

David M. Rose
Ronen Alon
Mark H. Ginsberg

Integrin modulation and signaling in leukocyte adhesion and migration

Authors' addresses

David M. Rose^{1,2}, Ronen Alon³, Mark H. Ginsberg¹

¹Department of Medicine, University of California, San Diego, CA, USA.

²VA Healthcare System, San Diego, CA, USA.

³Department of Immunology, Weizmann Institute of Science, Rehovot, Israel.

Correspondence to:

Mark H. Ginsberg, MD

University of California, San Diego

9500 Gilman Drive, MC 0726

San Diego, CA 92093-0726, USA

Tel.: 858-822-6432

Fax: 858-822-6458

E-mail: mhginsberg@ucsd.edu

Summary: The movement of leukocytes from the blood into peripheral tissues plays a key role in immunity as well as chronic inflammatory and autoimmune diseases. The shear force of blood flow presents special challenges to leukocytes as they establish adhesion on the vascular endothelium and migrate into the underlying tissues. Integrins are a family of cell adhesion and signaling molecules, whose function can be regulated to meet these challenges. The affinity of integrins for their vascular ligands can be stimulated in subseconds by chemoattractant signaling. This aids in inducing leukocyte adhesion under flow conditions. Further, linkage of these integrins to the actin cytoskeleton also helps to establish adhesion to the endothelium under flow conditions. In the case of $\alpha 4 \beta 1$ integrins, this linkage of the integrin to the cytoskeleton is mediated in part by the binding of paxillin to the $\alpha 4$ integrin subunit and the subsequent binding of paxillin to the cytoskeleton molecule talin. The movement of leukocytes along the vascular endothelium and in between endothelial cells requires the temporal and spatial regulation of small guanosine triphosphatases, such as Rac1. We describe mechanisms through which $\alpha 4 \beta 1$ integrin signaling regulates appropriate Rac activation to drive leukocyte migration.

Keywords: integrins, leukocyte migration, $\alpha 4 \beta 1$

Introduction

The movement of leukocytes from the blood into peripheral tissues is critical for immune surveillance and host defense. Further, aberrant leukocyte trafficking contributes to the pathogenesis of inflammatory and autoimmune diseases. Leukocyte trafficking is orchestrated and controlled by combinatorial inputs of adhesion and chemoattractant molecules located on both the leukocyte and the vascular endothelium. Numerous *in vivo* and *in vitro* studies have established that leukocytes circulating in the blood are recruited to target organs by a series of sequential steps mediated initially by leukocyte and endothelial selectins and selectin ligands or subsets of leukocyte integrins and their endothelial ligands of the immunoglobulin superfamily (1–3) (Fig. 1). These steps include initial contacts with the endothelium, which, in the presence of shear flow, result in

Immunological Reviews 2007

Vol. 218: 126–134

Printed in Singapore. All rights reserved

© 2007 The Authors

Journal compilation © 2007 Blackwell Munksgaard

Immunological Reviews

0105-2896

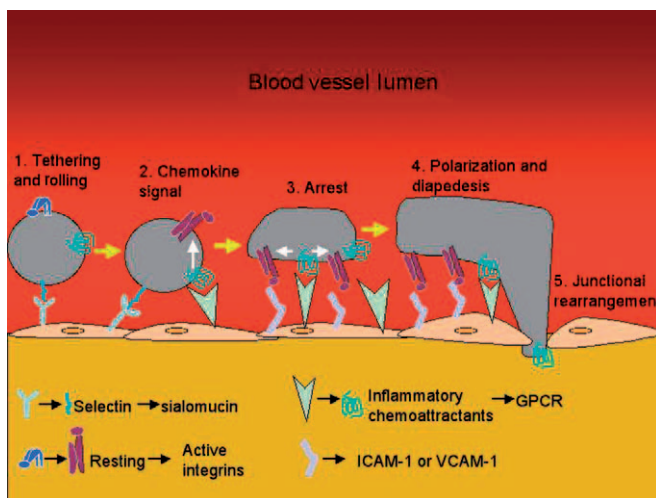


Fig. 1. Sequential steps in the movement of leukocytes from the blood into peripheral tissues. Step 1: transient interactions between leukocyte selectins and their vascular ligands result in tethering and rolling of the leukocyte along the vascular endothelium. Step 2: appropriate leukocyte activation (typically triggered by chemoattractants) induced transformation of leukocyte integrins into a high-affinity conformation. Step 3: leukocytes establish firm, integrin-mediated adhesion on the vascular endothelium. Step 4: lateral movement of leukocytes along the endothelium until they come to the junction of two or more endothelial cells. Step 5: leukocytes migrate in between the junctions of endothelial cells to reach the underlying tissues.

leukocyte rolling along the endothelium. Subsequently, stimulation by adhesive and chemoattractant agonists causes the activation of leukocyte integrins, resulting in arrest. Leukocytes then take up a polarized morphology and migrate both laterally along the endothelium and in between endothelial cells into the underlying tissue. As each adhesive step is conditional on the next, the diversity of potential interactions and their relative magnitude at each step provide the combinatorial diversity that lends high specificity to sites of leukocyte exit from the vasculature.

Leukocyte integrins are composed of α and β subunits and function as both adhesion and signaling molecules. Both integrin-mediated adhesion and signaling functions are regulated, and, in the case of leukocyte integrins, this regulation helps to meet the special challenges these cells face as they contact the vascular endothelium under shear flow conditions. These challenges require a mechanism for rapid activation of integrins to establish adhesion as well as means to temporally and spatially regulate integrin signal transduction for efficient migration across the endothelium. This review focuses on the mechanisms and signaling pathways that regulate integrin functions that contribute to movement of leukocytes from the blood into peripheral tissues.

Leukocyte capture: rolling and arrest

Leukocyte rolling

Rolling allows the leukocyte to examine the endothelial target for its repertoire of endothelial chemoattractants and integrin ligands. Rolling adhesions are primarily mediated by selectins, a three member highly conserved family of C-type lectins (4). L-selectin, expressed on most circulating leukocytes, is the key selectin that initiates most leukocyte capture events on lymph node venules and inflamed or injured vascular endothelium, which express specific fucosylated sialoglycoproteins (5). Leukocytes express P-selectin glycoprotein ligand-1; hence, once leukocytes are bound to endothelium, they can also capture L-selectin-expressing circulating leukocytes (6). With few exceptions, P- and E-selectins are expressed on both acutely and chronically stimulated endothelial beds in many inflammatory settings. Both selectins contribute to rolling, and either one, if adequately expressed, is sufficient for this process (7). Subsets of effector lymphocytes also use splice variants of CD44 to roll on endothelial surface hyaluronic acid before arresting on endothelial integrin ligands (7). Increasing evidence suggests that to resist detaching forces, both selectins and their ligands need to be properly anchored to the cytoskeleton (8–12). Recent evidence suggests that selectin–ligand interactions can be stabilized by low forces (13), and thus it appears that proper anchorage of both selectins and ligands within their respective cell membranes is mandatory for these counter receptors to load and resist detachment forces.

Chemokine signals to integrins underlying leukocyte arrest. The arrest of leukocytes rolling on target vascular beds involves rapid formation of shear-resistant adhesions by specialized leukocyte integrins. This arrest can be mediated by integrins that contain the β_2 subunit, e.g. $\alpha L\beta_2$ [leukocyte function-associated antigen-1 (LFA-1)] or α_4 subunit, e.g. $\alpha 4\beta 1$ [very late antigen-4 (VLA-4)]. Most circulating leukocytes maintain their integrins in largely low affinity state (14). Leukocyte integrins must undergo *in situ* modulation to develop high avidity for their endothelial ligands to establish shear-resistant adhesion and firm leukocyte arrest on the target endothelial site (15). Striking exceptions to this rule are T- and B-cell blasts and some myeloid subsets that maintain integrins in constitutively high affinity (16) or at intermediate affinity (17–19). Nevertheless, for most leukocytes, this dramatic change in integrin affinity is triggered when the rolling leukocyte encounters and rapidly responds to a proper chemoattractant signal presented on the apical endothelial surface (20, 21).

Lymphocyte and myeloid cells cease rolling and arrest on lymph node high endothelial venules as well as on peripheral

tissues upon activation of at least one of the four major leukocyte integrins: VLA-4, $\alpha 4\beta 7$, LFA-1, and the myeloid-specific integrin, Mac-1 (3). The molecular basis of integrin activation underlying leukocyte stoppage (sticking, arrest) on target endothelial sites is beginning to unfold (22, 23). New structural and functional data strongly suggest that bidirectional integrin signals involve rearrangements of their α and β subunit cytoplasmic tails and changes in the extracellular domain that follow binding of extracellular ligands (24–26). Changes in conformation initiated at the cytoplasmic domain and transmitted to the headpiece by the integrin leg domains are referred to as inside-out signaling. Recent *in vivo* and *in vitro* data in T cells indicate that integrin activation by immobilized chemokines is local and abrupt and involves inside-out signals initiated by G-protein-coupled receptors (GPCRs) and near simultaneous rearrangement of the *in situ*-activated integrin by a juxtaposed integrin ligand (27, 28). In light of the short time frame required for biologically significant integrin activation by chemokines, integrins may exist in preformed associations with different cytoskeletal adapters (29) and membrane proteins, such as tetraspanins (30), CD47 (31), CD98 (32), and CD44 (33).

Only specific pairs of chemokines and cognate GPCRs can activate integrins under shear flow, and these GPCRs activate specific Gi and Gq heterotrimeric proteins and their downstream effectors (34). The targets of these effectors and their mode of action in bidirectional integrin activation are still obscure. Two key guanosine triphosphatases (GTPases), RhoA and Rap1, have been implicated in chemokine activation of integrins (22, 35, 36). All leukocyte integrins link to the actin cytoskeleton by binding to cytoskeleton-associated proteins, such as talin. Talin plays a key role in rapid stimulation of integrin affinity by inside-out signals including chemokine signals through its ability to bind integrin cytoplasmic tails (28). Rap1 has been shown recently to activate integrins by a profilin and vasodilator-stimulated phosphoprotein-binding protein termed Rap-interacting adapter molecule (RIAM) (37), which recruits talin to the vicinity of target integrins in response to Rap1 activation and thereby triggers integrin activation (38). GPCR-activated Rap1 may also localize its effector, called regulator of adhesion and cell polarization enriched in lymphoid tissues (RAPL), nearby membrane proximal regions of particular vascular integrins to mediate integrin activation (39). Ligand-induced rearrangements of the integrin headpiece can follow these inside-out activation events and result in additional unclaspings of the integrin intracellular interface (40). Leukocytes do not need to accumulate chemoattractant signals over periods of rolling upstream of the arrest site, as their vascular integrins

can undergo this bidirectional conformational activation within milliseconds (41). Slow rolling, especially on endothelial selectins, exposes inaccessible integrins and GPCRs to endothelial signals through leukocyte flattening and collapse of microvilli (42). In addition to the rapid integrin activation mechanism, neutrophils and effector lymphocytes can use prolonged selectin-mediated rolling routes to activate their integrins (43, 44). This alternative mechanism may afford a stepwise integration over distance of weak endothelial signals distinct of the rapid *in situ* chemokine signals. New evidence also suggests that mechanical signals transduced by shear stress can further facilitate integrin activation by ligand (21, 45, 46), followed by post-arrest ligand-driven integrin microclustering and macroclustering (25, 47, 48).

Similar to selectins, GPCR-activated integrins may need to be properly anchored to the actin cytoskeleton to establish adhesion under shear flow conditions. Preformed associations of $\alpha 4$ integrins with the cytoskeleton adapter paxillin facilitate the ability of these integrins to mediate initial capture and rolling interactions on vascular cell adhesion molecule-1 (VCAM-1) and mucosal addressin cellular adhesion molecule-1 (49). The $\alpha 4$ -binding adapter paxillin not only appears important to link these $\alpha 4$ integrins to the actin cytoskeleton but also does so without altering the affinity of the $\alpha 4$ integrins under shear-free conditions (50). Other vascular integrins, like LFA-1 and Mac-1, may also use specific cytoskeletal adapters to facilitate activation by ligand and chemokine signals under shear forces, yet the relative importance of anchorage for specific $\beta 2$ and $\alpha 4$ members to adhesiveness is still open for further investigation. We suggest that moderate anchorage is ideally suited to translate ligand recognition into high-affinity and force-resistant binding. Unclassed integrins that are not tethered to the cytoskeleton may acquire high affinity for ligands but fail to support shear-resistant leukocyte arrest under shear flow because of their poor anchorage to the cytoskeleton. These subsets are expected to be more mobile than integrins specialized to generate immediate shear-resistant arrest. These mobile integrins would readily be recruited to sites stabilized by the cytoskeletally anchored integrins, where they may contribute to post-arrest adhesion strengthening.

Leukocyte polarization and migration

Once a leukocyte establishes firm adhesion to the vascular endothelium, it undergoes a morphological change known as polarization. Subsequently, the leukocyte migrates over the apical endothelial surface toward interendothelial junctions, where it then can transmigrate into the subendothelial tissues.

Polarization establishes the front (or leading edge) and the trailing edge (in leukocytes, an extended rear projection, termed the uropod, forms a specialized trailing edge) of a cell (51). After polarization is achieved, the cell is poised for directional movement. Depending on the adhesive and chemotactic cues, integrins on the polarized leukocyte generate highly dynamic adhesions. These integrins must constantly integrate both biochemical information from ligands and inside-out activation signals as well as mechanical signals in the form of external forces exerted by the shear stress experienced by the leukocyte at the endothelial contact site and internal forces generated by polymerized actin and actomyosin contractility (21, 52, 53).

Polarization

Polarization in leukocytes is primarily triggered by chemokines and involves reorganization of the actin cytoskeleton, Golgi, and microtubule-organizing center as well as redistribution of cell surface molecules (51, 54, 55). F-actin goes from being radially symmetric around the cell to being focused in areas such as the leading edge (56). Chemokine receptors redistribute to the leading edge, while other cell surface molecules, such as the adhesion molecule CD44, redistribute to the uropod (51). Integrin molecules also reorganize, forming clusters at the leading edge and the uropod. In addition, the activation state of integrins can change in a spatially distinct manner during polarization, with high and intermediate affinity integrins being primarily localized to the leading edge (57, 58). Several signaling pathways contribute to polarization including Rho family GTPases, protein kinases, and lipid kinases. Over the past few years, the small GTPase Rap1 has emerged as a major molecule contributing to integrin activation and redistribution during leukocyte polarization (22, 39, 57). Thus, we restrict our discussion to this molecule and its downstream effectors.

Chemokine stimulation causes guanosine triphosphate (GTP) loading of Rap1, which results in both integrin affinity regulation and leukocyte polarization (57). In particular, expression of a Rap1-specific GTPase-activating protein (GAP) (which inhibits Rap1 activation) inhibited chemokine-induced polarization, while expression of a constitutively active Rap1 (Rap1V12) induced all the characteristics of polarization, as seen with chemokine stimulation (57). Thus, Rap1 is a major signaling molecule mediating leukocyte polarization. The distribution of LFA-1 integrins to the leading edge of a polarized leukocyte is reported to proceed through a signaling cascade involving Rap1 and its downstream effectors RAPL and the threonine-serine kinase, Mst1 (39, 59) (Fig. 2). GTP-bound Rap1 associates with the protein RAPL, which is a non-kinase

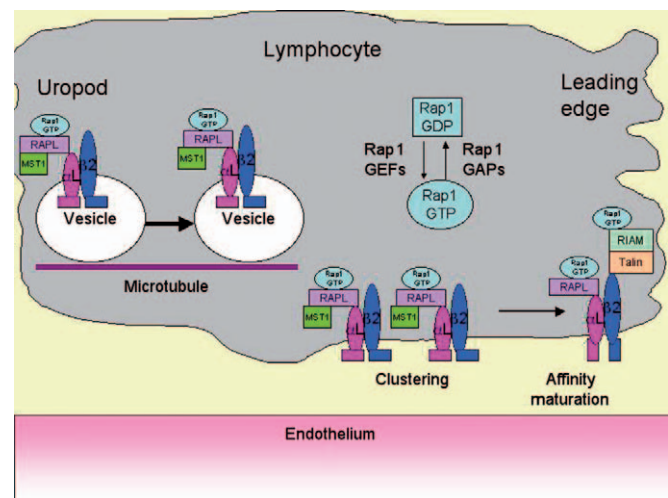


Fig. 2. Model for the role of the small GTPase Rap1 in the redistribution and activation of integrins during lymphocyte polarization. The stimulation of lymphocytes by endothelial surface-bound chemokines triggers the activation of the small GTPase Rap1. Active Rap1 (Rap1-GTP) and its effectors mediate the redistribution of integrins from the rear (uropod) to the front (leading) edge of the lymphocyte during polarization. Furthermore, Rap1 activates integrins at the leading edge. This integrin activation takes the form of both clustering (avidity changes) as well as increased affinity for ligands (affinity maturation). The trimolecular complex of Rap1 bound to the adapter molecule RAPL that associates with the serine-threonine kinase Mst1 mediates the redistribution of integrins from the rear to the front. This occurs by directional transport of integrin-containing vesicles along the cytoskeletal microtubule system. These vesicles containing clustered integrins are delivered to the leading edge of the lymphocyte. Leading edge integrins may be converted to their intermediate and high-affinity ligand-binding states by inside-out signaling mechanisms mediated by active Rap1. Two Rap1 effectors, RAPL and RIAM, act independently but perhaps cooperatively to change integrin affinity. RAPL associated with active Rap1 can bind to the cytoplasmic domain of α -integrin subunits. Such binding results in a conformational switch in the extracellular headpiece of the integrins in which the bent (low affinity) headpiece transitions to an extended (high affinity) position. A second mechanism that contributes to integrin activation mediated by Rap1 is the facilitation of talin binding to the intracellular domain of the β -integrin subunit. This talin binding is mediated by the second Rap1 effector RIAM. The binding of talin to the β integrin subunit also results in the opening of the extracellular integrin headpiece to promote high-affinity-ligand binding.

adapter molecule possessing a central Ras-binding domain mediating its interaction with Rap1 and a C-terminal coil-coil region for interaction with other effector molecules (39). RAPL is a Rassf (Ras suppressor factor) family member, and it is an alternative splice form of Rassf5 (Nore1) (60). The Rap1/RAPL complex alters both Rap1-dependent LFA-1 distribution and activation. Recently, RAPL has been reported to interact with the protein kinase, Mst1, and RAPL redistributes Mst1 from the Golgi to the leading edge (60). Furthermore, the movement of the RAPL/Mst1 complex is associated with transport of LFA-1 in vesicles to the leading edge. This vesicular redistribution

occurs along tracks of the microtubular system. Notably, however, Mst1 does not appear to directly interact with LFA-1, and thus, RAPL may be the direct LFA-1-binding partner in this complex (59).

Rap1 can stimulate integrin activation at the leading edge of a leukocyte through integrin avidity changes (clustering) in addition to affinity modulation (22). Rap1 acting through RAPL has been reported to alter integrin clustering, and RAPL overexpression appears to stimulate soluble ligand binding to LFA-1, suggesting that part of Rap1-induced changes in LFA-1 affinity is through RAPL binding (39) (Fig. 2). This alteration in LFA-1 affinity induced by RAPL is not dependent on Mst1 (59). Thus, the Rap1 effector Mst1 is responsible for redistribution but not activation of LFA-1 during polarization. However, overexpression of constitutively active Rap1 (Rap1V12) induced a greater change in LFA-1 affinity than RAPL, suggesting that Rap1 may alter LFA-1 affinity through additional effector molecules (39). Recently, a second Rap1 effector, RIAM, has been implicated in integrin affinity modulation (38). Rap1 activation results in RIAM association with the integrin α IIb β 3 and its subsequent activation. RIAM also contributes to LFA-1 activation by Rap1 (37). The activation of integrin α IIb β 3 by Rap1 signaling is ultimately dependent on the binding of talin to the β -integrin subunit (38) (Fig. 2). As talin binding also leads to activation of β 1 and β 2 integrins (61, 62), it is likely that the capacity of Rap1-RIAM to activate these integrins will also be mediated by talin. Integrin activation involves alterations in the transmembrane helix packing of integrin α and β subunits (63–65) initiated by interactions at the integrin cytoplasmic domain (66, 67). Talin binding to integrin β subunits activates integrins (68), and a recent study suggested that this event was mediated by a talin-specific interaction with a conserved membrane proximal region of the integrin β tails (69, 70). In sharp contrast, the reported activating effects of Rap1-RAPL on LFA-1 require a specific sequence motif in the α -subunit tail of LFA-1 and are reported to be insensitive to mutations in a conserved NPXF motif in the β 2 tail that is important in talin binding (39). Consequently, Rap1-RIAM-talin and Rap1-RAPL appear to activate integrins through distinct mechanisms (Fig. 2), with RAPL being apparently specific for LFA-1. In sum, the establishment of leukocyte polarity initiated by integrin ligands presented in combination with chemokine cues on the vascular endothelial surface is a key event that precedes transendothelial migration.

Transendothelial migration

Once a leukocyte establishes polarity on the endothelial surface, it can start the process of migrating across the endothelium to

enter the underlying tissues. For the most part, the leukocyte relies on cues in the form of chemokines, adhesive integrin ligands, and shear flow to maintain lateral migration along the endothelial surface until it reaches the junction of two or more endothelial cells. At that site, it migrates between the endothelial cells.

Over the past several years, a model has emerged that explains the overall mechanics of cell migration in extravascular space (70). Major aspects of this paradigm can be applied to the description of leukocyte locomotion over and across endothelial junctions. However, transendothelial migration also involves spatiotemporal bidirectional signaling between the leading edge of the transmigrating leukocyte and specific junctional molecules that reversibly remodel the endothelial junction through contractility events that allow the leukocyte passage and the sealing of the contracted endothelial junction soon after the leukocyte has terminated its passage (71, 72). The general model of cell motility involves repetitive cycles of new projections being sent out at the leading edge in the form of lamellipodia and filopodia (70). At these sites, new adhesions by integrins are laid down with the underlying substratum. This allows for traction as the bulk of the cell body is propelled forward, while at the same time adhesions are released at the rear of the cell. Integrins play key roles in cellular migration acting both as adhesive molecules that maintain locomotion over the apical endothelial surface and as signaling molecules, which, together with chemokine signals, maintain polarity and motility (22).

The signaling needed to coordinate migration is complex. Just as Rap1 activation plays a key role in integrin activation and leukocyte polarization, the activation of the small GTPase Rac has emerged as a key signaling event controlling cell motility on adhesive substrata (73–75). Rac activation drives actin polymerization and lamellipodia formation (76). However, efficient migration requires this Rac activation to be spatially and temporally restricted to the leading edge of the cell (77). A recently defined signaling pathway provides for spatial and temporal regulation of Rac activation during α 4 β 1-integrin-dependent cell migration on both the vascular ligand VCAM-1 and on the extracellular matrix protein fibronectin. This α 4 β 1-dependent modulation of Rac activation is mediated by the reversible binding of paxillin to the α 4 cytoplasmic domain. The α 4 integrin subunits bind directly to the signaling adapter molecule paxillin (78), and this interaction is regulated by selective phosphorylation of the α 4 cytoplasmic domain at serine988 in a protein kinase A-dependent manner (Fig. 3). Phosphorylation at this position leads to release of paxillin from the α 4 subunit, while dephosphorylation promotes the α 4

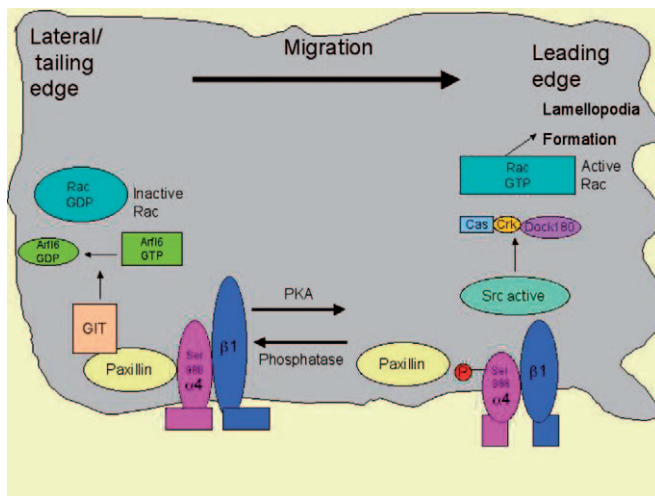


Fig. 3. Model for spatially restricted activation of the small GTPase Rac during $\alpha 4\beta 1$ integrin-dependent lymphocyte migration. A key process in cell migration is the projection of new cell extensions in the form of lamellipodia at the front or leading edge of the cell. Lamellipodia are formed by the polymerization of actin, which is largely driven by the activation of the small GTPase Rac. Thus, for efficient directional migration, Rac activation needs to be spatially limited to the leading edge. During $\alpha 4\beta 1$ integrin-dependent cell migration, the binding of the signaling adapter molecule paxillin to the $\alpha 4$ integrin subunit is spatially restricted, which acts to spatially restrict Rac activation. $\alpha 4$ integrin subunits at the lateral and trailing edges of the cell are bound to paxillin, which recruits the GAP molecule GIT1. GIT1 inhibits the activation of the small GTPase Arf6, which ultimately leads to inhibition of Rac activation, thus preventing lamellipodia formation at these sites. At the leading edge, the $\alpha 4$ integrin subunit is phosphorylated at serine988 in a protein kinase A-dependent fashion. Phosphorylation at this site disrupts the binding of paxillin to the $\alpha 4$ subunit and consequently removes the inhibition of Rac activation mediated by GIT1 recruitment. Furthermore, engagement of $\alpha 4\beta 1$ integrin triggers the activation of Src kinase, which can activate Rac-GEFs such as the Cas/Crk/DOCK180 complex to facilitate Rac activation and lamellipodia formation at the leading edge.

integrin-paxillin interaction (79). In a migrating cell, the phosphorylated $\alpha 4$ integrins are localized to the leading edge of the cell, while dephosphorylated $\alpha 4$ integrins are localized to the lateral and trailing edge of the cell (80). This spatial regulation of $\alpha 4$ integrin-paxillin binding promotes effective leukocyte migration, as either disrupting or enforcing the association of $\alpha 4$ with paxillin greatly impairs cell migration (81).

The $\alpha 4$ integrin-paxillin interaction contributes to effective cell migration by spatially regulating Rac activity. Paxillin bound to $\alpha 4$ integrin provides scaffolding for recruitment of additional signaling molecules to this site (82). One of these recruited signaling molecules is an adenosine diphosphate-ribosylation factor (Arf)-GAP, GIT1 (83) (Fig. 3). GIT1 ultimately leads to inhibition of Rac activation by inhibiting the activation of another small GTPase, Arf6 (84). Arf6 modulates Rac function through mechanisms involving

changes in cellular distribution of Rac1 by endosomal trafficking and recruitment of Rac activators such as a Rac-guanine nucleotide exchange factor (GEF), DOCK180/ELMO (85). Thus, during $\alpha 4$ -dependent migration, the $\alpha 4$ integrin-paxillin interaction contributes to the inhibition of Rac activation at the lateral and trailing edges through the recruitment of Arf-GAP.

$\alpha 4$ integrins can also regulate Rac activation and cell migration through Src kinases, independent of the $\alpha 4$ -paxillin interaction (86). Thus, $\alpha 4\beta 1$ integrin-dependent activation of Rac at the leading edge can proceed through activation of Src kinases (Fig. 3). Polarization and motility are also maintained by inhibition of Rac at the lateral and trailing edges mediated by the $\alpha 4$ integrin-paxillin complexes that recruit an Arf6 inhibitor. These two integrin-mediated signaling pathways can be complemented by chemoattractive signals presented to the leukocyte on the apical junctional and subluminal compartments of the endothelial barrier. When these chemoattractant signals are robust, the $\alpha 4\beta 1$ -Rac activation pathway may be less important for migration. For instance, T cells expressing an $\alpha 4$ -tail mutant that lacks paxillin binding can still respond to chemotactic cues from the chemokine stromal cell-derived factor-1 when crossing ligand-free barriers but show reduced migration toward the same chemoattractants when crossing VCAM-1- or fibronectin-coated barriers (50).

The above described paradigm for $\alpha 4\beta 1$ integrin-dependent regulation of Rac activation and migration is just one mechanism that contributes to leukocyte transendothelial migration. Certainly other cues that drive migration, such as chemoattractants and signals generated by mechanical force on the cell (such as shear flow), will come into play to regulate Rac activation and cell migration. The combined input of these cues will ultimately determine how and where leukocytes migrate. Furthermore, the relative importance of each of these cues may vary widely between different types of leukocytes. For example, while a fluid shear plays an important role in lymphocyte transendothelial migration, it plays a lesser role in neutrophil migration, especially on endothelium expressing high amounts of $\beta 2$ integrin ligands (87, 88).

Not all forms of leukocyte migration are the same with respect to mechanics and signaling. In lateral migration, leukocytes migrate to reach the junction of endothelial cells (89, 90), and in migration across the junction, the leukocyte moves between two endothelial cells. Junctional migration involves highly specialized adhesive interactions between the leukocyte and endothelial cells, which allows for the initial separation and reforming of endothelial junction as the leukocyte passes through (71, 91). These differences in

migration are reflected in different signaling pathways involved. For example, lateral migration of lymphocytes, driven by chemokines, requires DOCK2-dependent activation of Rac, while junctional migration of lymphocytes is DOCK2 independent (92).

Conclusions

Where and when leukocytes leave the blood and enter the peripheral tissues play key roles in immunity and inflammatory diseases. These locations are selected by repertoires of chemoattractant and adhesion molecules. The precisely regulated expression and functional state of these molecules allows for accurate specification of leukocyte trafficking throughout the body. Integrins are adhesion and signaling molecules involved

in the adhesive interactions between leukocytes and the vascular endothelium needed to resist the physical forces of blood flow and mediate effective migration across the endothelium. To meet the challenge of adhesion under shear flow conditions, leukocyte integrins link to the cytoskeleton to resist detachment under shear flow condition and undergo rapid reversible modulation of their ligand-binding affinities within subseconds of stimulation by chemoattractants. In addition, integrin-mediated signal transduction must be both spatially and temporally regulated in leukocytes to allow for effective migration across the endothelial barrier. Unraveling the molecular machineries that regulate adhesion, motility, and transendothelial migration in different subsets of leukocytes may help to identify therapeutic targets to selectively modify leukocyte trafficking.

References

- Springer TA. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. *Cell* 1994;**76**:301–314.
- Butcher EC, Picker LJ. Lymphocyte homing and homeostasis. *Science* 1996;**272**:60–66.
- Luster AD, Alon R, von Andrian UH. Immune cell migration in inflammation: present and future therapeutic targets. *Nat Immunol* 2005;**6**:1182–1190.
- McEver RP. Selectins: lectins that initiate cell adhesion under flow. *Curr Opin Cell Biol* 2002;**14**:581–586.
- Rosen SD. Ligands for L-selectin: homing, inflammation, and beyond. *Annu Rev Immunol* 2004;**22**:129–156.
- Sperandio M, et al. P-selectin glycoprotein ligand-1 mediates L-selectin-dependent leukocyte rolling in venules. *J Exp Med* 2003;**197**:1355–1363.
- DeGrendele HC, Estess P, Picker LJ, Siegelman MH. CD44 and its ligand hyaluronate mediate rolling under physiological flow: a novel lymphocyte-endothelial cell primary adhesion pathway. *J Exp Med* 1996;**183**:1119–1130.
- Dwir O, Kansas GS, Alon R. Cytoplasmic anchorage of L-selectin regulates leukocyte capture and rolling by controlling the mechanical stability of selectin:ligand tethers. *J Cell Biol* 2001;**155**:145–156.
- Ivetic A, Deka J, Ridley A, Ager A. The cytoplasmic tail of L-selectin interacts with members of the ezrin-radixin-moesin (ERM) family of proteins: cell activation-dependent binding of moesin but not ezrin. *J Biol Chem* 2002;**277**:2321–2329.
- Kansas GS, Ley K, Munro JM, Tedder TF. Regulation of leukocyte rolling and adhesion to high endothelial venules through the cytoplasmic domain of L-selectin. *J Exp Med* 1993;**177**:833–838.
- Setiadi H, Sedgewick G, Erlandsen SL, McEver RP. Interactions of the cytoplasmic domain of P-selectin with clathrin-coated pits enhance leukocyte adhesion under flow. *J Cell Biol* 1998;**142**:859–871.
- Snapp KR, Heitzin CE, Kansas GS. Attachment of the PSGL-1 cytoplasmic domain to the actin cytoskeleton is essential for leukocyte rolling on P-selectin. *Blood* 2002;**99**:4494–4502.
- Phan UT, Waldron TT, Springer TA. Remodeling of the lectin-EGF-like domain interface in P- and L-selectin increases adhesiveness and shear resistance under hydrodynamic force. *Nat Immunol* 2006;**7**:883–889.
- Carman CV, Springer TA. Integrin avidity regulation: are changes in affinity and conformation underemphasized? *Curr Opin Cell Biol* 2003;**15**:547–556.
- Alon R, Feigelson S. From rolling to arrest on blood vessels: leukocyte tap dancing on endothelial integrin ligands and chemokines at sub-second contacts. *Semin Immunol* 2002;**14**:93–104.
- Vajkoczy P, Laschinger M, Engelhardt B. $\alpha 4$ -integrin mediates G-protein independent capture of encephalitogenic T cell blasts on endothelial VCAM-1 in spinal cord white matter microvessels. *J Clin Invest* 2001;**108**:557–565.
- Henderson RB, et al. The use of lymphocyte function-associated antigen (LFA-1)-1-deficient mice to determine the role of LFA-1, Mac-1, and $\alpha 4$ integrin in the inflammatory response of neutrophils. *J Exp Med* 2001;**194**:219–226.
- Dunne JL, Collins RG, Beaudet AL, Ballantyne CM, Ley K. Mac-1, but not LFA-1, uses intercellular adhesion molecule-1 to mediate slow leukocyte rolling in TNF- α -induced inflammation. *J Immunol.* 2003;**171**: 6105–6111.
- Salas A, Shimaoka M, Kogan AN, Harwood C, von Andrian UH, Springer TA. Rolling adhesion through an extended conformation of integrin $\alpha_4\beta_2$ and relation to αI and βI -like domain interaction. *Immunity* 2004;**20**:393–406.
- Rot A, von Andrian UH. Chemokines in innate and adaptive host defense: basic chemokine grammar for immune cells. *Annu Rev Immunol* 2004;**22**:891–948.
- Alon R, Dustin M. Force as a facilitator of integrin conformation changes during leukocyte arrest on blood vessels and antigen-presenting cells. *Immunity* 2007;**26**:17–27.
- Kinashi T. Intracellular signaling controlling integrin activation in lymphocytes. *Nat Rev Immunol* 2005;**5**:546–559.
- Laudanna C, Alon R. Right on the spot. Chemokine triggering of integrin-mediated arrest of rolling leukocytes. *Thromb Haemost* 2006;**95**:5–11.
- Hynes RO. Integrins: bidirectional, allosteric signaling machines. *Cell* 2002;**110**:673–687.
- Kim M, Carman CV, Yang W, Salas A, Springer TA. The primacy of affinity over clustering in regulation of adhesiveness of the integrin $\alpha_4\beta_2$. *J Cell Biol* 2004;**167**:1241–1253.
- Ginsberg MH, Partridge A, Shattil SJ. Integrin regulation. *Curr Opin Cell Biol* 2005;**17**: 509–516.
- Grabovsky V, et al. Subsecond induction of $\alpha 4$ integrin clustering by immobilized chemokines stimulates leukocyte tethering and rolling on endothelial vascular cell adhesion molecule 1 under flow conditions. *J Exp Med* 2000;**192**:495–506.
- Shamri R, et al. Lymphocyte arrest requires instantaneous induction of an extended LFA-1 conformation mediated by endothelium-bound chemokines. *Nat Immunol* 2005;**6**:429–430.

29. Liu S, Claderwood DA, Ginsberg MH. Integrin cytoplasmic domain-binding proteins. *J Cell Sci* 2000;**113**:3563–3571.
30. Feigelson SB, Grabovsky V, Shamri R, Levy S, Alon R. The CD81 tetraspanin facilitates instantaneous leukocyte VLA-4 adhesion strengthening to VCAM-1 under shear flow. *J Biol Chem* 2003;**278**:51203–51212.
31. Ticchioni M, et al. Integrin-associated protein (CD47/IAP) contributes to T cell arrest on inflammatory vascular endothelium under flow. *FASEB J* 2001;**15**:341–350.
32. Suga K, et al. CD98 induces LFA-1 mediated cell adhesion in lymphoid cells via activation of Rap1. *FEBS Lett* 2001;**489**:249–253.
33. Nandi A, Estess P, Siegelman M. Bimolecular complex between rolling and firm adhesion receptors required for cell arrest; CD44 association with VLA-4 in T cell extravasation. *Immunity* 2004;**20**:455–465.
34. Ley K. Arrest chemokines. *Microcirculation* 2003;**10**:289–295.
35. Laudanna C, Kim JY, Constantin G, Butcher E. Rapid leukocyte integrin activation by chemokines. *Immunol Rev* 2002;**186**:37–46.
36. Bos JL. Linking Rap to cell adhesion. *Curr Opin Cell Biol* 2005;**17**:123–128.
37. Lafuente EM, et al. RIAM, an Ena/VASP and Profilin ligand, interacts with Rap1-GTP and mediates Rap1-induced adhesion. *Dev Cell* 2004;**7**:462–463.
38. Han J, et al. Reconstructing and deconstruction agonist-induced activation of integrin α IIb β 3. *Curr Biol* 2006;**16**:1796–1806.
39. Katagari K, Maeda A, Shimonaka M, Kinashi T. RAPL, a Rap1-binding molecule that mediate Rap1-induced adhesion through spatial regulation of LFA-1. *Nat Immunol* 2003;**4**:741–748.
40. Kim M, Carman CV, Springer TA. Bidirectional transmembrane signaling by cytoplasmic domain separation in integrins. *Science* 2003;**301**:1720–1725.
41. Neelamagham S, Taylor AD, Burns AR, Smith CW, Simon SI. Hydrodynamic shear shows distinct roles for LFA-1 and Mac-1 in neutrophil adhesion to intercellular adhesion molecule-1. *Blood* 1998;**92**:1626–1638.
42. Nijhara R, et al. Rac1 mediates collapse of microvilli on chemokine-activated T lymphocytes. *J Immunol* 2004;**173**:4985–4993.
43. Atarashi K, Hirata T, Matsumoto M, Kanemitsu N, Miyasaka M. Rolling of Th1 cells via P-selectin glycoprotein ligand-1 stimulates LFA-1 mediated cell binding to ICAM-1. *J Immunol* 2005;**174**:1424–1432.
44. Kunkel EJ, Dunne JL, Ley K. Leukocyte arrest during cytokine-dependent inflammation in vivo. *J Immunol* 2000;**164**:3301–3308.
45. Simon SI, Hu Y, Vestweber D, Smith CW. Neutrophil tethering on E-selectin activates β 2 integrin binding to ICAM-1 through a mitogen-activated protein kinase signal transduction pathway. *J Immunol* 2000;**164**:4348–4358.
46. Zwartz GJ, Chigaev A, Dwyer DC, Foutz TD, Edwards BS, Sklar LA. Real-time analysis of very late antigen-4 affinity modulation by shear. *J Biol Chem* 2004;**279**:38277–38286.
47. Constantin G, et al. Chemokines trigger immediate β 2 integrin affinity and mobility changes: differential regulation and roles in lymphocyte arrest under flow. *Immunity* 2000;**13**:759–769.
48. Green CE, Pearson DN, Camphausen RT, Staunton DE, Simon SI. Shear-dependent capping of L-selectin glycoprotein ligand 1 by E-selectin signals activation of high-avidity β 2-integrin on neutrophils. *J Immunol* 2004;**172**:7780–7790.
49. Alon R, et al. α 4 β 1-dependent adhesion strengthening under mechanical strain is regulated by paxillin association with the α 4-cytoplasmic domain. *J Cell Biol* 2005;**171**:1073–1084.
50. Rose DM, Liu S, Woodside DG, Han J, Schlaepfer DD, Ginsberg MH. Paxillin binding to the α 4 integrin subunit stimulates LFA-1 (integrin α 4 β 2)-dependent T cell migration by augmenting the activation of focal adhesion kinase/praline-rich tyrosine kinase-2. *J Immunol* 2003;**170**:5912–5918.
51. Sánchez-Madrid F, Angel del Pozo M. Leukocyte polarization in cell migration and immune interactions. *EMBO J* 1999;**18**:501–511.
52. Schwartz MA, Horwitz AR. Integrating adhesion, protrusion, and contraction during cell migration. *Cell* 2006;**125**:1223–1225.
53. Gupton SL, Waterman-Storer CM. Spatiotemporal feedback between actomyosin and focal adhesion systems optimizes rapid cell migration. *Cell* 2006;**125**:1361–1374.
54. Gomes ER, Jani S, Gunderson GG. Nuclear movement regulated Cdc42, MRCK, myosin, and actin flow establishes MTOC polarization in migrating cells. *Cell* 2005;**121**:451–463.
55. Magdalena J, Millard TH, Etienne-Manneville S, Launay S, Warwick HK, Machesky LM. Involvement of the Arp2/3 complex and Scar2 in Golgi polarity in scratch wound models. *Mol Biol Cell* 2003;**14**:670–684.
56. Coates TD, Watts RG, Hartman R, Howard TH. Relationship of F-actin distribution to development of polar shape in human polymorphonuclear neutrophils. *J Cell Biol* 1992;**117**:765–774.
57. Shimonaka M, et al. Rap1 translates chemokine signals to integrin activation, cell polarization, and motility across vascular endothelium under flow. *J Cell Biol* 2003;**161**:417–427.
58. Green GE, Schaff UY, Sarantos MR, Lum AF, Staunton DE, Simon SI. Dynamic shifts in LFA-1 affinity regulate neutrophil rolling, arrest, and transmigration on inflamed endothelium. *Blood* 2006;**107**:2101–2111.
59. Katagari K, Imamura M, Kinashi T. Spatio-temporal regulation of the kinase Mst1 by binding protein RAPL is critical for lymphocyte polarity and adhesion. *Nat Immunol* 2006;**7**:919–928.
60. Tommasi S, Dammann R, Jin S, Zhang X, Avruh J, Pfeifer GP. RASSF3 and NRE1: identification and cloning of two human homologues of the putative tumor suppressor gene RASSF1. *Oncogene* 2002;**21**:2713–2720.
61. Tadokoro S, et al. Talin binding to integrin β tails: a final common step in integrin activation. *Science* 2003;**302**:103–106.
62. Simonson WT, Franco SJ, Huttenlocher A. Talin1 regulates TCR-mediated LFA-1 function. *J Immunol* 2006;**177**:7707–7714.
63. Luo BH, Carman CV, Takagi J, Springer TA. Disrupting integrin transmembrane domain heterodimerization increases ligand binding affinity, not valency or clustering. *Proc Natl Acad Sci USA* 2005;**102**:3679–3684.
64. Partridge AW, Liu S, Kim S, Bowie JU, Ginsberg MH. Transmembrane domain helix packing stabilizes integrin α IIb β 3 in the low affinity state. *J Biol Chem* 2005;**280**:7294–7300.
65. Li W, et al. A push-pull mechanism for regulating integrin function. *Proc Natl Acad Sci USA* 2005;**102**:1424–1429.
66. Xie C, Shimaoka M, Xiao T, Schwab P, Klickstein LB, Springer TA. The integrin α subunit leg extends at a Ca²⁺-dependent epitope in the thigh/genu interface upon activation. *Proc Natl Acad Sci USA* 2004;**101**:15422–15427.
67. Nishida N, Xie C, Shimaoka M, Cheng Y, Walz T, Springer TA. Activation of leukocyte β 2 integrins by conversion from bent to extended conformations. *Immunity* 2006;**25**:583–594.
68. Ulmer TS, Calderwood DA, Ginsberg MH, Campbell ID. Domain-specific interactions of talin with the membrane-proximal region of the integrin β 3 subunit. *Biochemistry* 2003;**42**:8307–8312.
69. Wegener KL, et al. Structural basis of integrin activation by talin. *Cell* 2007;**128**:171–182.
70. Ridley AJ, et al. Cell migration: integrating signals from front to back. *Science* 2003;**302**:1704–1709.
71. Shaw SK, et al. Coordinated redistribution of leukocyte LFA-1 and endothelial cell ICAM-1 accompany neutrophil transmigration. *J Exp Med* 2004;**200**:1571–1580.
72. Ostermann G, Weber KA, Zernecke A, Schroder A, Weber C. JAM-1 is a ligand of the β 2 integrin LFA-1 involved in transendothelial migration of leukocytes. *Nat Immunol* 2002;**3**:151–158.
73. Srinivasan S, et al. Rac and Cdc42 play distinct roles in regulating PI(3,4,5)P3 and polarity during neutrophil chemotaxis. *J Cell Biol* 2003;**160**:375–385.

74. Xu J, et al. Divergent signals and cytoskeletal assemblies regulate self-organizing polarity in neutrophils. *Cell* 2003;**114**:201–214.
75. Fenteany G, Glogauer M. Cytoskeletal remodeling in leukocyte function. *Curr Opin Hematol* 2004;**11**:112.
76. Hall A. Rho GTPases and the control of cell behavior. *Biochem Soc Trans* 2005;**33**: 891–895.
77. Kraynov VS, Chamberlain C, Bokoch GM, Schwartz MA, Slabaugh S, Hahn KM. Localized Rac activation dynamics visualized in living cells. *Science* 2000;**290**:333–337.
78. Liu S, et al. Binding of paxillin to alpha4 integrins modifies integrin-dependent biological response. *Nature* 1999;**402**: 676–681.
79. Han J, Liu S, Rose DM, Schlaepfer DD, McDonald H, Ginsberg MH. Phosphorylation of the integrin alpha 4 cytoplasmic domain regulates paxillin binding. *J Biol Chem* 2001;**276**:40903–40909.
80. Goldfinger LE, Han J, Kiosses WB, Howe AK, Ginsberg MH. Spatial restriction of alpha4 integrin phosphorylation regulates lamellipodial stability and alpha4beta1-dependent cell migration. *J Cell Biol* 2003;**162**: 731–741.
81. Han J, Rose DM, Woodside DG, Goldfinger LE, Ginsberg MH. Integrin alpha4beta1-dependent T cell migration requires both phosphorylation and dephosphorylation of the alpha4 cytoplasmic domain to regulate the reversible binding of paxillin. *J Biol Chem* 2003;**278**:34845–34853.
82. Brown MC, Turner CE. Paxillin: adapting to change. *Physiol Rev* 2004;**84**:1315–1339.
83. Nishiya N, Kiosses W, Han J, Ginsberg MH. An α_4 integrin–paxillin–Arf-GAP complex restricts Rac activation to the leading edge of migrating cells. *Nat Cell Biol* 2005;**7**: 343–352.
84. Vitale N, Patton WA, Moss J, Vaughan M, Lefkowitz RJ, Premont RT. GIT proteins, a novel family of phosphatidylinositol 3, 4, 5-triphosphate-stimulated GTPase-activating proteins for ARF6. *J Biol Chem* 2000;**275**:13901–13906.
85. Santy LC, Ravichandran KS, Casanova JE. The DOCK180/Elmo complex couples ARNO-mediated Arf8 activation to the downstream activation of Rac1. *Curr Biol* 2005;**15**: 1749–1754.
86. Hsia DA, et al. Integrin $\alpha_4\beta_1$ promotes focal adhesion kinase-independent cell motility via α_4 cytoplasmic domain-specific activation of c-Src. *Mol Cell Biol* 2005;**25**:9700–9712.
87. Cinamon G, Shinder V, Alon R. Shear forces promote lymphocyte migration across vascular endothelium bearing apical chemokines. *Nat Immunol* 2001;**2**:515–522.
88. Cinamon G, Shinder V, Shamri R, Alon R. Chemoattractant signals and beta 2 integrin occupancy at apical endothelial contacts combine with shear stress signals to promote transendothelial neutrophils migration. *J Immunol* 2004;**173**:7282–7291.
89. Schenkel AR, Mamdouh Z, Muller WA. Locomotion of monocytes on endothelium is a critical step during extravasation. *Nat Immunol* 2004;**5**:393–400.
90. Phillipson M, Heit B, Colarusso P, Liu L, Ballantyne CM, Kubes P. Intraluminal crawling of neutrophils to emigration sites: a molecularly distinct process from adhesion in the recruitment cascade. *J Exp Med* 2006;**203**:2569–2575.
91. Carman CV, Springer TA. A trans migratory cup in leukocyte diapedesis both through individual vascular endothelial cells and between them. *J Cell Biol* 2004;**167**: 377–388.
92. Shulman Z, et al. DOCK2 regulates chemokine-triggered lateral lymphocyte motility but not transendothelial migration. *Blood* 2006;**108**:2150–2158.