Mechanisms of Transendothelial Migration of Leukocytes

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Abstract—A great deal of progress has been made recently in understanding the molecules and mechanisms that regulate transendothelial migration of leukocytes, or diapedesis, a critical step in the inflammatory response. This review focuses mainly on the active role of the endothelial cell in this process as it occurs at endothelial cell borders. It discusses some of the many molecules that have been reported to play a role in transendothelial migration and asks why so many molecules seem to be involved. The concept is emerging that diapedesis itself can be dissected into sequential steps controlled by specific molecule(s) at the endothelial cell border. Several mechanisms have been shown to play a critical role in transendothelial migration including signals derived from clustering of apically disposed intercellular adhesion molecule-1 and vascular cell adhesion molecule-1, disruption or loosening of adherens junctions, and targeted recycling of platelet/endothelial cell adhesion molecule and other molecules from the recently described lateral border recycling compartment. A hypothesis that integrates the various known mechanisms is proposed. (Circ Res. 2009;105:223-230.)

Key Words: transendothelial migration ■ inflammation ■ leukocyte ■ endothelial cell ■ transmigration

The inflammatory response is the stereotyped reaction of the body to tissue damage of any kind. It involves rapidly and transiently delivering preformed soluble elements in the blood to the site of injury followed by a more prolonged delivery of leukocytes. Because leukocytes cannot swim, they are recruited locally at the site of inflammation in a series of adhesive steps that allow them to attach to the vessel wall, locomote along the wall to the endothelial borders, traverse the endothelium and the subendothelial basement membrane, and migrate through the interstitial tissue.1,2 Transendothelial migration (TEM) or diapedesis is arguably the point of no return in the inflammatory response. The preceding steps of leukocyte rolling, activation, adhesion, and locomotion are all reversible, and most leukocytes that initiate contact with the postcapillary venule at the site of inflammation reenter the circulation. However, once the leukocyte commits to diapedesis, it does not go back, at least not as the same cell type.3 Most TEM takes place at endothelial borders. Recently, there has been a flurry of interest in TEM through the endothelial cell body (transcellular migration). Although this clearly can occur in vitro4 and in vivo,5 in the interests of space, this review focuses on transendothelial migration and specifically TEM at cell borders. Unfortunately, because of space limitations, a great many excellent publications on this subject cannot be cited.

Molecules Regulating Transmigration

A number of molecules have been implicated in transmigration because genetic deletion or antibody blockade of these molecules impairs diapedesis. All of these molecules belong to a recognized family of adhesion molecules, are expressed on endothelial cells, and are enriched at cell borders. In addition to adhesive functions, these molecules have signaling functions that contribute to their role in TEM. Intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 are also discussed in this section. Although they are not localized to cell borders or involved in the diapedesis step per se, they seem to be involved in events directly preceding diapedesis and are recruited to the endothelial cell border during transmigration.

ICAM-1 and VCAM-1 are involved in firm adhesion of leukocytes to the apical surface of endothelial cells through interactions with leukocyte CD11a/CD18 and/or CD11b/CD18 and CD49a/CD29, respectively. Once adherent, ICAM-1 becomes enriched under the leukocyte as it migrates to the endothelial cell border and continues to surround it during transmigration.6 The src-dependent phosphorylation of the actin-binding molecule cortactin is required for ICAM-1 clustering.7,8 VCAM-1 clustering has been observed in the steps leading up to diapedesis. Both ICAM-1 and VCAM-1 are concentrated in actin-rich “docking structures” that form before TEM.9,10

ICAM-2, another CD11a/CD18 ligand, is constitutively expressed on endothelial cells, where it is concentrated at cell borders. Antibodies against ICAM-2 do not seem to have a major effect on TEM in vitro. However, in some inflammatory models in vivo, blocking antibodies or genetic deletion of ICAM-2 inhibit transmigration of neutrophils,11,12 Junctional adhesion molecule (JAM)-A and JAM-C are concentrated at endothelial cell borders. Although JAM-A...
normally engages in homophilic adhesion, during inflammation it can bind to CD11a/CD18 on the leukocyte. Blocking JAM-A on human endothelial cells using a polyclonal antibody in vitro has been shown to reduce TEM; however, other investigators using polyclonal or monoclonal antibodies have seen no effect. On the other hand, in vivo studies show decreased inflammation and transendothelial migration when JAM-A is blocked. JAM-C can engage in homophilic adhesion, or heterophilic adhesion with JAM-B or CD11b/CD18. The latter interaction is implicated in transendothelial migration in vitro and in vivo. For an extensive review of the roles of JAM family members in the inflammatory response, the reader is referred to a recent review.

ESAM (endothelial cell–selective adhesion molecule) is molecularly related to the JAMs but has a long cytoplasmic domain. As its name implies, its distribution is mostly limited to endothelial junctions, but it is expressed on activated platelets. It binds homophilically, and a ligand on leukocytes has not been described. ESAM-deficient mice have no defect in lymphocyte extravasation but have a transient decrease in neutrophil emigration (marked decrease at 2 hours that had recovered by 4 hours). Platelet/endothelial cell adhesion molecule-1 (PECAM-1, PECAM, CD31) is an immunoglobulin superfamily member concentrated at the borders of endothelial cells, as well as expressed diffusely on platelets and leukocytes. Homophilic interaction of leukocyte PECAM with endothelial PECAM is required for transendothelial migration. Blockade with monoclonal antibody against the amino-terminal homophilic interaction domain, soluble PECAM-Fc chimeras, and genetic deletion of PECAM inhibit transendothelial migration in vitro and in vivo (reviewed elsewhere). When PECAM is transfected into cells that normally lack it, expression of PECAM imparts on them the ability to support TEM. This gain of function has not been demonstrated with other adhesion molecules. When PECAM–PECAM interactions are blocked, leukocytes are arrested tightly adherent to the apical surface of the cell and actively migrate along the junctions as if searching for a place to transmigrate. In vivo, leukocytes are able to get to the postcapillary venules at the site of inflammation, but are unable to exit efficiently. They are seen apparently adherent to the endothelial cell luminal surface, reminiscent of the block to TEM seen in vitro. This phenotype is seen with human cells and in all mouse strains examined except for C57BL/6. Interestingly, this mouse strain seems to be unique in that genetic deletion of PECAM or administration of blocking antibody or mouse PECAM-Fc to these mice has no effect in a variety of inflammatory models. Even the closely related C57BL/10 strain responds to anti-PECAM therapy. The ability to circumvent the need for PECAM in the thioglycollate peritonitis model of inflammation has been linked to a small locus at the proximal end of chromosome 2. Therefore, earlier studies carried out in C57BL/6 mice that found no role or only a minor role for PECAM in inflammation need to be reevaluated. A detailed discussion of the role of PECAM in various in vivo models is available elsewhere.

There is a role for leukocyte PECAM in traversing the basal lamina. C57BL/6 mice in which PECAM has been knocked out or blocked with antibody are defective in their ability to migrate across this extravascular barrier. CD99 is a relatively unique molecule unrelated to any other molecule in the human genome except the closely related paralog CD99-like 2 (CD99L2), which may have arisen from a common ancestral gene. Similar to PECAM, homophilic interaction between CD99 and the endothelial cell border and CD99 on monocytes and neutrophils is required for transmigration. However, CD99 regulates a later step in transmigration than PECAM. Leukocytes in which CD99 has been blocked can still be prevented from transmigrating if anti-CD99 is added after the anti-PECAM antibody has been washed away. Conversely, when CD99 interaction is first blocked, leukocytes can no longer be inhibited from transmigrating by anti-PECAM antibody when the anti-CD99 block is removed. In support of this, confocal images of leukocytes blocked in the act of transmigration by anti-CD99 show their leading edge under the endothelial cytoplasm, their cell body lodged at the border between endothelial cells, and the trailing uropod on the apical surface. As long as the block continues, they migrate along the junctions over the surface of the endothelium in this manner, unable to finish transmigration. There is indirect evidence that CD99 cannot function unless PECAM acts first. In mice, migration of T lymphocytes into skin and neutrophils and monocytes into the peritoneal cavity are blocked by anti-CD99 antibodies.

CD99L2 expression in mice seems similar to that of CD99. That is, it is expressed on vascular endothelium of all tissues examined and is expressed at the borders of endothelial cells. It is expressed to varying degrees on all circulating blood cells. Only polyclonal antibodies against murine CD99L2 have been tested in vivo. They block neutrophil and monocyte influx in the thioglycollate peritonitis model. It is tempting to speculate that the incomplete blockade of inflammation seen when interfering with either CD99 or CD99L2 is attributable partial redundancy of the function of these molecules.

Vascular endothelial cell cadherin (VE-cadherin, cadherin 5) is the major adhesion molecule of the adherens junction. It negatively regulates transmigration. Antibodies against VE-cadherin enhance early migration into a site of inflammation in vivo. In vitro studies show that VE-cadherin is transiently
removed from the site of transmigration at the cell junction.\textsuperscript{40,41} Mutation of the cytoplasmic tail of VE-cadherin so that it cannot interact with p120 or β-catenin or mutation of the latter prevents clearance of VE-cadherin from the cell border and blocks transmigration.\textsuperscript{42,43}

**Why So Many Molecules?**

Other endothelial molecules that have been shown to play a role in TEM by virtue of the inhibition of TEM by blocking antibodies include poliovirus receptor (CD155),\textsuperscript{44} MUC18 (CD146),\textsuperscript{45} activated leukocyte cell adhesion molecule (CD166),\textsuperscript{46} integrin-associated protein (CD47),\textsuperscript{47} and nepmucin/CLM-9.\textsuperscript{48} It seems that each month brings a new report of an endothelial cell or leukocyte molecule that is implicated in diapedesis. When added to the well-characterized molecules discussed in the previous section, this raises the question of why so many molecules are required for TEM. Is this just an artifact of clogging up the junction with antibody or turning the cell junctions into immune complexes? This is unlikely, because most of these studies used control antibody, Fab or F(ab\textsuperscript{′})\textsubscript{2} fragments, soluble recombinant adhesion molecules, small interfering RNA knockdown, or genetic deletion to buttress their claims.

The process of diapedesis itself can be further dissected into a series of molecularly defined steps controlled by specific molecules acting in sequence. Sequential blocking experiments demonstrated that PECAM regulates a step in diapedesis that is “upstream” of the step regulated by CD99.\textsuperscript{33} Sequential blockade analysis has not been performed with other pairs of molecules, but images of leukocytes blocked by antibodies in vivo in C57BL/6 mice show that ICAM-2 arrests neutrophils on the apical surface of the endothelium, anti-JAM-A arrests them at the cell junctions, and anti-PECAM arrests them between the endothelial cell and basal lamina.\textsuperscript{12} This raises the questions of whether each molecule controls its own defined step in the sequence, whether multiple molecules control each step, and how many steps are there? Until sequential blockade studies can be performed with each of these molecules, this question will remain unanswered. The answer is likely to be different for different leukocyte types, vascular beds, and inflammatory stimuli, as well as the time after the initiation of the stimulus. However, it seems unlikely that there is a separate unique step in diapedesis controlled by each molecule reported to be important for transmigration.

What if most of the endothelial molecules reported to control transmigration were part of a large multimolecular complex, or a series of multimolecular complexes (one for each successive step in diapedesis) that combined to make a platform to support transmigration analogous to the way that multiple transcription factors and coactivators combine to make DNA accessible to transcription? Loss of, or interference with, any one of the molecules in that case could make the complex less efficient at supporting diapedesis and could account for the published results.

**Mechanisms Regulating Transmigration**

**Clustering Surface ICAM-1 and VCAM-1**

The adhesion step immediately upstream of diapedesis is an obvious prerequisite for diapedesis, and there is reason to think that some of the events that occur during this step signal the events that regulate transmigration. Clustering of ICAM-1 and VCAM-1 on the endothelial cell has been observed as the leukocyte approaches the endothelial cell border.\textsuperscript{8,10} The initial leukocyte-facilitated clustering of ICAM-1 requires src-dependent phosphorylation of the actin-binding protein cortactin, which is also associated with actin filament remodeling that takes place during transmigration.\textsuperscript{7} On the other hand, ICAM-1 engagement or clustering induces src-dependent phosphorylation of cortactin.\textsuperscript{49} Rather than being results that are at odds, these observations may belie a self-amplification cycle: the initial recruitment of ICAM-1 and VCAM-1 may be attributable to adhesion to their leukocyte ligands. This clustering induces phosphorylation of cortactin, which leads to the actin polymerization and the recruitment of more ICAM-1 to the site of leukocyte adhesion, which induces more cortactin phosphorylation. Clustering of ICAM-1 and VCAM-1 stimulates signaling in the endothelial cells that promote diapedesis in ways that are discussed later.

Clustering of ICAM-1 and VCAM-1 may occur in 3 dimensions. Fingerlike projections of endothelial apical surface membrane have been reported to surround the lower portion of adherent leukocytes. The membrane is enriched in ICAM-1 and VCAM-1 and overlies cytoplasm enriched in f-actin and actin binding proteins. Sanchez-Madrid and co-workers first described these structures that engaged polyclonally activated lymphocytes and lymphoblasts adherent to cytokine-activated human umbilical vein endothelial cells.\textsuperscript{9} Subsequently, Carman et al\textsuperscript{50} demonstrated similar projections associated with transmigrating neutrophils, monocytes, and lymphocytes, at least under their experimental conditions that involved apical application of a chemokine or leukocyte activator and interaction with activated endothelium. They referred to these structures as “transmigratory cups.”\textsuperscript{51}

Disruption of the cytoskeleton abolished these structures but had no effect on leukocyte adhesion. The authors commented that this might belie a role in transmigration. Interestingly, however, Barreiro et al had found that the docking structures rapidly vanished as lymphocytes began to migrate through the monolayers.\textsuperscript{9} However, not everyone who reports rings of ICAM-1 enrichment around transmigrating leukocytes has seen docking structures or transmigratory cups. For example, Ridley and colleagues, using a similar system (but without application of apical stromal cell–derived factor 1 [CXCL12]) showed distinct ICAM-1 enrichment around transmigrating lymphoblasts but no docking structures.\textsuperscript{52} Luscinskas and colleagues also demonstrated local enrichment of ICAM-1 around transmigrating neutrophils undergoing transmigration\textsuperscript{8} and commented that they did not see such actin-rich microvilli.

What do these docking structures represent and why are they not universally seen? One possibility is that they represent a response of the endothelial cell to leukocytes that
are either highly activated or tightly adherent. The structures were seen under conditions where the leukocytes were adherent but could not transmigrate, allowing time for recruitment of additional ICAM-1 and/or VCAM-1 molecules or under which the leukocytes were additionally activated by the exogenous application of platelet activating factor or chemokines on the apical surface of the endothelial cells. One could easily imagine that under these conditions, enhanced leukocyte integrin activation could result in greater recruitment of counter-receptors from the endothelial surface. In contrast, under conditions where ICAM-1 enrichment was not accompanied by formation of transmigratory cups, the transmigrating neutrophils or lymphoblasts were activated only by interactions with the cytokine-activated endothelium.

**Loosening the Junctions**

Several lines of evidence show that loosening the endothelial cell junctions is important for efficient transmigration. Clustering of ICAM-1 and VCAM-1 on endothelial cells transmits a number of signals into the endothelial cell (reviewed elsewhere), some of which appear to be relevant to diapedesis. Cross-linking VCAM-1 and ICAM-1 on the endothelial cell stimulates an increase in cytosolic free calcium ions, which has long been known to be a requirement for diapedesis. The increase in cytosolic free calcium ion has been shown to activate myosin light chain kinase (MLCK), leading to actin–myosin fiber contraction. This is believed to help endothelial cells separate.

Stimulation of ICAM-1 leads to phosphorylation of VE-cadherin, which is a prerequisite for adherens junction disassembly. In HUVECs, the kinases Src and Pyk2 phosphorylate VE-cadherin on the p120 and β-catenin binding sites tyrosine residues 658 and 731, respectively. This inhibits the binding of p120 and β-catenin to VE-cadherin. Because the interaction of these proteins with VE-cadherin is critical for retaining VE-cadherin at the adherens junction, this destabilizes the junctions.

Cross-linking VCAM-1 also activates Rac1 and stimulates an increase in reactive oxygen species in endothelial cells that leads to loosening of adherens junctions. In other systems Rac1 activation leads to phosphorylation of VE-cadherin on serine 665, which signals its clathrin-dependent internalization. The net result is “loosening” of junctional structures.

Under resting conditions the vascular endothelial protein tyrosine phosphatase associates with VE-cadherin via plakoglobin (γ-catenin), maintaining it in a hypophosphorylated state at the junction. Interaction of leukocytes with cytokine-activated endothelial cells triggers rapid dissociation of vascular endothelial protein tyrosine phosphatase from VE-cadherin, allowing it to be phosphorylated on tyrosine, increasing junctional permeability and facilitating transendothelial migration. A role for another VE-cadherin accessory molecule, p120 catenin, has been demonstrated recently. Overexpression of p120 prevented VE-cadherin phosphorylation and the formation of “gaps” in VE-cadherin staining along the endothelial junction during engagement of leukocytes. (These gaps were not spaces between cells but disruption of the staining pattern of VE-cadherin.) This was associated with a significant decrease in transmigration. Interestingly, the authors did not find evidence for VE-cadherin internalization during gap formation.

In a similar manner, clustering of ICAM-1 activates RhoA, which activates Rho kinase (reviewed elsewhere). This in turn phosphorylates protein phosphatase 1c, the major phosphatase inactivating MLCK. The end result is potentiation of actin-myosin contraction. It is important to point out that, although intercellular gaps that are visible in the light microscope can be produced on endothelial cells cultured on glass coverslips, in vivo the gaps produced between endothelial cells by even the strongest inducers of vascular permeability (eg, histamine and serotonin) are in the order of hundreds of angstroms, and these are resealed by the time most leukocytes are recruited. This does not mean that these gaps are not important, but it means that leukocytes must still crawl through closely adherent endothelial cells; they do not fall into holes between endothelial cells.

**The Lateral Border Recycling Compartment**

Even under steady-state conditions, there is a considerable amount of membrane movement taking place at the endothelial cell borders. Membrane is internalized into and recycled from an interconnected reticulum of tubulo-vesicular structures (the components of which are ∼50 nm in diameter) that resides just beneath the plasma membrane of the endothelial cell borders. This compartment, the lateral border recycling compartment (LBRC), is distinct from caveolae, typical recycling endosomes, and vesiculovacuolar organelles. Approximately 30% of the PECAM of the cell resides in this compartment and recycles with a half-time of ∼10 minutes. This compartment also contains CD99 and JAM-A, but not VE-cadherin (Mamdouh, WA Muller, unpublished data, 2009). In high endothelial venule endothelium, the immunoglobulin superfamily molecule nepmucin (CLM-9), which promotes lymphocyte TEM, is in the LBRC. The purpose of the constitutive recycling is not known. However, when a leukocyte transmigrates, membrane from the LBRC is redirected. It is targeted to the position along the cell junction, where the leukocyte is transmigrating. Membrane from the LBRC is exteriorized along the endothelial cell border at this site, providing an increase in surface area to accommodate the leukocyte as well as a source of PECAM, CD99, and JAM-A to interact with. Blocking PECAM–PECAM interactions between leukocyte and endothelial cell blocks targeted recycling from the LBRC and blocks transmigration. Moreover, there is accumulating evidence that targeted recycling from the LBRC is an essential step in TEM: LBRC membrane is trafficked to the site of transmigration by kinesin molecular motors along microtubules. Disrupting or bundling microtubules, or inhibiting the motor domain of kinesin, blocks targeted recycling and blocks TEM. Activated lymphoblasts can transmigrate in a manner that cannot be blocked by anti-PECAM antibodies. Nevertheless, transmigration of lymphoblasts can be efficiently blocked by disrupting targeted recycling of the LBRC. A tyrosine→phenylalanine mutation on the cytoplasmic tail of PECAM blocks the ability of PECAM to support TEM. It turns out that this mutation interferes with the ability of PECAM to enter and leave the LBRC and...
markedly diminishes its ability to participate in targeted recycling. In confluent endothelial cell monolayers a minority of PECAM is phosphorylated; however, essentially all of the phosphorylated PECAM resides in the LBRC

Targeted recycling of LBRC membrane during TEM potentially solves many of the “problems” inherent in the process. Rather than having to “unzip” high-density homophilic adhesions of VE-cadherin, PECAM, JAM-A, CD99, etc., these molecules (and other structural components of the junction) may be pushed aside by membrane from the LBRC. This then presents unligated molecules with which the leukocyte must interact (e.g., PECAM, JAM-A, CD99, nepmucin) on its path across the endothelial cell while removing or diluting out those it needs to avoid (e.g., VE-cadherin). Hypothetically, once the leukocyte has moved across the junction, the LBRC may be pulled back into the cell, allowing

Figure. A, Clustering of ICAM-1 and VCAM-1 initiate activation of src, RhoA, and Rac-1 and increased cytosolic free calcium ion. B. These signals lead to activation of MLCK, inactivation of phosphatase 1c (PP1c), and phosphorylation of VE-cadherin, inducing release of the associated catenins. C. Signals from the leukocyte activate kinesin molecular motors and stimulate targeted trafficking of LBRC membrane to surround the leukocyte. See the text for a detailed description. CaM indicates calmodulin; circled P, phosphorylated state.
the other components to diffuse back into place, reestablishing the endothelial junction without having to reform all of the complex 3D interactions.

A Unifying Concept of Transmigration?
ICAM-1 and VCAM-1 signaling, cytosolic free calcium flux, RhoA and Rac1 activation, VE-cadherin removal from the junction, MLCK activation, and targeted recycling of the LBRC have all been shown to be necessary for efficient transmigration. How are these diverse phenomena related? Are they sequential links in a chain, or are they events occurring in parallel with all required for TEM to occur? Considering that many second messenger signaling systems interact with each other and feedback loops exist, this may be a question of semantics. However, the following undoubtedly oversimplified scheme seems to be consistent with all of the published data and at least provides a testable hypothesis (see the Figure).

Lymphocyte function associated antigen-1 (CD11a/CD18) preferentially binds to ICAM-1 dimers,66,67 which initiates clustering of ICAM-1. This stimulates phosphorylation of cortactin, enhancing the further actin-induced clustering of ICAM-1. This self-enhancing cycle leads to the enrichment of ICAM-1 around tightly adherent leukocytes. ICAM-1 multimerization leads to increases in cytosolic free calcium and activation of RhoA (Figure, A).

In the meantime, if the leukocytes express VLA-4 (very late antigen 4 [CD49d/CD29]) and the endothelial cells are expressing VCAM-1, clustering of VCAM-1 also stimulates an increase in cytosolic free calcium, activation of Rac-1, and production of reactive oxygen species in endothelial cells.68,69 The latter activates protein kinase Ca2+. The net result is loosening of endothelial cell junctions (Figure, A and B).

ICAM-1 and VCAM-1 signaling additionally result in weakening of the endothelial junctions because of effects on phosphorylation of VE-cadherin. This dissociates VE-cadherin from its links to the actin cytoskeleton and it potentially (but not necessarily) becomes subject to endocytosis in a clathrin-dependent manner (Figure, B).

The increase in cytosolic free calcium activates MLCK to induce tension in the endothelial cells. The activation of MLCK is augmented by the inactivation of protein phosphatase 1c mediated by the RhoA activation stimulated by signals originated through ICAM-1 clustering. The net result of contraction of the endothelial cell body against weakened junctions would be to allow easier passage of leukocytes (Figure, B).

With leukocytes poised over weakened adherens junctions, the other homophilic junctional adhesion molecules still hold the endothelial borders apposed. PECAM–PECAM interactions between leukocyte and endothelial cell19 or other signals83 stimulate targeted trafficking of LBRC membrane to the site of leukocyte transmigration (Figure, C). Targeted recycling of the LBRC may displace structural molecules of adherens junctions and other junctions laterally, allowing transmigration to proceed even if the disruption of these junctions is incomplete. The additional membrane brought by the LBRC provides increased surface area and unligated molecules that the leukocytes want to interact with. The signals that trigger targeted recycling are not known, nor is it clear how the membrane is directed to the site of transmigration. However, weakening of the endothelial cell adherens junctions by brief extracellular calcium chelation leads to diffuse exteriorization of the LBRC along the endothelial cell border (Mamdouth, WA Muller, unpublished data, 2009). It is possible that local weakening of the adherens junctions at the site of leukocyte engagement may allow for localized exteriorization of the LBRC.

It is possible, and even likely, that some of the many junctional molecules discussed earlier are also part of the LBRC or function to recruit it. That is, the LBRC may be one of the hypothetical multimolecular complexes controlling transmigration, and other multimolecular complexes may function to recruit it to the site of TEM and reinternalize it after TEM.

Unanswered Questions
Although much has been learned about the mechanisms that regulate TEM, many important unanswered questions remain. Why are so many endothelial cell molecules implicated in this process? Where is actin–myosin contraction tension exerted in vivo, because endothelial cells in postcapillary venules do not have stress fibers? What directs LBRC-targeted recycling? Finally, how are all of the molecules and mechanisms that have been identified to participate in transendothelial migration coordinated to ensure efficient leukocyte emigration?

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