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INTERACTIVE PROTRUSIVE STRUCTURES DURING LEUKOCYTE ADHESION AND TRANSENDOTHELIAL MIGRATION

Olga Barreiro, Miguel Vicente-Manzanares, Ana Urzainqui, María Yáñez-Mó, and Francisco Sánchez-Madrid

Servicio de Inmunología, Hospital de la Princesa, Universidad Autónoma de Madrid, 28006 Madrid, Spain

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1. ABSTRACT

Leukocyte transendothelial migration during homing and inflammation requires drastic cell morphological changes, involving cytoskeletal-directed clustering of adhesion receptors in specialized protrusive membrane structures in leukocytes and endothelial cells. Extravasation is an active process not only for leukocytes but also for endothelial cells, which promote the rapid and efficient entry of leukocytes to the target tissues, without disturbing the integrity of the endothelial barrier. Herein, we have revised the specialized protrusive structures (microvilli, endothelial docking structures, leukocyte lamellipodia and uropod) involved in the different stages of leukocyte extravasation. The adhesion receptor redistribution, cytoskeletal remodelling and intracellular signaling events that participate in this phenomenon are also discussed.

2. INTRODUCTION

Cell adhesion receptors regulate many cellular processes such as activation, migration, growth, differentiation and death (1, 2), by both signal transduction and the modulation of intracellular signaling cascades triggered by different growth factors (3). Cellular interactions are critical for regulation of hematopoiesis (4, 5) and inflammatory responses (6, 7). The coordinate function of adhesion receptors, cytoskeleton and signaling molecules is crucial for leukocyte extravasation, a central process in immunity. Hence, the correct integration of "outside-in" and "inside-out" signals in leukocytes and endothelium during each stage of extravasation is critical to allow the completion of this phenomenon, the so-called "multi-step paradigm" (6, 8). The present review focuses on the molecular mechanisms and the specialized protrusive structures that govern this dynamic process in both leukocytes and endothelial cells.

3. INITIAL INTERACTIONS BETWEEN CIRCULATING LEUKOCYTES AND THE ENDOTHELIUM: TETHERING AND ROLLING

Free-flowing leukocytes contact with and adhere to the vascular wall under shear forces to initiate an inflammatory response or to migrate into a secondary lymphoid organ (homing). Leukocyte tethering and rolling on activated endothelial cells are the first steps of the sequential process of extravasation, followed by the firm adhesion and transendothelial migration of leukocytes (9).

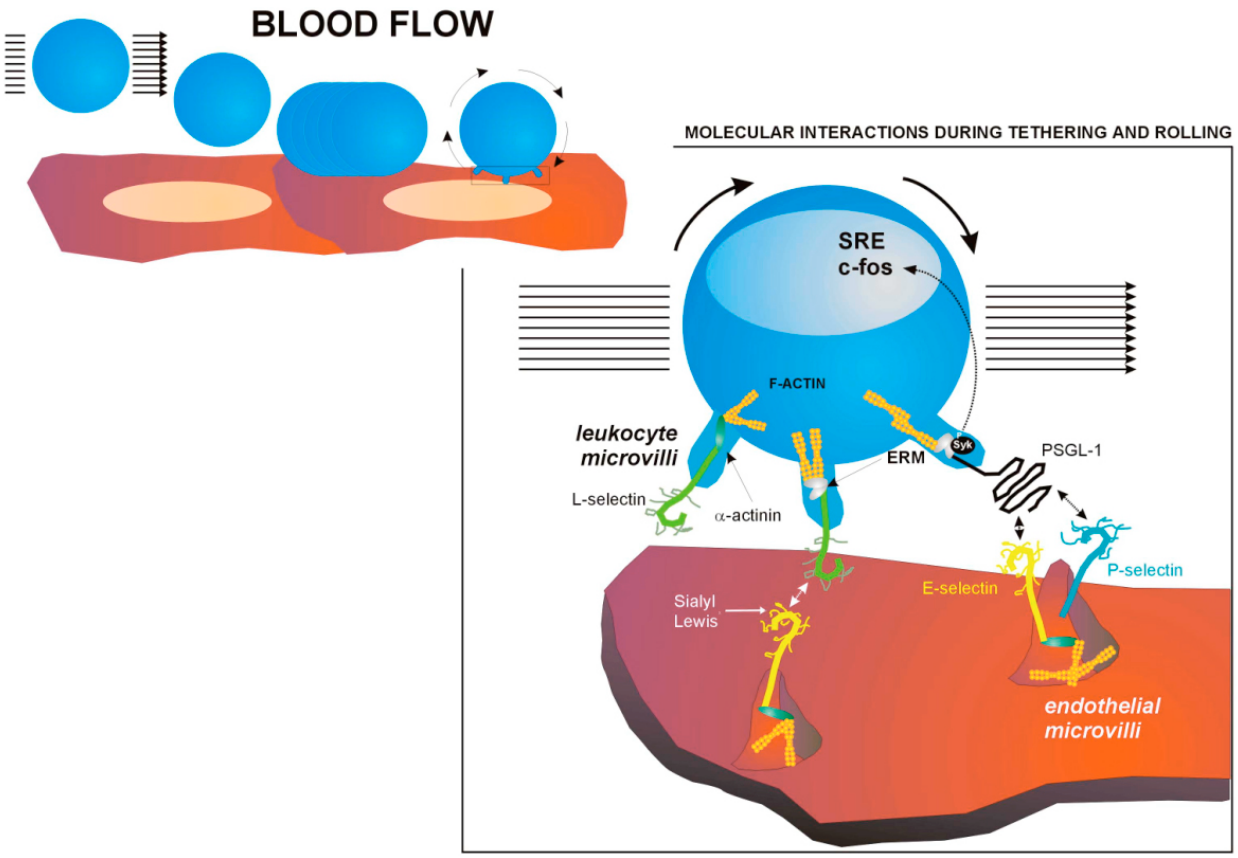

Figure 1. The first step of the extravasation process. As shown in the diagram, free-flowing leukocytes establish transient contacts with activated endothelial cells (tethering), being slowed down. These initial contacts allow leukocytes to roll on the endothelial wall to become activated and finally arrest. The main molecules that participate during this process are presented in detail in the inset: E- and P-selectin in endothelium as well as L-selectin and PSGL-1 in leukocytes are localized at specialized protrusions (microvilli) supported by the actin cytoskeleton, which is linked to the adhesion receptors via ERM proteins, alpha-actinin and other cytoskeletal components. All these molecules interact with their corresponding receptors through carbohydrate residues such as Sialyl Lewis^x. Thus, L-selectin binds to several endothelial counterreceptors (E-selectin among them) and leukocyte PSGL-1 interacts with endothelial E- and P-selectin. In addition, The binding of PSGL-1 with its ligands allows the recruitment of the tyrosine kinase Syk via ERM proteins, which triggers signaling cascades that culminate in the activation of expression of several genes (e.g., SRE and c-fos) in leukocytes.

These initial contacts are largely mediated by selectins and their ligands. All the issues addressed in this chapter are schematically depicted in Figure 1.

3.1. Selectins and their ligands as critical mediators of tethering and rolling

Selectins (P-, E- and L-selectin) are cell adhesion molecules that predominantly mediate the initial interactions of leukocytes with endothelium. They are type I transmembrane glycoproteins that bind to sialylated carbohydrate moieties present on ligand molecules in a calcium-dependent manner. Selectins and their ligands interact with variable affinity, and due to their rapid association and dissociation rates mediate transient contacts between leukocytes and endothelium ("tethering") (10, 11). Tethering results in the slowing of leukocytes in the bloodstream and their rolling on the surface of endothelium, which favors subsequent interactions with endothelial cells mediated by integrins and their ligands, increasing the adhesiveness of leukocytes, that leads to their final arrest on the vessel wall (12).

P-selectin, which is constitutively expressed by platelets and endothelial cells in secretory granules, is translocated to the cell surface within minutes upon cell activation (13). Once expressed on the surface of endothelial cells, P-selectin is rapidly internalized by endocytosis. Therefore, this adhesion receptor has an important role in early leukocyte recruitment during inflammation (14). E-selectin is also expressed by endothelial cells, but it is synthesized de novo upon cell activation (15). After stimulation with IL-1 or TNF-alpha, it is maximally expressed on the membrane at 4h, and then is slowly internalized and degraded (16). L-selectin is constitutively present in most leukocytes, but it is rapidly shed upon cell activation, thus facilitating the progression to adhesion and transendothelial migration (TEM) (17-19). It was first described as a lymphocyte homing receptor, but

it also participates in leukocyte recruitment at later stages of the inflammatory response (20).

The best characterized selectin ligand is PSGL-1 (P-selectin glycoprotein ligand-1), a homodimeric sialomucin expressed by almost all leukocytes (21) and platelets (22). PSGL-1 is an important functional ligand *in vivo* for all three selectins (23-26). In addition, CD24 and ESL-1 (E-selectin ligand-1) seem to be ligands in myeloid cells for P-selectin and E-selectin, respectively (27-29). Finally, it has been described that E-selectin, GlyCAM-1, MAdCAM-1, CD34 and Sgp200 specifically interact with L-selectin (reviewed in 30).

Although selectins and their ligands are the primary mediators of leukocyte rolling, alternative cell adhesion pathways are involved in this phenomenon. It has been described that alpha4beta1 (VLA-4) and alpha4beta7 integrins can also mediate leukocyte tethering, rolling and arrest through their interaction with VCAM-1 and MAdCAM-1 in the absence of selectins (31). On the other hand, the interaction of LFA-1(alphaLbeta2)/ICAM-1 cooperates with L-selectin in leukocyte rolling by stabilizing the tethering phase and decreasing the rolling velocity (32, 33). In addition, the chemokines CX3CL1 (fractalkine) and CXCL16 also mediate both rolling and firm adhesion by interacting with CX3CR1 and CXCR6, respectively (34-37).

3.2. Membrane topography of adhesion molecules involved in rolling

Adhesion receptor distribution on cell membrane has a key role in leukocyte interactions and is an important regulatory mechanism for leukocyte trafficking (38). Selectins are clustered at the tips of microvilli, and this localization is critical for tethering and rolling.

L-selectin is anchored to the actin cytoskeleton through the constitutive association of its cytoplasmic tail to alpha-actinin, and its cell activation-dependent binding to moesin (39, 40). Although the association with alpha-actinin is not essential for its targeting to microvilli (39), its cytoplasmic anchorage to the actin cytoskeleton is necessary to control L-selectin function (41, 42). PSGL-1 is also localized at the tips of microvilli, and this subcellular distribution has been found to be important for the initiation of tethering and rolling of leukocytes (43, 44). Furthermore, the capability of alpha4, but not beta2 integrins, to initiate leukocyte adhesion under flow is also explained by its selective topographic localization at microvilli (30). Finally, less is known about the involvement of cytoskeleton in the efficient presentation in microvilli of E- and P-selectin by endothelial cells. However, it has been described that leukocyte adhesion induces E-selectin linkage to the actin cytoskeleton through alpha-actinin, paxillin, vinculin, and FAK, but not talin (45).

3.3. Selectins and PSGL-1 as signaling receptors

It has been demonstrated that L-selectin activates multiple signaling pathways involved in the reorganization of the actin cytoskeleton, such as the MAPK cascade (46), the tyrosine kinase $p56^{lck}$ and Ras (47) or the Rho GTPase Rac2 (48). In this regard, it has been described that neutrophils from $Rac2^{-/-}$ mice show deficient actin polymerization and L-selectin-mediated rolling (49). On the other hand, PSGL-1 activates the MAPK pathway (50), and acts as a negative regulator of human hematopoietic progenitor cells (5). In addition, it has been demonstrated that PSGL-1 induces a rapid synthesis of uPAR and different cytokines such as TNF-alpha, IL-8 and MCP-1 in neutrophils, monocytes and T cells (51-54). Moreover, it has been shown that PSGL-1 induces activation of beta-2 integrins and binding to ICAM-1 in neutrophils (55, 56). Our group has also described the interaction of PSGL-1 with ERM proteins, which link membrane molecules with the actin cytoskeleton (57, 58). This interaction is of critical importance for the leukocyte activation that occurs before extravasation, because it allows the recruitment of the tyrosine kinase Syk by association to ERM proteins through their phosphorylated ITAM-like motifs. Therefore, after PSGL-1 ligation to P-selectin or E-selectin, Syk conveys rolling-emanating signals to the activation of gene expression programs (59).This phenomenon suggests that the intracellular signals induced through PSGL-1 have a priming effect on leukocyte activation, up-regulating the expression of different molecules further involved in extravasation and effector functions (60). Since it has been demonstrated that the cytoplasmic tail of L-selectin also interacts with moesin (40), it is very likely that selectins use a similar strategy to trigger intracellular signaling cascades. In this regard, it has been shown that E-selectin is dephosphorylated upon endothelial cell interaction with leukocytes, supporting its role as a signal transduction molecule (61). In addition, P-selectin also functions as a signaling receptor, mediating stimulation through its interaction with ligands expressed by leukocytes (62).

4. ACTIVATION, ARREST AND FIRM ADHESION OF LEUKOCYTES

During their rolling, leukocytes are stimulated by chemokines and integrin ligands expressed on the surface of endothelial cells. These outside-in signals induce an important increase in the affinity and/or avidity of leukocyte integrins (inside-out signals) that allows the shear-resistant arrest of these cells and their firm adhesion to activated endothelium. The adhesion mediated by integrins and their ligands, and their subsequent signaling processes involve a profound remodelling of cytoskeleton in both endothelial cells and leukocytes. In Figure 2, the major adhesive, structural and signaling molecules that participate in the leukocyte firm adhesion to endothelium are summarized.

4.1. Chemokines trigger the arrest of leukocytes on endothelium

The main *in situ* modulators of integrin function are chemokines. These chemotactic cytokines act through G-protein-coupled receptors (GPCR), and induce an array of activatory signals within fractions of seconds, leading to an enhancement of adhesion and shape changes in leukocytes (63, 64). Since it is unlikely that soluble chemokine gradients present in the blood flow regulate

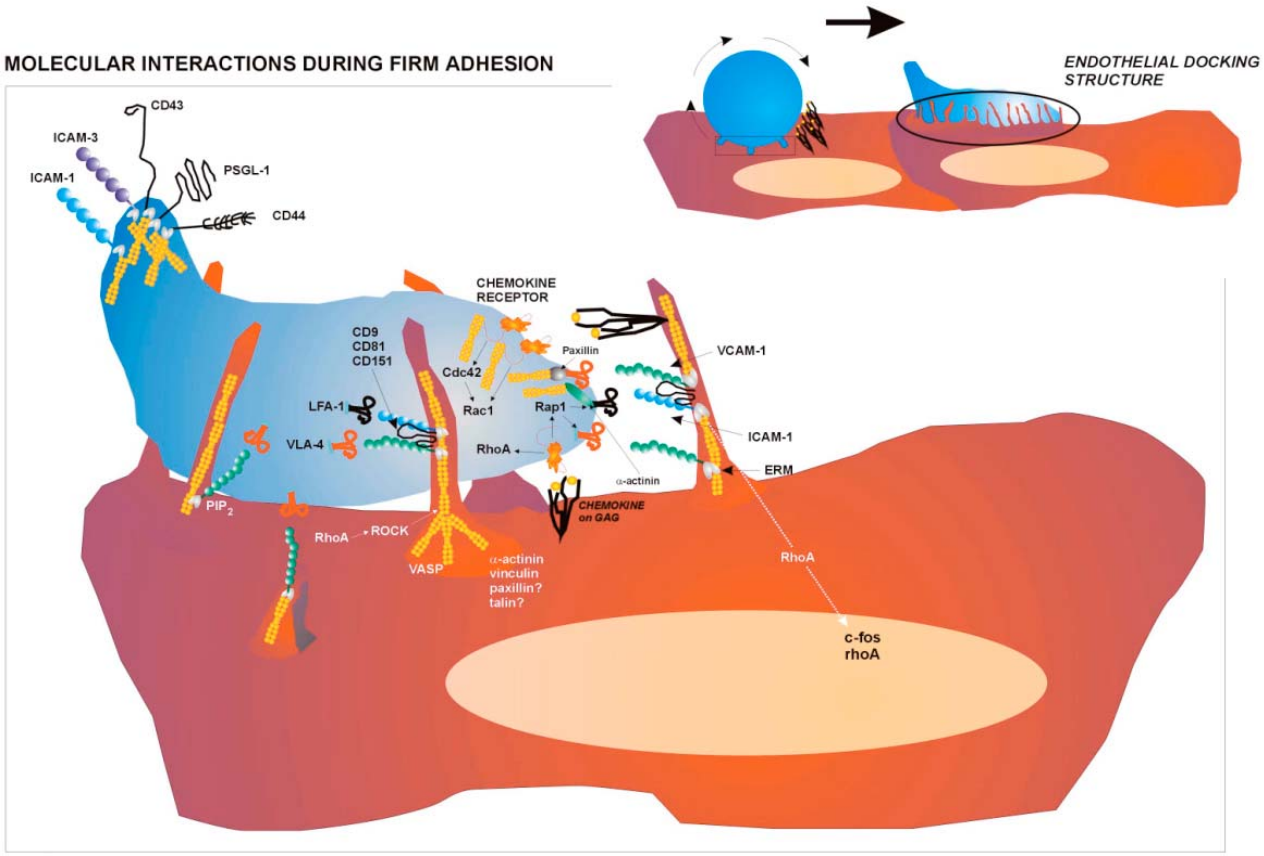

Figure 2. Activation, arrest and firm adhesion of leukocytes on endothelium: The slowing-down of leukocytes facilitates their interaction with chemokines exposed on endothelium, triggering the activation of leukocyte integrins by increasing their affinity and avidity to allow the final arrest of adherent leukocytes. This activated state involves a drastic morphological change from the round shape of circulating leukocytes to the polarized shape typical of migrating cells. The acquisition of polarity implies the segregation of adhesion molecules (ICAM-1, ICAM-3, CD43, CD44, PSGL-1, etc) to the rear pole of the cell (uropod), which is lumen-orientated for the recruitment of bystander leukocytes; whereas the integrins localized to the contact area with endothelium to allow the spreading of the cell body onto the vascular wall. On the other hand, the endothelium also plays an active role in firm adhesion by creating docking structures around the attached leukocytes. These endothelial docking structures are formed as a result of VCAM-1 and ICAM-1 engagement by their integrin counterreceptors (VLA-4 and LFA-1, respectively), and are supported by a cortical actin scaffold in which ERM proteins, alpha-actinin, vinculin,VASP and other actin-related proteins participate. In addition, members of the tetraspanin family also cooperate with the endothelial adhesion receptors in the docking structure formation. The principal regulatory molecules involved in the formation of the above-mentioned structure and in leukocyte integrin activation (as discussed in the text) are also shown in the inset.

leukocyte trafficking to specific target tissues, it is thought that chemokines mainly function immobilized at the emigration site (65). At sites of inflammation or in secondary lymphoid tissues, chemokines are present in the subendothelial tissues, as well as in the luminal surface of endothelium. These chemokines are synthesized or transported (transcytosis) by endothelial cells and bound to glycosaminoglycans (GAGs) and, possibly, to the Duffy antigen/receptor for chemokines (DARC) (66). Chemokine-binding sites are concentrated on endothelial microvilli, as occurs with chemokine receptors in leukocytes (67). The presence of particular subsets of chemokines on endothelium contributes to the selective recruitment of leukocytes, a critical phenomenon for the inflammatory response and lymphocyte homing (reviewed in 68). Chemokines may differentially modulate distinct integrins in the same microenvironment, leading to transient or sustained adhesion of leukocytes (69). Furthermore, CXCL12 (SDF-1alpha), CCL19 (MIP-3beta), CCL21 (SLC) and CCL20 (MIP-3alpha) have all been shown to induce LFA-1-mediated cell arrest in different lymphoid subpopulations (63, 65, 70). In addition, SDF-1alpha up-regulates VLA-4 affinity for VCAM-1, promoting monocyte arrest (71). The mechanism involved in such regulation is not clear, but may involve small Ras GTPases such as Rap1 (72-74), Rho GTPases (75) and other signaling molecules.

4.2. Integrin affinity and avidity changes as leukocyte strategies to achieve firm attachment and spreading

Integrins comprise a family of alpha-beta heterodimeric transmembrane molecules whose activation can be tuned to bind to different Ig-like or extracellular

matrix ligands. Integrin-mediated cell adhesion is tightly regulated by conformational changes (affinity) and clustering (avidity), being independent of surface expression levels (76). Circulating leukocytes avoid non-specific contacts with vascular walls by maintaining their integrins in non-adhesive states. The *in situ* activation of integrins during leukocyte rolling can be driven by multiple factors. Among affinity regulatory signals, divalent cations such as Mn^{2+}, Mg^{2+} or Ca^{2+} (77-79) rank as important elements at least *in vitro*. On the other hand, the binding of integrins to endothelial ligands can be enhanced independently of integrin affinity by increasing receptor density (avidity) at the contact area. Integrin avidity can be defined as a rapid interplay between preformed, ligand-induced, and chemokine-triggered avidity states (reviewed in 42). Other potential modulators of VLA-4 and LFA-1 avidity at dynamic contacts, requiring concomitant chemokine triggering, seem to be CD47 and L-selectin (80, 81). There is still another level of regulation known as integrin cross-talk. Thus, the interaction of high affinity VLA-4 with VCAM-1 may trigger LFA-1 clustering, enhancing its avidity to ICAM-1, and promoting leukocyte firm adhesion to endothelium. On the contrary, LFA-1/ICAM-1 engagement decreases the binding of VLA-4 to VCAM-1, allowing leukocyte migration towards the transmigration sites (82, 83).

The clustering of integrins is dependent on their release from the actin cytoskeleton. In this regard, there are relevant differences between VLA-4 and LFA-1. LFA-1 may preform microclusters stabilized by H-ras and cytohesin-1, which are activated via PI3-K or PKC (84-86). However, the macroclustering of LFA-1 prior to ligand engagement is prevented by cytoskeleton anchorage, with the involvement of non-phosphorylated PKC substrates and talin. Consequently, it is necessary the activation of PKC and calpain to release LFA-1 from the actin cytoskeleton (87). Conversely, VLA-4 clustering cannot be induced prior to ligand binding. Furthermore, although PKC signals participate in the release of VLA-4 from cytoskeleton, calpain or PI3-K are not implicated. In addition, it has been described that VLA-4 constitutively interacts with paxillin, while LFA-1 is able to interact with alpha-actinin (reviewed in 42).

Once integrin avidity has been up-regulated and an effective engagement with ligand occurs, the integrin anchorage to actin cytoskeleton is restored and outside-in signaling leads to actin remodelling and cell spreading. Then, leukocytes undergo a profound change in their morphology, acquiring a polarized, motility-related shape (reviewed in 88). This cell shape change favors leukocyte extravasation, and is also involved in the recruitment of bystander leukocytes through the trailing edge (uropod) (89).

4.3. The docking structure: the endothelial contribution to the leukocyte firm adhesion process

Endothelium had been considered as a mere physiological barrier, a passive partner for leukocytes during TEM. However, it is now evident that endothelium is a key active element in different physiological processes, including leukocyte extravasation. Our group has contributed to gain insight into the molecular mechanisms underlying the active role of endothelial cells in the TEM of leukocytes. We have found that activated endothelial cells generate "docking" structures that efficiently attach leukocytes, partially engulfing them. VCAM-1 and ICAM-1, together with cytoskeletal and signaling molecules, are essential constituents of these cup-like structures based on microspikes that emerge from the endothelial apical surface, and which are dynamically involved in capturing leukocytes prior to TEM (90).

4.3.1. VCAM-1 and ICAM-1 play an essential role in leukocyte capture

VCAM-1 and ICAM-1, members of the Ig superfamily, are the two major endothelial adhesion molecules involved in the binding to leukocyte integrins VLA-4 and LFA-1, respectively (91, 92). ICAM-1 but not VCAM-1 is expressed at low levels in resting endothelium, and both molecules are induced upon cell activation by pro-inflammatory cytokines such as IL-1 and TNF-alpha(93, 94). We have recently found that these integrin ligands are laterally associated with different tetraspanins (CD9, CD81 and CD151), forming protein microdomains in the apical surface of endothelium (Barreiro and Yáñez-Mó, unpublished data). Furthermore, it has been described that VCAM-1 and ICAM-1 are anchored to the actin cytoskeleton through members of the ERM family, mainly ezrin and moesin (90, 95, 96). All these molecules, which are clustered at endothelial microvilli and microspikes, contact and surround the adherent leukocyte, as key elements of the docking structure. The cytoskeletal linkage of VCAM-1 and ICAM-1 is critical for the generation of this structure upon leukocyte adhesion, but it is not necessary for the proper presentation of VCAM-1 and ICAM-1 at the apical surface, since this localization seems to be independent of ligand engagement and actin anchorage (Barreiro and Yáñez-Mó, unpublished data).

Dynamic experiments have demonstrated the involvement of ICAM-1 (through its interaction with LFA-1) not only in the firm adhesion of leukocytes but in their transendothelial migration and subsequent movement underneath the endothelial monolayer. Moesin has a similar behaviour, suggesting that ICAM-1 is anchored to the actin cytoskeleton via ERM proteins during the whole process. On the contrary, VCAM-1 is excluded of the late steps of leukocyte extravasation and only participates in the formation of the endothelial docking structure that firmly attaches the lymphocyte to the endothelium (90).

4.3.2. Structural components and signaling pathways involved in the generation and maintenance of the docking structure

The sequential steps involved in the generation of the endothelial docking structure could be as follows. The initial interaction of VCAM-1 and ICAM-1 with their ligands (VLA-4 and LFA-1 integrins, respectively) triggers their clustering at the leukocyte-endothelium contact area, together with phosphorylated activated ERM proteins. Then, these adaptor proteins in concert with alpha-actinin and vinculin participate in the rearrangement of the actin cytoskeleton to generate the docking structure. The

participation of other focal adhesion proteins such as talin or paxillin remains to be elucidated. On the other hand, it has been also described that VASP, which cooperates with the WASP-Arp2/3 complex in actin polymerization at nascent protrusions (97), is concentrated at the docking structure (90). These data suggest that this endothelial structure is supported by actin polymerization. However, microtubules do not appear to be involved in this process. Interestingly, the endothelial docking structure seems to be reminiscent of nascent complement receptor-mediated phagosomes, in that the subcellular distribution of all these structural proteins is similar in both structures (98).

Regarding the signaling pathways involved in the generation and maintenance of the docking structure, it has been described the preferential accumulation of $PI(4,5)P_2$ at the tips of the microspikes of this structure, where could participate in the activation of the ERM proteins. Furthermore, the essential role of the Rho/p160 ROCK signaling pathway in the formation of this protrusive structure, has been also documented (90). These results concur with the regulation of the VCAM-1, ICAM-1, and E-selectin clustering by the GTPase Rho during monocyte adhesion (99). Finally, further analyses are necessary to understand the mechanisms underlying the disruption of the endothelial docking structure to allow leukocyte diapedesis.

4.3.3. VCAM-1 and ICAM-1 outside-in signaling

VCAM-1 and ICAM-1 are capable of transducing signals after ligand binding. VCAM-1 is involved in the opening of the "endothelial passage" through which leukocytes can extravasate. In this regard, VCAM-1 ligation induces NADPH oxidase activation and the production of reactive oxygen species (ROS) in a Rac-mediated manner, with subsequent activation of matrix metalloproteinases and loss of VE-cadherin-mediated adhesion. This signaling pathway can be blocked by TGFbeta1 and IFNgamma (100-103). On the other hand, cross-linking of both VCAM-1 and ICAM-1 induces a rapid increase in intracellular Ca^{2+} concentration (62, 104). ICAM-1-mediated calcium signaling has been mostly studied in brain endothelial cells. In this cellular model, it has been found that ICAM-1-mediated calcium increase triggers activation of Src and subsequent phosphorylation of cortactin (104). ICAM-1 is also able to activate RhoA inducing stress fiber formation (105) and phosphorylation of FAK, paxillin and $p130^{Cas}$, which in turn trigger different signaling pathways involving JNK or p38 (106-108). Moreover, ICAM-1 cross-linking stimulate c-fos and rhoA transcription (105). As reported for PSGL-1 (59), ICAM-1 might enhance c-fos expression through the recruitment of Syk to the ICAM-1/ERM complex, but such possibility deserves further investigation. Finally, the ICAM-1 cross-linking can induce its own expression as well as that of VCAM-1, as a regulatory mechanism to facilitate leukocyte TEM (109).

5. TRANSENDOTHELIAL MIGRATION

The signals involved in the firm adhesion of leukocytes to endothelium must be reverted, weakening the original contact sufficiently to allow the migration and extravasation of leukocytes. During TEM, endothelial junctions must be loosen to a limited extent, thereby avoiding cell monolayer damage or important changes in permeability. Thus, the leukocyte and endothelium membranes are kept in close contact and show prominent associated cytoskeletal structures. Subsequently, the endothelial membranes reseal their connections over the trailing end of the leukocyte.

5.1. Leukocytes undergo drastic cytoskeletal rearrangements to extravasate across the endothelial barrier

In leukocytes, integrin-dependent adhesion is required for changes in cytoskeleton plasticity and cell motility (110). In addition, it has been recently described that immobilized chemokines play a pivotal role in this process, since SDF-1alpha presented on the apical surface of endothelial cells can trigger lymphoid TEM under shear stress conditions in the absence of a chemoattractant gradient across the endothelium, whereas soluble chemotactic gradients do not. This process has been designated as "chemorheotaxis" (111). However, monocytes (112) and neutrophils (113) do not require endothelial apical chemokines to undergo TEM, hence postulating this phenomenon as lymphocyte-specific.

The regulation of the deformation of the leukocyte cytoskeleton during TEM has not been well studied. The possible activation of regulators of the actin cytoskeleton such as small Rho GTPases by integrins or chemokines remains to be elucidated. However, an attractive hypothesis would comprise the activation of Cdc42 by integrins or chemokines, which would cause the extension of a thin exploratory pseudopodium between endothelial cells that, by sequential Rac1 activation, would evolve into a lamella squeezed within an endothelial monolayer gap. This leading lamella and the leukocyte membrane in contact with endothelium are enriched in LFA-1 (114, 115). Finally, the stretching of cell body and tail retraction would result from delayed, tail-oriented RhoA-ROCK activation and actomyosin-based contraction (116).

5.2. Endothelial cell lateral junctions regulation

The components of the endothelial lateral junctions can be divided in tight, adherens and gap junctions, each containing distinct molecular constituents, although they do not exhibit a well-organized basolateral organization as in epithelium. These molecular complexes are dynamically organized, associate with the actin cytoskeleton and, except for gap junctions, actively participate in leukocyte TEM. Recent reports strongly suggest that leukocytes and endothelium communicate each other during TEM. The most characterized intracellular signals generated by TEM in endothelium are the mobilization of intracellular Ca^{2+} and the reorganization of actin, myosin and associated molecules (117, 118).

Figure 3 establishes a comparison between the interendothelial junctions in the vascular wall and the heterotypic leukocyte-endothelium interactions during transendothelial migration. The morphological changes that leukocytes undergo to pass across the endothelial barrier, as

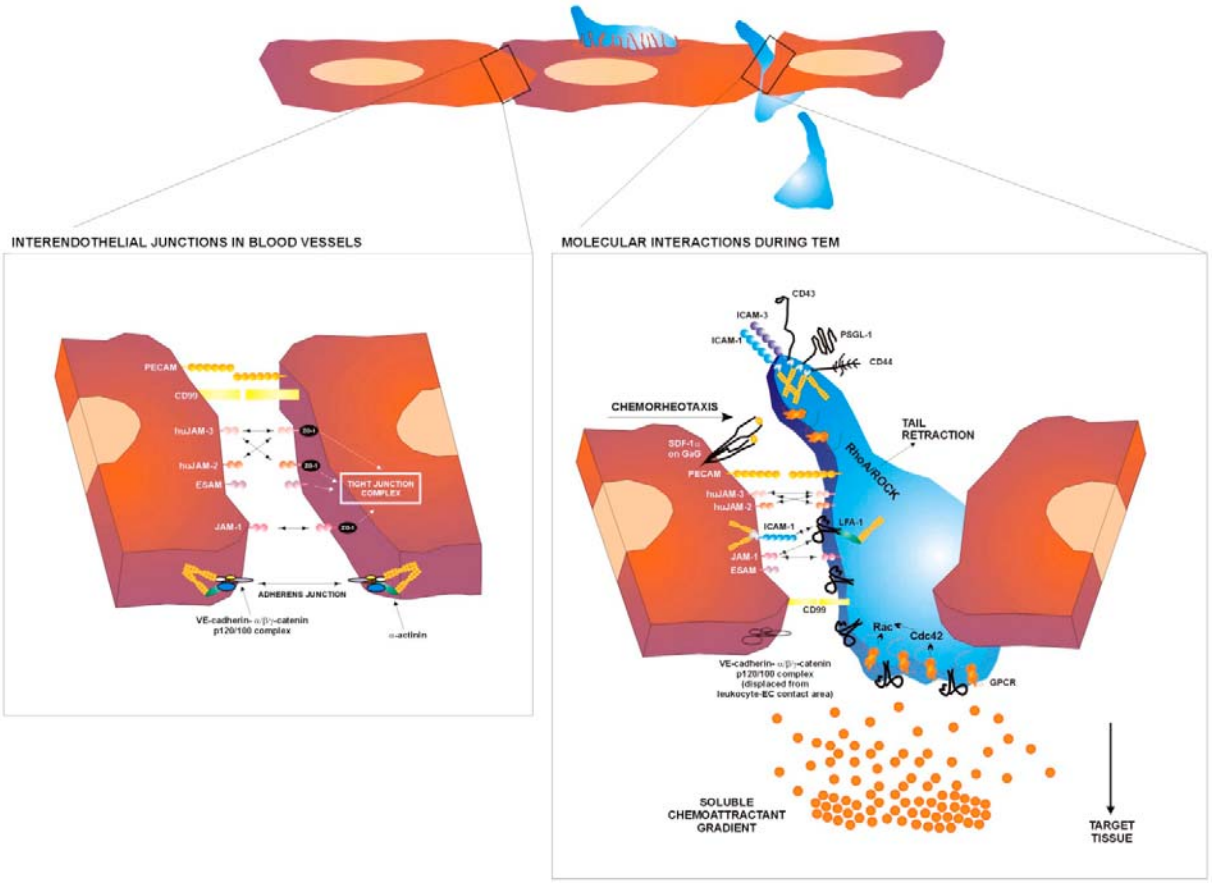

Figure 3. Comparison between the interendothelial junctions in the vascular wall and the heterotypic leukocyte-endothelium interactions during transendothelial migration. Only the molecules involved in both processes are shown, including members of the tight junctions (ESAM and the JAM family), the VE-cadherin complex and other molecules such as PECAM-1 and CD99. Most of them interact homophilically, being express by the endothelium as well as by leukocytes. On the other hand, the processes of lamellipodia formation at the leukocyte leading edge and tail retraction, the phenomenon of chemorheotaxis and the existence of a subendothelial soluble chemoattractant gradient to guide the extravasated leukocytes to the target tissue are also illustrated.

well as the effect of chemokines and chemoattractant gradients in this process, have been also specified.

5.2.1. Tight junction proteins: role of JAM family in transendothelial migration

Among the components of tight junctions (occludin, claudins, ZO-1, -2, -3, etc), two novel groups of adhesion molecules belonging to the Ig superfamily have been recently identified: ESAM (Endothelial cell-Selective Adhesion Molecule) and JAM (Junctional Adhesion Molecule) proteins. In contrast with other members of tight junctions, they play a role in paracellular permeability as well as in lymphocyte homing and TEM. ESAM is involved in the homotypic interaction of endothelial cells, but no heterophilic ligands have been described yet (119, 120).To date, three members of the JAM family have been identified: JAM-1, huJAM-2 (which corresponds to mJAM-3) and huJAM-3 (which corresponds to mJAM-2) (reviewed in 121). JAM-1 is expressed in epithelium, endothelium, erythrocytes, PMNs, monocytes, lymphocytes and platelets; huJAM2 is preferentially expressed in high endothelial venules (HEV); and JAM3 is detected in endothelium and activated T lymphocyte subsets (122-27). All members of the family contain a PDZ-binding motif in their cytoplasmic tail, which is involved in their association with components of the tight junctions such as ZO-1 and AF-6, suggesting a role for these molecules in recruiting and stabilizing JAMs to site of junction formation (128, 129). The JAM family proteins can interact homophilically at endothelial tight junctions to regulate paracellular permeability or heterophilically with counterreceptors from leukocytes to support TEM. In this regard, it has been described that JAM-1 is capable to interact with itself or with LFA-1 (130). huJAM-2 and huJAM-3 are binding partners, and this interaction could have relevance for lymphocyte homing, due to the restricted expression of huJAM-2 in high endothelial venules (124). Furthermore, huJAM-3 is also capable to interact with itself (131) and with alphaMbeta2 and alphaXbeta2 integrins (132). In turn, huJAM-2 interacts with alpha4beta1 integrin (133). The fact that all the members of the JAM family interact with leukocyte integrins and that are relocalized towards the apical surface upon endothelium activation argues for their fundamental role in the regulation of leukocyte adhesion.

5.2.2. Adherens junction disappearance at leukocyte contacts: proteolysis or displacement?

Adherens junctions are primarily involved in the regulation of endothelial cell monolayer permeability. The main component of these molecular complexes is VE-cadherin, which mediates Ca^{2+}-dependent homophilic adhesion with its extracellular domain and actin cytoskeleton linkage through the interaction of its cytoplasmic tail with alpha-, beta-, gamma-catenin, and p120/100 (134). During leukocyte TEM, VE-cadherin complexes are locally disrupted, generating a localized gap necessary for leukocyte passage, which reseals after TEM. According to these observations, VE-cadherin acts as a "gatekeeper" for leukocyte transmigration. Whether or not this disruption of adherens junctions involves protein degradation has been extensively discussed (135-137). However, the rapid recovery of adherens junctions after the passage of leukocytes seems to point to the existence of a zipper mechanism, which implies the local and transient displacement of molecular complexes from the leukocyte-endothelium area ("trapdoor model") (138, 139). However, the possibility of a certain degree of complex proteolysis cannot be excluded. Moreover, other alternative mechanisms such as transcytosis or preferential passage through tricellular corners, where tight junctions and adherens junctions are less organized, cannot be ruled out (140-142).

5.2.3. Other molecules with a major role in the passage of leukocytes across endothelium

Apart from the above-mentioned molecules, there are other endothelial proteins critical for leukocyte TEM that do not belong to tight junctions or adherens junctions complexes. PECAM-1 is another member of the immunoglobulin superfamily that is expressed in endothelium as well as in leukocytes, and that actively participates in TEM (143). It can associate homophilically or with alphavbeta3 in cis- (144, 145). PECAM-1 transduces negative intracellular signals via the ITIM motif of its cytoplasmic tail (146). In addition, PECAM-1 can regulate adherens junctions by associating to beta-catenin (147). Finally, a recent report describes the existence of a molecular network just below the endothelial plasma membrane that is connected at intervals with the junctional surface. PECAM-1 has been found in this compartment, constitutively recycling along the endothelium borders. During TEM, PECAM-1 recycling molecules are targeted to points of contact with leukocytes. This mechanism could explain how endothelial cells change their borders rapid and reversely, but remaining tightly apposed to leukocytes to allow their migration (148).

CD99, a highly O-glycosylated type I transmembrane protein, has been found to play a critical role in monocyte TEM, acting at a later stage than PECAM-1 (149). This protein is expressed in leukocytes, where triggers the activation of alpha4beta1 integrin and regulates the activity of LFA-1, and endothelium, interacting homophilically at interendothelial contacts (reviewed in 145). However, little is known about its precise function on endothelial cells or its involvement in signaling transduction .

6. CONCLUDING REMARKS AND PERSPECTIVES

Over the last decade, a huge effort has been made for the study of the basic adhesive mechanisms underlying vascular function. In this regard, the dissection of the phenomena involved in leukocyte extravasation has significantly improved our knowledge of different pathophysiological conditions. The recent description of new molecular complexes and subcellular structures implicated in this process, as well as the characterization of new intracellular signaling pathways or cytoskeletal components, have added more complexity to the extravasation mechanism and opened new insights to future investigations. Furthermore, molecules that are newly being involved in this process could constitute potential molecular targets for therapeutic intervention. Finally, some controversies and obscure points regarding, e.g., differences in the behaviour of monocytes, neutrophils or lymphocytes during TEM or the regulation of interendothelial junctions to allow the passage of leukocytes still remain partially unsolved.

7. ACKNOWLEDGEMENTS

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Abbreviations: ERM: ezrin-radixin-moesin proteins; ESAM: endothelial cell-selective adhesion molecule; ICAM-1: intercellular adhesion molecule-1; JAM: junctional adhesion molecule; LFA-1: lymphocyte function-associated antigen-1; PECAM-1: platelet endothelial adhesion molecule-1; PSGL-1: P-selectin glycoprotein ligand-1; TEM: transendothelial migration; VCAM-1: vascular cell adhesion molecule-1; VLA-4: very-late antigen-4.

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Send correspondence to: Dr. Francisco Sánchez-Madrid: Servicio de Inmunología, Hopital de la Princesa, Universidad Autónoma de Madrid, C/Diego de León 62, 28006 Madrid, Spain. Tel.: 34-91-3092115. Fax: 34-91-5202374. E-mail: fsanchez.hlpr@salud.madrid.org