

Brief Review

Endothelial Cell Junctional Adhesion Molecules Role and Regulation of Expression in Inflammation

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Abstract—Endothelial cells line the lumen of all blood vessels and play a critical role in maintaining the barrier function of the vasculature. Sealing of the vessel wall between adjacent endothelial cells is facilitated by interactions involving junctionally expressed transmembrane proteins, including tight junctional molecules, such as members of the junctional adhesion molecule family, components of adherence junctions, such as VE-Cadherin, and other molecules, such as platelet endothelial cell adhesion molecule. Of importance, a growing body of evidence indicates that the expression of these molecules is regulated in a spatiotemporal manner during inflammation: responses that have significant implications for the barrier function of blood vessels against blood-borne macromolecules and transmigrating leukocytes. This review summarizes key aspects of our current understanding of the dynamics and mechanisms that regulate the expression of endothelial cells junctional molecules during inflammation and discusses the associated functional implications of such events in acute and chronic scenarios. (*Arterioscler Thromb Vasc Biol.* 2016;36:00-00. DOI: 10.1161/ATVBAHA.116.307610.)

Key Words: adhesion molecule ■ blood vessel ■ endothelial cell ■ inflammation ■ vascular permeability

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Endothelial cells (ECs) line the inner wall of all blood vessels and are critical in maintaining the barrier function of the vasculature. Under inflammatory conditions, penetration of ECs by macromolecules and immune cells can be achieved via both transcellular mechanisms—possibly involving intracellular structures, such as vesiculo-vacuolar organelles—and paracellular mechanisms—involving breaching of tightly connected junctions between adjacent ECs.¹⁻³ With respect to the latter, it is now well accepted that strict regulation of expression, distribution, and function of EC junctional proteins is pivotal for maintaining steady-state stability, integrity, and barrier properties of vessel walls. Furthermore, in response to injury or infection, controlled opening/loosening of EC junctions plays a critical role in supporting an effective inflammatory response. This occurs through increased vascular permeability to macromolecules, facilitating leakage of essential blood-borne immunoregulatory and proinflammatory proteins to the extravascular tissue (eg, immunoglobulins and components of the complement cascade) and also enabling breaching of venular walls by transmigrating immune cells.¹⁻³ Because of their essential role in such biological functions, there is immense interest in the signaling properties of EC junctional molecules under both physiological and pathological conditions.¹⁻³ In addition, there is increasing awareness and, indeed, evidence for altered cell surface expression of EC junctional molecules in inflammation, phenomena that are currently under investigation in terms of their associated mechanisms and biological implications. This review summarizes the key findings related to this topic.

Expression and Function of EC Junctional Molecules

The single-cell EC layer of blood vessels is held together via complex structures comprising numerous transmembrane proteins that interact both with binding ligands on adjacent cells and with associated intracellular partners.¹⁻⁴ Two key junctional structures are tight junctions that incorporate members of the junctional adhesion molecule (JAM) family, EC-selective adhesion molecule, and claudins and adherens junctions that include VE-Cadherin.¹⁻⁵ Numerous other adhesion molecules are also present at EC contacts, such as platelet endothelial cell adhesion molecule-1 (PECAM-1), CD99, CD47, activated leukocyte cell adhesion molecule-1, and ICAM-2, molecules that contribute to junction formation and properties¹⁻⁶ (Figure). A growing body of evidence also indicates the expression of EC junctional molecules in a variety of EC intracellular compartments, such as the membranous lateral border recycling compartment (LBRC), endosomes, and vesicle-type structures (Figure). Recent developments strongly support the concept that intracellular stores of EC junctional molecules contribute to maintaining the integrity and function of the endothelium (discussed later).⁴

The 2 key principal roles of EC junctions during inflammation are regulation of leukocyte migration out of the vascular lumen and regulation of vascular permeability to macromolecules. With respect to the former, ECs are critical in attracting and facilitating the transmigration of immune cells, both for tissue surveillance and in direct response to sterile and

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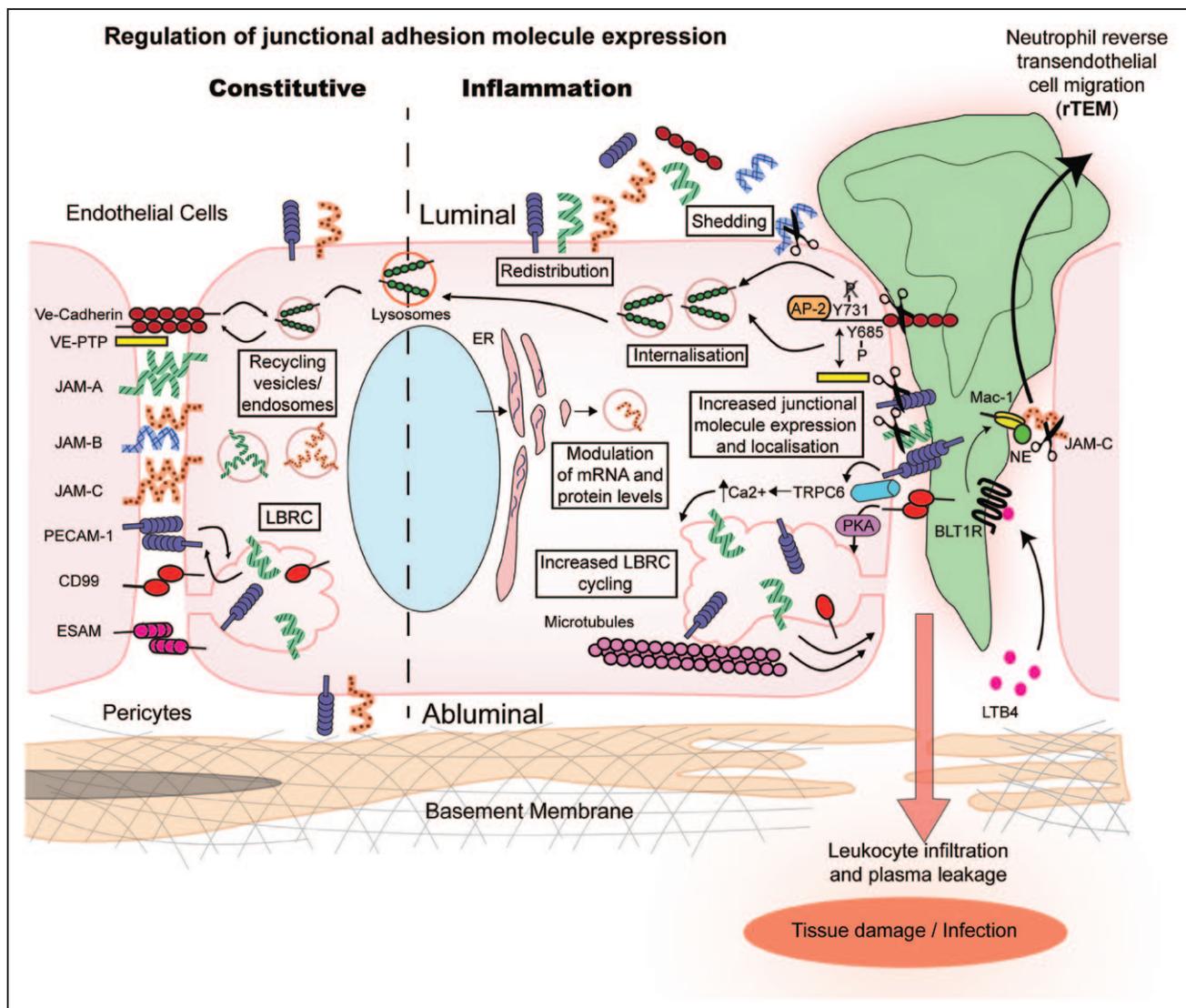


Figure. Mechanisms associated with regulation of expression of key endothelial cell junctional adhesion molecules. Constitutive junctional adhesion molecule regulation involves protein internalization in endosomes and vesicular structures, their recycling back to the cell surface, and degradation (eg, via lysosomes). Certain proteins, such as PECAM-1, JAM-A, and CD99, can also cycle between the cell membrane and the LBRC under basal conditions. Under inflammatory conditions, the expression of adhesion molecules at endothelial cell junctions is regulated through additional mechanisms. Namely, increased gene transcription and mRNA translation leading to upregulation of total junctional molecule expression at protein level in endothelial cells. JAM-A, JAM-C, and PECAM-1 can be redistributed away from cell junctions to nonjunctional membranes, whereas internalization from the plasma membrane of certain molecules, such as VE-Cadherin, is increased. Cycling of the LBRC increases during inflammation, allowing the recruitment of additional PECAM-1 and CD99 molecules to junctional sites. Enzymatic cleavage and shedding from the cell surface reduces the expression of junctional adhesion molecules at endothelial cell junctions. The mechanisms illustrated in the diagram can regulate inflammatory responses, such as vascular leakage, and the magnitude and profile of leukocyte transendothelial cell migration (TEM). For example, with respect to the latter, NE cleavage of JAM-C is known to promote neutrophil rTEM. ER indicates endoplasmic reticulum; ESAM, endothelial cell-selective adhesion molecule; JAM, junctional adhesion molecule; LBRC, lateral border recycling compartment; NE, neutrophil elastase; PECAM, platelet endothelial cell adhesion molecule; and rTEM, reverse transendothelial cell migration.

infectious insults. Specifically, at sites of inflammation, leukocytes exhibit several luminal interactions with ECs, initiating with leukocyte rolling along the endothelium, followed by leukocyte arrest and crawling. These events are mediated by a cascade of intricate molecular and cellular interactions between immune cells and ECs as described by the leukocyte adhesion cascade.⁷ During leukocyte crawling, leukocytes engage with EC junctions and begin breaching venular walls.^{2,6,7} Migration through the EC barrier (transendothelial cell migration; TEM) can occur via both transcellular and

paracellular modes. Although significant use of the transcellular route has been reported across the blood–brain and blood–retinal barriers during inflammatory pathologies,⁸ paracellular diapedesis seems to be the most prevalent mode of breaching ECs both in vitro and in vivo ($\approx 70\%$ to 90%).^{3,6} This was directly demonstrated in vivo through the application of high-resolution 3-dimensional intravital imaging of inflamed mouse cremaster muscle venules where paracellular TEM was found to account for $\approx 90\%$ of all observed neutrophil TEM events as induced by multiple inflammatory stimuli.⁹ Furthermore,

a mouse strain in which the EC junctions were stabilized through expression of a VE-Cadherin- α -catenin fusion complex (replacing endogenous VE-Cadherin) exhibited reduced leukocyte (neutrophil and lymphocyte) infiltration in several models of inflammation, providing strong supportive evidence for the involvement of EC junctions in leukocyte trafficking.¹⁰ Paracellular leukocyte TEM is mediated by an elaborate series of interactions between leukocytes and EC JAMs, including PECAM-1, JAMs, CD47, activated leukocyte cell adhesion molecule-1, EC-selective adhesion molecule, ICAM-2, and CD99 (for reviews on this topic, see references Nourshargh et al,² Vestweber,³ Muller,⁴ Nourshargh and Alon,⁶ and Gerhardt and Ley¹¹), and there exists some evidence to suggest that EC junctional molecules can also support leukocyte transcellular TEM.^{4,6} In addition, there is strong evidence to indicate distinct and sequential roles for these molecules in different stages of leukocyte movement through venular walls.^{2,4,6} However, care needs to be exercised in formulating general concepts because roles of different molecules in different stages of leukocyte transmigration seem to be governed to a large extent by the nature of the inflammatory reaction being studied (eg, the tissue and genetic background of animals in which it is elicited) and the leukocyte subtype under observation.^{3,4,6,7,11} In addition, EC phenotype, morphology, and junctional composition can vary between different vascular beds, differences that may well impact the profile and dynamics of vascular permeability and leukocyte-EC interactions.¹²

Vascular permeability to plasma proteins, and the subsequent formation of tissue edema, is another important protective physiological reaction to tissue injury. Although it is well established that neutrophils can mediate vascular permeability¹³ and numerous neutrophil-derived factors have been implicated in this response,^{13,14} the precise molecular basis of this reaction remains unclear. Of importance, recent developments in this area have provided compelling evidence for the existence of distinct molecular pathways in induction of vascular permeability and leukocyte TEM.^{1,15,16} Specifically, the works of Vestweber and colleagues have shed new insights into the mechanism through which VE-Cadherin can mediate these responses.^{3,10,16-18} Briefly, through generation of knock-in mice in which specific tyrosine residues of VE-Cadherin were mutated to phenylalanine, evidence was obtained for the phosphorylation state of VE-Cadherin in maintaining functional EC junctions.¹⁶ Importantly, this study showed that the phosphorylation status of 2 distinct tyrosine residues of VE-Cadherin can selectively and exclusively regulate either vascular permeability or leukocyte diapedesis.¹⁶

Other junctional molecules involved in the regulation of vascular permeability include JAM-A and JAM-C. Despite the high percentage of homology between the 2 proteins, these molecules seem to have opposing roles in regulating barrier function of ECs. For example, although genetic deletion and blockade of JAM-A generally results in increased EC permeability,¹⁹ knocking down JAM-C decreases EC permeability *in vitro*.²⁰ Of note, as opposed to cultured macrovascular ECs (eg, human umbilical vein ECs) that constitutively express JAM-C at cell-cell contacts, quiescent microvascular ECs in culture express JAM-C predominantly within intracellular stores that can be mobilized to junctions after cellular

stimulation. The latter seems to provide a mechanism through which EC permeability is regulated.²⁰ However, the role of EC JAM-C in vascular permeability *in vivo* remains unclear and may depend on the nature of the inflammatory model. Although treatment of wild-type mice with soluble recombinant JAM-C (used as a competitive blocker of JAM-C interactions) reduces vascular permeability in response to histamine or VEGF,²⁰ specific antibody blockade of endothelial JAM-C leads to increased vascular leakage in a model of cutaneous infection by *Leishmania major*.²¹ Of note, genetic inactivation of the related tight junction-associated protein EC-selective adhesion molecule attenuates the induction of vascular permeability and delays leukocyte diapedesis *in vivo*.²²

Collectively, because there is undisputed evidence for the involvement of EC junctional molecules in regulation of endothelial barrier function both at rest and during tissue injury, better understanding of the mechanisms that regulate remodeling of EC junctions, and the associated biological impact, could shed light on the intricacies of many vascular and inflammatory processes.

Mechanisms Regulating the Surface Expression of EC Junctional Molecules in Inflammation

Several studies have reported on altered expression of EC junctional molecules *in vitro* and *in vivo*, with some studies associating such changes with altered functional readouts.^{1,4} At present, investigations into regulation of EC junctional molecule expression are at an early stage, but to date, several diverse mechanisms have been suggested as discussed later (see also Tables 1 and 2).

Internalization and Redistribution

Internalization and redistribution of molecules to intracellular compartments are commonly reported means through which the expressions of a wide range of cell surface-presented transmembrane proteins are regulated in many cell types. Such responses are also emerging as important mechanisms in control of EC barrier functions. Several EC junctional proteins, such as PECAM and VE-Cadherin, undergo some form of intracellular recycling or degradation.^{3,4} In addition, many others, such as JAM-C, JAM-A, and CD99, are also present in intracellular stores and are, therefore, susceptible to mobilization to and from EC junctions post stimulation (Figure).⁴ In this context, ECs show a unique array of intracellular compartments that are associated with junctional molecule internalization, storage, and recycling. These include the membrane invagination structure termed LBRC (commonly found in close proximity of EC junctional lateral borders), components of the vesicular system, vesiculo-vacuolar organelles and caveolae, and endosomes that are additionally associated with delivery of proteins to lysosomes for destruction. Current evidence suggests that the nature of the carrier system is relatively specific to the cargo molecule, with some molecules showing intracellular recycling both at rest and also during acute inflammation, as discussed later.

The LBRC represents an internalization organelle, apparently unique to ECs and containing key junctional molecules, including PECAM-1, JAM-A, CD99, and the poliovirus

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Table 1. Key Reported Changes in Expression of EC Junctional Molecules Post Inflammation, Excluding Cleavage-Induced Changes (Listed in Table 2)

Molecule	Inflammatory Stimuli/Model	Change in Expression	Proposed Mechanism	Functional Implications	References
In vitro					
PECAM-1	TNF+IFN γ (HUVECs)	↓ mRNA	mRNA destabilization	↓ leukocyte TEM No effect on leukocyte TEM	Ozaki et al ²³ , Rival et al ²⁴ , Shaw et al ²⁵ , and Stewart et al ²⁶
		↓ total and junctional expression	Protein internalization and degradation		
			Redistribution to the cell surface		
VE-Cadherin	TNF (HUVECs/primary lung EC)	↓ junctional expression	Tyrosine phosphorylation	↑ permeability	Wessel et al ¹⁶ , Alcaide et al ²⁷ , Allport et al ²⁸ , Shaw et al ²⁹
		Gap formation curtain effect		Facilitate neutrophil TEM	
	VEGF	↑ internalization	Endocytosis through a VEGFR-2-Src-Vav2-Rac-PAK signaling axis	↑ permeability	Gavard and Gutkind ³⁰
	TNF (primary lung EC)	↓ junctional expression	Leukocyte induced SHP2-mediated Y731 dephosphorylation and endocytosis through AP-2	↑ leukocyte TEM	Wessel et al ¹⁶
JAM-A	bFGF	Redistribution to cell membrane	Dissociation from integrin $\alpha_v\beta_3$	↑ angiogenesis	Naik et al ³¹
	OxLDL (human and mouse aortic ECs)	Redistribution to apical membrane	Statin-dependent	↑ monocyte adhesion and TEM	Schmitt et al ³²
JAM-C	OxLDL	↑ total protein expression	None described	↑ monocyte adhesion and TEM	Keiper et al ³³
		↑ expression at nonjunctional sites			
	Thrombin	Relocalization to junctions	None described	Binding and modulation of β_3 integrin activity	Li et al ³⁴
				↑ permeability	
VEGF, Histamine	Relocalization to junctions	None described	Regulates actomyosin contractility	Orlova et al ²⁰	
			Stabilization of VE-Cadherin		
↑ permeability					
In vivo					
JAM-A	Murine model of atherosclerosis	↑ mRNA and protein in arterial ECs and atherosclerotic plaques	Disturbed flow and upregulation of miR-145	↑ monocyte recruitment into the arterial wall	Ostermann et al ³⁵ , Babinska et al ³⁶ , Schmitt et al ³⁷
		Luminal redistribution			
	Murine model of hepatic I-R injury	Protein upregulation	None described	Supports neutrophil TEM	Khandoga et al ³⁸
JAM-C	Murine model of atherosclerosis	↑ protein in atherosclerotic plaques	None described	None described	Keiper et al ³³
	Human rheumatoid arthritis and osteoarthritis	↑ protein in synovial tissue	None described	None described	Rabquer et al ³⁹
	Murine cremaster muscle I-R injury	↓ junctional expression	ROS-mediated loss of JAM-C	Polarized neutrophil TEM	Woodfin et al ⁹ , Scheiermann et al ⁴⁰ , Colom et al ⁴¹
		Redistribution to nonjunctional membrane			
Mice injected IV with anti-JAM-C mAb	Redistribution to nonjunctional membrane	Loss of JAM-C/JAM-B interaction	↑ monocyte adhesion to lymph nodes	Lamagna et al ⁴²	

bFGF indicates basic fibroblast growth factor; EC, endothelial cell; HUVECs, human umbilical vein endothelial cells; IFN γ , interferon- γ ; I-R, ischemia-reperfusion; IV, intravenously; JAM, junctional adhesion molecule; mAb, monoclonal antibody; OxLDL, oxidized low-density lipoprotein; PECAM, platelet endothelial cell adhesion molecule; TEM, transendothelial cell migration; and TNF, tumor necrosis factor.

Table 2. Cleavage of EC Junctional Molecules During Inflammation

Molecule	Model	Mechanism	Functional Implications	References
PECAM-1	Serum starvation (HUVECs, EOMA, BAECs)	Cleavage by caspases and shedding by MMPs	None described	Ilan et al ⁴³
VE-Cadherin	fMLP stimulated neutrophils (HUVECs)	Cleavage by neutrophil elastase and cathepsin G	↑ permeability Support neutrophil TEM	Hermant et al ⁴⁴
	Thrombin stimulation (HUVECs)	Cleavage by ADAM-10	↑ permeability Support T-cell migration	Schulz et al ⁴⁵
	TNF stimulation (HUVECs)	Cleavage-dependent on tyrosine kinases, Src kinase, and MMPs	None described	Xiao et al ⁴⁶
JAM-A	PMA, TNF α + IFN γ , PAF (HUVECs)	Cleavage by ADAM-10, ADAM-17	↓ EC migration	Koenen et al ⁴⁷
			↓ neutrophil TEM	
JAM-B	HBMEC incubation with tumor cell-secreted supernatant	Cleavage by cathepsin S	↑ TEM of brain metastatic tumor cells	Sevenich et al ⁴⁸
JAM-C	LPS, IL1- β , IL-18, MIF, IL-17, TNF, PMA (HUVECs)	Cleavage by ADAM-10, ADAM-17	Supports angiogenesis	Rabquer et al ⁴⁹ , Colom et al ⁴¹
	Murine cremaster muscle I-R and local LTB4	Cleavage by NE as supported by presentation of NE to JAM-C via neutrophil Mac-1	↑ neutrophil rTEM	

BAECs indicates bovine aortic endothelial cells; bFGF, basic fibroblast growth factor; EOMA, mouse hemangioendothelioma endothelial cells; HBMEC, human brain microvascular endothelial cells; HUVECs, human umbilical vein endothelial cells; IFN γ , interferon- γ ; IL, interleukin; JAM, junctional adhesion molecule; NE, neutrophil elastase; OxLDL, oxidized low-density lipoprotein, PECAM, platelet endothelial cell adhesion molecule; TEM, transendothelial cell migration; and TNF, tumor necrosis factor.

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receptor (CD155), but not VE-Cadherin, which is actively excluded from this domain.^{4,50,51} This compartment is believed to provide a means through which adhesion molecules and additional membrane can be efficiently recruited to sites of leukocyte diapedesis on demand.⁴ In support of this concept, changes in the intracellular pool of PECAM-1 have been located to sites where the LBRC is redirected toward the cell border to support paracellular migration⁵² or toward the cell body during transcellular migration.⁵³ Although there is now ample evidence to support a role for LBRC in regulation of leukocyte TEM, there remain many unanswered questions regarding the trafficking of this structure, an area that has been developed in recent studies by Muller et al.⁴ For example, vesicles of the LBRC compartment are moved by kinesin molecular motors along microtubules.^{4,50,52} Furthermore, although it has been known for >20 years that a transient increase in EC cytosolic-free calcium is required for TEM, Weber et al⁵⁴ have recently demonstrated that the Ca²⁺ channel, transient receptor potential canonical 6 (TRPC6), mediates this response and have linked this reaction with LBRC trafficking. Specifically, homophilic interaction of leukocyte and EC PECAM triggered the activation of TRPC6 and promoted its colocalization with PECAM-1 during TEM and trafficking of the LBRC. Of note, selective inactivation of EC TRPC6 blocked neutrophil TEM while its activation rescued TEM during conditions of PECAM blockade, suggesting that TRPC6 acts downstream of PECAM-1 ligation. The importance of this pathway in regulation of leukocyte transmigration was also demonstrated in vivo, where chimeric mice deficient in EC TRPC6 exhibited defective neutrophil TEM in a model of acute inflammation.⁵⁴ Downstream of the pathway triggered by PECAM engagement, trafficking of the LBRC is also regulated by EC CD99

through activation of PKA, a mechanism involving ezrin and soluble adenylyl cyclase.⁵⁵ Collectively, such studies have shed much light on the mechanism through which LBRC is recruited to sites of leukocyte TEM and have strengthened the evidence for the functional importance of this trafficking toward and away from EC junctions.⁴

Changes in VE-Cadherin junctional expression can also have profound impact on EC barrier function.⁵ VE-Cadherin displays constitutive endosomal internalization from the EC surface via a clathrin-dependent pathway⁵⁶ and can then be lysosomally degraded⁴⁶ or recycled back to the EC surface through Rab11a-mediated trafficking to recover EC barrier properties after junctional challenge.⁵⁷ VE-Cadherin maintenance at EC junctions is regulated by different cytoplasmic binding partners of this molecule, such as p120-catenin and Src kinase. Briefly, p120 dissociation from VE-Cadherin intracellular tail regulates cadherin levels, preventing its endocytosis and degradation.^{46,56} In addition, more recent works identified a specific motif of VE-Cadherin intracellular tail that is required for p120 binding and VE-Cadherin internalization. Mutations in this region strongly affect EC migration, although the impact of such effects on EC barrier function remains to be elucidated.⁵⁸ On the contrary, changes in VE-Cadherin phosphorylation status destabilize EC contacts, a response that supports vascular leakage and leukocyte migration.^{3,5,16,27,59} VE-Cadherin phosphorylation can be regulated by p120²⁷ and the kinases Src and Pyk2 in response to ICAM-1 ligation.⁵⁹ This is necessary for leukocyte TEM but does not imply internalization of the molecule and so will not be discussed further. Phosphorylation of residues Y658 and Y685 have, however, been shown to be responsible for internalization and ubiquitination of the protein after bradykinin

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and histamine-induced permeability.⁶⁰ This phenomenon occurs specifically in veins and not arteries, possibly because of the shear stress-dependent activation of junctional Src in the former, and regulates loosening of venular EC junctions and leakage.⁶⁰ More recently, heterotrimeric G protein G α 13 binding to VE-Cadherin has been shown to regulate Src-mediated phosphorylation of VE-Cadherin and its internalization, identifying a unique role for G α 13 in mediating EC barrier disruption in vivo in response to vascular permeability factors, such as histamine and bradykinin.⁶¹ Internalization of the molecule can also be regulated by phosphorylation of S665 through the VEGFR-2–Src–Vav2–Rac–PAK signaling axis after VEGF stimulation.³⁰ This promotes VE-Cadherin association with β -arrestin2 and its internalization through clathrin-coated vesicles.

As well as responding to inflammatory mediators, VE-Cadherin is also transiently displaced from EC junctions by transmigrating leukocytes, a reaction described as the curtain effect because it rapidly reseals behind emigrating cells.^{28,29} In addition, VE-Cadherin can be internalized in response to leukocyte–EC interactions.¹⁶ New findings have identified the molecular basis of this phenomenon by demonstrating how leukocyte–EC interactions trigger SHP2-mediated dephosphorylation of Y731 and further endocytosis of VE-Cadherin through binding of adaptin AP-2.¹⁶ Although Y731 dephosphorylation seemed essential for leukocyte TEM in vivo, it was not necessary for inflammation-induced vascular permeability. Conversely, phosphorylation of Y685 was required for junction destabilization but was dispensable for leukocyte diapedesis, indicating the intricacies of regulating VE-Cadherin phosphorylation and its functional implications.¹⁶ Under resting conditions, VE-Cadherin binds to VE-PTP, a phosphatase that maintains VE-Cadherin and its associated catenins in a nonphosphorylated state critical for optimal adhesive functions of VE-Cadherin and EC contact integrity. After engagement of leukocytes with ECs or stimulation with VEGF, VE-PTP dissociates from VE-Cadherin, promoting its phosphorylation, subsequent loss of VE-Cadherin interactions, and hence, loosening of EC junctions.¹⁸ Of note, VE-PTP has also recently been described to regulate EC junctional stability by a VE-Cadherin-independent mechanism involving its interaction with Tie-2. Briefly, VE-PTP–Tie 2 interaction can dampen the tyrosine kinase activity of this receptor and, hence, its ability to stabilize EC junctions. Pharmacological or genetic ablation of VE-PTP leads to increased EC junctional stability in vivo via Tie-2, counteracting vascular leakage and leukocyte transmigration induced by inflammatory mediators. Thus, activation of Tie-2 via inhibition of VE-PTP protects endothelial junctions against inflammation-induced destabilization and overrides the negative effect of VE-PTP inhibition on the adhesive function of VE-cadherin.⁶² VE-Cadherin association with one or another partner is reversible and can be spatially and temporally regulated. Of note, the actin-binding protein EPS8 has recently been identified as a binding partner of VE-Cadherin. EPS8 promotes VE-Cadherin ubiquitination and phosphorylation, leading to increased internalization and enhanced cell surface turnover of the molecule.⁶³ This interaction mediates transduction of signals impinging on the regulation of

the transcriptional cofactor Yes-associated protein and, as a result, modulates vascular permeability.⁶³

Similar to internalization, redistribution of adhesion molecules away from EC junctions and onto the cell body may represent a means through which an inflammatory reaction is regulated. Such a response may facilitate the development of an inflammatory event through promotion of leukocyte adhesion to the EC surface (via increased expression of adhesion molecules on the EC apical membrane) or its termination through inhibiting TEM (via reduced expression of molecules at junctions between adjacent cells). Redistribution of EC junctional molecules has been reported within in vitro and in vivo models of acute inflammation with respect to several proteins (Figure and Table 1). For example, expressions of PECAM-1 and JAM-A are reduced from junctions of human umbilical vein ECs treated with the cytokine combination interferon- γ and tumor necrosis factor, with no apparent reduction in total cellular protein levels.^{23,64} A similar phenomenon was noted for JAM-A in brain ECs stimulated with CCL2 and LPS, a response that was associated with increased adhesion of monocytes and neutrophils.⁶⁵ This occurred via internalization of the molecule by macropinocytosis and its transient storage in recycling endosomes before being recruited back to the apical side of ECs. These findings indicate that redistribution may be supported by internalization pathways. JAM-A redistribution to the apical membrane of aortic ECs also occurs in response to proatherogenic oxidized lipoproteins in vitro^{32,65} and in vivo in murine models of atherosclerosis in regions of disturbed flow.³⁷ The latter response was associated with increased monocyte recruitment into the arterial wall and enhanced atherosclerotic lesion formation.³⁷

A significant body of work has investigated the regulation of expression of JAM-C and its functional implications. In vitro, human umbilical vein ECs treated with oxidized low-density lipoprotein, but not tumor necrosis factor, interleukin-1 β , VEGF, or histamine, showed redistribution of JAM-C from junctions to the cell surface.^{20,33} This redistribution resulted in the ability of JAM-C to mediate both leukocyte adhesion and TEM as compared with JAM-C on unstimulated ECs that only supported leukocyte diapedesis.^{20,33} As previously mentioned, in quiescent microvascular ECs, JAM-C is mainly intracellularly expressed and is recruited to junctions after short-term stimulation with stimuli, such as VEGF or histamine. This induced expression of JAM-C at EC junctions was shown to support vascular permeability, a response mediated through modulation of actomyosin-based endothelial contractility and regulation of VE-Cadherin-mediated cell–cell contacts in a Rap1-dependent manner.²⁰ In contrast to in vitro studies, in vivo JAM-C is expressed at EC junctions, as indicated through analysis of numerous murine tissues.⁴⁰ However, in line with in vitro works, there is evidence for the presence of JAM-C in intracellular vesicles within microvascular ECs in vivo.⁴⁰ This intracellular store of JAM-C appeared to be available for mobilization under inflammatory conditions, in that redistribution of JAM-C from intracellular vesicles and EC junctions to EC nonjunctional plasma membrane regions was noted in a murine model of ischemia–reperfusion injury. The in vivo redistribution of EC JAM-C was associated with

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enhanced luminal neutrophil–venular wall interactions.^{9,40} As well as mediating leukocyte adhesion and diapedesis, there is also evidence from both *in vitro* and *in vivo* works for the ability of EC JAM-C to mediate polarized migration of leukocytes through EC monolayers.^{9,66} Specifically, Bradfield et al⁶⁶ showed that inhibition of EC JAM-C can lead to enhanced monocyte reverse TEM (rTEM) through tumor necrosis factor–stimulated human umbilical vein ECs, that is, increased frequency of monocyte movement in an abluminal-to-luminal direction. *In vivo*, our studies provided the first direct evidence for the occurrence of neutrophil rTEM in a mammalian model (inflamed mouse cremaster muscle), a phenomenon that was significantly enhanced under conditions of reduced EC junctional expression or functionality of JAM-C. This was achieved through the use of EC JAM-C-deficient mice, antibody blockade of JAM-C, or after induction of inflammatory reactions, such as ischemia–reperfusion injury, that cause reduced expression of junctional EC JAM-C.^{9,41} The underlying mechanism through which EC JAM-C supports luminal-to-abluminal TEM is at present unclear but maybe related to the role of JAM-C in maintaining EC polarity.⁶⁷ Although the pathophysiological relevance of neutrophil rTEM requires further investigations, our current data suggest that rTEM neutrophils stemming from a primary site of injury may contribute to dissemination of systemic inflammation and second organ damage.^{9,41}

Overall, it is becoming increasingly clear that altered surface localization and expression of EC junctional molecules, as mediated via multiple different modes, can lead to altered functional properties of molecules with respect to both leukocyte trafficking and regulation of vascular permeability to macromolecules.

Enzymatic Cleavage and Shedding From the Cell Surface

Several EC junctional molecules have been reported to be enzymatically cleaved by leukocyte and EC-derived proteases. Although such events have been implicated to several vascular responses, including permeability, immune cell recruitment, vascular repair, and angiogenesis (Table 2),^{68,69} this aspect of the field requires further exploration and critical assessment. Of importance, caution is required when linking the shedding of a certain cell surface protein to a specific functional readout(s) because, commonly, the study cannot rule out the possibility that the observed effects was mediated via the shedding of other cell surface proteins that were not analyzed. Thus, much of the studies cited below are correlations that do not necessarily link a defined shedding phenomenon with the reported biological observation.

To date, 3 families of proteases have been associated with such responses, namely ADAMs, MMPs, and serine proteases. For example, PECAM-1 is shed by MMPs from the cell surface during EC apoptosis,⁴³ and VE-Cadherin is reportedly cleaved by ADAM10,⁴⁵ neutrophil elastase (NE), and cathepsin G.⁴⁴ Although the latter studies have associated enzymatic cleavage of VE-Cadherin with its role as a regulator of vascular permeability and leukocyte TEM, additional investigations are needed here.

Numerous studies have investigated enzymatic cleavage of members of the JAM family. Specifically, JAM-A can be cleaved by ADAM17 and, to a lesser extent, by ADAM10 post stimulation of ECs by certain inflammatory stimuli.⁴⁷ Functionally, soluble JAM-A blocked migration of cultured ECs and reduced neutrophil TEM *in vitro* and decreased neutrophil infiltration in a murine air pouch model *in vivo*.⁴⁷ Hence, generation of soluble JAM-A, as mediated through ADAM17/10, may regulate JAM-A-mediated functions through destabilization of JAM-A homophilic interactions at sites of inflammation. More recently, Sevenich et al⁴⁸ proposed that cathepsin S-mediated cleavage of endothelial JAM-B promotes transmigration of metastatic cells across brain microvascular ECs. In addition, genetic or pharmacological targeting of cathepsin B impaired brain metastasis in a model of breast cancer, suggesting that proteolytic processing of JAM-B at the blood–brain barrier can modulate site-specific metastasis,⁴⁸ although the cleavage of endothelial JAM-B at specific sites of tumor cell TEM was not addressed. Findings from our laboratory have demonstrated that NE can cleave JAM-C.⁴¹ In line with our previous works showing that loss of EC JAM-C can promote neutrophil rTEM,⁹ NE cleavage of EC JAM-C promoted neutrophil rTEM.⁴¹ Under conditions of ischemia–reperfusion injury, this response was driven by endogenously generated LTB₄, and exogenous LTB₄ was highly efficacious at causing specific loss of venular JAM-C without affecting the expression of other EC junctional molecules.⁴¹ The impact of LTB₄-NE axis on JAM-C cleavage was totally neutrophil-dependent, with NE governing the cleavage of EC JAM-C at sites of intense neutrophil infiltration.⁴¹ Collectively, our findings demonstrated that NE is presented to EC JAM-C via activated neutrophil Mac-1, and because the latter is a ligand for JAM-C,⁷⁰ Mac-1 seems to act as a molecular bridge between NE and JAM-C (Figure). Finally, because the activation of local LTB₄-NE axis could drive remote organ damage, the findings of this study provided additional evidence for the involvement of neutrophil rTEM in propagation of a local sterile inflammatory response toward a systemic multiorgan phenomena.⁴¹

After enzymatic cleavage, junctional molecule ectodomains can be shed into the bloodstream. Several such soluble forms have been quantified in plasma of patients with inflammatory conditions, including trauma, atherosclerosis, and rheumatoid arthritis, with the levels commonly correlating with the severity of the disease.^{41,71–74} In our studies, elevated concentrations of sJAM-C were detected in plasma of trauma patients as compared with healthy controls, a parameter that further increased in patients who developed acute respiratory distress syndrome post admission.⁴¹ Because increased plasma content of sJAM-C is associated with trauma-induced organ failure⁴¹ and is also elevated in serum or synovial fluid from rheumatoid arthritis, psoriatic arthritis, osteoarthritis, and systemic sclerosis patients,^{49,75} sJAM-C may be a useful vascular-derived biomarker for assessing the extent of a systemic inflammatory response.

Apart from their potential role as biomarkers, relatively little is known about the biological consequences of released soluble ectodomains in pathophysiological scenarios, and

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there is evidence for both pro- and anti-inflammatory roles. Of note, it has been reported that generation of sVE-Cadherin contributes to inflammation-induced breakdown of endothelial barrier function through inhibition of VE-Cadherin binding⁷⁶ and as such promotes leukocyte TEM via increased vascular permeability.⁴⁵ In contrast, exogenous administration of soluble forms of PECAM-1, JAM-A, and JAM-C suppresses leukocyte transmigration in several rodent models of inflammation.^{35,40,47,54} The mechanism through which these pharmacological interventions act is at present unclear but is likely because of competitive binding of the soluble molecules with their ligands on either ECs or circulating leukocytes.

Impact of Acute Versus Chronic Inflammatory Insults on EC Junctional Molecule Expression and Function

The mechanisms associated with altered expression of EC JAMs (discussed earlier) may differ between acute and chronic inflammatory scenarios. Numerous *in vitro* and *in vivo* studies have investigated the impact of short-term acute inflammatory insults on expression of EC regulatory molecules. In such scenarios, the expression of molecules on the apical side of ECs that facilitate leukocyte adhesion to the endothelium, such as E-selectin, P-selectin, ICAM-1, and VCAM-1, is generally elevated. This supports increased luminal leukocyte–EC interactions (eg, rolling, crawling, and firm adhesion) and overall capture of leukocytes from the blood stream.^{7,77} Conversely, the expression of EC adhesion molecules at cell–cell junctions is commonly reduced under such conditions (Tables 1 and 2), potentially leading to decreased barrier properties of EC junctions. One manner in which this occurs is redistribution of adhesion molecules away from the junctions to the luminal side of the venule, a response that may provide a means through which leukocytes are guided to EC junctions in a haptotactic manner. Although the highly regulated changes in junctional molecule expression are necessary for the appropriate development of the acute inflammatory response, and may indeed play a role in its outcome, evidence suggests that they can also have pathogenic implications, as discussed earlier for JAM-C and its role in modulating rTEM and second organ damage.

As opposed to acute inflammatory responses, chronic inflammatory states allow time for further molecular pathways to become activated, such as upregulation of junctional protein expression at transcriptional and translational level.^{33,35,36,39} This can result in increased junctional protein expression levels, compared with resting states, as noted, for example, in the context of EC JAM-C in atherosclerosis,³³ rheumatoid arthritis, and osteoarthritis³⁹ and EC JAM-A in atherosclerotic vessels.^{35–37} In line with this, increased concentrations of soluble junctional molecules have been found in plasma from several chronic inflammatory pathologies, such as stroke-induced ischemia,⁷³ rheumatoid arthritis,^{49,72} atherosclerosis, and hypertension.^{71,78} Enhanced expression of EC junctional molecules may account for increased or prolonged recruitment of leukocytes and their retention during chronic inflammation.

Conclusion

The EC barrier allows regulated and selective passage of appropriate solutes and immune cells during resting and inflammatory conditions. This vital function is mediated by interactions between ECs through junctional molecules, such as VE-Cadherin, JAMs, and PECAM-1. Remodeling of the EC membrane during inflammation includes reorganization of junctional molecules, a response that is pivotal for regulation of vascular permeability and leukocyte extravasation. Changes in expression levels of junctional molecules can also be temporally and spatially regulated by inflammatory mediators and leukocyte TEM. These mechanisms include cell surface redistribution and internalization of key cell border structures, the recycling of intracellular pools of molecules, and their enzymatic cleavage. Such changes may also have a role in orchestrating the inflammatory response under chronic conditions and affecting its resolution. Collectively, better understanding of the molecular mechanisms that mediate the spatiotemporal expression and trafficking of EC junctional molecules could identify novel means of targeting both acute and chronic inflammatory pathologies.

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Disclosures

None.

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