic increase in transendothelial flux of water and large solutes (Curtis et al., 1995; Qiao et al., 1995; Wheatley et al., 1993). Direct evidence that underscores the physiological significance of integrin-matrix interactions comes from a study in intact exchange microvessels showing that RGD peptides that inhibit integrin binding to either fibronectin or vitronectin dose-dependently increase albumin permeability by 2- to 3-fold (Wu et al., 2001). The RGD-induced hyperpermeability is time-dependent and reversible upon clearance of the peptides, indicating that the effect is not merely due to a permanent disruption of the endothelium, consistent with the note that focal adhesion-dependent permeability regulation is a dynamic process (Yuan, 2000; Yuan, 2003).

Not only do focal adhesions maintain the endothelial barrier property, their assembly and activity also mediate hyperpermeability under stimulated conditions or in the presence of angiogenic factors, inflammatory mediators, or physical forces. For example, integrins are essential to the mechanotransduction of endothelial responses to shear stress which is a known modulator of endothelial permeability (Chien et al., 2005; Ingber, 2002; Yuan et al., 1992). The $\beta 5$ subunit of integrins has been identified as a key molecule involved in the recruitment of kinases to focal adhesions in endothelial cells upon stimulation by vascular endothelial growth factor (VEGF) (Avraham et al., 2003), and mice deficient in $\beta 5$ integrin display a reduced vascular permeability response to the growth factor (Eliceiri et al., 2002). Most recently, an *in vivo* experiment demonstrated that plasma leakage across microvessels in the presence of fibrinogen degradation products is greatly attenuated in $\beta 1$ integrin knockout mice (Guo et al., 2009), further supporting a role for integrins in mediating endothelial hyperpermeability.

Endothelial Barrier Response to Neutrophils

As indicated above, activated neutrophils cause endothelial barrier dysfunction mainly by releasing permeability-increasing factors and by activating intracellular signaling reactions triggered by adhesion receptor occupancy. Neutrophil transendothelial migration *per se* does not physically disrupt endothelial cell-cell junctions. Transmigration may be accompanied by hyperpermeability; however, it is not necessarily required for neutrophil-induced microvascular leakage.

Neutrophil-derived factors cause endothelial barrier dysfunction by acting on barrier structural components, including the cytoskeleton, junctions, and focal adhesions. For example, ROS have been shown to induce endothelial junction disorganization by

promoting tyrosine phosphorylation of VE-cadherin/catenins or inhibiting phosphatase activity (Alexander et al., 2001; Sallee et al., 2006). In other studies, ROS are capable of not only decreasing junction protein expression but also enhancing stress fiber formation and actomyosin contractility (Bouez and Hassoun, 2009; Schreiber et al., 2007). Furthermore, ROS activate transient receptor potential cation channels responsible for calcium influx, where increased cytosolic Ca^{2+} serves as a second messenger that triggers further intracellular signaling, such as activating the extracellular regulated kinase (ERK1/2) cascade (Fischer et al., 2005; Hecquet et al., 2010; Hecquet et al., 2008; Hecquet and Malik, 2009). In addition, digestive enzymes secreted from neutrophil granules, including elastase, serine proteases and metalloproteinases, contribute to endothelial hyperpermeability by degrading matrix components that constitute focal adhesions, cleaving and thereby activating cytokines (e.g. IL-8), and releasing vasoactive growth factors (e.g., VEGF) from the matrix (Heissig et al., 2010). Serine proteases secreted by neutrophils also activate MT1-MMP and subsequently MMP-2, which is capable of cleaving endothelial junction proteins (Rosenberg and Yang, 2007).

Unlike transmigration, adhesion of neutrophils to microvascular endothelium is considered to have direct impact on endothelial hyperpermeability during inflammation. In particular, adhesion brings neutrophil-derived factors into close proximity with the endothelium, thereby increasing their targeting efficiency. Further, binding of neutrophil adhesion molecules (mainly $\beta 2$ integrins) to their receptors on the endothelial surface can trigger intracellular signaling events that lead to barrier responses. For example, neutrophil $\beta 2$ integrin binding to endothelial ICAM-1 increases endothelial cytosolic calcium, ROS production, and cytoskeleton reorganization (DiStasi and Ley, 2009; Gerthoffer and Gunst, 2001; Nguyen et al., 2004; Wang and Doerschuk, 2000; Wang and Doerschuk, 2002) (Figure 2). An array of intracellular molecules is activated after ICAM-1 ligation, including the non-receptor tyrosine kinases Src and Pyk2 (Allingham et al., 2007), the small GTPases RhoA and its downstream effector ROCK (Thompson et al., 2002; Wittchen, 2009), and myosin light chain kinase, an essential element of the actomyosin contractile machinery (Yuan et al., 2002). Similar signaling events have been identified in endothelial cells upon leukocyte $\beta 1$ -integrin binding to VCAM-1, and such signaling is believed to facilitate neutrophil transendothelial migration (Hordijk, 2006; van Wetering et al., 2003).

FAK in PMN-dependent barrier dysfunction

A non-receptor tyrosine kinase located in focal adhesions, FAK is composed of a central kinase domain flanked by large N- and C-terminal domains. The C-terminal non-catalytic domain, also referred to as FAK-related non-kinase (FRNK), contains a focal adhesion targeting (FAT) sequence that not only directs FAK to adhesion complexes, but also provides docking sites for other signaling molecules (Parsons, 2003; Schaller, 2001; Schlaepfer et al., 2004). In particular, the FAT sequence recruits p190RhoGEF and activates RhoA, conferring an essential role in stress fiber formation (Schlaepfer and Mitra, 2004; Zhai et al., 2003). In addition, phosphorylation of FAK at Tyr-925 exposes an SH2-domain binding site for Grb2, which subsequently activates the Ras-ERK1/2 cascade. This reaction is considered an initiating event in cell-matrix dissociation as well as MLCK-dependent actomyosin contraction (Klemke et al., 1997; Lodish, 2000; Playford and Schaller, 2004). Also, the ERK1/2 cascade promotes focal contact turnover through upregulation of both extracellular (MMPs) and intracellular (calpain) protease activities (Mitra et al., 2005; Playford and Schaller, 2004). These proteases can further degrade matrix components and intracellular FAK, respectively.

Abundant FAK is identified in vascular endothelial cells. Many studies demonstrate its critical role in vascular network stability, cell migration, and survival (Braren et al., 2006;

Peng et al., 2004; Wu, 2005). Spatial and temporal correlation between FAK activation and altered endothelial barrier morphology has been reported (Wu et al., 2003). However, the precise molecular mechanisms by which FAK regulates endothelial barrier function remains poorly understood. Even less is known about the specific role of FAK in neutrophil-elicited permeability responses. As FAK is also expressed in neutrophils, the relative contribution of neutrophilic vs. endothelial FAK to barrier regulation represents an interesting area of investigation.

With respect to neutrophil FAK, a previous study reports that FAK-mediated focal adhesions is an initiating factor for NADPH oxidase assembly in human neutrophils (Umanskiy et al., 2003). Engagement of β 1-integrin cooperates with CXCL8/CXCR-induced FAK-mediated paxillin phosphorylation and directed cell migration (Cohen-Hillel et al., 2009; Cohen-Hillel et al., 2006). Most recently, a study using myeloid-specific conditional FAK knockout mice suggests that FAK is required for neutrophil adhesion to ICAM-1, but not for transendothelial migration or chemotaxis (Kasorn et al., 2009). Moreover, FAK is critical for NADPH oxidase-mediated superoxide production, suggesting a potential role of neutrophil FAK in the regulation of vascular endothelial barrier function (Kasorn et al., 2009).

To characterize the role of endothelial FAK in neutrophil-elicited microvascular hyperpermeability, we measured the apparent permeability coefficient of albumin in isolated porcine coronary venules perfused with C5a-activated neutrophils (Guo et al., 2005). Compared with many *in vitro* permeability models, the isolated venule approach provides a more physiologically relevant analysis of microvascular permeability with the endothelium exposed to its native environment where the physical forces and chemical conditions are tightly controlled (Yuan et al., 1992). Further development of this model in combination with a polyamine-assisted protein/peptide transfer technique (Tinsley et al., 1998; Tinsley et al., 2004; Tinsley et al., 2001) enables specific identification and characterization of endothelial molecules (FAK) in regulating microvascular permeability. A series of experiments using this model produced data demonstrating that C5a-activated neutrophils induced a time- and concentration-dependent increase in venular permeability (Guo et al., 2005). The hyperpermeability was significantly attenuated during inhibition of endothelial FAK via intravascular perfusion of FRNK, a specific FAK inhibiting peptide. However, FRNK did not change the basal barrier function in intact microvessels. These results suggest that FAK plays an active role in mediating the microvascular permeability response to neutrophils. In support of the microvessel data, C5a-activated neutrophils produced paracellular hyperpermeability in cultured endothelial cell monolayers (Tinsley et al., 1999), and neutrophil-induced transendothelial flux of albumin was diminished in FRNK-transfected endothelial cells (Guo et al., 2005). Further biochemical and imaging studies showed that neutrophils promoted tyrosine phosphorylation and redistribution of FAK in endothelial cells over a time course consistent with that of hyperpermeability (Guo et al., 2005). Based on these data, we hypothesize that during inflammation, circulating neutrophils interact with microvascular endothelium causing endothelial FAK activation (presumably via inside-out signaling triggered by PMN-derived factors), which subsequently induces focal adhesion reorganization and strengthened integrin-matrix binding. The changes in focal adhesions may provide structural support for endothelial cytoskeleton contraction and junction dissociation that simultaneously occur in response to neutrophil stimulation (Yuan, 2000). We and others have previously shown that activated neutrophils produce contractile morphology and junction dissociation in endothelial cells (Breslin and Yuan, 2004; Garcia et al., 1998; He et al., 2000; Kaslovsky et al., 1995; Tinsley et al., 2002; Tinsley et al., 1999; Yuan et al., 2002).

Consistent with this hypothesis, a previous study by Alexander *et al* shows that increased transendothelial flux occurs in parallel with increased cell-matrix adhesions and decreased lateral intercellular adhesions in response to inflammatory mediators (Alexander et al., 2001).

The mechanisms by which neutrophils activate FAK endothelial cells are under investigation. It is now known that FAK phosphorylation (indicator of activation) can occur by way of phosphorylation independent of integrin clustering. Many neutrophil-derived inflammatory mediators (e.g. cytokines, growth factors, oxidants) are capable of increasing FAK tyrosine phosphorylation (Gozin et al., 1998; Schaphorst et al., 1997; Vepa et al., 1999; Wu et al., 2003; Yuan et al., 2002; Yuan et al., 1998). Activated neutrophils release VEGF (Scapini et al., 1999; Taichman et al., 1997), which in turn phosphorylates endothelial FAK and induces FAK-dependent hyperpermeability (Eliceiri et al., 2002; Wu et al., 2003). In addition, neutrophil adherence to TNF α -activated endothelial cells (via binding to ICAM-1) initiates ROS production within endothelial cells (Wang and Doerschuk, 2000), and ROS production is associated with intracellular protein tyrosine phosphorylation. Indeed, ROS induce a time- and dose-dependent increase in FAK phosphorylation, accompanied by cytoskeleton reorganization and stress fiber formation (Natarajan et al., 1998; Vepa et al., 1999). At the molecular level, it has been shown that neutrophils induce FAK tyrosine phosphorylation via the Src family kinases, which are activated downstream of calcium influx and calcium-dependent PKC activation (Etienne-Manneville et al., 2000).

Accumulating evidence suggests feedback control or cross-talk exists between FAK and other protein kinases. For example, Src and PKC can activate FAK, and FAK in turn activates Src and PKC (Bruce-Staskal and Bouton, 2001; Etienne-Manneville et al., 2000; Thomas and Brugge, 1997; Yamamoto et al., 2003). Of particular note is that FAK activation is not only associated with focal adhesion dynamics, but is also associated with the transcriptional upregulation of cell surface adhesion molecules, such as ICAM-1 (Taglia et al., 2007), which may serve as a positive feed-back loop to facilitate neutrophil transmigration. After neutrophils cross the endothelium, sub-endothelial transverse migration near focal adhesions is frequently observed before neutrophils invade into the perivascular stroma. In most cases, neutrophils are in direct contact with the basal side of endothelial cells with the majority of focal adhesions not substantially disrupted (Su et al., 2007). When neutrophils migrate transversely at a low speed, FAK appears to fade away from the endothelial focal adhesions (Su et al., 2007). During highspeed migration, paxillin, a FAK-associated focal adhesion protein, dissociates rapidly from focal contacts at the sites where endothelial cells interact with the leading edge of migrating neutrophils, and recovers afterwards (Su et al., 2007). This response may be related to paxillin activation of the p130CAS/Crk/Dock/Rac-1 cascade (Petit et al., 2000; Valles et al., 2004). Because Rac-1 is generally believed to be a barrier protector or mediator of junction re-annealing after hyperpermeability (Mehta and Malik, 2006; Wojciak-Stothard and Ridley, 2002), the Rac-1-related focal adhesion response during the phase of neutrophil transverse migration may represent a potential mechanism of barrier recovery.

In summary, integrin-supported focal adhesions are crucial in maintaining endothelial integrity under physiological conditions. Focal adhesion assembly or reorganization can either strengthen or weaken endothelial barrier function, depending on the biological status of the extracellular matrix, dynamic balance between cell-cell/matrix adhesion and cytoskeleton contraction, and the presence and nature of stimuli. In response to neutrophil stimulation, endothelial FAK is activated and signals through complex intracellular cascades leading to conformational changes in endothelial cytoskeleton and cell junctions. Increased paracellular permeability further facilitates neutrophil transmigration. Given the central role of FAK in the endothelial barrier response to neutrophil activation and neutrophil-released

products, further studies shall be directed towards the downstream structural or signaling molecules that are activated by FAK in the mediation of microvascular hyperpermeability. Live cell imaging in combination with molecular manipulation via gene silencing, mutation or knockout will continue to provide excellent tools for identification and characterization of key signaling molecules. Dynamic monitoring of neutrophil TEM, focal adhesion activities, and endothelial barrier properties at subcellular resolution in real-time represents a desirable experimental approach for future studies.

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Highlights

Neutrophil activation induces endothelial barrier dysfunction.

Focal adhesion kinase (FAK) is involved in neutrophil elicited barrier dysfunction.

Endothelial FAK is activated and triggers multiple intracellular signaling cascades.

FAK activation alters structure and function of cytoskeleton and cell-cell junctions.

Figure 1.

Schematic process of neutrophil adhesion and transendothelial migration. In response to inflammatory stimuli, adhesion molecules such as selectins are upregulated on endothelial cells and neutrophils, and neutrophils roll along the vascular endothelial wall through selectin-mediated weak interactions (I). This is followed by firm adhesion of neutrophils to endothelium through binding of integrins on the neutrophil surface to ICAM-1 or VCAM-1 on the endothelial cell surface (II). Subsequently, neutrophils transmigrate through the microvascular endothelium via a process involving complex interactions with endothelial cell-cell junction molecules, including VE-cadherin, JAMs, and PECAM-1 (III).

Figure 2.

Signaling mechanisms involved in neutrophil transendothelial migration and endothelial hyperpermeability. Activated neutrophils release vasoactive or cytotoxic agents, such as ROS, cytokines, proteases, and leukotrienes, which in turn act on endothelial cells causing barrier injury. In parallel, neutrophil adhesion to the endothelium via $\beta 2$ integrin ligand (e.g., LFA-1, MAC-1) binding to endothelial receptors (e.g., ICAM-1 or VCAM-1) triggers multiple endothelial signaling cascades causing elevated intracellular calcium, cytoskeleton contraction, and junction protein phosphorylation and dissociation. These responses act in concert leading to widened intercellular space and facilitating plasma leakage and neutrophil transmigration.