Immunity
Review

Force as a Facilitator of Integrin Conformational Changes during Leukocyte Arrest on Blood Vessels and Antigen-Presenting Cells

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Integrins comprise a large family of cell-cell and cell-matrix adhesion receptors that rapidly modulate their adhesiveness. The arrest of leukocyte integrins on target vascular beds involves instantaneous conformational switches generating shear-resistant adhesions. Structural data suggest that these integrins are maintained in low-affinity conformations and must rapidly undergo conformational switches transduced via cytoplasmic changes ("inside-out" signaling) and simultaneous ligand-induced rearrangements ("outside-in"). This bidirectional activation is accelerated by signals from endothelial chemoattractants (chemokines). Recent studies predict that shear forces in the piconewton (pN) range per integrin can facilitate these biochemical switches. After extravasation, antigen recognition involves smaller internal forces from cytoskeletal motors and actin polymers forming the immune synapse. In this review, we address how forces facilitate allosteric integrin activation by biochemical signals. Evidence suggests that preformed cytoskeletal anchorage rather than free integrin mobility is key for force-enhanced integrin activation by chemokines and TCR signals.

Introduction

Leukocytes circulating in the blood are recruited to lymphoid organs and peripheral sites of injury, infection, and inflammation by a series of sequential but overlapping steps mediated by members of two major adhesion receptor families, selectins and integrins (Butcher, 1991). Multiple molecular options at each step provide large combinatorial diversity, generating high specificity and tissue selectivity in leukocyte-endothelial cell recognition. In order to appreciate the contributions of integrins to leukocyte rolling and arrest on blood vessels, we will first introduce concepts initially elucidated from the selectin family.

Selectins Are Structurally Adapted to Mediate the Capture and Rolling of Circulating Leukocytes on Target Endothelial Sites

Selectins are the main receptors that mediate the initial capture of circulating leukocytes to ligands expressed on the endothelium (McEver, 2002). Leukocyte capture is followed by rolling adhesions, which are maintained on a timescale of seconds to a few minutes, depending on the selectin type involved (Figure 1). The selectins comprise a three-member family (L-, P-, and E-selectins) sharing highly conserved N-terminal C-type lectin domains followed by regulatory epidermal growth factor (EGF)-like domains and short consensus repeats. All selectins recognize sialyl Lewis X and related carbohydrate ligands, presented mainly by sialomucin-like surface molecules (McEver, 2002; Rosen, 2004). The leukocyte selectin, L-selectin, is expressed on most circulating leukocytes and is the key receptor that initiates leukocyte capture events in secondary lymphoid tissues and at peripheral sites of injury and inflammation (Rosen, 2004). P- and E-selectins are inducibly expressed in both acutely and chronically stimulated endothelial beds and are critical mediators of slow rolling adhesions (Ley et al., 1995; McEver, 2002). Selectin-mediated adhesions are characterized by fast on and off rates and exceptional resistance to disruptive shear forces exerted on the leukocyte at the vessel wall (Alon et al., 1995). Selectins and their ligands are localized to microvilli-like projections, favorable sites for leukocyte-endothelial collisions (von Andrian et al., 1995). Selectin bonds undergo conformational changes that decrease their off rate under low tensile forces, giving them properties of "catch bonds" (Thomas et al., 2002; Marshall et al., 2003; Sarangapani et al., 2004; Zhu et al., 2005), and their lectin-EGF interdomain hinge may critically regulate this key mechanical property (Lou et al., 2006; Phan et al., 2006). The interdomain hinge may also control the rotational freedom of the selectin lectin domain (Lou et al., 2006). At high forces, however, there is a consistent but mild increase in the off rate of selectin bonds, a behavior characteristic of "slip bonds" (Dembo et al., 1988). The mechanical stability of selectin interactions is also conferred by selectin or ligand dimerization, which disperses high shear forces applied on the leukocyte-endothelial contact over multiple bonds (Chen and Springer, 1999; Li et al., 1998; Ramachandran et al., 2001).

Evidence suggests that, in order to resist shear-derived detaching forces, both selectins and their ligands also need to be properly anchored to the cytoskeleton (Kansas et al., 1993; Setiadi et al., 1998; Dwir et al., 2001; Ivetic

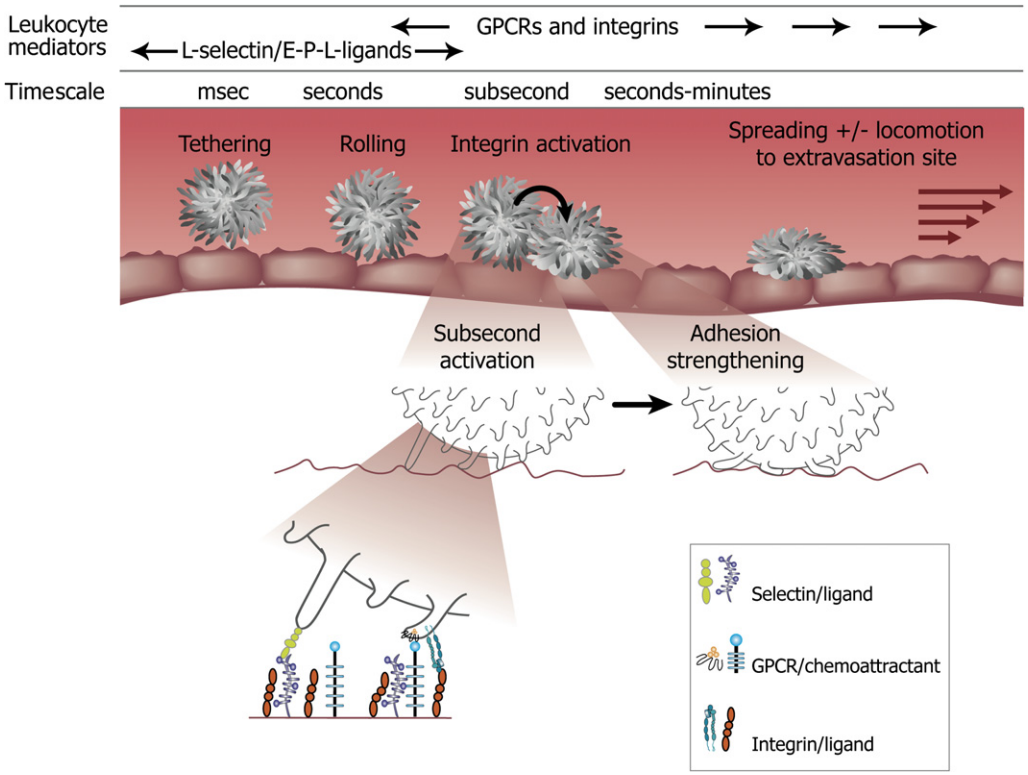

Figure 1. Integrin Activation on Selectin-Occupied Leukocytes Rolling on Vessel Walls Presenting Chemoattractants
Adhesive cascades generated by leukocytes recruited to different endothelial sites and their timescales. Leukocyte microvilli are preferential sites of collision and serve as elastic projections that reduce the forces applied to given selectin or integrin bonds. Selectin-mediated adhesions bring leukocytes into proximity with clusters of chemoattractants (chemokines) and integrin ligands. Initial activation of integrins by the chemoattractant receptor GPCR occurs within a subsecond period, possibly within singular microvillar contacts. For simplicity, selectin and integrin bonds are shown to form on two separate microvilli, but at high densities of integrin ligands and activating chemokines, a given microvillus may simultaneously occupy both selectin and integrin bonds. Microvillar flattening during rolling or following initial integrin activation can bring more integrins and chemoattractant receptors into contact with their endothelial ligands and facilitate adhesion strengthening after the initial arrest.

et al., 2002; Snapp et al., 2002). Thus, conformations of selectin-ligand pairs that are stabilized by force (Phan et al., 2006) may require anchorage of selectins and ligands within their respective cell membranes. In agreement with this possibility, bonds mediated by tailless L-selectin fail to stabilize adhesive interactions even under low-force conditions and break within milliseconds (Dwir et al., 2001; Dwir et al., 2003). Microvillar localization of L-selectin, which enhances the selectin availability on the leukocyte surface (von Andrian et al., 1995), may also disperse the forces exerted on the stiff selectin-ligand bond (Fritz et al., 1998) since microvilli are highly elastic projections (Shao et al., 1998). To disperse forces along the microvilli axis, ligand-occupied selectins must be properly anchored to the leukocyte microvilli (Figure 1). Indeed, chemical stiffening of microvilli destabilizes selectin-mediated adhesions under shear flow (Yago et al., 2002).

Leukocyte Integrin Activation: Structural Basis

The arrest of tethered or rolling leukocytes on target endothelium is nearly exclusively mediated by members of the integrin superfamily and their endothelial immunoglobulin superfamily (IgSF) ligands (Hynes, 2002). Integrins constitute a family of over 20 heterodimers whose ligand-binding activity can be rapidly regulated by conformational changes, clustering, and redistribution from surface and intracellular pools (Carman and Springer, 2003; Dustin et al., 2004). The most relevant integrins for leukocyte adhesion to endothelial targets are members of the β_2 subfamily, especially LFA-1 (CD11a/CD18 or $\alpha_L\beta_2$) and the myeloid-specific integrin Mac-1 ($\alpha_M\beta_2$), as well as the two α_4 integrins, $\alpha_4\beta_1$ (VLA-4) and $\alpha_4\beta_7$ (Hynes, 2002). With the exception of T cell and B cell blasts (Vajkoczy et al., 2001) and subsets of innate immune cells that express highly adhesive integrins, circulating leukocytes maintain their integrins in largely inactive states. They must therefore undergo in situ modulation to develop high-affinity and shear-resistant adhesiveness for their specific endothelial ligands. Both in vitro and in vivo data suggest that integrin adhesiveness can be increased during subsecond contacts with stimulatory endothelial signals (Alon et al., 2003).

New insights into the structural and functional regulation of LFA-1, the most thoroughly investigated leukocyte integrin, have been provided by studies of its main

ligand-binding domains and detailed structural analysis of related integrins, including the platelet integrin $\alpha_{IIb}\beta_3$, the vitronectin receptor $\alpha_v\beta_3$, the β_2 integrins Mac-1 and $\alpha_x\beta_2$, and the fibronectin receptor $\alpha_5\beta_1$ (Adair et al., 2005; Shimaoka et al., 2003; Takagi et al., 2001; Xiong et al., 2001, 2002; Nishida et al., 2006). These studies took advantage of X-ray-based structural analysis, NMR, or ultrastructural imaging by negative stain electron microscopy. Use of monoclonal antibodies that recognize neoepitopes induced by conformational switches on native and mutated integrins has also provided key insights into integrin rearrangements and activation states (Beglova et al., 2002; Xie et al., 2004). The α and β subunit of all integrin heterodimers fold to form an extracellular headpiece connected to the membrane by two "legs," followed by a short transmembrane and cytoplasmic tail (Figure 2). The headpiece of all integrins is formed by a β-propeller domain of the α subunit, which closely interacts with a dinucleotide fold domain within the β subunit, termed the β I domain (also known as the I-like domain). The β I domain is linked to the hybrid domain, part of the upper leg that acts as a lever, and the angle between these two β subunit domains controls the β I domain conformation and the ligand-binding affinity of the headpiece (Figure 2). The integrin legs are formed by multiple globular domains that incorporate two knee-like joints referred to as a "genu."

Inactive integrins are compact and bent (Xiong et al., 2001; Takagi et al., 2002), with their genu folded and the headpiece only 5 nm from the membrane (Figure 2, top). Separation of the α and β subunit legs, a critical step in inside-out integrin activation, destabilizes their interface with the headpiece, converting the bent structure to an overall extended conformation and relieving constraints on headpiece activation (Figure 2, top). This separation is driven by unclasping intersubunit associations between the transmembrane and cytoplasmic integrin domains. When the genu is straightened through this switchblade-like unbending, the integrin headpiece can project 20–25 nm above the membrane, becoming readily available for surface-presented ligand (Beglova et al., 2002; Salas et al., 2004; Zhang et al., 2005; Nishida et al., 2006) NMR and X-ray structures of the EGF-like domains C-terminal to the β subunit hybrid domain suggest that these domains, and in particular the EGF-like domain 2 (Figure 2, middle right ribbon diagram), are key in translating cytoplasmic tail rearrangements into separation of the α and β subunit legs and straightening of the integrin ectodomain (Beglova et al., 2002; Xiong et al., 2001).

Affinity states of all integrins are also tightly controlled by local rearrangements of their headpiece. Notably, these rearrangements can be initiated both by extrinsic ligands and by binding of specific adaptors to the cytoplasmic domain clasp (Arnaout et al., 2002; Carman and Springer, 2003). The conformation of a critical ligand-binding site termed the metal-ion-dependent adhesion site (MIDAS) dictates the affinity of ligand binding to the integrin headpiece. The metal ion bound at this site is coordinated by a key carboxylate residue shared by all integrin ligands (Hynes, 2002), and the coordination of this metal dictates the overall conformation of the MIDAS loops and the headpiece affinity to ligand. Whereas the ligand-binding sites of most integrins are composed of residues from both their α subunit β-propeller and β I domains (Figure 2, lower row), the ligand-binding site of LFA-1, as well as of other β_2 integrins and subsets of β_1 and β_7 integrins, is located in an α I domain, inserted atop the α subunit β-propeller domain (Shimaoka et al., 2003) (Figure 2, middle row). The β I and the α I domains share a similar fold and MIDAS motifs. Lateral movements of loops that form the MIDAS upon ligand binding result in allosteric (outside-in) rearrangements in the C-terminal α7 helix (Figure 2, middle and bottom rows). Activation and opening of the β I MIDAS can result from molecular interactions within the cytoplasmic domains (Takagi et al., 2001) that exert a swing-out of the β subunit hybrid domain (Figure 2, inside-out). This critical conformational switch can pull on the C-terminal α helix of the β I domain in a manner similar to a bell rope. Notably, in α I domain-containing integrins, the β I domain does not directly participate in ligand binding but can pull the C-terminal α helix of the α I domain downward through an intramolecular bond between an invariant glutamate on the α subunit and the β I MIDAS (Figure 2, middle row). This in turn exerts a second downward pull on the seventh α helix of the β I domain (Shimaoka et al., 2003).

To summarize, changes in conformation initiated at the cytoplasmic clasp and transmitted to the headpiece MIDAS domain (or domains) via the leg domains are referred to as inside-out activation (Dustin and Springer, 1989). When ligand binding to the headpiece domain induces changes in the headpiece as well as in the orientation of the leg domains and the cytoplasmic regions, this is referred to as outside-in activation. Consistent with this model, LFA-1 tail unclasping, a marker of integrin activation, can also be induced by integrin occupancy with its ligand, ICAM-1 (Kim et al., 2003). Intersubunit interactions including the cytoplasmic clasp, transmembrane domains, and leg interactions will tend to maintain the integrin in its low-affinity closed state. Although high-affinity integrin states can be artificially induced by chemically locking extended and open integrin conformations or by freezing these states with monoclonal antibodies prior to ligand binding (Carman and Springer, 2003), physiological activation of integrins appears to involve simultaneous bidirectional activation induced by both inside-out and outside-in rearrangements (Figure 2).

VLA-4 and $\alpha_4\beta_7$

In contrast to the great advances in our understanding of β_2 and β_3 integrin affinity modulation, there is still limited information regarding how α_4 integrins undergo conformational activation by inside-out and outside-in signals. Although there is no current evidence for overall extension of these two integrins, ligand binding by three non-β_2 integrins, VLA-5, $\alpha_{IIb}\beta_3$, and $\alpha_v\beta_3$, induces a canonical swing-out of their β subunit hybrid domain (Chen et al., 2004). Since α_4 integrins on both resting lymphocytes

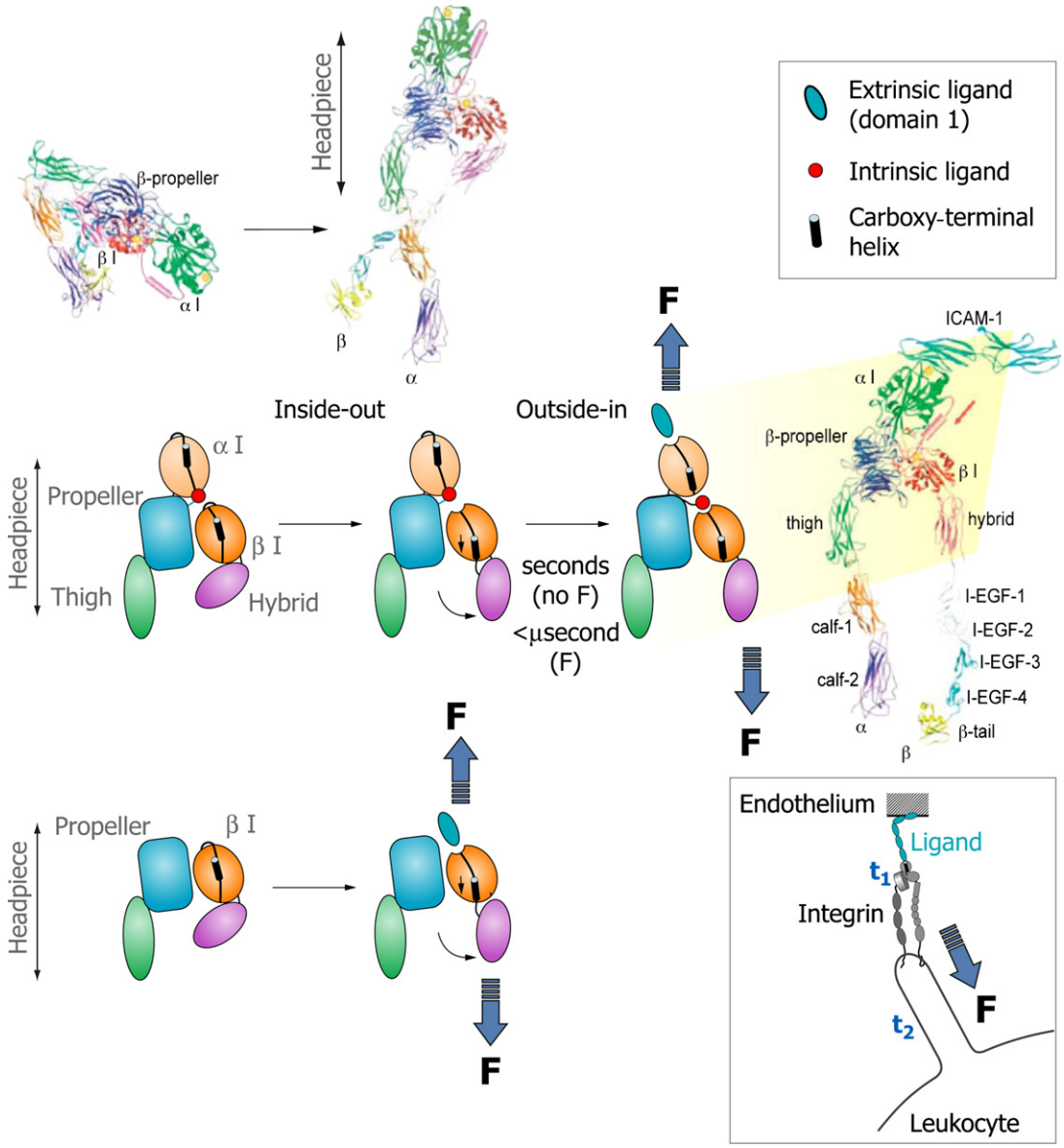

Figure 2. Proposed Role for External (Shear) Forces Applied on Ligand-Integrin Complexes in α and β I Domain Activation
Integrin activation is triggered by a switchblade-like extension of its bent form that increases headpiece accessibility to surface-bound ligand (top row). The middle row depicts bidirectional headpiece activation of the α I domain-containing integrin LFA-1 as a prototype. A partial opening of the β I domain is driven by a swing-out of the β subunit hybrid domain (purple) through inside-out activation signals. The extrinsic ligand then occupies and further activates (outside-in) the α I domain. In addition, the β I domain must be occupied with an intrinsic ligand to enable maximal stabilization. Bottom row: Bidirectional activation of an integrin lacking an α I domain. When loaded with low forces (F, blue arrows, middle and bottom rows), the various ligand-occupied I domains are predicted to undergo these activation events within less than a microsecond (Puklin-Faucher et al., 2006), whereas in the absence of force, these events take seconds. Bottom right: Integrin anchorage is required for the integrin to load low forces (<30 pN) and undergo instantaneous activation by surface-bound ligand (t_1, millisecond time range). Subsequent dispersion of the applied forces across the much softer microvillus may take place via microvillus extension (t_2, subsecond range). At t > 1 s, the ligand-occupied integrin can break apart from the cytoskeleton (not shown), allowing a long membrane cylinder to extend before final bond dissociation (Shao et al., 1998) (Heinrich et al., 2005). (Modified from Carman and Springer, 2003; Zhang et al., 2005).

and monocytes can spontaneously interact with their respective endothelial ligands when present at high density (Berlin et al., 1995), it is possible that these integrins exist in overall extended conformations with high accessibility to their endothelial ligands. Accessibility of α_4 integrins may also result from their relatively high occupancy on microvilli (Berlin et al., 1995). Notably, chemokines that potently stimulate T cell VLA-4 adhesiveness to VCAM-1 under shear flow fail to trigger activation epitopes on VLA-4 that report high affinity to ligands (Grabovsky et al., 2000) but do trigger LFA-1 extension and activation epitopes (Shamri et al., 2005). α_4 integrin adhesiveness under shear forces can be therefore augmented by conformational switches in their cytoplasmic domains that do not alter

integrin affinity to ligand in shear-free conditions (Alon et al., 2005). Recent findings also indicate that VLA-4 can undergo direct conformational activation by shear stress generated by circular stirring (Zwartz et al., 2004). Taken together, externally applied shear force is an additional factor in VLA-4 activation by ligand overlooked by ligand-binding assays conducted in shear-free conditions.

Force Exerted on Leukocyte Integrins at Vessel Walls: A Barrier or a Positive Regulator?

Low forces (<30 pN) appear to stabilize extended headpiece conformations of selectins associated with strengthened selectin-ligand bonds (Marshall et al., 2003; Sarangapani et al., 2004; Phan et al., 2006). Since full α I domain integrin activation requires sequential pull-down steps with a simultaneous swing-out of the β subunit hybrid domain, these rearrangements may also benefit from low forces applied on the ligand-headpiece interface (Figure 2). Even though rupture analysis of single integrin-ligand bonds using atomic force microscopy has not detected net stabilization of these bonds by low forces (Zhang et al., 2002) as was recently found for selectin bonds (Zhu et al., 2005), there are accumulating data suggesting that low shear forces exerted on cells expressing an isolated LFA-1-derived α I domain augment I domain adhesiveness to ICAM-1 by stabilizing the open conformation of this domain (Salas et al., 2002) (Astrof et al., 2006). The $\alpha_4\beta_7$ integrin also acquires high adhesiveness to its ligand MadCAM-1 above a critical threshold of shear stress (de Chateau et al., 2001). Optimal integrin activation by chemokines in T cells is also greatly facilitated by externally applied forces (E. Woolf, A. Sagiv, Z. Shulman, V. Grabovsky, R. Pasvolsky, S. Feigelson, M. Sixt, and R.A., unpublished data). Notably, recent computer simulation of integrin dynamics also predicts that the ligand-bound $\alpha_v\beta_3$ transitions into a high-affinity open headpiece state within nanoseconds if force is applied to the ligand-headpiece complex (Puklin-Faucher et al., 2006). In contrast, ligand-driven head rearrangements of integrins may take seconds to complete in the absence of force (Bednar et al., 1997). Thus, low forces may theoretically accelerate ligand-driven outside-in activation of the integrin headpiece by up to nine orders of magnitude.

To load forces, whether externally applied by shear stress or internally generated by filamentous actin, integrin-ligand complexes must be properly anchored within the plasma membrane. The role of preexisting cytoskeletal integrin links in integrin adhesiveness of leukocytes under shear flow has only begun to unfold. One line of evidence that leukocyte integrin anchorage is essential for mechanical strengthening of integrin bonds is based on recent findings indicating that strong α_4 integrin anchorage to the cytoskeleton is key for subsecond stabilization of VLA-4-VCAM-1 bonds under applied forces, but not for VLA-4 adhesiveness under shear-free conditions (Alon et al., 2005). Although preformed integrin anchorage was considered to be antiadhesive due to its negative effects on lateral integrin mobility to contact sites (Kucik et al., 1996), for initial mechanical activation of integrin-ligand bonds at leukocyte-vessel contacts, the opposite may be true. A recent study using single-molecule tracking on total, closed (low-affinity), and extended forms of LFA-1 identified a large fraction of open and extended LFA-1 conformations as being preanchored to the cytoskeleton (Cairo et al., 2006). This analysis revealed that the population of anchored and extended LFA-1 conformations increases upon inside-out activation by either phorbol esters or T cell receptor (TCR) ligation, two key inside-out modalities of LFA-1 activation (Cairo et al., 2006). Ligand-engaged LFA-1 molecules showed the greatest degree of cytoskeletal association, consistent with ligand-induced outside-in conformational changes that favor integrin cytoskeletal associations (Shamri et al., 2005). In contrast, closed low-affinity LFA-1 conformations were found to be poorly anchored to the cytoskeleton and appeared to further decrease their cytoskeletal associations upon activation. These low-affinity unanchored LFA-1 species may therefore serve as a reserve pool that can diffuse to sites of adhesion, initiated by the anchored extended LFA-1 subsets. The properly anchored and extended integrin appears to be ideally suited to translate ligand recognition into high-affinity and shear-resistant binding necessary for the rapid arrest of the rolling leukocyte (Shamri et al., 2005). Released and unclasped integrins with high affinity to ligand may, on the other hand, fail to generate the mechanically stable ligand complexes necessary for initial arrests under shear flow. These integrins are still likely to be recruited by ligand-bound integrins and contribute to postarrest adhesion strengthening.

Increasing evidence suggests that subsets of circulating leukocytes express a fraction of their integrins in preformed intermediate-affinity states. These integrins may contain partially closed I domains that temporarily arrest and/or slow down rolling leukocytes, especially in vascular beds lacking endothelial selectins (Berlin et al., 1995; Henderson et al., 2001; Salas et al., 2004). In lymphocytes homing to Peyer's patch HEV, $\alpha_4\beta_7$-MadCAM-1 interactions decelerate rapid L-selectin-mediated rolling prior to GPCR activation (Bargatze et al., 1995); in eosinophils, VLA-4-VCAM-1 interactions slow down eosinophil rolling on inflamed venules (Sriramarao et al., 1994); and in neutrophils, both LFA-1 and Mac-1 have been reported to retard selectin-mediated rolling (Dunne et al., 2003). These integrin states may be preanchored to the cytoskeleton and stabilized by membrane effectors such as tetraspanins (Feigelson et al., 2003) and surface receptors such as CD44 (Nandi et al., 2004).

Chemoattractants May Stimulate Abrupt Integrin Adhesiveness by Accelerating Bidirectional Conformational Changes of Anchored Integrins

In situ activation of integrins on leukocytes rolling on endothelial targets is rapidly triggered by the binding of specialized chemoattractants, or chemotactic cytokines (chemokines), to cognate G protein-coupled receptors (GPCRs) (Bargatze and Butcher, 1993; Campbell et al., 1998). A recent study suggests that subsets of GPCRs, when occupied by endothelial-presented ligands but not

by soluble ligands, can trigger, within subseconds, both inside-out and outside-in activation of LFA-1 in T lymphocytes (Shamri et al., 2005). A critical step in this LFA-1 activation is the instantaneous stabilization of the extended integrin state through a chemokine signal, which must be immediately coupled to an ICAM-1-induced activation (Figure 2). Juxtaposition between the GPCR signal and the integrin ligand (Figure 1) and an intact actin cytoskeleton appear to be also required for chemoattractant-triggered activation in multiple types of leukocyte and integrin systems (Alon et al., 2003).

Numerous downstream effectors have been so far suggested to mediate this integrin activation step (Kinashi, 2005). However, many integrin-associated adhesive processes have been assessed in the absence of shear forces or have involved late adhesive and spreading steps downstream of the initial bidirectional integrin activation described above (Alon et al., 2003). So far, only a few regulators, mainly GTPases, have been implicated in rapid integrin activation at leukocyte-endothelial contacts. The small GTPase RhoA was originally shown to be involved in rapid integrin activation by CXCL8 in neutrophils (Laudanna et al., 1996) as well as LFA-1 activation by the CCL21 and CXCL12 chemokines in lymphocytes (Giagulli et al., 2004). The downstream target of chemokine-activated RhoA in rapid integrin activation is still unclear. A second GTPase, Rap1, has emerged as another key regulator of early integrin activation by chemokine signals and shear stress signals (Katagiri et al., 2004; Shimonaka et al., 2003). The role of Rap1 in integrin triggering was further demonstrated by the recent description of a human genetic defect, LAD III, in which a deficiency in chemokine-triggered integrin activation correlates with impairment of Rap1 activation (Kinashi et al., 2004).

A key potential adaptor capable of translating these and other GPCR signals to conformational changes of properly anchored integrins is talin (Shamri et al., 2005). Talin is a large and extended homodimer that links integrins to the actin cytoskeleton (Critchley, 2000). Talin has an amino-terminal FERM head domain that, when exposed, binds an NPXY/F tail motif shared by all major β-integrin subunits. Rap1 has been recently shown to link inside-out signals to talin activation of integrins (Han et al., 2006). Talin head can bind β subunits of multiple integrins and unclasp their tails, driving integrin extension or reinforcing integrin activation by extracellular ligand (Tadokoro et al., 2003). As talin exists in multiple conformational states, inactive talin may link integrin to the cytoskeleton in a manner that restricts their tail unclasping (Sampath et al., 1998), whereas, when properly activated by interaction with the phosphoinositide PI(4,5)P2 or by phosphorylation, talin can productively trigger conformational integrin activation (Kim et al., 2003; Tadokoro et al., 2003). Talin may also crosslink correctly spaced ligand-occupied integrins and thereby further strengthen adhesions (Jiang et al., 2003). Notably, proteolytic talin cleavage and release of the head domain, although reported to induce integrin activation, may release the activated integrin from the actin cytoskeleton, a counterproductive outcome for optimal stabilization of the integrin bond under tensile forces. Intact talin is therefore most suited to both anchor and unclasp the integrin cytoplasmic domains upon chemoattractant activation of leukocytes under shear stress conditions.

In conclusion, multiple features are required for an integrin to successfully generate firm adhesion at endothelial contacts. Selectin- and integrin-mediated rolling may slow down freely flowing leukocytes and thereby allow them to survey the endothelial target for proper arrays of endothelial chemoattractants (chemokines) and integrin ligands (Figure 1). Chemoattractant signals may either induce or stabilize extended integrin subsets by transducing integrin unclasping. Preformed binding of extended integrins to the cortical cytoskeleton (directly or via transmembrane associations with other anchored proteins or with cytoskeleton-tethered lipids) will allow anchored integrins to load low forces and undergo rapid (subsecond) ligand-induced activation (Figure 2, outside-in). Integrins presented on leukocyte microvilli are more likely to participate in these rapid steps (Figure 1). Integrins may also need to oligomerize prior to ligand binding in order to generate firm adhesion (Cambi et al., 2006), and endothelial integrin ligands are more adhesive in their dimeric states (Miller et al., 1995), which rapidly drive integrin microclustering (Kim et al., 2004). Positive feedback loops between chemoattractant-driven integrin unclasping, ligand-induced headpiece activation, and dimerization of integrin-ligand complexes may also be possible. Once nascent adhesion is generated, pools of recruited high-mobility integrins can further enhance leukocyte adhesion through multivalent interactions (Giagulli et al., 2004; Kim et al., 2004).

Earliest LFA-1 Activation Events Triggered in the Immune Synapse: A Role for Intracellular Forces?

LFA-1 is used for arrest not only on the blood-vessel wall but within the T cell zones of secondary lymphoid tissues (Dustin, 2004). In the steady state, T cells undergo rapid amoeboid locomotion in a variety of tissue contexts (Geissmann et al., 2005; Miller et al., 2003). This motility is thought to be critical in the search for rare antigen-expressing cells. Once T lymphocytes locate cells with antigenic MHC-peptide complexes, they decelerate from >10 μm/min to <2 μm/min in order to form stable interactions with antigen-presenting cells (APCs) (Shakhar et al., 2005). Such interactions include both priming of naive T cells and activation of effector cells, both of which can involve rapid arrest (Mempel et al., 2006). While slow by comparison to the blood-flow-induced velocities at the vessel wall, the amoeboid motion of T cells within tissue is rapid, reaching speeds of up to 30 μm/min. Unlike fibroblasts, which contract collagen gels by generating high levels of force through focal adhesion-like structures, cells undergoing amoeboid locomotion do not contract collagen gels and do not dramatically remodel their environment (Wolf et al., 2003).

Integrins are key components of the immunological synapse, and LFA-1 is the best-studied molecule involved in this process (Grakoui et al., 1999; Monks et al., 1998).

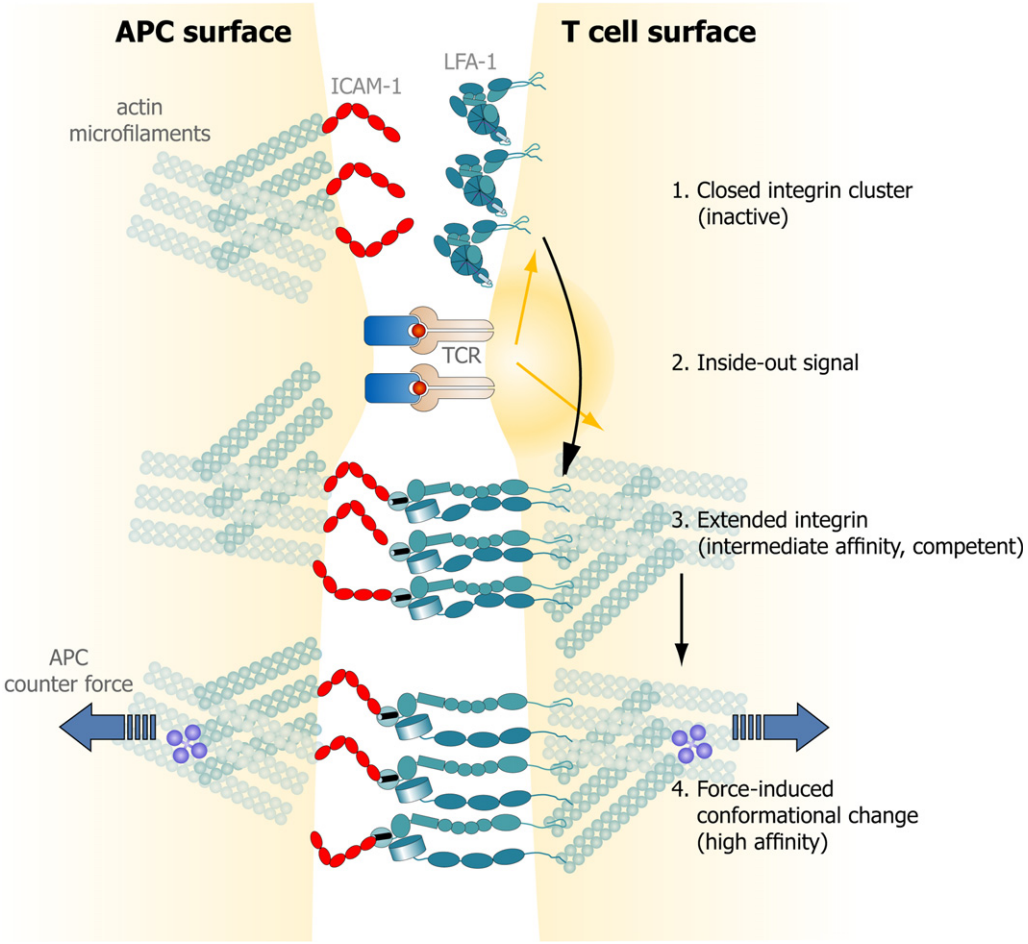

Figure 3. A Proposed Role for Internal, Cytoskeleton-Driven Forces in the Interactions of TCR and Integrin Clusters in the Immunological Synapse

Schematic representation of a region in the pSMAC of the immunological synapse. Integrins on lymphocytes appear to be extensively preclustered (Cambi et al., 2006). These clusters may be inactive on resting lymphocytes (1); following TCR microcluster activation, inside-out signaling (2) induces cytoskeletal association of LFA-1 and LFA-1 extension (3), rendering the integrin competent for ICAM-1 binding. Within seconds, myosin II-mediated contraction or retrograde actin flow exerts low forces on the integrin-ligand complexes to induce rapid and full outside-in activation in a ligand- and anchorage-dependent manner (4). The LFA-1 and ICAM-1 clusters may also work against each other across the synapse to maintain tension and fully arrest the T cell as it locomotes over the APC.

Ligand-induced LFA-1 activation may take place more readily when LFA-1 is subjected to internally applied forces during immunological synapse formation (Varma et al., 2006). The observation that supported planar bilayers, in which proteins have free lateral mobility, support immunological synapse formation and full T cell activation would seem to indicate that cytoskeletal anchorage of molecules in the APC may not be important. However, unlike biological membranes from which nonanchored proteins pull long elastic membrane tethers when subjected to ~10 pN forces, the planar bilayer does not allow pulling of such tethers and instead is rigid in the vertical dimension due to trapping at the glass surface. Thus, molecules in the bilayer have the physical signature of cytoskeletally anchored proteins when pulled vertically. The earliest LFA-1 activation events underlying the initiation of firm T cell-APC contacts coincide with rapid rises in Ca^{2+} (Campi et al., 2005; Dustin et al., 1997; Negulescu et al., 1996) but involve weak inside-out conformational switches prior to ligand binding (R. Pasvolsky and R.A., unpublished data). These events may therefore involve the local triggering of anchored LFA-1 subsets, which are prone to load internally generated forces via Ca^{2+}-stimulated myosin II activation and contraction (Figure 3).

The immunological synapse formed on round APCs or on planar substrates is a radially symmetric contact interface with an actin-rich peripheral ring, an integrin/talin-rich intermediate ring, and a core containing accumulated TCRs and protein-sorting and secretory compartments (Grakoui et al., 1999; Monks et al., 1998; Stinchcombe et al., 2001). Kupfer and colleagues (Monks et al., 1998) defined the central TCR/sorting/secretory region as the central supramolecular activation cluster (cSMAC). The LFA-1 ring is also known as the peripheral SMAC (pSMAC)

(Figure 3). The outer F-actin ring is also enriched in CD45 and has been defined as the distal SMAC (dSMAC). The dSMAC undergoes cycles of actin polymerization-dependent extension and myosin II-mediated contraction, which move around the periphery of the synapse in circular waves (Dobereiner et al., 2005). This periodic extension/contraction activity, together with intermediate filaments and microtubules, may allow diverse cell types to sense the physical properties of the substrate, whether soft or rigid (Giannone and Sheetz, 2006). Peak forces evolve during the contraction phase, and integrins engaged during the actin-extension phase would be subjected to vertical and lateral forces. The magnitude of these forces would depend upon the rigidity of the substrate and the cytoskeletal anchorage of ICAM-1 and stimulatory molecules on the APC surface. LFA-1-ICAM-1 complexes that are formed in the dSMAC couple to the retrograde actin flow and translocate to the pSMAC, where they accumulate and generate forces that are significant at the level of single receptor-ligand pairs. Therefore, while there is little translocation of cells during immunological synapse formation, continuous force sensing and generation occurs through LFA-1-ICAM-1 complexes. Force-dependent conformational changes in LFA-1 may therefore play an important role in transduction of costimulatory signals in T cells during the process of T cell activation. The ability of the cell to exert forces on molecules held by the APC may also be important in evaluating the affinity of antigen receptors (Fleire et al., 2006).

Conclusion

Leukocyte integrins are versatile adhesion molecules known to play important functions in both extravasation and tissue interactions. Conformational flexibility of integrins has been appreciated traditionally through studies with conformation-sensitive antibodies and more recently from structural studies and molecular dynamic simulations. Force is an accelerator of integrin-mediated lymphocyte adhesion in the context of arrest from flow and immunological synapse formation. This new awareness of force as a critical signal in rapid integrin activation by its own ligand may change the model for LFA-1 activation by inside-out signals. When external (shear-based) or internal (cytoskeleton-based) forces on individual bonds are negligible during leukocyte motility, freely mobile LFA-1 may contribute to adhesion by clustering and interacting with ligand and only then undergoing various cytoskeletal associations. However, in the presence of external shear forces, cytoskeletal anchorage of LFA-1 and other integrins can be essential even prior to ligand binding. We propose that in the immunological synapse, forces internally applied by the cytoskeleton to nascent LFA-1-ICAM-1 interactions could also facilitate LFA-1 activation. Thus, we can no longer study how GPCRs and TCRs transmit inside-out signals to integrins without also fully dissecting how cytoskeletal assemblies of these integrins promote ligand-induced force-facilitated integrin activation. Future studies will need to resolve how different cytoskeletal assemblies are regulated in distinct types of immune cells to translate external and internal forces into rapid generation of integrin adhesions in endothelial and immunological synapses.

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