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

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Abstract

Endothelial cells (ECs) 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 ECs is facilitated by interactions involving junctionally expressed transmembrane proteins including tight junctional molecules, such as members of the junctional adhesion molecule (JAM) family, components of adherence junctions, such as VE-Cadherin, and other molecules such as platelet endothelial cell adhesion molecule (PECAM). 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 summarises key aspects of our current understanding of the dynamics and mechanisms that regulate the expression of EC junctional molecules during inflammation, and discusses the associated functional implications of such events in acute and chronic scenarios.

Introduction

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 (VVOs), and paracellular mechanisms; involving breaching of tightly connected junctions between adjacent ECs1–3. 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

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role in supporting an effective inflammatory response. This occurs through increased vascular permeability to macromolecules, facilitating leakage of essential blood born immunoregulatory and pro-inflammatory proteins to the extravascular tissue (eg immunoglobulins and components of the complement cascade), and also enabling breaching of venular walls by transmigrating immune cells1–3. Due to their essential role in such biological functions, there is immense interest in the signalling properties of EC junctional molecules under both physiological and pathological conditions1–3. 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 summarises 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 partners1–4. Two key junctional structures are tight junctions that incorporate members of the junctional adhesion molecule (JAM) family, endothelial cell-selective adhesion molecule (ESAM) and claudins, and adherens junctions that include VE-Cadherin1–5. Numerous other adhesion molecules are also present at EC contacts, such as PECAM-1, CD99, CD47, activated leukocyte cell adhesion molecule-1 (ALCAM-1) and ICAM-2, molecules that contribute to junction formation and properties1–6 (Figure 1). 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 1). Recent developments strongly support the concept that intracellular stores of EC junctional molecules contribute to maintaining the integrity and function of the endothelium (discussed below) 4.

The two 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 infectious insults. Specifically, at sites of inflammation leukocytes exhibit a number of 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 walls2, 6, 7. Migration through the endothelial cell barrier (transendothelial cell migration; TEM) can occur via both transcellular and paracellular modes. Whilst significant use of the transcellular route has been reported across the blood-brain and blood-retinal barriers during inflammatory pathologies8, paracellular diapedesis appears to be the most prevalent mode of breaching ECs both *in vitro* and *in vivo* (~70-90%)3, 6. This was directly demonstrated *in vivo* through the application of high resolution 3D intravital imaging of inflamed mouse cremaster muscle venules where paracellular TEM was found to account for ~90% of all observed neutrophil TEM events as

induced by multiple inflammatory stimuli9. Furthermore, a mouse strain in which the EC junctions were stabilised 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 trafficking10. Paracellular leukocyte TEM is mediated by an elaborate series of interactions between leukocytes and EC junctional adhesion molecules including PECAM-1, JAMs, CD47, ALCAM-1, ESAM, ICAM-2 and CD99 (for reviews on this topic see references2–4, 6, 11) and there exists some evidence to suggest that EC junctional molecules can also support leukocyte transcellular TEM4, 6. In addition, there is strong evidence to indicate distinct and/or sequential roles for these molecules in different stages of leukocyte movement through venular walls2, 4, 6. However care needs to be exercised in formulating general concepts as roles of different molecules in different stages of leukocyte transmigration appear 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 sub-type 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 interactions12.

Vascular permeability to plasma proteins, and the subsequent formation of tissue oedema, is another important protective physiological reaction to tissue injury. Whilst it is well-established that neutrophils can mediate vascular permeability13, and numerous neutrophil-derived factors have been implicated in this response13, 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 TEM1,15, 16. Specifically, the works of Vestweber and colleagues has 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 junctions16. Importantly, this study showed that the phosphorylation status of two distinct tyrosine residues of VE-Cadherin can selectively and exclusively regulate either vascular permeability or leukocyte diapedesis16.

Other junctional molecules involved in regulation of vascular permeability include JAM-A and JAM-C. Despite the high percentage of homology between the two proteins, these molecules appear to have opposing roles in regulating barrier function of ECs. For example, whilst genetic deletion and/or blockade of JAM-A generally results in increased EC permeability19, knocking down JAM-C decreases EC permeability *in vitro*20. Of note, as opposed to cultured macrovascular ECs (eg HUVECs) that constitutively express JAM-C at cell-cell contacts, quiescent microvascular ECs in culture express JAM-C predominantly within intracellular stores that can be mobilised to junctions following cellular stimulation. The latter appears to provide a mechanism through which endothelial cell permeability is regulated20. 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 VEGF20, specific antibody blockade of endothelial JAM-C leads to increased vascular leakage in a model of cutaneous infection by Leishmania major21. Of note, genetic inactivation of the related tight junction-associated protein ESAM, attenuates the induction of vascular permeability and delays leukocyte diapedesis *in vivo*22.

Collectively, as 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 remodelling 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

A number of studies have reported on altered expression of EC junctional molecules *in vitro* and *in vivo*, with some studies associating such changes with altered functional read-outs1, 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 below (see also Tables 1 and 2).

Internalisation and redistribution

Internalisation and/or redistribution of molecules to intracellular compartments are commonly reported means through which the expression 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, under-go some form of intracellular recycling or degradation3, 4. In addition, many others such as JAM-C, JAM-A and CD99 are also present in intracellular stores and are therefore susceptible to mobilisation to and from EC junctions post stimulation (Figure 1)4. In this context, ECs show a unique array of intracellular compartments that are associated with junctional molecule internalisation, storage and re-cycling. These include the membrane invagination structure termed LBRC (commonly found in close proximity of EC junctional lateral borders), components of the vesicular system, VVO's and caveolae, and endosomes which 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 re-cycling both at rest and also during acute inflammation, as discussed below.

The LBRC represents an internalisation organelle, apparently unique to ECs and containing key junctional molecules including PECAM-1, JAM-A, CD99 and the poliovirus receptor (PVR; CD155), but not VE-Cadherin, which is actively excluded from this domain 4, 23, 24. 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 demand4. In support of this concept, changes in the intracellular pool of PECAM-1 have

been located to sites where the LBRC is re-directed towards the cell border to support paracellular migration25, or towards the cell body during transcellular migration26. Whilst there is now ample evidence to support a role for LBRC in regulation of leukocyte TEM, there remain many un-answered question regarding the trafficking of this structure, an area that has been developed in recent studies by Muller and colleagues4. For example, vesicles of the LBRC compartment are moved by kinesin molecular motors along microtubules 4, 23 25. Furthermore, whilst it has been known for more than 20 years that a transient increase in EC cytosolic free calcium is required for TEM, Weber et al have recently demonstrated that the Ca^{2+} channel, transient receptor potential canonical 6 (TRPC6), mediates this response and have linked this reaction with LBRC trafficking27. Specifically, homophilic interaction of leukocyte and EC PECAM triggered the activation of TRPC6 and promoted its co-localization with PECAM-1 during TEM and trafficking of the LBRC. Of note, selective inactivation of EC TRPC6 blocked neutrophil TEM whilst its activation rescued TEM during conditions of PECAM blockade, suggesting that TRPC6 acts down-stream 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 inflammation27. Down-stream 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 cyclase28. 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 towards and away from EC junctions4.

Changes in VE-Cadherin junctional expression can also have profound impact on EC barrier function5. VE-Cadherin displays constitutive endosomal internalisation from the EC surface via a clathrin-dependent pathway29 and can then be lysosomally degraded30 or recycled back to the EC surface through Rab11a mediated-trafficking in order to recover EC barrier properties after junctional challenge31. 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 degradation29, 30. In addition, more recent works identified a specific motif of VE-Cadherin intracellular tail that is required for p120 binding and VE-Cadherin internalisation. Mutations in this region strongly affect EC migration, although the impact of such effects on EC barrier function remains to be elucidated32. On the other hand, changes in VE-Cadherin phosphorylation status destabilise EC contacts, a response that supports vascular leakage and leukocyte migration3, 5, 16, 33, 34. VE-Cadherin phosphorylation can be regulated by p12033 and the kinases Src and Pyk2 in response to ICAM-1 ligation34. This is necessary for leukocyte TEM but does not imply internalisation of the molecule and so will not be discussed further. Phosphorylation of residues Y658 and Y685 have however been shown to be responsible for internalisation and ubiquitinisation of the protein following bradykinin and histamine-induced permeability35. This phenomenon occurs specifically in veins and not arteries, possibly due to the shear-stress dependent activation of junctional Src in the former, and regulates loosening of venular EC junctions and leakage35. More recently, heterotrimeric G protein Gα13 binding to VE-Cadherin has been shown to regulate Src-mediated phosphorylation of VE-Cadherin

and its internalisation, identifying a unique role for Gα13 in mediating EC barrier disruption *in vivo* in response to vascular permeability factors such as histamine and bradykinin36. Internalisation of the molecule can also be regulated by phosphorylation of S665 through the VEGFR-2–Src–Vav2–Rac–PAK signalling axis following VEGF stimulation37. This promotes VE-Cadherin association with β-arrestin2 and its internalisation 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” since it rapidly reseals behind emigrating cells38, 39. In addition, VE-Cadherin can be internalized in response to leukocyte-EC interactions16. 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-216. Although Y731 dephosphorylation appeared 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 implications16. Under resting conditions VE-Cadherin binds to VE-PTP, a phosphatase that maintains VE-Cadherin and its associated catenins in a non-phosphorylated state critical for optimal adhesive functions of VE-Cadherin and EC contact integrity. Following 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 junctions18. 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-cadherin40. 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 internalisation and enhanced cell surface turnover of the molecule41. This interaction mediates transduction of signals impinging on the regulation of the transcriptional cofactor Yes-associated protein (YAP) and as a result modulates vascular permeability41.

Similar to internalisation, 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 1 and Table 1). For example, expressions of PECAM-1 and JAM-A are reduced from junctions of HUVECs treated with the cytokine combination IFNγ and TNF with no apparent reduction in total cellular protein levels42, 43. 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 neutrophils44. 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 pro-atherogenic oxidized lipoproteins *in vitro*44, 45 and *in vivo* in murine models of atherosclerosis in regions of disturbed flow46. The latter response was associated with increased monocyte recruitment into the arterial wall and enhanced atherosclerotic lesion formation46.

A significant body of work has investigated the regulation of expression of JAM-C and its functional implications. *In vitro*, HUVECs treated with oxidised LDL, but not TNF, IL1β, VEGF or histamine, showed redistribution of JAM-C from junctions to the cell surface20, 47. This redistribution resulted in the ability of JAM-C to mediate both leukocyte adhesion and TEM as compared to JAM-C on unstimulated ECs that only supported leukocyte diapedesis20, 47. As previously mentioned, in quiescent microvascular ECs JAM-C is mainly intracellularly expressed and is recruited to junctions following 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 manner20. In contrast to *in vitro* studies, *in vivo* JAM-C is expressed at EC junctions, as indicated through analysis of numerous murine tissues48. However in line with *in vitro* works, there is evidence for the presence of JAM-C in intracellular vesicles within microvascular ECs *in vivo*48. This intracellular store of JAM-C appeared to be available for mobilisation under inflammatory conditions in that re-distribution of JAM-C from intracellular vesicles and EC junctions to EC non-junctional plasma membrane regions was noted in a murine model of ischemia-reperfusion injury. The *in vivo* redistribution of EC JAM-C was associated with enhanced luminal neutrophil-venular wall interactions9, 48. 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 polarised migration of leukocytes through endothelial cell monolayers9, 49. Specifically, Bradfield and colleagues showed that inhibition of EC JAM-C can lead to enhanced monocyte reverse TEM (rTEM) through TNF-stimulated HUVECs, ie increased frequency of monocyte movement in an abluminal-to-luminal direction49. *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 following induction of inflammatory reactions, such as ischemia-reperfusion injury, that cause reduced expression of junctional EC JAM-C9, 50. The underlying mechanism through which EC JAM-C supports luminal-to-abluminal TEM is at present unclear but

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maybe related to the role of JAM-C in maintaining EC polarity51. Although the pathophysiological relevance of neutrophil rTEM requires further investigations, our current data suggests that rTEM neutrophils stemming from a primary site of injury may contribute to dissemination of systemic inflammation and second organ damage9 50.

Overall it is becoming increasingly clear that altered surface localization and/or 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

A number of EC junctional molecules have been reported to be enzymatically cleaved by leukocyte and/or EC derived proteases. Although such events have been implicated to several vascular responses, including permeability, immune cell recruitment, vascular repair and angiogenesis (Table 2)52, 53, 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 read-out(s) as 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 analysed. 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, three 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 apoptosis54 and VE-Cadherin is reportedly cleaved by ADAM1055, neutrophil elastase (NE) and Cathepsin G56. 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 stimuli57. 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*57. Hence, generation of soluble JAM-A, as mediated through ADAM17/10, may regulate JAM-A-mediated functions through destabilisation of JAM-A homophilic interactions at sites of inflammation. More recently, Sevenich and colleagues proposed that Cathepsin S-mediated cleavage of endothelial JAM-B promotes transmigration of metastatic cells across brain microvascular ECs58. In addition, genetic or pharmacological targeting of Cathepsin B impaired brain metastasis in a model of breast cancer, suggesting proteolytic processing of JAM-B at the blood-brain barrier can modulate site-specific metastasis58, although the cleavage of endothelial JAM-B at specific sites of tumour cell TEM was not addressed. Findings from our laboratory have demonstrated that NE can cleave JAM-C50. In line with our previous works showing that loss of EC JAM-C can promote neutrophil reverse TEM (rTEM)9, NE cleavage of EC JAM-C promoted neutrophil rTEM50. Under conditions of ischemia-reperfusion injury, this response was driven by endogenously generated LTB_4 and exogenous LTB_4 was highly

efficacious at causing specific loss of venular JAM-C without affecting the expression of other EC junctional molecules50. The impact of LTB_4-NE axis on JAM-C cleavage was totally neutrophil dependent, with NE governing the cleavage of EC JAM-C at sites of intense neutrophil infiltration50. Collectively, our findings demonstrated that NE is presented to EC JAM-C via activated neutrophil Mac-1, and since the latter is a ligand for JAM-C59, Mac-1 appears to act as a molecular “bridge” between NE and JAM-C (Figure 1). Finally, since the activation of local LTB_4-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 towards a systemic multi-organ phenomena50.

Following enzymatic cleavage, junctional molecule ectodomains can be shed into the bloodstream. A number of 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 disease50,60–63. In our studies elevated concentrations of sJAM-C were detected in plasma of trauma patients as compared to healthy controls, a parameter that further increased in patients that developed acute respiratory distress syndrome (ARDS) post-admission50. As increased plasma content of sJAM-C is associated with trauma-induced organ failure 50, and is also elevated in serum or synovial fluid from rheumatoid arthritis, psoriatic arthritis, osteoarthritis and systemic sclerosis patients64, 65, 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 patho-physiological scenarios, and 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 binding66 and as such promotes leukocyte TEM via increased vascular permeability55. In contrast, exogenous administration of soluble forms of PECAM-1, JAM-A and JAM-C suppress leukocyte transmigration in several rodent models of inflammation27, 48, 57, 67. The mechanism through which these pharmacological interventions act is at present unclear but is likely due to competitive binding of the soluble molecules with their ligands on either ECs or circulating leukocytes.

Impact of acute vs chronic inflammatory insults on EC junctional molecule expression and function

The mechanisms associated with altered expression of EC junctional adhesion molecules (discussed above) 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/or firm adhesion) and overall capture of leukocytes from the blood stream7,68. 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 re-distribution 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 above 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 up-regulation of junctional protein expression at transcriptional and translational level47, 67, 69, 70. This can result in increased junctional protein expression levels, compared to resting states, as noted for example in the context of EC JAM-C in atherosclerosis47, rheumatoid arthritis and osteoarthritis70 and EC JAM-A in atherosclerotic vessels46, 67, 69. In line with this, increased concentrations of soluble junctional molecules have been found in plasma from a number of chronic inflammatory pathologies such as stroke-induced ischemia62, rheumatoid arthritis61, 65, atherosclerosis and hypertension60, 71. Enhanced expression of EC junctional molecules may account for increased or prolonged recruitment of leukocytes and/or their retention during chronic inflammation.

Conclusion

The endothelial cell 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. Remodelling of the EC membrane during inflammation includes reorganisation 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 internalisation 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 impacting 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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Abbreviations

LBRC	Lateral Border Recycling Compartment
ER	endoplasmic Reticulum
NE	Neutrophil Elastase
HUVECs	Human Umbilical Vein Endothelial Cells
BAECs	Bovine Aortic Endothelial Cells
HBMEC	Human Brain Microvascular Endothelial Cells
EOMA	Mouse Hemangioendothelioma Endothelial Cells
bFGF	Basic Fibroblast Growth Factor
OxLDL	Oxidised Low Density Lipoprotein
i.v.	intravenously
mAb	monoclonal antibody

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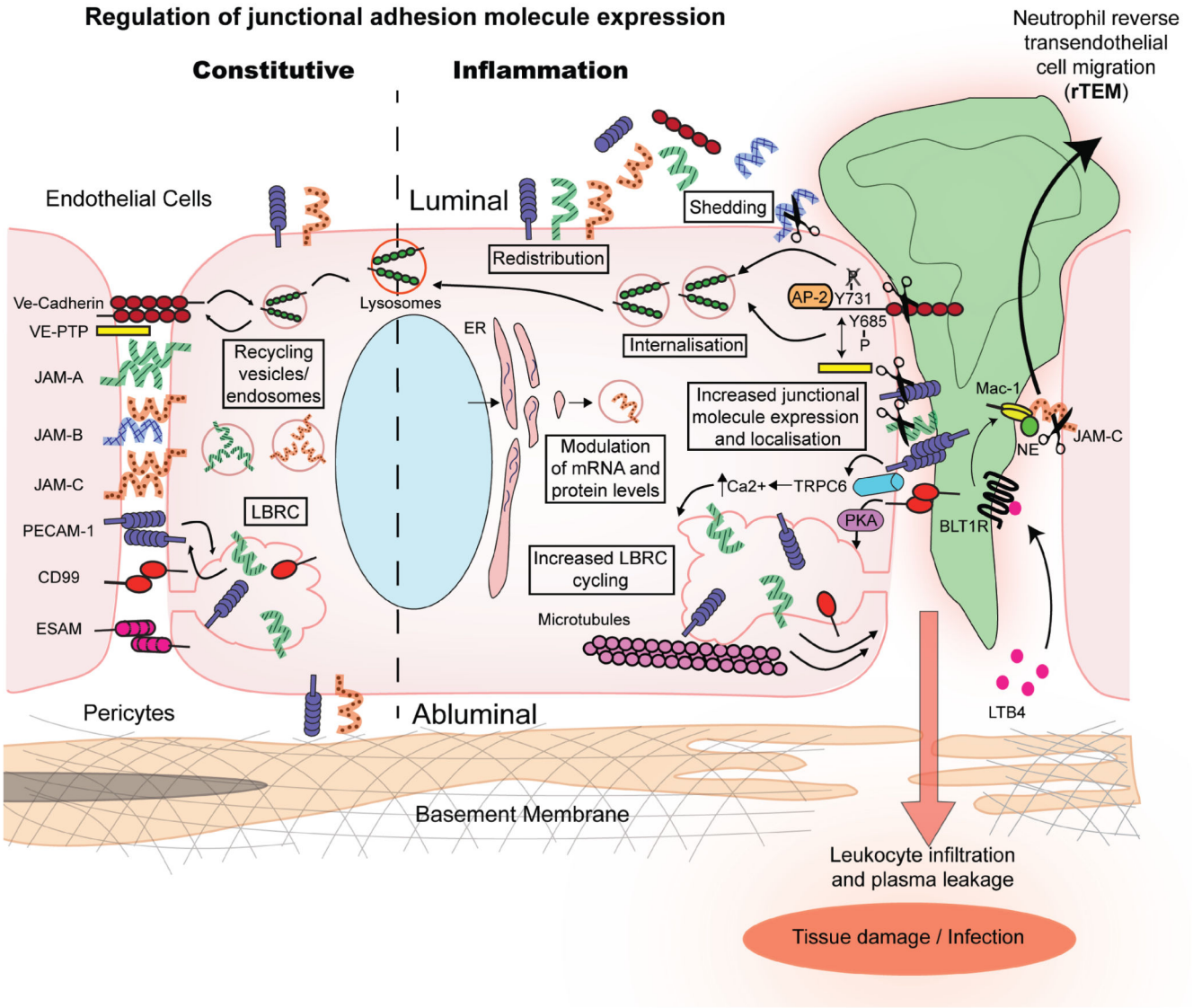

Figure 1. Mechanisms associated with regulation of expression of key endothelial cell junctional adhesion molecules.

Constitutive junctional adhesion molecule regulation involves protein internalisation in endosomes and vesicular structures, their recycling back to the cell surface and/or degradation (e.g. 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 up-regulation 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 non-junctional membranes, whilst internalisation 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 TEM. For example with respect to the latter, NE cleavage of JAM-C is known to promote neutrophil rTEM.

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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	Refs
In vitro					
PECAM-1	TNF+IFNγ (HUVECs)	- ↓ mRNA - ↓ total and junctional expression	- mRNA de-stabilisation - Protein internalisation & degradation - Redistribution to the cell surface	- ↓ leukocyte TEM - No effect on leukocyte TEM	43, 72–74
VE-Cadherin	TNF (HUVECs/Primary lung EC)	- ↓ junctional expression - Gap formation "curtain effect"	- Tyrosine phosphorylation	- ↑ permeability - Facilitate neutrophil TEM	16, 33, 38, 39
	VEGF	- ↑ internalisation	- Endocytosis through a VEGFR-2-Src-Vav2-Rac-PAK signalling axis	- ↑ permeability	37
	TNF (Primary lung EC)	- ↓ junctional expression	- Leukocyte induced SHP2 mediated Y731 de-phosphorylation and endocytosis through AP-2	- ↑ leukocyte TEM	16
JAM-A	bFGF	- Redistribution to cell membrane	- Dissociation from integrin $\alpha_v\beta_3$	- ↑ angiogenesis	75
	OxLDL (Human and mouse aortic ECs)	- Redistribution to apical membrane	- statin dependent	- ↑ monocyte adhesion and TEM	45
JAM-C	OxLDL	- ↑ total protein expression - ↑ expression at non junctional sites	- None described	- ↑monocyte adhesion & TEM	47
	Thrombin	- Re-localisation to junctions	- None described	- Binding and modulation of β_3 integrin activity - ↑ permeability	76
	VEGF, Histamine	- Re-localisation to junctions	- None described	- Regulates actomyosin contractility - Stabilisation of VE-Cadherin - ↑ permeability	20
In vivo					
JAM-A	Murine model of atherosclerosis	- ↑ mRNA and protein in arterial ECs & atherosclerotic plaques - luminal redistribution	- Disturbed flow and upregulation of miR-145	-↑monocyte recruitment into the arterial wall	67, 69,46
	Murine model of hepatic I-R injury	- Protein upregulation	- None described	- Supports neutrophil TEM	77
JAM-C	Murine model of atherosclerosis	- ↑ protein in atherosclerotic plaques	- None described	- None described	47
	Human rheumatoid arthritis & osteoarthritis	- ↑ protein in synovial tissue	- None described	- None described	70
	Murine cremaster muscle I-R injury	- ↓ junctional expression-	- ROS-mediated loss of JAM-C	- Polarised neutrophil TEM	9, 48, 50

Molecule	Inflammatory stimuli/model	Change in expression	Proposed mechanism	Functional implications	Refs
		Redistribution to non junctional membrane		- Regulation of systemic inflammation	
	Mice injected *i.v.* with anti-JAM-C mAb	- Redistribution to non-junctional membrane	- Loss of JAM-C/JAM-B interaction	- ↑ monocyte adhesion to lymph nodes	78

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Table 2

Cleavage of EC junctional molecules during inflammation

Molecule	Model	Mechanism	Functional implications	Refs
PECAM-1	Serum starvation (HUVECs, EOMA, BAECs)	Cleavage by caspases & shedding by MMPs	- None described	54
VE-Cadherin	fMLP stimulated neutrophils (HUVECs)	Cleavage by neutrophil elastase & Cathepsin G	- ↑ permeability - Support neutrophil TEM	56
	Thrombin stimulation (HUVECs)	Cleavage by ADAM-10	- ↑ permeability - Support T-cell migration	55
	TNF stimulation (HUVECs)	Cleavage dependent on tyrosine kinases, Src kinase & MMPs	- None described	30
JAM-A	PMA, TNFα+ IFNγ, PAF (HUVECs)	Cleavage by ADAM-10, ADAM-17	- ↓ EC migration - ↓ neutrophil TEM	57
JAM-B	HBMEC incubation with tumour-cell secreted supernatant	Cleavage by Cathepsin S	- ↑ TEM of brain metastatic tumour cells	58
JAM-C	LPS, IL1-β, IL-18, MIF, IL-17, TNF, PMA (HUVECs)	Cleavage by ADAM-10, ADAM-17	- Supports angiogenesis	65
	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	50