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Leukocyte rolling and adhesion via ICAM-1 signals to endothelial permeability. Focus on "Leukocyte rolling and adhesion both contribute to regulation of microvascular permeability to albumin via ligation of ICAM-1"

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THE VASCULAR ENDOTHELIUM plays a vital role in the inflammatory response by induction and surface expression of adhesion molecules and chemokines. At sites of acute inflammation, blood flow is increased and postcapillary venules exhibit increased permeability (leakiness) and support the influx of blood leukocytes, primarily neutrophils. The relationship between neutrophil influx and increased vessel permeability has been a topic of investigation for decades. Many investigations have reported a direct neutrophil-dependent increase in vessel permeability during inflammation using in vivo and in vitro models (reviewed in Ref. 4). One aspect that has received increased attention is whether endothelial cell adhesion molecules contribute to the regulation of vessel permeability, in addition to their well-characterized role in neutrophil rolling and adhesion.

Intercellular adhesion molecule-1 (ICAM-1, also known as CD54) is an immunoglobulin superfamily member that is abundantly expressed on the endothelial cell surface and is enriched at endothelial cell borders in vivo and in vitro (2, 12, 14, 17). ICAM-1 surface expression is upregulated in endothelial cells by proinflammatory cytokines like TNF-α, IL-1β, IFN-γ, or bacterial endotoxins, and ICAM-1 serves as a receptor for leukocyte β_2-integrins (LFA-1 and Mac-1) (reviewed in Ref. 15). In an experiment of nature, patients with leukocyte adhesion deficiency-1 (LAD-1) have a severe primary immune deficiency in which blood neutrophils fail to localize to sites of inflammation or injury. This defect occurs because patients' neutrophils either lack β_2-integrins or contain mutations in these molecules. Studies using neutrophils from LAD-1 patients or β_2-integrin-deficient mice clearly demonstrate that these cells have normal selectin-dependent rolling on activated endothelium but fail to stably arrest and transmigrate. Additional studies have demonstrated that endothelial-expressed ICAM-1 contributes to both kinetics of leukocyte rolling and arrest in vivo as determined by intravital microscopy of the cremaster muscle microcirculation in ICAM-$1^{-/-}$ mice (16).

In this issue of *American Journal of Physiology-Cell Physiology*, Sumagin and colleagues (18) use elegant in vivo studies to demonstrate that microvascular permeability in the cremaster muscle model is regulated by leukocyte engagement of ICAM-1. The authors monitored permeability (P_s) in cremaster microcirculation by efflux of luminal fluorescent-tagged albumin by fluorescence confocal intravital microscopy. Prior studies by Sarelius and colleagues (19) in the same model demonstrated that ICAM-1 engagement induced a localized increase in vessel permeability in unstimulated arterioles and in TNF-α-stimulated venules. In agreement with this finding, 4 h of TNF-α stimulation of arterioles and venules in ICAM-1 knockout (KO) animals did not result in increased vessel permeability as compared with wild-type (WT) mice under identical conditions. Similar results were seen with β_2-integrin KO mice.

In the current study, the authors provide further evidence linking ICAM-1 expression to regulation of endothelial permeability. Comparisons between unstimulated venules and TNF-α-treated venules reveal that neutrophil rolling regulates venule permeability in unstimulated venules while leukocyte adhesion regulates venule permeability in TNF-α-stimulated venules. The β_2-integrin-blocking antibodies, which inhibit neutrophil arrest on endothelium, reduced the vessel permeability, and this was further reduced by treatment with a combination of β_2-integrin and P-selectin blocking antibodies that inhibits both rolling and arrest. Interestingly, the reduction in venule permeability observed by blockade of P-selectin and β_2-integrin function were suggested to be due to insufficient ICAM-1 engagement due to the absence of rolling leukocytes. To examine the role that neutrophils play, circulating neutrophils were depleted by antineutrophil GR-1 antibody treatment. Notably, depletion of neutrophils caused a marked decrease in permeability of unstimulated venules and permeability was returned to "baseline" following antibody ligation of ICAM-1 in neutrophil-depleted venules. Although suggestive, these data do not conclusively rule out a contribution of neutrophil-secreted products in regulation of vessel permeability.

Further evidence for the importance of ICAM-1 engagement in regulation of venule permeability was provided by injecting secondary antibodies to cross-link ICAM-1 antibody in unstimulated venules in the presence of rolling leukocytes. Antibody cross-linking of ICAM-1 is a widely used approach to mimic ICAM-1 clustering that occurs beneath stably adherent and transmigrating neutrophils in vivo and in vitro (14, 17). Secondary mAb cross-linking of ICAM-1 induced increases in permeability in unstimulated venules to levels seen in TNF-α-stimulated venules. This observation indicates that the degree of ICAM-1 cross-linking is commensurate with the level of vessel permeability. Thus, as more leukocytes interact with the venule wall and begin to adhere, the more venule permeability increases. Cross-linking of VCAM-1, an inducible adhesion molecule expressed in unstimulated cremaster venules and arterioles that minimally supports neutrophil interactions, did not alter permeability in unstimulated venules or arterioles (Fig. 5 in Ref. 18). These data establish an important role for neutrophil interactions with ICAM-1 in regulating vessel permeability and also provide evidence that the degree of

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ICAM-1 cross-linking may play an important role in regulating vessel permeability. The authors' findings beg the question, do other endothelial adhesion molecules phenocopy ICAM-1 effects on vessel permeability? Several adhesion molecules are involved in leukocyte rolling, arrest, and transmigration, including VCAM-1, platelet endothelial cell adhesion molecule-1 (PECAM-1; CD31), CD47, CD99, JAM-A, E- and P-selectins, mucosal addressin cell adhesion molecule-1 (MAdCAM-1), and endothelial selective adhesion molecule (ESAM) (1, 11). While many of these molecules have been reported to transmit signals upon ligation in endothelium (5, 21), most have not been interrogated for an effect on permeability in appropriate in vivo models. In vitro studies by Clark and colleagues (3) have also found that increased ICAM-1 expression in dermal microvascular endothelial monolayers reduced transelectrical resistance, rearrangement of the cytoskeleton, and alterations in adherens junctions (AJs) and tight junctions (TJs). Thus, ICAM-1 alone stands out as a key effector adhesion molecule that contributes to vessel permeability. Future studies are necessary to determine whether other adhesion molecules contribute to vessel permeability.

Further evidence for the role of ICAM-1 engagement in regulating vessel permeability was provided using TNF-α receptor 1 (TNFR1) KO mice. TNF-α treatment of venules in TNFR1 KO mice failed to induce ICAM-1, failed to increase leukocyte adhesion, and failed to increase venule permeability. Additional studies are required to determine whether other proinflammatory cytokines and agents that upregulate ICAM-1, such as IFN-γ or IL-1β, also regulate vessel permeability through ICAM-1 engagement.

Sumagin and colleagues also examined the effect of ICAM-1 engagement in arterioles and found that the responses had many similarities to venules. Although leukocytes do not roll in unstimulated arterioles, these vessels are responsive to TNF-α by increasing ICAM-1 and P-selectin surface expression and leukocyte rolling. Similar to venules, TNF-α stimulated-arterioles had increased permeability versus unstimulated arterioles, and this increase was inhibited by either β_2-integrin or P-selectin antibodies, which prevent leukocyte rolling and adhesion. Neutrophil depletion had no effect on unstimulated arterioles, because leukocytes do not normally interact with these vessels; however, ICAM-1 cross-linking of unstimulated arterioles increased permeability in a manner similar to that seen in venules. Interestingly, incubation with a membrane-permeable peptide to block the ICAM-1 cytoplasmic tail interactions also reduced the permeability of unstimulated arterioles. These results and the above mentioned in vitro studies by Clark and colleagues (3) suggest that, even in the absence of leukocyte interactions, ICAM-1 actively participates in regulation of vessel permeability. The authors argue that "leukocyte-EC interactions are a more important contributor to regulation of P_s than are the established differences in structure and EC morphology between arterioles and venules." While this pathway of regulating vessel permeability plays an important role, recent studies suggest that other pathways are also physiologically relevant. For example, other investigators have observed that endothelium derived from venous (human umbilical vein endothelial cells; HUVEC) versus arterial (human umbilical artery endothelial cells; HUAEC) beds have differential responses to TNF-α, and they have showed that cell culture conditions (organ versus cell culture models) play a crucial role in these phenotypic responses (10). The differences were ascribed to multiple factors including transcriptional regulation through an effect of Kruppel-like factor 2 expression in arterial endothelium, which is upregulated in arterial endothelium and suppresses TNF-mediated adhesion molecule gene expression (13).

Previous studies have shown that clustering of ICAM-1 by antibody cross-linking or leukocyte adhesion initiates outside in signaling. These signaling events include rapid association of the ICAM-1 cytoplasmic tail with cytoskeletal adapter proteins (reviewed in Ref. 20), activation of Src family kinases (SFK), Pyk2 kinase, RhoA, RhoG, p38 kinase, and a transient increase in cytosolic Ca^{2+}, leading to stress fiber formation and cytoskeletal reorganization. Through an incompletely understood mechanism, these events trigger tyrosine phosphorylation of VE-cadherin by SFK and Pyk2 (1). Sumagin and coworkers showed that blockade of the ICAM-1 cytosolic tail to prevent outside-in signaling also decreased venule permeability, but had no effect on leukocyte rolling and adhesion. To gain insight into the mechanisms underlying ICAM-1-dependent regulation of vessel permeability, the authors used pharmacological inhibitors. Their results showed that, in unstimulated arterioles, vessel permeability was dependent on SFK. However, in unstimulated venules, cross-linking ICAM-1 by a primary antibody induced PKC-α-dependent and SFK-independent increases in venule permeability. Addition of a secondary antibody to cross-link ICAM-1 induced further increases in venule permeability in a SFK-dependent and PKC-α-independent fashion. These data suggest that different signaling pathways are involved in regulating venule and arteriole permeability and that these pathways can switch depending on the level of ICAM-1 engagement. More specific interventions (e.g., in vitro knockdown or knockout animals) are needed to corroborate these findings.

Vascular permeability is regulated by two pathways, paracellular (endothelial cell-to-cell borders) and transcellular transport (reviewed in Refs. 8 and 9). An important question raised by the work of Sumagin and colleagues is whether one or both pathways are involved in ICAM-1-triggered regulation of vessel permeability. The paracellular pathway is maintained by AJs and TJs, and the AJs are considered the dominant contributor to vessel permeability in most vascular beds (9). AJs are composed of VE-cadherin associated with its cytosolic binding partners, β-, γ-, and p120-catenins, and this complex is further strengthened through its association with the cytoskeleton (22). Several studies have implicated the SFK in the regulation of AJ function. SFK activation leads to increased tyrosine phosphorylation of VE-cadherin and β-catenin, which triggers disruption of the AJ and actin-myosin contractility. Interestingly, SFK are also regulators of the endothelial cell transcellular transport pathway (reviewed in Ref. 6). The pathway is described as a receptor-mediated transport of albumin and other macromolecules from the luminal to the abluminal surface and is an energy-dependent process involving formation and trafficking of caveolae (reviewed in Ref. 9). The role of the transcellular pathway in ICAM-1-mediated regulation of permeability has not been investigated as intensively as the paracellular pathway. Nonetheless, recent studies by Hu and colleagues (7) reported that, in an in vitro model of rat lung microvascular endothelial cells, ICAM-1 cross-linking induced

increased transcellular albumin permeability. These data implicate a role for the transcellular pathway, but additional studies are needed to better explore this avenue of inquiry.

This rich set of studies by Sarelius and colleagues together with work from other investigators provide convincing evidence that leukocyte-endothelial cell communication during acute inflammation plays an important role in vessel permeability and that endothelial ICAM-1 plays a key role in this process. Future investigations are needed to identify the molecular mechanisms downstream of ICAM-1 signaling that regulate permeability. An understanding of these pathways will facilitate the design of therapeutic strategies to inhibit or impair ICAM-1 signaling, and these approaches may prove useful in treatment of acute and chronic inflammatory diseases including atherogenesis, stroke, ischemia-reperfusion injury, and sepsis-induced acute respiratory distress syndrome.

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