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BRIEF REVIEWS

# Regulation of Leukocyte Transmigration: Cell Surface Interactions and Signaling Events[1]

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A fundamental event in the inflammatory response is recruitment of blood leukocytes to a site of injury or infection, often resulting in tissue dysfunction and damage. The steps in this recruitment process are leukocyte adhesion to the endothelial lining of the vessel wall, diapedesis, or transmigration across the endothelial monolayer, followed by directed migration to a site of infection or injury that often involves transmigration across epithelia. This review will highlight recent advances in our understanding of the mechanisms of leukocyte-endothelial and leukocyte-epithelial cell adhesive interactions at the level of cell surface protein-protein binding events, and in intracellular signal transduction pathways that regulate leukocyte egress out of the vascular space and into epithelial-lined tissues.

*Exodus of leukocytes from blood vessels: cell surface events during transendothelial migration*

In the initial phase of an acute inflammatory response, circulating polymorphonuclear leukocytes (PMN)[3] respond to locally produced proinflammatory factors or inflammatory mediators (e.g., cytokines, chemokines, TNF-$\alpha$, IL-1, and LPS), leading to their sequestration and rolling along the surface of endothelial cells lining postcapillary venules. Further stimulation by endothelial cell-bound chemokines leads to rapid activation of leukocyte $\beta_1$ and $\beta_2$ integrins, resulting in arrest of leukocyte on the endothelium surface and subsequent transmigration (or diapedesis) into tissues. Thus, the current paradigm of blood leukocyte transendothelial migration under physiological levels of laminar shear flow involves a sequential, multistep adhesion cascade between leukocyte and endothelial cell adhesion molecules that mediate leukocyte attachment and rolling (endothelial E- and P-selectin, leukocyte L-selectin; integrin $\alpha_4\beta_1/\alpha_4\beta_7$) (step 1); subsequent leukocyte stable adhesion ($\beta_1$ and $\beta_2$ integrins) (step 2); and, ultimately, transmigration across the endothelium (step 3) (1) (see Fig. 1). Although the mechanisms underlying leukocyte rolling and chemoattractant-triggered firm adhesion via $\beta_1$ and $\beta_2$ integrins to peripheral vascular endothelium are reasonably understood in a global sense, the endothelial-dependent steps that regulate leukocyte transendothelial migration (step 3) and their subsequent migration to and retention in tissues and organs (step 4), remain less well understood. In addition, we will discuss the events and mechanisms that mediate PMN transepithelial cell migration (step 5) that ultimately allow for circulating blood PMN to access inflammatory stimuli (step 6) (see Fig. 1).

A comparison of in vivo and in vitro models of leukocyte transmigration of vascular endothelium reveals morphologic differences that remain to be resolved. In particular, prior investigations of leukocyte transmigration through the vessel wall using in vivo models of inflammation using detailed electron microscopic analyses suggest that leukocytes can migrate across endothelial cells in a transcellular fashion (2). Others (3) have reported that transmigration occurs at junctions (paracellular) as well as at nonjunctional (transcytosis) locations in a variety of tissues examined. However, more recently, Feng et al. (2) have elegantly shown by serial sectioning and computer-aided reconstruction neutrophil transcytosis or nonjunctional transmigration, in response to localized injection of chemoattractant, fMLP. Clearly, further studies are necessary using recent advances in imaging technology to address the site of leukocyte transmigration in vivo.

In contrast to the above in vivo data, most in vitro experimental systems have reported that transendothelial migration of leukocytes occurs predominantly at endothelial junctions along a paracellular pathway (4, 5) (step 3 in Fig. 1). Because leukocytes encounter multiple endothelial cell junctional molecules and molecular complexes during paracellular transmigration, the field has focused on cell surface molecules that localize to cell-cell junctions (reviewed in Ref. 6). CD99 and CD31 (platelet endothelial cell adhesion molecule-1 (PECAM-1)) are expressed on endothelium and are enriched at cell-cell lateral junctions. These molecules also are expressed on most leukocyte types (6). Both molecules interact through homophilic interactions, that is, CD99 on one endothelial cell binds to the

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[3] Abbreviations used in this paper: PMN, polymorphonuclear leukocyte; PECAM-1, platelet endothelial cell adhesion molecule-1; TJ, tight junction; AJ, adherens junction; VE-cadherin, vascular endothelial-cadherin; JAM, junctional adhesion molecule; IgSF, Ig superfamily; PI3K, phosphatidylinositol 3-kinase; SIRP, signal regulatory protein; PLC, phospholipase C; PTK, protein tyrosine kinase.



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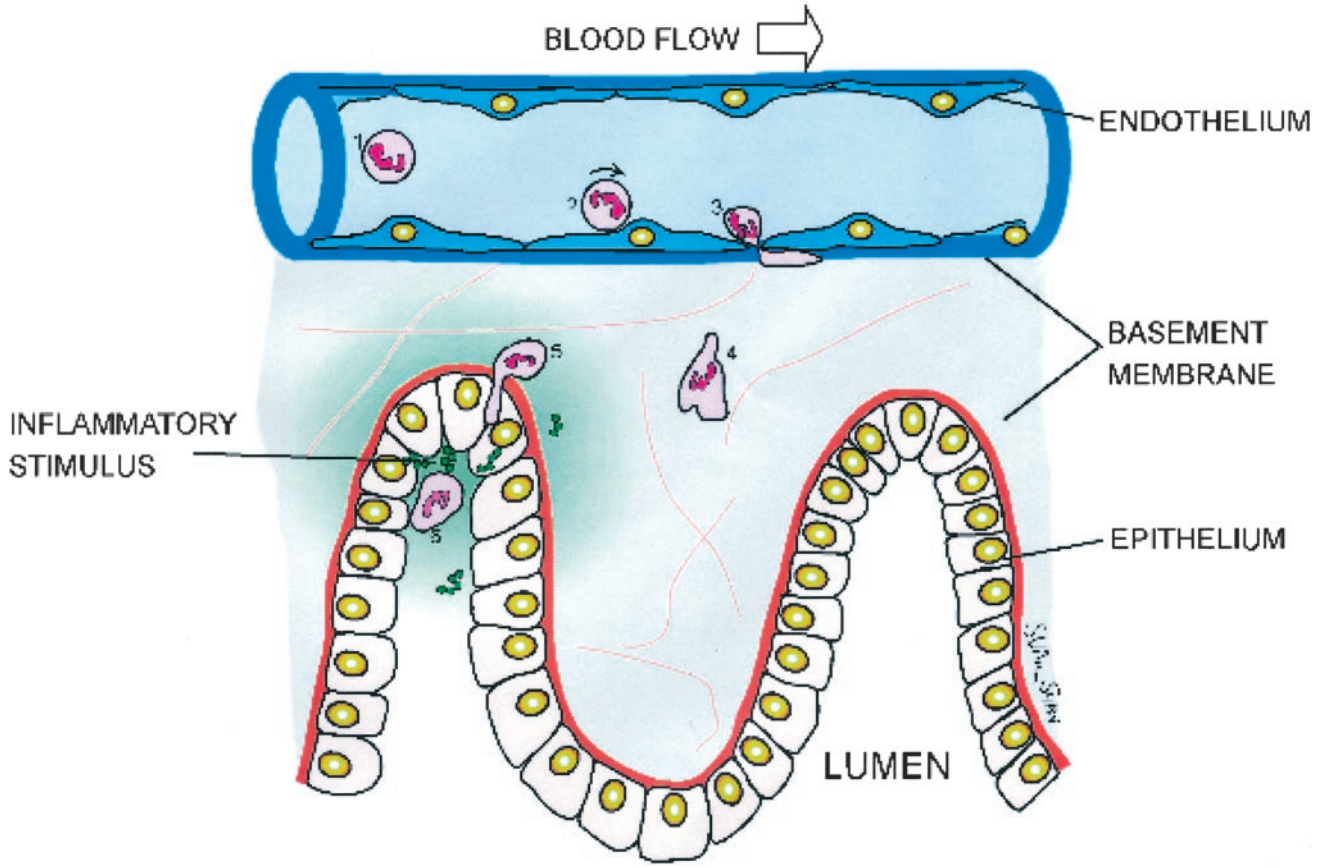


**FIGURE 1.** PMN are recruited from blood vessels to inflamed tissues (steps 1–6), which requires directed migration through the endothelium (steps 1–3) and interstitium (step 4) and across the epithelium (step 5) to reach sites of injury and inflammation (step 6), the lumen of the gut in this example. This figure identifies a blood PMN (step 1) that attaches, rolls (step 2), and arrests, and ultimately transmigrates across the single-cell thick endothelium (step 3) to gain access to the interstitial space (step 4). This interstitial PMN then migrates toward a chemotactic gradient of bacteria and inflammatory cytokines to approach the basement membrane below the epithelium that lines the villus of the intestine (step 5). Finally, the PMN reaches its initial goal and localizes to the bacteria that have colonized a crypt in the small intestine.

same molecule on adjacent endothelial cells. PECAM-1 homophilic interactions work in an analogous fashion. In addition, PECAM-1 can also function as a signaling molecule (7). During leukocyte transendothelial migration, both leukocyte PECAM-1 and CD99 engage in homophilic binding of the same molecules on endothelium. Transmigrating leukocytes must cross endothelial tight junctions (TJ) and adherens junctions (AJ), which contain numerous proteins involved in selective permeability, growth control, and cell-cell adhesion (reviewed in Ref. 8). Endothelial AJ contain the transmembrane protein vascular endothelial-cadherin (VE-cadherin), which forms a complex with cytosolic molecules $\alpha$-catenin, $\beta$-catenin, plakoglobin (or $\gamma$-catenin), and p120 (9). The VE-cadherin complex then links to the actin cytoskeleton, providing stability. Recent studies have suggested that the VE-cadherin complex regulates the passage of blood leukocytes (reviewed in Refs. 10 and 11). Recently, the effects of leukocyte transendothelial migration on VE-cadherin in endothelium were directly visualized using live-cell imaging of GFP-tagged VE-cadherin under defined laminar flow (5). Neutrophils and monocytes triggered transient and reversible displacement (4- to 6-$\mu$m gaps) in VE-cadherin-GFP during transmigration. Others (12) have reported similar alterations in VE-cadherin during PMN transmigration. The mechanism underlying displacement of VE-cadherin during leukocyte transendothelial migration is currently unknown but may involve a localized and reversible uncoupling of the VE-cadherin from the actin cytoskeleton. These findings suggest that transmigration of leukocytes across intercellular junctions is regulated by complex signaling events within both cell types. Burns et al. (13) have also reported that neutrophils transmigrate at tricellular or multicellular endothelial junctions that exhibit small gaps in VE-cadherin staining, although the mechanism is unknown.

Junctional adhesion molecule (JAM)-A (14) (formerly named JAM-1 and F-11 Ag) is another transmembrane intercellular junction protein implicated in leukocyte transendothelial migration (reviewed in Ref. 10). Murine JAM-A was originally identified as an Ig superfamily (IgSF) member concentrated at the TJ of both epithelial and endothelial cells, which was implicated in monocyte and neutrophil transmigration in vivo in the mouse (14). Subsequently, the human JAM-A homolog was found to have a similar pattern of expression on endothelial and epithelial cells, but also was expressed on the surface of all human blood cells. Recently, Ostermann et al. (15) have shown that endothelial JAM-A serves as a ligand for leukocyte LFA-1 integrin and that a murine antiserum to JAM-A blocked LFA-1-JAM-A interactions in transfected cell lines and reduced lymphocyte transendothelial migration. We have further investigated the role of JAM-A in leukocyte transendothelial migration by directly visualizing JAM-A using live-cell imaging under flow (16) and observe a unique JAM-A ring-like structure that forms transiently in endothelial cells

surrounding a transmigrating PMN. This JAM-A-enriched ring structure is transient and subsequently closes after completion of leukocyte transmigration. Simultaneous analysis of both endothelial JAM-A and VE-cadherin during transmigration revealed clear differences in their behavior, with ring formation by endothelial JAM-A and, in contrast, displacement/gap formation by VE-cadherin-GFP. Visualization of leukocyte JAM-A and its putative leukocyte counterreceptor LFA-1 during transmigration showed that both molecules segregate in discrete regions: JAM-A accumulated at the point of transmigration in junctions and on the posterior tip (tail) of the PMN as it completed transmigration, whereas LFA-1 was distributed adjacent to the tail region. It is possible that such ring-like structures in conjunction with cytoskeletal components and regulatory molecules act as transmigration tunnels in the endothelium to assemble appropriate endothelial and leukocyte adhesion molecules that play a role in organizing the transmigration machinery in space and time for this complex process to occur.

*Cell surface events during transepithelial migration*

After transendothelial migration, under many conditions, PMN negotiate the interstitium to migrate across epithelial surfaces (see Fig. 1, steps 5 and 6). In contrast to the initial PMN interactions with apical endothelial cell surface that occur in the presence of laminar shear flow, PMN transmigration across epithelial surfaces is initiated in the absence of blood flow and after PMN adhere to the basolateral epithelial membrane. In addition, after transmigration across endothelium, the PMN is no longer a naive blood cell, but rather has been acted on and influenced by the environments of the endothelium and the interstitium. These considerations raise the question of how endothelial transmigration and migration through the tissue interstitium affects transepithelial migration. Transepithelial migration is stimulated by chemokines (e.g., IL-8) or other factors derived from epithelial cells under inflammatory conditions, or after infection with pathogens. In addition to stimulated release of chemotactic factors, exposure of epithelial cells to pathogens or proinflammatory agents also results in altered protein expression such as cell surface protein CD47 (discussed below) that may facilitate leukocyte transepithelial migration (17). Hence, another consideration, perhaps not as dramatic as the above-mentioned ones, concerns the absolute role of a chemotactic gradient. For transendothelial migration, new paradigms for certain leukocyte types, especially T cells and eosinophils, suggest that apically presented chemokines in combination with physiological levels of shear flow may strongly influence transmigration, and that a traditional chemotactic gradient per se is not required (18). In contrast, it is widely thought that transepithelial migration requires the existence of a chemotactic gradient. It should be pointed out here that, in this review, chemotaxis is considered a component of transmigration, because there are binding and signaling interactions that are clearly independent of those required for pure chemotaxis.

Compared with PMN transendothelial migration, the cell surface molecules and the molecular mechanism(s) by which PMN transmigrate across epithelia are less well understood. However, a number of similarities and differences between PMN transepithelial and transendothelial migration have been identified. One key distinction between the two processes lies in dependence on $\beta_2$ integrins. Whereas PMN transmigration across vascular endothelium uses both CD11a/CD18 and CD11b/CD18, PMN transepithelial migration requires only CD11b/CD18 (19). There are exceptions to this. PMN recruitment in certain lung infections is $\beta_2$ integrin independent (reviewed in Ref. 20).

In a fashion analogous to that observed in transmigration across endothelium, PMN are likely to use multiple heterogeneous epithelial ligands, because multiple adhesive steps are required to cross the epithelial paracellular space (Fig. 1). However, it is clear that known endothelial ligands such as ICAM-1, PECAM-1, and selectins are not involved (reviewed in Ref. 21). ICAM-1, for example, is expressed in many epithelia on the apical membrane only under inflammatory conditions (22). Because PMN transmigrate from the basolateral to apical direction across epithelial surfaces, ICAM-1 is not accessible until after transmigration. Although this has been convincingly demonstrated for PMN transmigration across gut epithelial monolayers (22), it is possible that PMN may use ICAM-1 under specific circumstances in the lung (23). Although classical selectins do not serve as epithelial ligands for CD11b/CD18, recent data suggest that cell surface proteoglycans decorated with sulfated fucose moieties are attractive candidates (24). Other candidate epithelial ligands for CD11b/CD18 comprise more recently discovered IgSF proteins including JAMs, nectins, coxsackie adenovirus receptor, and related proteins that have been implicated in cell adhesion and migration. A number of these IgSF proteins are expressed on the basolateral epithelial membrane in the region of the AJ and TJ, making them ideal candidate receptors for transmigrating PMN. Indeed, reports of a role of JAM-A in murine leukocyte transendothelial migration support this. However, in humans, the role of JAM-A as an adhesive ligand for both transepithelial and transendothelial migration of PMN remains to be demonstrated despite studies using multiple functionally inhibitory Abs and soluble forms of JAM-A (25, 26). Although these observations raise questions as to the role of JAM-A in regulating human PMN transepithelial migration, they are not inconsistent with this protein in the maintenance of a barrier (forming a seal around PMN) during transepithelial migration. Our observations in endothelia and those of Rescigno et al. (27) support this. The latter presented data suggesting that dendritic cells can selectively express TJ proteins during penetration of the intestinal epithelium to sample lumenal Ags, which might help to form a seal between the leukocyte and epithelial cell. Another attractive candidate epithelial receptor for transmigrating PMN is JAM-C (formerly named JAM-2 in the mouse and JAM-3 in the human), which has recently been reported to enhance leukocyte transendothelial migration and facilitate platelet binding to PMN via CD11b/CD18 (28, 29). We have observed basolateral expression of JAM-C in human intestinal epithelial cells. Thus, further studies of JAM-C in PMN transepithelial migration will answer these important questions.

Another cell surface protein that has been shown to play an important role in modulating PMN transmigration across both endothelia and epithelia is CD47. We and others (17, 30) have shown that anti-CD47 mAbs strongly inhibit PMN transmigration across vascular endothelium, cell matrix, and intestinal epithelium. CD47 is richly expressed on PMN, epithelia, and nearly all other cell types. Our studies have indicated that CD47 expressed on both PMN and epithelial cells regulate PMN transepithelial migration (31). In PMN, stimulation

with fMLP results in redistribution of CD47 to the cell surface with kinetics much slower than those observed for CD11b/CD18 (31).

Recently, we obtained experimental evidence suggesting that CD47 regulates the rate of PMN transepithelial migration but does so after initial adhesion events have occurred (17, 31). This is consistent with observations in CD47-deficient mice where intra-abdominal challenge with *Escherichia coli* resulted in death due to delayed PMN accumulation at the infection site (32). These data suggest that CD47 serves to fine-tune the rate of migration and ensure the timely arrival of PMN to inflammatory sites in vivo. Although the precise mechanism(s) is not known, recent studies have suggested that ligation of PMN cell surface CD47 after fMLP stimulation triggers downstream tyrosine phosphorylation events that are phosphatidylinositol 3-kinase (PI3K) independent (31). In other systems, CD47 has been shown to regulate $\beta_3$ integrin function and interact with thrombospondin (reviewed in Ref. 33). However, these interactions, do not appear to play a significant role in the regulation of PMN transepithelial migration. In contrast, recent reports of CD47 binding to an IgSF member, signal regulatory protein (SIRP)$\alpha$ (33), suggest that CD47-SIRP interactions are important in regulating PMN transmigration. SIRPs are transmembrane glycoproteins with extracellular Ig-like loops and are abundantly expressed in leukocytes (34). SIRP$\alpha$ has a long intracellular domain containing four tyrosine residues forming two immunoreceptor tyrosine-based inhibitory motifs and has been shown to bind Src homology domain 2-containing tyrosine phosphatase-1 or -2 (35). We recently observed inhibitory effects by anti-SIRP Abs and soluble CD47 fusion proteins in PMN transepithelial migration assays (36) with similar observations reported in a study on transendothelial migration of monocytes (37). The details of how CD47 interacts with SIRP in leukocytes to regulate migration remain to be determined.

As PMN transmigrate across epithelia, there is an increase in permeability at sites where PMN cross (38). We have further observed an increase in epithelial permeability (reduced barrier function) when PMN contact the epithelial basolateral surface in the presence of a transepithelial gradient of fMLP (39). This observation suggests that active signaling events between PMN and epithelial cells at early stages of the transepithelial migration, although independent of $\beta_2$ integrins, may serve to facilitate PMN transmigration. It should be noted that there are reports suggesting that PMN can transmigrate across epithelia in vivo without causing gross changes in permeability (40). Thus, it is likely that PMN can efficiently crawl across TJ at densities that are low enough to allow for coordinated regulation of barrier function. Under this scenario, dysregulation of transmigration or transmigration of large numbers of PMN would still result in disruption of barrier.

*Signaling pathways that regulate leukocyte migration*

As detailed above, many complex cell surface protein-protein interactions serve to regulate leukocyte transmigration. It is widely held that leukocyte transmigration is dependent on the ability of migrating cells to sense gradients of chemokines and chemoattractants, although T cells and eosinophils also require shear flow (41). Binding of chemoattractants to leukocyte receptors triggers activation of complex intracellular signaling cascades, leading to a series of events that include cell surface protein-protein/carbohydrate-mediated cell adhesion, cytoskeletal remodeling, and lamellar protrusion at the leading edge of the migrating cell, and both intracellular and cell surface protein interactions that modulate detachment at the rear of the migrating cell. Classical chemoattractants for PMN include bacterial products such as *N*-formylated peptides (e.g., fMLP), host-derived products including chemokines (such as IL-8), phospholipids such as leukotriene $B_4$, platelet-activating factor, and lipoxins, and products of immune activation such as complement fragments (C3 or C5a). In addition to these classical chemoattractants, other agents that induce chemotaxis include macrophage-inflammatory proteins 1 and 2, cytokine-inducible neutrophil chemoattractant, substance P, and others. These chemoattractants have been suggested to play important functional roles in inflammation and infections in multiple organs. Interestingly, the classical chemoattractants not only elicit directed motility but also induce PMN degranulation, $Ca^{2+}$ mobilization, and release of $O_2^-$, whereas the latter act as pure chemoattractants with minimal activation of other cellular functions (42).

Soluble chemoattractants work through binding to leukocyte cell surface receptors, and most, if not all, such receptors are of the seven-membrane-spanning type with similar structure and topology. Ligation of chemoattractant receptors activate pertussis toxin-sensitive, heterotrimeric G proteins of the $G_i$ subfamily by triggering exchange of GDP to GTP binding in the $\alpha$ subunit and dissociation of the $\beta$ and $\gamma$ subunits. Despite these structural similarities, different types of chemoattractants possessing distinct structures bind to specific receptors, although cross-recognition has been observed within certain subfamilies of chemokines (43). This high recognition specificity is crucial for directing PMN migration in the context of a complex chemoattractant field that is envisioned to be present during an inflammatory response in vivo. Indeed, there is evidence that, when exposed to multiple chemoattractant sources in different temporal and spatial configurations, PMN can manage to discriminate and selectively migrate toward specific chemoattractants. This is accomplished, in part, through a combination of cross-desensitization of a particular receptor-mediated response and dominance exerted by certain signaling pathways. For example, it has been shown that exposure of PMN to IL-8 has no significant effect on further fMLP-stimulated adhesion and migration, whereas prestimulation of cells with subsaturating concentrations of fMLP totally abrogated further chemoattractant responses to IL-8 (44). More recent studies have further confirmed that dominant hierarchies of chemoattractants exist because PMN migrate preferentially toward end target chemoattractants, such as fMLP and C5a, rather than intermediary chemoattractants, such as IL-8 and leukotriene $B_4$ (45).

To date, the activation mechanisms coupling leukocyte migration and chemotactic responses to chemoattractants are not completely understood. N-Formylated peptide-mediated responses are, by far, the best characterized, and have provided the most insights into PMN chemotaxis. After fMLP-mediated G protein activation and dissociation of $\beta\gamma$ subunits, free G$\alpha$ subunits interact with downstream effectors including phosphatidylinositol-specific phospholipase C (PLC), that subsequently induce inositol trisphosphate-mediated release of intracellular $Ca^{2+}$ from the endoplasmic reticulum and diacylglycerol-mediated activation of protein kinase C. Activation of protein kinase C results in activation of many cell signaling pathways that

regulate PMN adhesion, while transient increases in intracellular $Ca^{2+}$ lead to cytoskeletal reorganization and actin filament uncapping/polymerization to push the plasma membrane forward and induce crawling. Dissociated $\beta\gamma$ subunits, in contrast, have been reported to interact with PI3K and isoforms of PLC-$\beta$, leading to activation of these proteins and their attachment to plasma membrane domains (46). However, the role of PLC-$\beta$ in PMN migration appears to be less important, because PMN from PLC-$\beta$-deficient mice have a defect in stimulated superoxide production but not in chemotaxis (47).

Activation of PMN by fMLP produces transient increases of phosphatidylinositol 3,4,5-trisphosphate and phosphatidylinositol 3,4-bisphosphate, implicating a role of PI3K in fMLP-driven chemotaxis. These lipid products, along with the activated downstream effector protein kinase B (PKB/Akt), become localized to the leading edge of lamellar protrusions where active actin polymerization, branching, and rearrangement of actin occur during PMN chemotaxis and thus indicate an important role in PMN polarization during transmigration (reviewed in Refs. 48 and 49). However, the actual role of PI3K in regulating leukocyte transmigration is more controversial. There are at least three families of PI3Ks that have different tissue expression patterns (50). PI3K$\gamma$, in particular, is expressed mainly in hemopoietic cells. Leukocytes from PI3K$\gamma$-deficient mice produce no detectable phosphatidylinositol 3,4,5-trisphosphate in response to G protein-coupled receptor agonists, and show diminished leukocyte chemotaxis in vitro (reviewed in Ref. 50), although PI3K($\gamma$)-independent regulatory pathways exist, as indicated by a low level of PMN recruitment in PI3K$\gamma$-deficient mice. In humans, inhibitor experiments examining the role of PI3K in PMN chemotaxis have yielded conflicting results. In particular, some reports on treatment of PMN with either wortmannin or LY294002 have shown only minimal inhibitory effects on PMN chemotaxis toward fMLP by under agarose assays (45), whereas others have observed enhanced PMN chemotaxis in Transwell migration assays in the presence of the same inhibitors (31, 51). Roles for other PI3Ks in PMN migration have also been inferred. For example, in under agarose chemotaxis assays using ICAM-1-coated surfaces, it appears that a newly described isoform-specific inhibitor for PI3K$\delta$ is able to inhibit fMLP-induced human PMN chemotaxis (52). These observations suggest that the role of PI3K in PMN chemotaxis may be dependent on the substrate; thus PI3K may function to regulate adhesive aspects of transmigration.

Studies on the molecular mechanisms of PMN chemotaxis in the presence of multiple chemoattractants have also provided evidence for requirement of distinct intracellular signaling pathways. In particular, Heit et al. (45) reported that p38 mitogen-activated protein kinase mediates PMN migration toward end target chemoattractants, fMLP and C5a, whereas a PI3K/Akt pathway is involved in regulating PMN migration toward intermediate chemoattractants such as IL-8. In the presence of both chemoattractants, the results suggested that p38 mitogen-activated protein kinase pathways were activated while PI3K/Akt pathways were suppressed. Although these analyses used in vitro system, the results suggest complicity of multiple signaling pathways and their cross talks in directing leukocyte migration in the context of complex chemoattractant arrays within tissues.

Among the recent focus of studies on various signal transduction pathways involved in regulating leukocyte migration, tyrosine kinase-mediated phosphorylation events have gained increased attention. Although the link between the G protein-coupled chemoattractant receptors and the activation of tyrosine kinases is poorly characterized, it is widely accepted that chemoattractant-mediated protein tyrosine phosphorylation is among the earliest events in leukocyte migration responses. Two families of tyrosine kinases, receptor-coupled tyrosine kinases (such as growth factors) and nonreceptor tyrosine kinases, have been described in PMN. The contribution of receptor-coupled tyrosine kinases to PMN chemotaxis appears to be minor, because agonists for these receptors trigger only nonchemotactic functions. In contrast, the importance of soluble (nonreceptor) protein tyrosine kinases (PTKs) were first suggested by the observations of inhibitory effects on chemotaxis and motility by specific inhibitors of PTKs. Such observations have been substantiated by gene knockout studies in mice.

Of the >10 subfamilies of PTKs, several members from Src family including Fgr, Hck, Fyn, Src, Lyn, and Yes have been described in PMN (53). Hck and Fgr localize to the azurophil (primary) and specific (secondary) granules in resting PMN, while isoforms of Hck are also found associated with specific membrane compartments. Stimulation of PMN with fMLP has been shown to activate Fgr kinase activity that is further enhanced by interactions with fibrinogen-coated surfaces (54), suggesting that Fgr kinase may play a downstream role in $\beta_2$ integrin-mediated adhesion and signaling. Indeed, studies of $hck^{-/-}fgr^{-/-}$ double-knockout mice display a deficiency in PMN $\beta_2$ and $\beta_3$ integrin-mediated functions (55, 56). However, whether adhesion-defective PMNs from these mice are capable of migration is unclear. Although there is a report of reduced PMN infiltration into livers of $hck^{-/-}fgr^{-/-}$ mice after high-dose administration of LPS (57), others (58) have reported that integrin-dependent migration of PMN from mice deficient in these Src-family kinases is unaffected. Another Src family kinase Lyn also has been reported to be activated after fMLP stimulation. It seems that Lyn mediates a signaling pathway that involves activation of PI3K, which in turn regulates PMN polarity and migration (59).

Other PTK have been studied in PMN including Syk/ZAP70 and Tec family tyrosine kinases Btk, Tec, and Bmx. Syk tyrosine kinase is most abundant in cells of hemopoietic lineages, and a role of this kinase in regulating cellular migration has been reported in monocytes and T cells (60, 61). Similar to hck and fgr, a functional role of Syk in adhesion-mediated signaling has been demonstrated (62), and PMN from Syk-deficient mice show impaired $\beta_2$ integrin-mediated functions in response to proinflammatory stimuli (58). Although the role of Syk in PMN adhesion is convincing, its deficiency does not appear to impair PMN transmigration in response to multiple chemoattractants (58, 63). Recent reports have demonstrated G protein- and PI3K-dependent activation of Tec family tyrosine kinases, including Tec, Btk, and Bmx, in human PMN after stimulation with fMLP (64). Inhibition of Btk appears to influence both chemotaxis and adhesion (65), suggesting a role distinct from those of Syk and Src. These studies raise an old but unresolved question regarding the regulation of PMN migration: How do cell surface-based adhesive events contribute to cell migration?

*Concluding remarks*

The emigration of leukocytes from the blood across vascular endothelium, interstitial matrix, and epithelia to an inflammatory site is a central component of the innate immune response. During this journey, there is continuous communication between leukocytes and different cellular and matrix environments in the form of multiple adhesive interactions and cell signaling events that result in cellular membrane protrusion followed by detachment and directed migration. There are both similar and distinct interactions of leukocytes with vascular endothelial cells and epithelia that are likely to provide tissue-specific inflammatory responses. A major challenge for transmigrating leukocytes also lies in correctly interpreting chemotactic gradients in the context of multiple stimuli, and ultimately finding the correct migration path. Studies focused on these topics will likely aid in development of new anti-inflammatory therapies.

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