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Mechanism of Diapedesis: Importance of the Transcellular Route

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Abstract

The neutrophil transmigration across the blood endothelial cell barrier represents the prerequisite step of innate inflammation. Neutrophil recruitment to inflamed tissues occurs in a well-defined stepwise manner, which includes elements of neutrophil rolling, firm adhesion, and crawling onto the endothelial cell surface before trans-migrating across the endothelial barrier. This latter step known as diapedesis can occur at the endothelial cell junction (paracellular) or directly through the endothelial cell body (transcellular). The extravasation cascade is controlled by series of engagement of various adhesive modules, which result in activation of bidirectional signals to neutrophils and endothelial cells for adequate cellular response. This review will focus on recent advances in our understanding of mechanism of leukocyte crawling and diapedesis, with an emphasis on leukocyte–endothelial interactions and the signaling pathways they transduce to determine the mode of diapedesis, junctional or non-junctional. I will also discuss emerging evidence highlighting key differences in the two modes of diapedesis and why it is clinically important to understand specificity in the regulation of diapedesis.

Keywords

Leukocyte extravasation cascade; Diapedesis; Locomotion; Transcellular migration; Rho GTPases; Rap1b; PI3K

1. INTRODUCTION

Neutrophils are the first line of cellular defense against invading microorganisms and play a central role in innate immunity and inflammatory processes (Ley et al., 2007; Phillipson & Kubes, 2011). These white blood cells circulate into the blood stream but must cross the endothelial barrier to reach inflamed tissues. This rapid migration from the blood to site of infections is critical for pathogen elimination and tissue repair in response to acute inflammation. However, when uncontrolled, excessive accumulation of activated neutrophils into tissue leads to tissue damage during hyper-inflammatory disorders, including acute lung injury, multiple organ failure syndrome, vascular inflammation, or arthritis.

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The initial step of the inflammatory response is a reorganization of the endothelial cell surface to capture floating neutrophils. The release of inflammatory cytokines and bacteria-derived peptides stimulates the upregulation of adhesive molecules, on the endothelial luminal surface, which locally promote weak and transient adhesive interactions between neutrophils and the endothelium, known as “rolling.” The deposition of chemokines on the endothelial luminal surface, then, triggers the activation of leukocyte integrins that promote their firm adhesion and arrest via interactions with their ligand counterpart expressed on the endothelial surface. Subsequently, the activated neutrophils further respond to chemokines and undergo a drastic cell shape change from round to flat and highly polarized—defining a cell “front” and “rear or uropod.” The adoption of this polarized shape permits the cells to migrate or crawl on the endothelial lumen surface to find a nearby site to cross the endothelial cells lining the blood vessels. This latter process is called diapedesis. Once passed the endothelial barrier, the cells must cross the pericyte layer within the venular basal membrane before to reach the inflamed interstitial tissues. This sequence of events represents the paradigm of the extravasation cascade and is summarized in Fig. 1 (Ley et al., 2007; Muller, 2011; Nourshargh et al., 2010). Although long debated, it is now accepted that leukocytes can breach the endothelial barrier by two distinct routes (Carman et al., 2007; Feng et al., 1998; Mamdouh, Mikhailov, & Muller, 2009; Marchesi, 1961; Millan et al., 2006; Muller, 2011; Phillipson et al., 2006; Yang et al., 2005). The leukocytes can find their way between two endothelial cells (the paracellular route). This is facilitated by the disruption of endothelial vascular endothelial (VE)-cadherin contacts, which form a paracellular gap through which the cells migrate. Alternatively, the leukocytes can transmigrate directly through individual endothelial cell (the transcellular route). In this case, the endothelial cell junctions remain intact. Instead, the membrane of neutrophils and endothelial cells fuse and remodel into a transcellular channel, forming a path for leukocytes. The leukocyte extravasation is a highly regulated process that involves the engagement of complex interactions between the leukocyte and the endothelium, including via selectins, integrins, intercellular adhesion molecule (ICAM), junctional adhesion molecule (JAM), and platelet endothelial cell adhesion molecule (PECAM). These interactions are well coordinated and are known to occur in a sequential manner (Muller, 2011). Beyond promoting cell–cell interactions, adhesive molecules send bidirectional signaling from the leukocytes to the endothelial cells and vice versa that participate in the establishment of leukocyte polarity, their ability to crawl on the endothelium, and that are instrumental in guiding the mode of leukocyte diapedesis (Herter & Zarbock, 2013; Muller, 2011).

This review will summarize key mechanisms and leukocyte signaling pathways that control the extravasation cascade. It will focus on emerging evidences of new pathways that specifically control transcellular migration, underscoring that, after all, paracellular and transcellular are regulated by separate mechanisms. It will then discuss the impact transmigration route may have on the immune response and why it is clinically important to understand specificity in the regulation of diapedesis.

2. LEUKOCYTE INTERACTIONS WITH THE ENDOTHELIUM

The extravasation cascade has been well studied, in particular in the context of paracellular migration. It is mediated by a series of complex and sequential interactions between the leukocytes and the endothelial apical surface via various adhesion receptors. These receptors have been extensively reviewed elsewhere (Ley et al., 2007; Luo, Carman, & Springer, 2007; Muller, 2013; Nourshargh & Alon, 2014). Endothelial (E)- and platelet (P)-selectin that are expressed on the endothelial apical surface upon inflammatory insults capture leukocytes and mediate their rolling onto the endothelium via leukocyte (L)-selectin. Subsequently, firm adhesion is controlled by adhesion receptors of the immunoglobulin family, namely leukocyte integrins (LFA-1 [lymphocyte function-associated antigen-1 also $\alpha_L\beta_2$ integrin or CD11a/CD18], Mac-1 [macrophage-1 antigen also $\alpha_M\beta_2$ integrin or CD11b/CD18], and VLA-4 [very late antigen-4 also $\alpha_4\beta_1$ integrin]), which bind to their endothelial ligands, including ICAM (ICAM-1 and -2) and vascular cell adhesion molecule 1 (VCAM-1), respectively. Following firm adhesion, leukocytes adopt a polarized shape and crawl onto the endothelial apical surface in search for a permissive site of extravasation. Locomotion of leukocytes is strictly dependent on β_2 integrins (Phillipson et al., 2006; Schenkel, Mamdouh, & Muller, 2004). In neutrophils, which express both LFA-1 and Mac-1, genetic ablation of LFA-1 and Mac-1 has established that LFA-1 and Mac-1 play sequential roles in the extravasation cascade. LFA-1 mediates neutrophil firm adhesion whereas Mac-1 controls their crawling onto the endothelial apical surface (Phillipson et al., 2006; Sumagin et al., 2010). Subsequently, the leukocytes engage a sequence of interactions to cross the endothelial barrier, which involve JAM-1/A/C (junctional adhesion molecule-1/A/C), PECAM-1 (platelet endothelial cell adhesion molecule), CD99, and ESAM (endothelial cell adhesion molecule; Muller, 2013; Nourshargh et al., 2010). In this chapter, I will mostly focus on mechanisms of leukocyte crawling and diapedesis.

2.1 Docking Structures and Crawling

Arrest of leukocytes on the endothelium is mediated by a shift from intermediate affinity to high-affinity (HA) β_2 -integrins (Shaw et al., 2004). Adhesion molecules including leukocyte integrins and endothelial ICAM redistribute into dense clusters located at the leukocyte–endothelial cell interface and surrounding the cells (Shaw et al., 2004). These dense clusters stabilize and strengthen leukocyte–endothelial cell interactions. Following arrest and firm anchorage onto apical endothelial surface, the leukocytes flatten and adopt a highly polarized shape enabling their lateral migration or crawling for several microns on the vascular endothelium in search for permissive site of transmigration (Phillipson et al., 2006). Leukocyte motility or crawling depends on asymmetric rearrangement of the leukocyte cytoskeleton in response to chemokines, which is coordinated with a dynamic cycle of assembly and disassembly of adhesive points binding the leukocyte to the endothelium. During this process, filamentous actin (F-actin) polymerizes asymmetrically forming the cell leading edge, and providing the protrusive forces to propel the cell membrane forward, whereas a network of actomyosin assembles along the cell sides and the trailing edge or uropod, and prevents lateral membrane protrusions to occur (Ridley et al., 2003; Stephens, Milne, & Hawkins, 2008; Williams et al., 2011). Maintaining cell polarity and a single leading edge are critical for persistent migration in one direction. At the same time, an active

reorganization of the cell plasma membrane occurs and involves the polarized redistribution of membrane receptors, including integrins. In migrating neutrophils, the plasma membrane becomes organized into lipid-rich domains that are different at the front and at the rear (Bodin & Welch, 2005; Pierini et al., 2003). The transmembrane receptor CD45 accumulates at the front, whereas the uropod and sides of the cells are enriched in CD44, L-selectin, heavily glycosylated proteins—e.g., PSGL-1 and integrins (Barreiro et al., 2007; Bodin & Welch, 2005; Pierini et al., 2003; Zhang et al., 2006). During active migration on endothelial cells, LFA-1 and Mac-1 are being excluded from the protrusive leading edge; instead, they actively redistribute into punctuated regions of clustered integrins that are located underneath the cells as well as along the sides and at the uropod of the leukocytes (Cinamon et al., 2004; Kumar et al., 2012; Smith et al., 2005; Zhang et al., 2006). These spatial changes are also accompanied by changes in both affinity and avidity of the integrins for their ligands. In lymphocytes, HA-LFA-1 can be seen enriched toward the uropod. In addition, dense clusters of HA-LFA-1–ICAM develop in the ventral part of the leukocytes in close contact with the endothelial apical surface (Shulman et al., 2009; Smith et al., 2005). The clusters of HA-LFA-1, and Mac-1, generated by crawling leukocytes are dynamic focal assemblies to modulate the strength of leukocyte/endothelial interactions and are necessary for leukocyte crawling on vascular endothelium (Shulman et al., 2009; Smith et al., 2005). *In vitro* studies have shown that blocking Mac-1 or CD18 with monoclonal antibodies significantly blocked monocyte crawling onto HUVEc (Shulman et al., 2009). Intravital microscopy *in vivo* demonstrated that CD11b-null neutrophils failed to crawl in the vessel lumen (Phillipson et al., 2006).

Interestingly, integrins not only provide dynamic adhesion points, they are also important to regulate the intracellular cytoskeleton and to maintain leukocyte polarization during crawling. Live imaging indicated that monocytes treated with functional blocking antibody to CD18 would adhere, polarize, and extend protrusions; they would then often retract existing protrusions and extend new protrusions in several directions (Schenkel et al., 2004). Monocytes would rotate on their uropod, unable to reach endothelial junctions (Schenkel et al., 2004). In neutrophils, CD11b-deficiency caused the cells to extend inappropriate lateral protrusions, which induced a systematic change in direction (Szczer, Zheng, & Filippi, 2009). This inability to travel in one direction was due to defective assembly of the actomyosin network at the uropod, indicating that Mac-1 plays a specialized role in maintain the cell polarity axis (Szczer et al., 2009). Hence, ICAM- β 2 integrin clusters are critical for maintaining leukocyte polarity and efficient crawling. These studies underscore the critical role integrins play during leukocyte locomotion that goes well beyond their role in attachment.

In addition to forming dense clusters underneath the cells, ICAM clusters are also consistently seen surrounding the cells prior diapedesis (Fig. 2A; Barreiro et al., 2002; Carman et al., 2003; Millan et al., 2006; Shaw et al., 2004). These clusters are regulated by endothelial cell cortical cytoskeleton, which regulates the membrane localization of adhesion receptors through ezrin, radixin, and moesin (ERM) proteins, to anchor the leukocytes to the endothelial surface (Barreiro et al., 2002). The recruitment of VCAM or ICAM to cell–cell contact on the apical endothelial membrane is dependent on endothelial tetraspanin (Barreiro et al., 2002, 2005) and cortactin (Yang et al., 2006). Some studies have

also reported the existence of actin-rich microvilli-containing ICAM clusters arising from the endothelial surface, which seem to embrace the leukocytes (Barreiro et al., 2002; Carman et al., 2003). These docking structures are also known as “transmigratory cup.” They require intracellular calcium, intact actin, and microtubule filaments in endothelial cells (Carman et al., 2003) and are mediated by endothelial RhoG signaling (van Buul et al., 2007). The formation of the transmigratory cup-containing actin-rich microvilli was seen on firmly adherent leukocytes, and preceding both the paracellular and transcellular route. It remains unclear whether this structure represents an adhesive platform that is formed to firmly anchor the leukocytes onto the endothelial surface prior to emigration or whether it actively participates in leukocyte crawling (Carman & Springer, 2004). Nevertheless, these studies consistently support the essential functions of dynamic integrin–ICAM bonds during leukocyte crawling *in vitro* and *in vivo*.

2.2 Leukocyte Invasive Protrusions

During lateral crawling, leukocytes extend highly dynamic membrane protrusions, constantly protruding and retracting onto the endothelial cell surface prior to emigration. Initially observed in neutrophils by Cinamon et al. (2004), they also occur during lymphocyte crawling (Carman et al., 2007; Millan et al., 2006; Shulman et al., 2009). Live-cell imaging combining with immunofluorescence demonstrated that crawling leukocytes generated numerous finger-like protrusions that extended underneath the cell and at the cell periphery, concentrated at the uropod (Carman et al., 2007; Millan et al., 2006; Shulman et al., 2009). These projections are assumed to be cell-autonomous as they are equally observed in leukocyte crawling on EC-free substrate but are stimulated under shear stress conditions (Shulman et al., 2009). They create deep invaginations onto the endothelial cells away from the junctions, and through endothelial junctions; and, so were named “invasive protrusions” (Carman et al., 2007; Millan et al., 2006; Shulman et al., 2009). Although one study observed invasive protrusions preceding transcellular migration only (Carman et al., 2007), others have demonstrated their occurrence during the initial stage of transmigration both at and away from the junction (Martinelli et al., 2014; Shulman et al., 2009). These protrusions may coincide with the integrin-enriched focal zones described above. Immunofluorescence indicated HA-LFA-1 situated at the base of individual invasive filopodia; and they were observed both in the ventral part of the leukocyte and at the cell periphery (Shulman et al., 2009). Carman et al. reported that these structures closely resemble podosomes classically seen in myeloid cells, as these protrusions were rich in actin, and were surrounded by rings of integrins (Carman et al., 2007).

Due to their dynamic nature, constantly protruding and retracting onto the endothelial surface, it was hypothesized that they are important to guide transmigration by scanning the endothelial surface to find a site permissive for transmigration. This hypothesis was recently demonstrated (Martinelli et al., 2014). Carman and colleagues propose that these podosomes serve as “mechanosensors” to “probe” the endothelial cell surface in order to find permissive sites for transcellular migration (Carman, 2009; Carman et al., 2007). Using atomic force microscopy-enabled nanoindentation along with electron and fluorescence microscopy, they show that lymphocyte protrusions sense the levels of resistance of endothelial cell junctions

and stiffness of endothelial cells, and, as a result, can identify area of weak endothelial actin density where the cells then transmigrate (Martinelli et al., 2014).

2.3 Paracellular Diapedesis

Paracellular or junctional diapedesis is itself a multistep process, which is controlled by the sequential involvement of ICAM-1/2, VCAM-1, JAM-1/A/C, PECAM-1, CD99, and ESAM (Muller, 2013; Nourshargh et al., 2010). One essential component of the paracellular route is the opening of the endothelial junction. It has been established that leukocyte–endothelial cell interactions via ICAM- β 2 integrin trigger the activation of signals to endothelial cells, which lead to the phosphorylation of VE-cadherin—a necessary step for loosening the adherent endothelial cell junctions and facilitating the passage of leukocytes (Vestweber, 2008). Then, leukocytes migrate and cross the endothelial junction via sequential interactions with several adhesive molecules. JAM-A/C (Woodfin et al., 2007, 2009) and PECAM (Muller et al., 1993) are critical for leukocyte diapedesis (Fig. 2B). The use of genetic deletion mouse models combined with intravital microscopy to identify the exact location where leukocyte transmigration was blocked established that heterophilic interactions between endothelial JAM-A/C and leukocyte β 2 integrins control transmigration upstream of PECAM. Indeed, the main site of arrest of JAM-A-deficient neutrophils was found to be at the level of the endothelium. In contrast, PECAM-deficient neutrophils were mostly arrested between endothelial cells and below the endothelial cell basement membrane (Woodfin et al., 2007, 2009). Elegant experiments using sequential addition and removal of anti-PECAM and anti-CD99 blocking antibody or vice versa further demonstrated that CD99 is required at a later stage of the transmigration process than PECAM (Lou et al., 2007; Schenkel et al., 2002). Interestingly, PECAM-1 interactions stimulate the recruitment of unligated adhesion molecules (e.g., PECAM, JAM-A, CD99) that leukocytes can interact with within the endothelial junction, likely to guide leukocytes moving across the junction. Unligated molecules are recruited to the endothelial cell border via certain types of vesicles called the endothelial lateral border recycling compartment (LBRC; Mamdouh et al., 2003). At the same time, the LBRC is thought to allow high-density adhesive interactions to be pushed aside to remove structural barrier to transmigration and open the endothelial junction. This compartment is trafficked to the site of transmigration by kinesin molecular motor along microtubules (Mamdouh, Kreitzer, & Muller, 2008). It is distinct from caveola and vesiculo-vacuolar organelles (VVO). Finally, once past the endothelial cell layer, neutrophils transmigrate through pericytes and the vascular basement membrane in ICAM-1/Mac-1–LFA-1- and PECAM-1-dependent manners (Dangerfield et al., 2002; Proebstl et al., 2012; Voisin & Nourshargh, 2013).

2.4 Transcellular Diapedesis

A number of studies have now provided convincing evidence for the occurrence of transcellular migration *in vivo*, as reviewed in Sage and Carman (2009). Earlier studies using transmission electron microscopy of tissue sections demonstrated that neutrophils migrated almost exclusively via the transcellular route in skin tissues in response to the bacterial chemoattractant formyl-Met-Leu-Phe (fMLP), *in vivo* (Feng et al., 1998). More recently, serial-section confocal fluorescence microscopy indicated that 15% of neutrophils migrated transcellularly in macrophage inflammatory protein 2-alpha (MIP2-alpha)-

challenged cremaster muscle, *in vivo* (Phillipson et al., 2006). Finally, the transcellular migration seems to prevail when the endothelial cell junctions are too tight, such as the blood–brain barrier (Lossinsky & Shivers, 2004; Wolburg, Wolburg-Buchholz, & Engelhardt, 2005). Hence, it has become clear that the transcellular route is a regulated process *in vivo*. In this regards, several factors have recently been shown to favor transcellular migration, including the stiffness of endothelial cells, the tightness of endothelial cell junctions, or the density of integrin ligands at the endothelial apical surface; these factors will be discussed later (Martinelli et al., 2014; Schaefer et al., 2014; Yang et al., 2005).

Transcellular migration is a fascinating process enabling leukocytes to cross the endothelial cell barrier away from the endothelial cell junctions. For this, the membrane of leukocytes and endothelial cells fuses to form a transcellular channel between the apical and basal membrane facilitating leukocyte transmigration while leaving the endothelial cell junctions intact (Carman et al., 2007). Surprisingly, the adhesive molecules and mechanisms that guide transcellular migration are very similar to those controlling junctional migration. Like for paracellular migration, transcellular diapedesis is always preceded by ICAM-dependent lateral leukocyte crawling onto the endothelial surface during which the cells extend “scanning/invasive” protrusions (Carman et al., 2007; Gorina et al., 2014; Martinelli et al., 2014; Shulman et al., 2009); the formation of a transmigratory cup made of ICAM-1 clusters and of docking structures as well as the recruitment of PECAM-1, CD99, and JAM-A to leukocyte–endothelial cell contact via the LBRC are also necessary for transcellular diapedesis (Carman et al., 2003, 2007; Mamdouh et al., 2009; Millan et al., 2006).

Detailed epifluorescence and total internal reflection fluorescence microscopy time-lapse imaging provided important information on the transcellular process (Carman et al., 2007; Millan et al., 2006). First, these studies confirmed that transmigrating leukocytes extended active protrusions and were surrounded by rings enriched in LFA-1. ICAM-1 and VCAM-1 on endothelial cells localize to F-actin-rich docking structures around adherent leukocytes (Carman et al., 2007; Millan et al., 2006). Intermediate filaments, such as vimentin, participate in forming a robust docking structure at the interface between the leukocyte and endothelial cells (Nieminen et al., 2006). Interestingly, endothelial caveolin-1 was found distributed in ICAM-1-rich areas at the endothelial cell periphery and surrounding actively transmigrating leukocytes away from the endothelial junction (Millan et al., 2006). As the lymphocytes extended dynamic protrusions into endothelial cells, ICAM-1 clusters then internalized into caveolin-1 and F-actin-rich membrane invaginations and vesicles and translocated to the basal plasma membrane, such that the cells seemed to glide through the LFA-1 ring leaving a small cluster of LFA-1 on the endothelial apical surface (Millan et al., 2006). Interestingly, caveolin-1 localized more frequently around lymphocytes taking the transcellular route than paracellular. Knockdown of caveolin-1 in endothelial cells specifically reduced transcellular migration suggesting a specialized role for caveolin-1 in the nonjunctional migration (Millan et al., 2006). Consistently, another group reported high levels of caveolin-1 in endothelial cells favored the transcellular path whereas its downregulation promoted the paracellular route (Marmon et al., 2009). Hence, once the site for transcellular migration has been decided, podosomes/protrusions can extend into long “invasive-like” protrusions to facilitate the transcellular channel likely via recruitment of

specialized endothelial cell vesicles providing cellular membrane as well as cytoskeleton components and adhesive molecules (Carman et al., 2007; Millan et al., 2006). Indeed, EM studies have shown the recruitment of membrane enriched vesicles at site of transcellular migration as well as occurrence of membrane fusion between the leukocyte and endothelial cells. Membrane fusion depended on SNARE-containing membrane fusion complexes and involved the recruitment of actin and lipid raft-rich membranes via displacement of endothelial cell caveola and vesicular vacuolar organelles (Carman et al., 2007; Millan et al., 2006). Caveola and vesicular vacuolar organelles were not observed during paracellular migration, although the LBRC was involved during both junctional and nonjunctional migration. Hence, these studies highlight some key differences between the two modes of diapedesis. The nature of the vesicles to be recruited to the site of migration differs between the two routes. Caveola- and VVO-mediated membrane fusion between leukocytes and endothelial cells appear to be unique to transcellular migration (Fig. 2B). The mechanisms controlling these events are likely key determinant factors of paracellular and transcellular migration and require further investigations.

3. SIGNALING MECHANISM

3.1 Signaling in Leukocyte Transmigration

3.1.1 Regulation of Integrin Activation—The extravasation cascade is mainly regulated by a coordinated cellular response to chemokines and adhesive molecules (Gambardella & Vermeren, 2013; Mocsai, Walzog, & Lowell, 2015; Williams et al., 2011). Here, I will mostly focus on firm adhesion, crawling, and transmigration. In resting state, leukocytes are freely floating in the blood stream owing to their low affinity for the endothelial apical surface. This is due to a low expression of integrin ligands on the endothelial apical surface, and to the bent conformation of the leukocyte integrin, which offers low binding affinity for ligands (Herter & Zarbock, 2013; Luo et al., 2007). In response to inflammatory insult, chemokines are released and immobilized onto the endothelial apical surface. In addition, the expression of endothelial integrin ligand increases. Cells initially respond to immobilized endothelial chemokines through leukocyte heterotrimeric guanine nucleotide-binding regulatory proteins (G-proteins)-coupled receptors (GPCR), which then transmit intracellular signals that coordinate active rearrangement of the cytoskeleton and integrin activation. These intracellular signals trigger a change in integrin conformation, which increases integrin affinity for ligands, known as “inside-out signaling.” The intracytoplasmic tail of integrins is bound to the cytoskeleton, and integrin activation requires active rearrangement of these bounds via interaction with cytoskeletal proteins alpha-actinin, talin-1, and kindlin-3 (Herter & Zarbock, 2013; Luo et al., 2007). In addition, the reorganization of single integrin molecule into clusters of several molecules via lateral movement of integrin within the plasma membrane enables multiple integrin ligand interactions, known as avidity changes, which strengthens leukocyte–endothelial cell interactions. Binding of ligands to integrin in turn triggers signaling cascades, called “outside-in signaling,” further regulating leukocyte behavior. Ras proximity 1 (Rap1) is an evolutionary conserved protein of the Ras-like GTPase superfamily that cycles between GTP-bound active and GDP-bound inactive forms through guanine exchange factors (GEFs) and GTPase-activating proteins (GAPs; Caron, 2003; M’Rabet et al., 1998).

The mammalian genome encodes two Rap1 genes, Rap1a and Rap1b, which are highly homologous, although they have both redundant and specific functions (Caron, 2003; Chrzanowska-Wodnicka et al., 2005; Li et al., 2007; Wittchen, Aghajanian, & Burridge, 2011). Rap1 has emerged as a key regulator of integrin activation through inside-out signaling (Katagiri et al., 2003; Sebzda et al., 2002). In immune cells, Rap1 promotes lymphocyte adhesion and migration. Rap1 is activated at the plasma membrane and recruits its effector RAPL to the integrin α subunit tail (Katagiri et al., 2003). In addition, Rap1 recruits talin to the integrin β subunit tail, which is sufficient to open integrins into high ligand binding affinity. Another actin binding protein kindlin-3 binds to the integrin tail and directly participates in integrin conformational changes (Svensson et al., 2009). These tensions further control lateral mobility of the integrin within the plasma membrane and play critical role in integrin clustering. The integrin inside-out signaling cascade involves other signaling molecules, including notably Src kinases, PLC, and PI3K, which will not be described here. This canonical pathway has mostly been described and validated using genetic models for LFA-1 activation and firm adhesion. Its involvement in Mac-1 activation and neutrophil adhesion is less clear. Recent work suggested that Rap1b may be dispensable for inside-out integrin activation in neutrophils. Rather, it seems important for outside-in integrin signaling and a limiting factor of integrin activation (described in detail below; Kumar et al., 2014).

3.1.2 Regulation of Cell Polarity and Locomotion—Leukocyte locomotion and transmigration result from a coordinated reorganization of the cytoskeleton and cycles of adhesion and de-adhesion, called adhesion turnover, in response to chemokines and clusters of adhesion molecules (Gambardella & Vermeren, 2013; Mocsai et al., 2015; Williams et al., 2011). These responses are mediated by signals emanating from GPCR and integrins, respectively. Mechanisms of adhesion turnover have been well studied and reviewed in the context of cancer cell migration and will not be discussed here. It should be noted that while leukocyte rolling and firm adhesion have been extensively examined including in *in vivo* studies, little is known about signaling mechanisms of leukocyte locomotion leading to transmigration *in vivo*. This is due to the technical complexity of examining these steps *in vivo* or in *in vitro* model systems of cells migrating through endothelial cells under shear flow. Further, the same signaling molecules are involved at each step of the extravasation cascade, although in different network organization, so that their specific roles during locomotion and diapedesis are difficult to appreciate *in vivo* using genetic models. Hence, most of our knowledge of leukocyte polarity and locomotion derive from *in vitro* studies of cells plated on various substrates and in transwell assays, which have yet to be validated by *in vivo* intravital microscopy studies.

Nevertheless, these studies have provided important information on how leukocyte polarity and motility are controlled. They are orchestrated by class I phosphoinositide 3 kinase (PI3K γ , δ) and its second messenger phosphoinositol(3,4,5)tri-phosphate (PIP3), lipid phosphatases (PTEN and SHIP), and small GTPases, which together coordinate the asymmetric assembly of the cytoskeleton with adhesion turnover via bidirectional integrin signaling (Gambardella & Vermeren, 2013; Mocsai et al., 2015). PI3 kinases convert phosphatidyl inositol 4,5-bisphosphate [PI(4,5)P₂] to PI(3,4,5)P₃; in turn PIP3 levels are

regulated by two phosphatases—PTEN converts PI(3,4,5)P3 to PI(4,5)P2 whereas SHIP converts PI(3,4,5)P3 to PI(3,4)P2. PIP3 is known to recruit to the membrane proteins containing a pleckstrin homology (PH) domain, including PKB/Akt and regulators of the small GTPases. There are three main subfamilies of small GTPases, Ras (Ras and Rap), Arf (Arf3–6), and Rho (Rac, Cdc42, RhoA). They cycle between an inactive, GDP-bound and active, GTP-bound forms via guanine nucleotide exchange factors (GEFs) and GAPs. For instance, PIP3 recruits Vav, a RacGEF, to the plasma membrane. A key event of cell polarity is the formation of a gradient of PIP3 to PIP2 between the cell front and the back. Activation of the $\beta\gamma$ subunit of the GPCR at one pole of the cell triggers PI3K activation, which amplifies PIP3 and subsequently promotes Rac activity and actin polymerization via WAVE-Arp2/3. This defines the leading edge (Affolter & Weijer, 2005; Ridley et al., 2003; Van Haastert & Devreotes, 2004). At the same time, G α subunit activates PTEN along the sides of the cells and at the rear, which amplifies PIP2 and as a result prevents Rac activation. Instead, RhoAGEF is recruited in this domain and triggers RhoA-mediated actomyosin contraction. RhoA and its effector ROCK stimulate the formation of myosin filaments at the uropod via phosphorylation of myosin light chain (p-MLC; Worthylake & Burridge, 2003; Xu et al., 2003). Since Rac facilitates PIP3 formation, which further promotes the local activation actin polymerization, and since RhoA can recruit PTEN at the rear, these two cascades offer positive feedback loops to reinforce the segregation of actin protrusion and actomyosin contraction at the leading edge and the rear, respectively (Fig. 3; Affolter & Weijer, 2005; Ridley et al., 2003; Van Haastert & Devreotes, 2004). In addition, these pathways antagonize one another through the recruitment of GAPs, such that Rac activation inhibits RhoA at the front within the leading F-actin protrusions, whereas RhoA activity outside the leading edge inhibits Rac-mediated actin polymerization. While this model may be globally true, genetic models have drawn a more complex picture, and details in mechanisms will need to be further defined in specific context. PI3K clearly controls neutrophil functions (Ferguson et al., 2007; Stephens et al., 2008); however, which PI3K isoform controls neutrophil locomotion and transmigration *in vivo* remains to be established. Genetic models have revealed that in neutrophils, which express three Rac proteins—Rac1, Rac2, and Rac3—Rac2 but not Rac1 mainly controls actin polymerization (Glogauer et al., 2003; Gu et al., 2003). Rac1, however, positively controls the distribution of RhoA to the cell rear and is important for RhoA-mediated tail retraction and cell spreading (Filippi et al., 2007; Pestonjamas et al., 2006). Two RacGEFs, Vav1 and DOCK2, appears to be important for neutrophil migration. Vav1 is required for neutrophil intraluminal crawling *in vivo* (Phillipson et al., 2009). DOCK2 controls PIP3-dependent F-actin polarization and lateral migration, at least in lymphocyte (Fukui et al., 2001; Shulman et al., 2006). Recent genetic studies have confirmed that RhoA is a key regulator of myosin-dependent uropod formation and tail retraction (Jennings et al., 2014).

The canonical Rac–RhoA polarity axis is further regulated by another small GTPase, Cdc42. Cdc42 controls where the lamellipodia forms, likely by determining Rac activity to the leading edge (Cau & Hall, 2005). In addition, Cdc42 appears to play unique and distinctive roles in maintaining front–back polarity, acting from a distance using so-called long-range signaling pathways. Whereas, it is located at the front of the cells, Cdc42 amplifies RhoA signaling at the uropod, in HL-60 neutrophilic cells (Srinivasan et al., 2003; Van Keymeulen

et al., 2006). Genetic loss of Cdc42 in primary mouse neutrophils demonstrated that Cdc42 controls the activation of p-MLC and an actomyosin rich uropod in order to prevent the formation of inappropriate actin protrusions outside the leading edge (Szczur et al., 2009). Interestingly, Cdc42 regulates the uropod via Mac-1 signaling. Cdc42 controls Mac-1 distribution and clustering along the side of the neutrophils both *in vitro* and *in vivo* (Sreeramkumar et al., 2014; Szczur et al., 2009), in a manner dependent on the Cdc42 effector WASp (Kumar et al., 2012); in turn, Mac-1 is necessary for proper activation and distribution of p-MLC at the uropod (Fig. 3; Szczur et al., 2009). As a result, Cdc42-deficient or Mac-1-deficient neutrophils have dramatic defects in polarity, chemotaxis, and transmigration (Szczur et al., 2009). Interestingly, these cells fail to reach endothelial cell junction in an *in vitro* 3D migration assay (S. Kumar & M.-D. Filippi, unpublished observation) and fail to properly crawl onto the endothelial cell surface *in vivo* (Sreeramkumar et al., 2014), and as a result were not able to cross the endothelial barrier (Kumar et al., 2012). Together, these studies provide important insights on how neutrophil intraluminal crawling is likely regulated at a molecular level.

The investigation of lipid phosphatases using genetic models has led to interesting findings. Not surprisingly, genetic loss of PTEN enhanced PIP3 levels and increased the amount of polymerized actin (Li et al., 2009; Sarraj et al., 2009). This was associated with abnormal actin protrusions and loss of polarity axis in response to GPCR activation. However, it resulted in an increase in speed of neutrophil intraluminal crawling and increased in neutrophil extravasation *in vivo* (Li et al., 2009; Sarraj et al., 2009). Likewise, the 5' PIP3 phosphatase SHIP1 also altered cell polarity and increased PIP3 levels in neutrophils (Mondal et al., 2012; Nishio et al., 2007). But, in this case, it led to an increase in integrin-mediated neutrophil adhesion, which resulted in impaired migration *in vitro* (Mondal et al., 2012; Nishio et al., 2007). Hence, PTEN and SHIP are both negative regulators of PIP3 levels, but with distinct functions as PTEN controls PIP3 levels along the polarity axis whereas SHIP regulates PIP3 at the site of cellular attachment, although the consequence this has on neutrophil extravasation *in vivo* was not examined. It would be interesting to do so, as SHIP inhibition in zebrafish is associated with increase neutrophil tissue infiltration *in vivo* (Lam et al., 2012). Together, these studies definitely imply that controlling PIP3 levels is a central event during neutrophil crawling and adhesion.

3.1.3 Regulation of Specific Route of Diapedesis: A Unique Role for Leukocyte Integrin-Rap1b Signaling?—

Recently, Rap1b—the predominant Rap1 isoform expressed in neutrophils—was shown to be a critical factor of diapedesis route. Unexpectedly, in neutrophils, Rap1b is a negative regulator of neutrophil migration by specifically suppressing the transcellular migratory route while leaving the paracellular route unaffected (Kumar et al., 2014). Rap1b loss induced an increase in neutrophil transmigration across endothelial cells both *in vitro* in response to chemokines and *in vivo*. When plated onto LPS-activated endothelial cells, Rap1b-deficient neutrophils remained away from the endothelial cell junctions, contrary to WT neutrophils; yet, they form a transcellular pore and transmigrated through endothelial cells more efficiently than WT cells. A combination of electron microscopy and immunofluorescence images revealed that Rap1b neutrophils extended long protrusions that penetrated deeper into endothelial surfaces than those formed

by WT cells. Hence, Rap1b limits neutrophil migration by specifically suppressing the transcellular migration process, and this effect protects mice from developing hyperinflammatory reaction (Kumar et al., 2014).

Interestingly, Rap1b^{-/-} neutrophils exhibited enhanced PIP3 levels and phosphorylated Akt compared to WT cells, both in response to chemokine and integrin stimulation, which was essential to their functions since Akt inhibition selectively suppressed transcellular migration of Rap1b^{-/-} neutrophils without affecting their paracellular migration. Akt inhibition also suppressed Rap1b^{-/-} neutrophil invasive protrusions. However, Akt inhibition had no effect on WT cell paracellular transmigration (Kumar et al., 2014). Hence, PI3K–Akt signal intensity robustness downstream of Rap1b favors transcellular migration, at least *in vitro* (Fig. 2B). These findings provide evidence for the existence of signaling mechanisms that separately control junctional and nonjunctional migration. Noteworthy, this regulation is quite different from the canonical function of Rap1 proteins described in lymphocytes in which Rap1 proteins positively regulate integrin inside-out activation (Katagiri et al., 2003; Sebzda et al., 2002). In neutrophils, Rap1b was found enriched in detergent-resistance plasma membrane (DRM) domains at the uropod, where CD11b redistributes, and this distribution was strictly dependent on functional CD11b (Kumar et al., 2014). Indeed, anti-CD11b cross-linking caused Rap1b to translocate to DRM, whereas functional blocking anti-CD11b antibody and CD11b-deficiency prevented Rap1b DRM recruitment. Likewise, Akt signaling intensity of Rap1b^{-/-} neutrophils was dependent on CD11b activity. These findings suggest that in neutrophils, Rap1b acts downstream of integrin and limits transcellular migration by limiting CD11b–PI3K–Akt signaling. This signaling mechanism may represent a central leukocyte signaling mechanism that controls the transcellular pathway.

This notion is supported by compelling additional evidences. First, the Rap1b effector Tiam1 has been involved in transcellular migration (Gerard et al., 2009). In this case, loss of Tiam1 in lymphocytes led to a shift in route of diapedesis toward transcellular migration, but without changing the overall number of migrated cells. Tiam1-deficient lymphocytes exhibited increased transcellular migration across endothelial cells *in vitro* and increased in transcellular pore formation away from the endothelial junctions (Gerard et al., 2009). Another interesting study reported the role of ARAP3—a PIP3-dependent Rap-activated RhoAGAP—in neutrophil migration *in vivo*. ARAP3-deficient neutrophils exhibited increased β 2-integrin affinity and avidity, and hyperresponsive responses to several adhesion-dependent neutrophil functions (Gambardella et al., 2011). ARAP-3-deficient neutrophils adhered more firmly under flow conditions *in vitro* and to the luminal vessel wall *in vivo*. Yet, they penetrated into the perivascular space more efficiently than WT cells. Finally, several studies have shown that signaling molecules, which suppress CD11b integrin outside-in signaling, limit neutrophil extravasation, including nonreceptor protein-tyrosine phosphatase SHP-1 (Abram et al., 2013; McMillan et al., 2013; Zhang et al., 2005). SHP-1 is a negative regulator of innate immune cell functions known to limit signaling pathways, including Src and Akt signalings (Tsui et al., 2006). SHP-1 is classically recruited to the plasma membrane by immunoreceptor tyrosine-based inhibition motifs (ITIMs) bearing receptors, including Siglec-E, PIR-B, or Ly49d, and limits integrin signaling (McMillan et al., 2013; Sasawatari et al., 2010; Zhang et al., 2005). Siglec-E and PIR-B have been shown

to limit neutrophil functions *in vitro* and *in vivo* (McMillan et al., 2013; Zhang et al., 2005), although their exact role during the extravasation cascade was not examined in detail. Genetic deletion of Siglec-E caused exaggerated neutrophil recruitment into tissue that was reversed by blockade of CD11b. Interestingly, Siglec-E appears to be constitutively associated with SHP-1 in neutrophils (McMillan et al., 2013). The combined deletion of Hck and Fgr in neutrophils led to an increase in Ca²⁺ flux and MAPK signaling associated with increased migration responses *in vivo* and *in vitro*. This phenotype was due to impaired PIR-B phosphorylation and association with SHP-1 (Zhang et al., 2005). Interestingly, Rap1b may control PI3K–Akt signaling intensity via SHP-1 (Kumar et al., 2014). These data are consistent with increased integrin-dependent PIP3 signaling intensity and enhanced transmigration. It would be interesting to examine the role of these molecules in the route of diapedesis.

Together, these findings reveal the existence of a neutrophil signaling pathway that specifically controls transcellular migration. Rap1b and integrin-dependent PIP3 signaling intensity may represent a “switch” signal favoring transcellular migration or paracellular migration depending on its intensity. This is likely controlled by external cues since increased endothelial ICAM can increase the degree of neutrophil transcellular diapedesis (Yang et al., 2005). These findings imply that the route of neutrophil diapedesis is a regulated process *in vivo* that may be modulated by the nature and intensity of inflammatory cues. It will thus be important to validate these findings *in vivo* a real time imaging.

3.1.4 Regulation of Invasive Protrusions—The exact nature of these protrusions and how they elongate and “invade” endothelial cells to form the transcellular channel remain unclear. We know that they are actin-rich, require Mac-1–ICAM-1 interactions. They depend on functional Src kinase activity and the actin regulatory protein WASp (Carman et al., 2007). Since loss of Rap1b causes neutrophils to extend invadopodia-like protrusions and makes use of the transcellular pathway at higher frequency than WT cells in otherwise similar endothelial environment suggests that invadopodia-like protrusions have a direct role during transcellular migration beyond mechanical sensing (Kumar et al., 2014). An interesting observation is that Rap1b-deficient neutrophils have enhanced protease degrading activity, as seen on fluorescent gelatin surface, which was completely dependent on increased Akt activity (Kumar et al., 2014). Further, it seems that blocking matrix metalloproteinase (MMP) activity using pharmacological inhibitor suppressed Rap1b neutrophil transmigration (S. Kumar & M.-D. Filippi, unpublished data). The role of MMP activity during neutrophil transendothelial remains ill-understood. Yet, several studies have provided convincing evidence they may still participate in this process since inhibitors of proteases activity clearly blocked neutrophil transmigration *in vitro* and *in vivo* (Lerchenberger et al., 2013; Young et al., 2004). Together these findings still raise an interesting possibility that MMPs may play a specialized role in transcellular migration. MMP activity is a hallmark of invadopodia found in cancer cells (Boateng & Huttenlocher, 2012; Hoshino, Branch, & Weaver, 2013; Poincloux, Lizarraga, & Chavrier, 2009). MMPs help cellular invasion through interstitial tissues, via extracellular matrix (ECM) degradation. Interestingly, in addition to ECM degradation, MMPs can induce shedding of chemokine and adhesive receptors (Kajita et al., 2001; Marco, Fortin, & Fulop, 2013). Thus,

neutrophil MMPs could modify neutrophil–endothelial cell interactions and modulate signaling cross talk between these cells, favoring transcellular migration. In this case, mechanisms that control MMP release [or not] could be a critical factor to switch noninvasive into invasive protrusions and promote transcellular migration.

Hence, knowledge on the exact protein composition and regulation of neutrophil invadopodia-like protrusions will provide critical insights on how transcellular migration is regulated.

4. FACTORS FAVORING TRANSCELLULAR MIGRATION

4.1 The Role of the Endothelium Environment

The nature of the vascular bed is a critical factor of migration route. Leukocytes cross the tight blood–brain barrier exclusively via nonjunctional migration (Lossinsky & Shivers, 2004; Wolburg et al., 2005). Two recent studies have shown that endothelial cell stiffness and the tightness of the endothelial cell junctions are important factors of route choice (Martinelli et al., 2014; Schaefer et al., 2014). The Carman's group showed that altering junctional integrity with agents enhancing or disrupting the tightness of the endothelial junctions was sufficient to change migration route (Martinelli et al., 2014). In addition, increasing endothelial cell stiffness and endothelial junction tightness shifted the route of leukocyte migration to transcellular mode. In this model, using atomic force microscopy, it was nicely showed that leukocyte invadopodia-like protrusions could “sense” endothelial cell stiffness and the resistance of endothelial cell junctions to identify endothelial cell area of “least resistance” for transmigration (Martinelli et al., 2014). This very well explains why protrusions are seen preceding both paracellular and transcellular migration (Carman et al., 2007; Shulman et al., 2009). Consistently, another study found that increasing endothelial cell stiffness stimulated ICAM-1 expression and ICAM-1-mediated neutrophil transmigration (Schaefer et al., 2014), although specific routes of migration were not examined. These studies support earlier findings that endothelial integrin ligand density or endothelial cell caveolin-1 is important for the route of migration. High expression of ICAM on endothelial cell surface or endothelial caveolin-1 expression favors transcellular migration *in vitro* (Marmon et al., 2009; Yang et al., 2005). These findings imply that after scanning, the endothelial surface via “invasive protrusions” and identifying the “path of least resistance,” leukocytes may in turn receive signals from endothelial cells that trigger transmigration wherever their location might be, at or away from the junction. Hence, transcellular migration could be a compensatory mechanism allowing emigration when the cells are unable to reach the junction. Several studies have observed that impaired crawling was correlated with increased transcellular migration. Leukocytes deficient in MAC-1 expression or the expression of the Rac GTPase regulator TIAM-1 failed to crawl onto endothelium but the cells that successfully cross the endothelium did so transcellularly (Gerard et al., 2009; Phillipson et al., 2006). However, other determinant factors exist since transcellular migration is observed in vascular beds with loose junctions (Feng et al., 1998).

4.2 Neutrophil-Intrinsic Control of Transcellular Migration

The fact that Rap1b-deficiency increases the ability of neutrophils to extend invasive protrusions and exploit the transcellular pathway (Kumar et al., 2014) strongly suggest that transcellular migration is a specialized and regulated process that can be promoted when neutrophils are highly activated. Earlier studies from the Muller's group suggested that direct leukocyte activation with fMLP increases transcellular migration events, at least *in vitro* (Mamdouh et al., 2009). Other studies have reported that intradermal injection of fMLP into ear skin where endothelial cell junctions are not tight stimulates transcellular migration *in vivo* (Feng et al., 1998). Hence, there is strong evidence to suggest that the level of leukocyte activation is sufficient to determine the route of migration. Because of this, understanding the factors that control leukocyte signaling intensity will certainly shed light on how diapedesis is modulated *in vivo*. How a signaling axis such as CD11b-Rap1b-Akt is regulated during inflammation is unclear, but likely modulated by the nature of the external stimuli, including chemokine concentration and integrin ligand density. Which signaling pathways other than Rap1b control transcellular migration and which inflammatory conditions favor transcellular migration *in vivo* will need to be examined.

5. WHY DOES TRANSCELLULAR MIGRATION EXIST?

The need for transcellular migration and the impact this migratory behavior has on inflammation remain a matter of debate. *In vivo* imaging has elegantly shown that the transcellular channel and the "hole" the cells make into endothelial cells while migrating through is very transient (Woodfin et al., 2011). In fact, since the endothelial cell junctions remain intact during transcellular migration, this mode of migration could reduce vascular permeability. However, there is no consistent evidence indicating that neutrophil transmigration causes a breach in the endothelial cell barrier and vascular leakage (He, 2010); instead Petri et al. reported that endothelial cells formed a "dome" engulfing leukocytes during paracellular migration, which is thought to minimize vascular leakage (Petri et al., 2010). As discussed previously, transcellular migration may represent the alternate mode of migration when endothelial cell junctions are too tight. Yet, transcellular migration has been observed in tissues with "leaky" endothelial cell junctions (Feng et al., 1998). An interesting possibility would be that inflammatory outcome differs depending on the route of leukocyte migration. Rap1b-deficiency causes drastic increased susceptibility to endotoxin shock in mice (WT mice whose bone marrow was reconstituted with Rap1b-deficient blood cells) with 90% mortality in a model in which control mice fully recover (Kumar et al., 2014). But the level of neutrophil migration in tissue is only twofold increased. Rap1b-deficiency also causes a middle increase in production of reactive oxygen species, and propensity to higher degranulation (Kumar et al., 2014), which certainly contribute to the inflammatory reaction. Yet, it also seems insufficient to account for the rapid death of Rap1b^{-/-}-reconstituted mice upon challenge. Hence, it is possible that transcellular migration itself aggravates inflammation. This could be achieved via transfer of specific membrane proteins onto neutrophils during the transcellular passage, in a manner similar to the transfer of ICAM-2 to neutrophils seen during paracellular migration (Woodfin et al., 2011). Such phenomenon could directly modulate signaling cross talk and further influence endothelial and/or neutrophil functions. If true, this notion has important clinical

implications. Neutrophils are double-edge swords. Finding a therapeutic window for treating patients with inflammatory diseases without compromising their anti-infection defense mechanisms has been an unreachable challenge. Since transcellular and paracellular migration appear to be, after all, separately regulated, targeting only one mode of migration could offer some level of specificity in treating hyperinflammation while leaving some host defense mechanism intact, at least in some conditions. On the other hand, if transcellular migration increases inflammatory reactions, this could be used to increase neutrophil responses in neutropenic patients. If so, Rap1b and/or PI3K signaling could be useful clinical targets.

6. CONCLUSION

Tremendous knowledge has been gained on the cellular and molecular interactions that take place during leukocyte extravasation cascade. However, diapedesis remains a poorly understood, yet critical, step of leukocyte tissue infiltration. Diapedesis is likely controlled by complex molecular interactions and signaling cross talks between leukocytes and endothelial cells that remain to be investigated in detail. Advances in live imaging either *in vitro* or in whole animal *in vivo* and investigation of signaling cross talk between leukocytes and endothelial cells will be instrumental and its physiological purpose. There is significant need for the development of new strategies for the treatment of inflammatory disorders. Understanding how diapedesis is regulated and how it is being used to modulate inflammation may offer unique opportunity for specificity in the development of novel pharmacological intervention to inflammation.

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Figure 1.

The leukocyte extravasation cascade is controlled by sequential adhesive interactions between leukocytes and endothelial cells. This schema depicts various steps and the adhesive molecules that are involved at each step. The neutrophil extravasation cascade involves a sequence of tethering and rolling along the endothelium, followed by firm adhesion and arrest onto the endothelium. Subsequently, neutrophils undergo lateral migration or crawling on endothelial cells to find a permissive site for transmigration. It should be noted that subsequent to moving across the endothelial barrier, leukocytes undergo abluminal crawling between endothelial cells and pericytes before crossing the basement membrane and migrating within interstitial tissues (Nourshargh, Hordijk, & Sixt, 2010).

Figure 2.

The leukocyte diapedesis. (A) Representation of the transcellular cup made of clusters of leukocyte integrins and endothelial ICAM. Some studies have observed the formation of actin-microvilli embracing the transmigrating leukocyte. (B) It is now accepted that leukocytes can transmigrate at the junction between two endothelial cells (paracellular migration depicted in left panel) or directly through endothelial cells (transcellular migration depicted in right panel). Paracellular migration is accompanied by the disruption of the endothelial cell junction to form a gap through which the cells migrate. This is accompanied by the reorganization of an adhesive platform and the recycling of adhesive molecules via the LBRC. On the other hand, during transcellular migration, the endothelial cell junctions remain intact. Instead, neutrophil–endothelial cell contacts fuse (represented in blue (gray in the print version)) and remodel into a transcellular channel forming a path for leukocytes. This necessitates the recruitment of actin-rich membrane, ICAM-enriched caveola and vesicle, vesicular vacuolar organelles as well as the recruitment of various adhesive molecules via the LBRC. In addition, the involvement of MMP activity is likely and may help remodeling the leukocyte–endothelial cell interaction to facilitate the formation of the transcellular channel. Several signaling mechanisms important for invasive protrusions and transcellular have been identified. High ICAM density, high integrin signaling, low Rap1b, and subsequent high PI3K/Akt signaling trigger neutrophil invasive protrusions and transcellular migration.

Figure 3.

Signaling mechanism of leukocyte locomotion. Upon firm adhesion, leukocyte flattens and adopts a polarized shape with a cell front enriched in polymerized F-actin protrusions and a rear enriched in actomyosin contractile filaments. This asymmetric shape is controlled by two major signaling networks. One is activated at the front leading to high levels of PI3K–PIP3 and subsequent Rac1-mediated actin polymerization formation. One at the cell rear leads to PTEN-mediated PIP2 and RhoA-driven actomyosin contraction. In addition, Cdc42 signaling at the front communicates with the rear of the cells by sending WASp signals to the uropod to activate integrins and RhoA signaling. Lastly, invasive protrusions mediated by Src signaling develop at the ventral and lateral part of the leukocyte to scan for permissive site of transmigration.