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The endothelium is the first barrier that leukocytes have to overcome during recruitment to sites of inflamed tissues. The leukocyte extravasation cascade is a complex multistep process that requires the activation of various adhesion molecules and signaling pathways, as well as actin remodeling, in both leukocytes and endothelial cells. Endothelial adhesion molecules, such as E-selectin or ICAM-1, are connected to the actin cytoskeleton via actin-binding proteins (ABPs). Although the contribution of receptor–ligand interactions to leukocyte extravasation has been studied extensively, the contribution of endothelial ABPs to the regulation of leukocyte adhesion and transendothelial migration remains poorly understood. This review focuses on recently published evidence that endothelial ABPs, such as cortactin, myosin, or α-actinin, regulate leukocyte extravasation by controlling actin dynamics, biomechanical properties of endothelia, and signaling pathways, such as GTPase activation, during inflammation. Thus, ABPs may serve as targets for novel treatment strategies for disorders characterized by excessive leukocyte recruitment. The Journal of Immunology, 2015, 194: 3535–3541.

During inflammation, the endothelium actively contributes to leukocyte extravasation by expression of adhesion molecules, release of cytokines, presentation of chemokines, and by accommodating leukocyte crawling on its apical surface and transmigration across its cell body or intercellular contacts by “customized” actin dynamics controlling endothelial cell (EC) functionality.

The leukocyte extravasation cascade is a complex multistep process that requires adhesive interactions and dynamic actin remodeling in both transmigrating immune cells and ECs (Fig. 1). When an inflammatory stimulus arises, proinflammatory cytokines are produced that induce surface expression of selectins to mediate leukocyte tethering and rolling (1). Expression of ICAM-1 and VCAM-1 on the endothelium and activation of the β2-integrins lymphocyte function-associated Ag-1 and macrophage-1 Ag or the β1-integrin very late Ag-4 on leukocytes mediate firm adhesion. Subsequently, leukocytes reach the site of transmigration by intraluminal crawling. Leukocytes can crawl as much as 60 μm in a macrophage-1 Ag/ICAM-1–dependent fashion before they transmigrate (2). Transmigration (also known as diapedesis) occurs either transcellularly or paracellularly, with the majority of transmigration events being across paracellular junctions both in vivo and in vitro (3, 4). Finally, leukocytes have to cross pericytes and the basement membrane (BM) to conclude extravasation. This occurs through gaps in the pericyte layer that coincide with regions of the BM that contain less extracellular matrix proteins and, therefore, pose a thinner barrier for the transmigrating leukocyte (5, 6). All of these steps require cell movement and actin cytoskeletal remodeling in both cell types involved. Thus, it seems logical that actin-binding proteins (ABPs) play a central role in the control of the cellular movements involved in transmigration. Several ABP-mediated mechanisms in immune cells have been described to regulate cellular interactions during infection and inflammation (7), but endothelial ABPs have been largely neglected because the endothelium has long been considered a passive barrier that needs to be breached by leukocytes during extravasation. However, it becomes increasingly clear that the endothelium plays a more active role than previously anticipated and actively supports immune cells during transmigration. For example, ECs can extend membrane structures that engulf adhering leukocytes. Several groups identified such structures that were termed docking structures (8), transmigration cups (9), endothelial apical cups (10), or domes (11). They are enriched in clustered ICAM-1 and VCAM-1, actin, and ABP (12, 13). Docking structures are believed to strengthen leukocyte–endothelial interactions and guide emigrating leukocytes, but their exact physiological relevance remains elusive.

The leukocyte extravasation cascade is continuously the topic of excellent reviews; however, most reviews focused on receptor–ligand interactions and subsequent signaling mechanisms (3, 6, 14, 15). Few reviews highlighted the importance of endothelial actin remodeling and endothelial ABP for leukocyte transmigration (16–18). This review provides an update on the...
emerging importance of endothelial ABP and actin dynamics for the leukocyte extravasation cascade (Table I).

**Endothelial actin dynamics during leukocyte extravasation**

The actin cytoskeleton is crucial for endothelial functionality. Depending on the type of vasculature, ECs contain different forms of F-actin. Under basal conditions, large arterioles of the rat mesentery are characterized by a circumferential actin rim, capillaries show diffuse actin staining, and postcapillary venules, a main site of leukocyte extravasation, display a thin peripheral actin ring with few central fibers (19). Under inflammatory conditions, the endothelial actin cytoskeleton needs to be constantly remodeled to accommodate leukocyte movement on and across the endothelium. Inflammatory

**FIGURE 1.** Endothelial ABPs control leukocyte recruitment at different steps of the leukocyte extravasation cascade. The central panel shows the different steps of the entire leukocyte extravasation cascade. The surrounding panels show, in detail, the receptor–ligand interactions, involved ABPs, and actin-remodeling processes that contribute to each step of the cascade. Numbers in the boxes refer to the respective steps of the cascade as they appear in the central panel. (1–4) Mechanisms by which endothelial ABPs support leukocyte rolling and adhesion. (1) Cortactin binds to E-selectin to support tethering of leukocytes to the endothelial apical surface. (2 and 3) Cortactin, filamin B, α-actinin, and ERM are recruited to ligand-bound ICAM-1 and VCAM-1 to slow down rolling leukocytes and mediate firm adhesion. This may include binding of ERM to contractile actomyosin stress fibers. (4) It has not been studied whether ABPs also support intraluminal crawling. (5) Cortactin, filamin B, α-actinin, and ERM facilitate transmigratory cup formation by supporting ICAM-1/VCAM-1 clustering, RhoG activation [also Rac1 (12), not depicted], and actin remodeling for protrusion formation. VASP and ERM may connect docking structures to actomyosin stress fibers. (6a) Wave-2– and Arp2/3-dependent actin remodeling is required for opening and closure of transmigratory pores to enable transcellular migration. Actin depolymerization occurs to create low-resistance regions within the cell. ICAM-1 gets internalized within caveolin-1–enriched caveolae to stabilize the transmigratory pore. ICAM-1 is transcytosed to the basal membrane, where it may serve as receptor for transmigrated leukocytes. (6b) ABPs, such as ZO-1 and α-catenin, which stabilize the endothelial barrier by connecting TJs and AJs to the actin cytoskeleton, need to be disassembled from their adhesion receptors and the cytoskeleton to allow for junction opening. This is accompanied by the formation of contractile actomyosin stress fibers to exert pulling forces on junctions. It is not known whether abluminal crawling (7) and crossing of pericytes and BM (8) require endothelial ABPs. Cav-1, caveolin-1; LER, low expression region.
mediators, such as TNF-α, induce stress fiber formation independent of the presence of leukocytes (20). In contrast, adherent leukocytes coincide with a peripheral endothelial actin ring that surrounds adherent leukocytes in vivo (21). Adhesion of lymphocytes is enabled by recruitment of ICAM-1 and VCAM-1 to tetraspanin-enriched microdomains termed “endothelial adhesive platforms” (22). This is followed by activation of Ca2+-dependent signaling cascades, src family kinases, and small GTPases, leading to cytoskeletal remodeling, ABP recruitment, and ABP-mediated connection of adhesion molecules to the actin cytoskeleton (23, 24).

Another emerging concept of actin-dependent endothelial support of leukocyte adhesion and transmigration is the biomechanical control of ECs and/or endothelial substrate stiffness. Neutrophils induce actin-dependent changes in endothelial stiffness and substrate stiffness, and they can sense differences in endothelial stiffness that are important because different tissues (e.g., brain versus muscle) provide substrates of different stiffness for their blood vessels (25–28). By cultivating HUVECs on fibronectin-coated polyacrylamide gels of varying concentrations to emulate different substrate stiffnesses of physiological relevance, neutrophil transmigration was found to be increased with increasing substrate stiffness (29). Although ICAM-1 expression and cell morphology did not change with substrate stiffness, the increased transmigration rate on a stiff substrate was dependent on actomyosin contractility. Inhibition of myosin L chain kinase (MLCK) or myosin II normalized the increased transmigration rate on stiff substrates. This is of significance for cardiovascular diseases because atherosclerotic vessels are characterized by increased stiffness that may contribute to excessive leukocyte recruitment into atherosclerotic lesions. Data from the same group corroborated this notion. Treatment with oxidized low-density lipoprotein, a substance promoting atherosclerosis, further increased transmigration of neutrophils across endothelial monolayers on stiffer substrates in an actomyosin-dependent manner (30).

Leukocyte-induced cytoskeletal remodeling is also required to open transmigratory pores through ECs. Combined atomic force and immunofluorescence microscopy studies revealed that firmly adherent neutrophils caused depolymerization of endothelial actin filaments below the adhesion site, resulting in a spot of lower resistance that can be traversed more easily (31) (Fig. 1). Another study from the same group corroborated this notion. Treatment with oxidized low-density lipoprotein, a substance promoting atherosclerosis, further increased transmigration of neutrophils across endothelial monolayers on stiffer substrates in an actomyosin-dependent manner (30).

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whereas barrier-stabilizing substances, such as histamine, or src-inhibition induced stress fiber formation and VE-cadherin gaps and triggered paracellular migration (35). Moreover, application of shear stress resulted in stronger cortical actin accumulation and a reduction in stress fibers in conjunction with a preference for transcellular migration. In agreement with the above-mentioned studies, ECs grown on soft substrates had more cortical actin and supported transcellular migration of lymphocytes, but with an overall reduced rate of diapedesis. Thus, dynamic endothelial actin remodeling is required to support leukocyte transmigration per se, and it determines the preferred route for leukocytes. Future in vivo studies need to corroborate the physiological relevance of spatiotemporal actin dynamics during diapedesis to explore the possibility of pharmacological interference at this molecular step to prevent organ dysfunction caused by excessive leukocyte recruitment. In agreement with the above-mentioned atomic force microscopy study, endothelial spots of lymphocyte transmigration were consistently low in actin filaments, whereas microtubules and vimentin remained unaffected in these areas (31, 35). It will be interesting to examine whether these low-actin spots are in the vicinity of pericYTE gaps and low-expression regions in BMs (5). This would minimize the distance of abluminal crawling during transcellular migration and shorten the overall transmigration time. If true, what molecular mechanisms would coordinate the appearance of these low-resistance regions in space and time?

Different ABPs act during different steps of the leukocyte extravasation cascade

All of the mentioned actin remodeling is not possible without ABP. A detailed description of the different classes of ABPs and their impact on the actin cytoskeleton can be found in a very recent review (37). The biomechanical properties of endothelial monolayers are differentially regulated by ABPs. In a comprehensive small interfering RNA–based study, Schaefer et al. (38) investigated the contributions of the endothelial ABPs cortactin, filamin B, and α-actinin-4 to the leukocyte extravasation cascade. Downregulation of cortactin, filamin B, or α-actinin-4 alone reduced neutrophil spreading, adhesion, and transmigration, with α-actinin-4 having the strongest effects. All of these ABPs bound to clustered ICAM-1, with α-actinin-4 being recruited first, followed by cortactin and filamin-B. However, it is unlikely that all proteins bind at the same time, because neither could compensate for the lack of the other, and downregulation of one of these ABPs did not prevent binding of the others to ICAM-1 (38). Thus, a spatiotemporal regulation of ICAM-1–ABP complexes seems likely. The investigators envisioned that the functional consequences of these different complexes lie in the formation of different types of actin networks. Cortactin is known to support Arp2/3-mediated branching, α-actinin-4 cross-links actin filaments into strong bundles, and filamin B triggers cross-linking into looser meshworks. Loss of α-actinin-4 reduced F-actin bundles and F-actin–dependent ICAM-1 clustering (38). These data likely explain the strongly reduced transmigration in the absence of α-actinin-4, and they are in agreement with other studies showing that leukocytes require ABP-dependent ICAM-1 clustering for transmigration. Moreover, α-actinin-4 is upregulated in atherosclerotic plaques, making it an interesting candidate as a molecular biomarker for atherosclerosis (38).

Initial apical adhesive interactions supported by ABPs. Leukocytes are captured on the endothelial apical surface when an inflammatory stimulus arises. They interact with various endothelial adhesion molecules that are connected to the actin cytoskeleton by different ABPs (17). Tethering is mediated by E-selectin that is connected to actin upon leukocyte engagement via a complex of ABPs, including α-actinin, filamin, vinculin, paxillin, and focal adhesion kinase, molecules that also connect focal adhesions to the cytoskeleton (39). Cortactin binds directly to E-selectin, gets phosphorylated by src, and triggers E-selectin clustering and leukocyte adhesion (40). ICAM-1 also interacts with cortactin in a src-dependent manner to induce actin linkage and ICAM-1 clustering into ring-like structures that support neutrophil transmigration (23, 40–42). In vivo, cortactin regulates rolling, adhesion, and transmigration (43). In fact, we identified cortactin as the first endothelial ABP that regulates leukocyte rolling in the TNF-inflamed cremaster. Cortactin depletion caused defective RhoG activation, leading to reduced ICAM-1 clustering and firm adhesion and transmigration of neutrophils. Overexpression of constitutively active RhoG in cortactin-deficient ECs rescued the defects in ICAM-1 clustering and transmigration (43). ICAM-1 also directly interacts with Filamin B to induce actin linkage, ICAM-1 clustering, and neutrophil transmigration by regulating the lateral mobility of this complex (44). α-Actinin binds to E-selectin and ICAM-1, as well as to ICAM-2, connecting it to the actin cytoskeleton (45, 46). Although ICAM-1 binding to α-actinin is required for proper transmigration, the functional consequence of ICAM-2 binding to α-actinin has not been characterized.

ABPs trigger docking structure formation. Endothelial protrusive structures surrounding and guiding transmigrating leukocytes (Fig. 1) were independently identified by several groups. First, Barreiro et al. (8) discovered docking structures enriched in ICAM-1, VCAM-1, and various ABPs, including moesin, ezrin, α-actinin, talin, paxillin, vinculin, and VASP. Docking structures were dependent on ROCK-mediated actin remodeling, likely into stress fibers that are known to be supported by VASP and ezrin/radixin/moesin (ERM) proteins. ERM proteins within docking structures were phosphorylated in a ROCK-dependent manner, and ROCK inhibition significantly reduced docking structures and lymphocyte transmigration (8). Because ROCK1 also induces actomyosin contractility, and ERM proteins are known to associate with stress fibers, it is tempting to speculate that contractile stress fibers contribute to the formation and motility of docking structures. Myosin phosphorylation is important for transmigration. Inhibition or absence of kinases that phosphorylate myosin to induce actomyosin contractility (MLCK and ROCK) reduced transendothelial migration of both neutrophils and monocytes (47–51). Similar three-dimensional structures were observed and termed transmigratory cups, which were suggested to increase the available membrane surface and, thus, guide transmigrating lymphocytes (9). Transmigratory cups also were enriched in ICAM-1, VCAM-1, and F-actin and were required for both trans- and paracellular transmigration. Transcellular migration pores were associated with caveolin-1, but other ABPs were not investigated. Van Buul et al. (10) identified a similar apical cup structure that formed in a RhoG-dependent manner downstream of ICAM-1 engagement by neutrophils. However, they did not explicitly investigate the role of ABP in that study. We later showed that ICAM-1–induced RhoG activation depended
on the presence of cortactin (43). The guanylate exchange factor required for RhoG activation and docking structure formation was recently identified to be Trio. Inhibition of Trio reduced cortactin recruitment to ICAM-1 (12). Conversely, it seems possible that cortactin also may contribute to Trio activation, because it also acts upstream of RhoG activation (43). Cortactin was required for ICAM-1 clustering into ring-like structures surrounding adherent neutrophils (41, 43). Later, it was shown that cortactin-deficient ECs produced fewer docking structures (13). Wave2-dependent recruitment and activation of the Arp2/3 complex and Arp2/3-dependent actin remodeling also were required for docking structure formation around adherent lymphocytes (52).

In vivo, so-called “endothelial domes” were observed that completely covered transmigrating neutrophils. These domes were proposed to serve as a closure mechanism after completion of transmigration to prevent plasma leakage (11). Domes were dependent on the expression of leukocyte specific protein-1 (LSP-1) in the endothelium, where it was recruited from the cytosol and nucleus to the actin cytoskeleton under inflammatory conditions (53). LSP-1 deficiency prevented dome formation and neutrophil transmigration and was associated with increased vascular permeability.

**ABPs in transcellular diapedesis.** The actin cytoskeleton plays an important role in determining weak spots that serve as exit points for leukocytes, and ABPs are required for adhesion receptor clustering and docking structure formation. But how are transmigratory pores opened? A first hint came from a study showing that clustered apical ICAM-1 is translocated to caveolin-1–rich microdomains that are connected to actin stress fibers during lymphocyte transcellular migration (54). ICAM-1 was internalized, enriched around the transmigratory pore, and transcytosed to the basal membrane in caveolae (Fig. 1). Lymphocytes extended protrusions into these caveolin-1–rich domains, and a ring of caveolae and F-actin formed the transmigratory pore. Downregulation of caveolin-1 reduced transcellular, but not paracellular, migration. Wave2 and Arp2/3 also were recruited to docking structures, perhaps to induce Arp2/3-dependent actin remodeling necessary for pore opening and pore stabilization (52). Wave2 depletion caused a strong reduction in transcellular migration. These mechanisms were examined using lymphocytes, and it remains to be proven whether other types of leukocytes exploit similar mechanisms during transcellular migration.

**ABPs in paracelluar diapedesis.** Docking structures also were found to precede paracellular transmigration (9). Paracellular transmigration is initiated by remodeling and the opening of tight junctions (TJs) and adherens junctions (AJs). Transmigrating leukocytes exploit a multitude of homo- and heterotypic adhesive interactions during paracellular transmigration in a sequential manner, including molecules such as JAM-A, PECAM-1, and CD99, which are, in large part, provided by recruitment of the lateral border recycling compartment (LBRC) (15). The role of ABPs in LBRC recruitment and these adhesive interactions remains elusive. However, ABPs that connect integral membrane receptors of TJs and AJs to the actin cytoskeleton need to be dissociated from their adhesion receptors to allow for junction opening. For example, neutrophil and monocyte transmigration across the blood–brain barrier (an endothelium with strong TJs) required reduction in ZO-1 expression, leading to occludin translocation and TJ opening (55, 56). During monocyte transmigration, occludin internalization depended on Arp2/3 activity (57). IQGAP was shown to regulate junction remodeling in a microtubule-dependent fashion. Downregulation of IQGAP caused a strong reduction in lymphocyte transendothelial migration (58). In contrast, VE-cadherin, which is not included in the LBRC and does not serve as a counterreceptor for transmigrating leukocytes, needs to be moved out of the way. Transmigrating leukocytes disassemble the VE-cadherin/catenin complex to break the connection of AJs with the actin cytoskeleton (4). Mice expressing a VE-cadherin–α-catenin fusion protein, instead of endogenous VE-cadherin, showed strongly reduced inflammatory leukocyte recruitment into various tissues in conjunction with stronger AJ connections to the actin cytoskeleton and a more stable endothelial barrier (59). These data provide physiological evidence for the importance of the paracellular route for leukocyte recruitment in different inflammation models.

**Conclusions**

It becomes increasingly evident that ECs are not only a physical barrier for immune cells but that they actively support leukocytes during extravasation. One major feature of this active support is the remodeling of the endothelial actin cytoskeleton to allow for the morphological changes that enable leukocyte diapedesis. These actin dynamics are made possible by the orchestrated action of ABPs. Several endothelial ABPs have been implicated in the leukocyte extravasation cascade, but many more exist that have not been investigated in this context. The availability of (endothelial-specific) knockout mice for ABPs will be invaluable tools to study the physiological significance of ABP functions during leukocyte extravasation. Another important aspect is the spatio-temporal regulation of ABP recruitment and activation, because different steps of the extravasation cascade need different modes of actin remodeling. It also will be important to unravel differential mechanisms that different types of leukocytes induce in ECs during extravasation. Although some steps of the leukocyte cascade, such as ICAM-1 clustering and docking structure formation, have been reported for most types of leukocytes, other steps have only been reported in certain types of leukocytes (e.g.,
caveolin-mediated ICAM-1 transcytosis during lymphocyte transcellular migration). It will be important to know whether such mechanisms are really specific for a certain leukocyte type or have simply not been investigated for other leukocytes. Identifying unique extravasation mechanisms for a given leukocyte type will enable researchers and clinicians to specifically intervene in the recruitment of a specific leukocyte subset. A better understanding of these processes may unveil the usefulness of ABPs as targets for the treatment of acute and chronic inflammatory diseases characterized by excessive leukocyte recruitment. Certainly, we are only at the beginning of appreciating the true importance of endothelial ABPs for the recruitment of immune cells from the blood into inflamed tissues.

Disclosures

The author has no financial conflicts of interest.

References