CAMs and Rho small GTPases: gatekeepers for leukocyte transendothelial migration. Focus on “VCAM-1-mediated Rac signaling controls endothelial cell-cell contacts and leukocyte transmigration”

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WHEN FLOWING BLOOD LEUKOCYTES interact with activated endothelium, the initial rolling event is mediated by selectins, which bind to their carbohydrate-based ligands, and the later firm adhesion event is mediated by integrins, which bind to cell adhesion molecules (CAMs). Specifically, neutrophils firmly adhere to activated endothelial cells (ECs) through the binding of $\alpha_{L/M}\beta_2$-integrins to intercellular adhesion molecule (ICAM)-1, whereas monocytes and lymphocytes utilize $\alpha_{L/M}\beta_2$- and $\alpha\beta_1$-integrins to bind to ICAM-1 and vascular cell adhesion molecule (VCAM)-1, respectively, on the activated EC surface (12, 23). Leukocyte transendothelial migration (also called transmigration or extravasation) is mediated by $\alpha$, $\beta_3$ binding to vitronectin, as well as by platelet endothelial cell adhesion molecule (PECAM)-1 on adjacent ECs (30). Leukocyte transmigration is crucial for an effective immune response, and understanding its molecular mechanisms is one of the goals of current biomedical research. Although it is clear that the leukocyte activation state in part controls migration (17), studies have suggested that endothelial CAMs are also involved (16), and intracellular signaling pathways in ECs are required to further facilitate the passage of bound leukocytes across the vessel wall (2, 15). Specifically, adhesion of preactivated lymphocytes to ICAM-1 on rat brain ECs or ICAM-1 cross-linking on cytokine-activated rat brain ECs (which mimics lymphocyte adhesion to the endothelium) was each shown to activate the endothelial small GTP binding protein Rho, and Rho activity was required for endothelial actin reorganization and lymphocyte transendothelial migration (2). According to another study, lymphocyte adhesion to VCAM-1 on EC lines that constitutively express VCAM-1 stimulated an endothelial NADPH oxidase-dependent production of reactive oxygen species (ROS), which was necessary for endothelial actin restructuring and lymphocyte transmigration (15). NADPH oxidase-dependent ROS production also occurred upon VCAM-1 cross-linking on cytokine-activated primary human umbilical vein ECs (HUVEC) (15).

Earlier work by Hordijk and colleagues (10) showed that vascular endothelial (VE)-cadherin colocalizes with actin stress fibers at the adherens junctions between HUVEC, and inhibition of VE-cadherin results in loss of cell-cell adhesion and increased neutrophil transmigration (10). It was also shown that activating Rac1, a member of the Rho family of small GTPases, induces stress fiber formation and loss of VE-cadherin-mediated cell-cell contact. Although Rho activity is necessary for Rac1-dependent stress fiber and intercellular gap formation, activated Rho by itself just induces stress fiber formation without loss of cell-cell contact (26). In the current article in focus (Ref. 27; see p. C343 in this issue), the same group examined the relative involvement of Rac1 and Rho GTPases in signaling pathways that are initiated by either VCAM-1 cross-linking or monocyte binding to activated pHUVEC and result in actin stress fiber formation, loss of VE-cadherin function, and monocyte transmigration. It was found that, after VCAM-1 cross-linking, Rho activity is sufficient for induction of stress fibers, but both Rac1 (and Rac1-dependent ROS production) and Rho activities are required for loss of cell-cell contact. In agreement with the above, Rac1 inhibition (or ROS scavenging) was more efficient than Rho inhibition in blocking the migration of monocytes across activated HUVEC. Hence, it was proposed that upon integrin-mediated monocyte adhesion to activated ECs and VCAM-1 clustering, a Rac1/ROS signaling pathway accompanied by Rho-mediated actin stress fiber formation may regulate monocyte transendothelial migration (27).

The proposal that Rac1 activity may control monocyte transmigration is consistent with an earlier study (15), because Rac1-dependent ROS production is believed to occur via activation of NADPH oxidase (1, 24) and VCAM-1 cross-linking was found to stimulate NADPH oxidase-dependent ROS formation, which was required for leukocyte transmigration (15). Differences in the design and/or conclusions between this and other studies are as follows: Matheny et al. (15) found that the NADPH oxidase-dependent ROS production mediates a coalescence of actin in immortalized ECs (that constitutively express VCAM-1) around a bound lymphocyte or an anti-VCAM-1-coated bead, whereas the rest of the actin structure remains unchanged. In
contrast, the study by Hordijk and colleagues (27) shows that when VCAM-1 cross-linking occurs in activated pHUVEC, it induces actin stress fiber formation that is Rac1/ROS independent. Besides the difference in ECs in the two studies, the study in focus employed an incubation with monoclonal anti-VCAM-1 antibody followed by cross-linking F(ab)_2 antibodies to achieve VCAM-1 cross-linking, and different cross-linking methods may have differential effects on cytoskeleton dynamics and cell signaling. In another report, Adamson et al. (2) found that upon lymphocyte binding to ICAM-1, Rho activity is necessary for lymphocyte transendothelial migration. They studied, though, spontaneous migration of preactivated lymphocytes across primary rat brain ECs or immortalized ECs, whereas the study in focus analyzed chemokine-driven migration of monocytes across pHUVEC. Because each study analyzed cell signaling following cross-linking of a different CAM, it is also possible that ICAM-1 and VCAM-1 signal differentially to the Rho small GTPases. Alon and colleagues (8) recently employed an in vitro flow model that allowed them to study adhesion of flowing lymphocytes on, and transmigration across, cytokine-activated HUVEC monolayers with apically overlaid chemokines. They found that flow-induced shear stress, although restrictive for leukocyte recruitment on ECs, positively regulates the subsequent transendothelial migration of bound leukocytes. The Rho family of small GTPases is known to be involved in shear stress-induced signal transduction and cytoskeleton reorganization: At the onset of steady laminar shear stress (or after an acute change in shear stress), Rac1 mediates the shear-stress-induced redox changes and activation of protein tyrosine phosphorylation (31), as well as the translocation of the actin-binding protein cortactin and activation of actin polymerization at the cell periphery, whereas the Rho-associated kinase and the myosin light chain (MLC) kinase contribute to actomyosin assembly in the cortical actin ring (7). Sustained exposure to flow induces a second phase of cytoskeletal remodeling that results in EC orientation in the direction of flow and is thought to be regulated by the Rho-associated kinase (14) and the p38 mitogen-activated protein kinase (p38 MAPK) (4). Steady laminar shear stress does not induce intercellular gap formation, but upon exposure to shear, there is a transient increase in monolayer permeability, followed by a slow decline that recovers to starting values by the time the cell orientation in the flow direction is complete (21). Because in vivo ECs and leukocytes are continuously exposed to shear stresses that vary spatially and temporally, and shear stress was shown to regulate the function of Rho small GTPases, monolayer permeability, and leukocyte transmigration, the physiological relevance of the findings of the article in focus remains to be determined in an appropriate experimental model.

Despite these limitations, the present study provides valuable information on signaling pathways that are initiated by monocyte adhesion to VCAM-1 on activated ECs and, via activation of Rho small GTPases, lead to intercellular gap formation and monocyte migration across the EC barrier. The Rho small GTPases, Rho, Rac1, and Cdc42, are known to be involved in signal transduction linking extracellular stimuli to the organization of the actin cytoskeleton, and activation of Rho, in particular, induces the formation of stress fibers (25). The Rho/Rho-associated kinase pathway, through inhibition of MLC phosphatase activity, results in increased MLC phosphorylation, actin stress fiber formation, and EC barrier dysfunction (11). However, p21-activated protein kinase (PAK)-2, a member of the PAK family, which are downstream effectors of Rac1 and Cdc42, was also shown to phosphorylate MLC and produce EC contraction (32). The major finding of the study in focus is that after CAM cross-linking, the Rho-mediated contractility is only partially responsible for the increase in monolayer permeability; the primary pathway for intercellular gap formation is Rac1/ROS-dependent with p38 MAPK as a downstream effector. One possible way that ROS may regulate cell shape and cell-cell contact is by inhibiting phosphatase activity (9), because phosphatase inhibitors are known to induce cell constriction, reorganization of actin and microtubules, and cell-cell junction separation (5, 22). The phosphatase inhibitor pervanadate, for example, caused tyrosine phosphorylation of catenins, dissociation of catenins from the cadherin-catenin complex, and, finally, loss of cell-cell contact in tumor cells (18) and in bovine aortic ECs (3). Another possible way that ROS may regulate monolayer permeability is by activation of matrix metalloproteinases (MMPs), which may degrade extracellular matrix at cell junctions, thus altering cell shape and cell-cell contact (19). Lymphocyte adhesion to VCAM-1 on immortalized rat microvascular ECs was shown to induce lymphocyte MMP2 enzyme activity that was required for lymphocyte transmigration across the EC barrier (20). Similarly, Matheny et al. (15) showed that EC-derived NADPH oxidase-dependent ROS are required for lymphocyte transmigration. Future studies need to focus on whether EC- and/or leukocyte-derived ROS inhibit vascular phosphatases and/or activate MMPs and, thus, regulate leukocyte transmigration.

The recognition of p38 MAPK as a downstream effector of Rac1/ROS partly agrees with another study (28), in which activation of p38 MAPK after ICAM-1 cross-linking on cytokine-activated pulmonary microvascular ECs was mediated by ROS, but xanthine oxidase was identified as the source. The mechanism of action of p38 MAPK, which plays an important role in EC alignment under flow (4), involves the phosphorylation of heat shock protein (HSP)27, which dissociates HSP27 from actin microfilament caps to promote assembly (6). Another potential way that activated Rac1 and Cdc42 may negatively impinge on barrier function is by regulating the IQ GTPase-activating protein (IQGAP)1, a cytoskeleton-associated protein that destabilizes the adherens junctions by competing with α-catenin for β-catenin binding (13). In addition, cortactin may play a role in regulation of vascular permeability and leukocyte migration, because it is associated with...
the actin-related protein (Arp)2/3 complex, an effector of Rac1 and Cdc42 (29), and it was shown to be phosphorylated upon ICAM-1 cross-linking (2). Thus future studies are needed to elucidate the complex pathways that regulate leukocyte transmigration across the EC barrier, to identify the relative importance of each Rho small GTPase effector in ECs from different vascular sites, and to determine how signals triggered by different CAMs are functionally integrated inside these ECs.

**DISCUSSIONS**

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**REFERENCES**


