SDF-1α (CXCL12) regulation of lateral mobility contributes to activation of LFA-1 adhesion

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Wu X, Yu T, Bullard DC, Kucik DF. SDF-1α (CXCL12) regulation of lateral mobility contributes to activation of LFA-1 adhesion. Am J Physiol Cell Physiol 303: C666–C672, 2012. First published August 8, 2012; doi:10.1152/ajpcell.00190.2012.—Regulation of integrin activity enables leukocytes to circulate freely, avoiding inappropriate adhesion while maintaining the ability to adhere quickly at sites of infection or inflammation. This regulation involves at least two components: affinity for ligand and affinity-independent avidity effects such as lateral mobility. Using lymphocyte function associated antigen-1 (LFA-1) as a model, we investigated the role of integrin release from cytoskeletal motion constraints in response to the chemokine stromal cell-derived factor-1 (SDF-1α) in this process. All experiments were done in primary T cells to avoid nonphysiological activation processes often seen with the use of cell lines. We found that SDF-1α releases LFA-1 from cytoskeletal constraints as effectively as does cytochalasin D. The resultant increased diffusion is correlated with a robust increase in LFA-1-mediated adhesion under physiological shear stress. We further investigated the role of the highly conserved GFFKR sequence in the LFA-1 cytoplasmic domain. We report that the GFFKR sequence is both necessary and sufficient for regulation of the SDF-1α-triggered proadhesive release from cytoskeleton interactions. While this does not address the role of transient SDF-1α-induced conformational changes in the activation process, these results strongly suggest that any model of chemokine-induced LFA-1 activation must take into account chemokine-induced integrin lateral mobility. In addition, these results have ramifications for models of differential binding of LFA-1 to surface-bound vs. soluble intercellular adhesion molecule-1.

chemokines; integrins; avidity; lateral mobility; single particle tracking

ACTIVATION OF ADHESION, leading to arrest of leukocytes at target endothelium, is a key, early step in inflammation, without which there can be no effective immune response. A major adhesion molecule involved in rapid adhesion of T cells is lymphocyte-function-associated antigen-1, or LFA-1 (also known as CD11a/CD18 and as the integrin αLβ2). Although inactive on most circulating T cells, LFA-1 can rapidly become active in response to binding of specific endothelium-displayed chemokines and other signals. There has been some feeling in the field that a model consisting exclusively of multiple LFA-1 affinity states can completely explain activation of adhesion (2). Currently, there are thought to be five different LFA-1 conformations, termed closed, basal, primed, intermediate, and open (15). These correspond to affinity states, with open having the highest affinity for ligand. A high affinity state of LFA-1 can be induced by adding exogenous Mg2+, resulting in binding of soluble intercellular adhesion molecule-1 (ICAM-1), and this alone is sufficient to activate cell adhesion. This profound affinity change generally does not occur, however, in response to physiological stimuli. For example, T-cell receptor crosslinking, a physiological stimulus, increases LFA-1-mediated cell adhesion but not binding of soluble ICAM-1 (18). To date, affinity changes in response to T-cell receptor crosslinking or chemokine stimulation have only been demonstrated in artificial systems by stabilizing each conformation with Fab antibody fragments and using as the ligand ii3-ICAM-1, an engineered ICAM-1 with 20-fold higher affinity for LFA-1 than wild-type ICAM-1 (15). In addition, a model consisting solely of integrin conformational changes is insufficient to explain all aspects of adhesion activation. For example, conformational changes alone cannot explain why the measured affinity is lower for soluble ligand than for that presented on a surface (15).

A second mechanism of activation of LFA-1-mediated adhesion is avidity adjustment (10). Activation of leukocytes by the phorbol ester phorbol-12,13 dibutyrate does not result in soluble ICAM-1 binding (19), but in a release of LFA-1 from cytoskeletal constraints, increasing LFA-1 lateral mobility, which in turn leads to increased adhesion (11). This is thought to be because increased lateral mobility facilitates encounters between the integrin and its ligands, consistent with a diffusion-limited adhesion theory (1). In addition, release of LFA-1 from cytoskeletal constraints on its movements, even by disruption of integrin-cytoskeleton connections by cytochalasin D (22) or by truncation of the β2-cytoplasmic domain (20), also leads to LFA-1 clustering. Theory predicts that clustering alone can lead to increased avidity, due to cooperative effects on resistance to bond breakage (21), and this has been confirmed by atomic force microscopy (4). Recently, it was shown that increased binding kinetics due to enhanced lateral mobility, clustering, and avidity adjustment can each increase cell adhesion independently (22). Since LFA-1 activation includes components of both affinity and avidity modulation, any comprehensive model requires an understanding of both.

Little, however, is known about the cytoskeletal linkages that regulate proadhesive LFA-1 mobility. Since the particular cytoplasmic proteins involved in the integrin-cytoskeleton interaction that regulate adhesion-associated LFA-1 movements remain to be identified, a logical first step is to identify which sequences in the LFA-1 cytoplasmic domain are involved. To do this, we used a mutational approach, guided by previous work that identified an important target in the LFA-1 αL-subunit cytoplasmic domain: the membrane-proximal GFFKR sequence (Fig. 1), which is highly conserved, not just between human and mouse,
but across a wide variety of species. This sequence, which is important for activation in both human and mouse integrins, has long been implicated in control of leukocyte adhesion. Most studies so far, however, have focused on either affinity changes or on cell adhesion without regard to mechanism and have not addressed control of LFA-1 lateral mobility.

Although integrin lateral mobility has sometimes been inferred as an adhesion mechanism when results are not readily explained by affinity changes (5), it is rarely measured directly. In the current study, single particle tracking (SPT) was used to directly measure LFA-1 lateral mobility in real time on living T cells. The basis of the SPT technique is that when a small bead coated with antibody is placed on a cell and allowed to bind specifically to a membrane protein, displacements of the bead reflect the motion of the membrane protein to which the bead is attached (7, 14, 16). Even a cytoskeletonally anchored integrin will have some thermal motion, and this can be quantified by calculating a diffusion coefficient (even though the integrins are not actually diffusing). However, when integrins are freed from cytoskeletal constraints, their thermal motion (and thus their diffusion coefficient) is dramatically higher (11, 13, 16), reflecting a lateral mobility consistent with diffusion in the plane of the membrane. This higher lateral mobility has been shown by multiple groups to be proadhesive.

To understand the role of GFFKR in LFA-1 lateral mobility, primary T cells were used in this study. This is because, while work in cell lines has yielded important advances in our understanding of integrin activation, it is limited by the fact that activation of adhesion in transformed cells in response to physiological stimuli is not completely normal. For example, nonleukocyte cell lines, such as COS or CHO, can express functional LFA-1, but extrapolation to integrin behavior in a leukocyte is often not reliable. Leukocyte cell lines lacking native LFA-1 have also been used, but even they fail to accurately mimic physiological adhesion activation when LFA-1 is expressed in them. It is thought that this may be due to the fact that the cytoplasmic machinery for regulation of LFA-1 function (cytoskeletal linker proteins plus signaling pathways) may be incomplete or defective in these transformed cells. For example, while much about LFA-1 function has been learned using the K562 erythroleukemia cell line, LFA-1 expressed in those cells has abnormally high affinity on resting K562 cells compared with resting primary lymphocytes (15). Despite its relatively high affinity, however, LFA-1 in K562 cells tends to mediate rolling adhesion to ICAM-1 under physiological shear stress, rather than the firm adhesion typical of LFA-1 in primary leukocytes (17).

While primary leukocytes can be expected to have a complete, normal activation system, a disadvantage is that they generally express their own LFA-1, which can mask any functional differences of transfected integrin constructs. This was overcome by using T cells from gene-targeted mice lacking the αL-subunit of LFA-1 (since human T cells naturally lacking the αL-subunit of LFA-1 are not available), allowing expression of engineered integrin constructs without a background of wild-type integrin. Unlike the case with human LFA-1, however, a suite of antibodies to report LFA-1 conformational states does not exist for mouse. Therefore, this study is focused on activation effects on LFA-1 lateral mobility and their consequences for overall adhesion.

Here we demonstrate increased LFA-1 lateral mobility on primary T cells in response to a physiological stimulus, the chemokine stromal cell-derived factor-1 (SDF-1α; also known as CXCL12). Moreover, we demonstrate that the GFFKR sequence in the LFA-1 cytoplasmic domain that regulates adhesion also regulates lateral mobility. Regulations of both lateral mobility and adhesion by SDF-1α closely mimic those induced by cytochalasin D (which does not induce high-affinity; Ref 22). While this does not address the role of affinity adjustment, it suggests that regulation of lateral mobility plays an important part in SDF-1α-induced cell adhesion and provides important information regarding the basis for differential adhesiveness of LFA-1 for soluble vs. anchored ICAM-1.

MATERIALS AND METHODS

Materials

ICAM-1/Fc, P-selectin/Fc chimera recombinant proteins, and IL-2 recombinant protein were purchased from R&D Systems (Minneap-
olis, MN). Protein A and human serum albumin were purchased from Sigma (St. Louis, MO). The mouse T-cell negative isolation kit was purchased from Invitrogen. The mouse T-cell Nucleofector kit was purchased from Lonza amaxa (VPA-1006). The FITC-conjugated anti-mouse CD11a mAb, PE-conjugated anti-mouse CD3ε mAb, and NA/LE anti-mouse CD3ε mAbs were purchased from BD Biosciences.

**DNA Constructs**

A mouse CD11a full-length cDNA was purchased from ATCC (ATCC 7421401) and was subcloned into pCR2.1 vector by PCR for sequencing and mutagenesis. Mutant and wild-type constructs (see Fig. 1) were created by site-directed mutagenesis. In brief, the cytoplasmic domain truncation mutant (TM) was made using primers moaL91F (GAG ATC GAA GCT TCT GTC CGG ACC CAC AGG TCC CTC) and TT ATA C TC GAG GCC TTA GTA GAG CGC GAG GAA AAT C with a stop codon (italic) right after the transmembrane domain. The GFFKR construct, which has the cytoplasmic domain truncated following the membrane-proximal GFFKR, was made using primers moaL91F and TT ATA C TC GAG GCC TTA CCG TTT GAA GAA GCC AAC C. The ΔGFFKR construct, where the GFFKR sequence was deleted leaving the rest of the cytoplasmic domain intact, was made with the primers 5' dKVFFGR: CTGTATTTTCCTGGGCGTTTAC/AACTGTAAGGAGAAGATG; and 3' dKVFFGR: CATCCTTCTCCTCAAGGTTACAGGAC. The products were confirmed by DNA sequencing.

**Mice**

CD11a mutant mice have been previously described (8). For all transfection experiments, we used T cells isolated from CD11a-/- mice (both male and female) at an N16 backcross generation onto C57BL/6. T cells from inbred C57BL/6 mice (both male and female) were used as wild-type controls. All animal protocols were submitted to, and approved by, the University of Alabama, Birmingham, Institutional Animal Care and Use Committee.

**Transfection and FACS Sorting and Analysis**

Primary T cells were isolated from the spleens of wild-type or αL (CD11a) -/- mice using a negative T-cell isolation kit (Invitrogen) according to the manufacturer’s protocol and cultured in the supplied T-cell medium. The transfection was performed using the Amaza Nucleofector technology (Lonza). Cells were labeled with BD monoclinal anti-mouse CD11a antibody (Clone M17/4) conjugated with FITC or PE (BD Biosciences) and FACS sorted to match expression levels in the University of Alabama, Birmingham flow cytometry core facility.

**Purified Substrate Coating**

Tissue culture dishes (35 mm) were marked with a diamond pen to outline a small circle of 0.5 cm in diameter in the center. Then, 10 μl of 20 μg/ml protein A (Sigma) in PBS were placed in the marked area and spread with the pipette tip, incubated at 37°C for 1 h, and then washed three times with PBS. Nonspecific binding was blocked with 2% human serum albumin (Sigma) in PBS for 2 h. At 4°C followed by three washes with pH 8.0 PBS. The marked areas were then double-coated with 50 μl purified substrate mix of 10 μg/ml recombinant mouse ICAM-1/Fc chimera and 10 μg/ml recombinant mouse P-selectin/Fc chimera in PBS at pH 8.0 overnight in 4°C (R&D Systems).

**Cell Cultures and Treatments**

Transfected primary T cells were cultured overnight and then suspended in HBSS (CellGro) at a concentration of 0.5 × 10⁶/ml. The cells were then treated with SDF-1α (3 nM, 1 min), cytochalasin D (1.0 μg/ml, 30 min at 37°C), or HBSS only (controls). Adhesion to the substrate-coated dishes was then measured using a flow chamber adhesion assay.

**In Vitro Rolling and Adhesion Assay**

For flow experiments, a custom-made flow chamber insert and gasket was inserted into a 35-mm dish to form a laminar flow chamber that can be viewed on a microscope. Cells were injected into the flow chamber in HBSS at a controlled shear stress of 1.0 dyn/cm² using a programmable syringe pump (KD Scientific, New Hope, PA). Cells were viewed on an Zeiss Axiovert 100 microscope equipped with a JAI CV-53200 CCD camera (Edmund Optics, Barrington, NJ) and viewed as brightfield images. Video was recorded onto a computer using a Video Advantage capture card (Turtle Beach, Valhalla, NY), and the newly adherent cells were counted.

**Analysis of Adhesion**

Cell adhesion was assayed by viewing digital movies of adhesion experiments and scoring cells that were captured and adhered in the field of view during the period of observation (expressed as adherent cells/min). All figures represent pooled data from at least three independent experiments conducted on separate days.

**Definition of firm adhesion**. An objective definition of firm adhesion was used in this project. A cell that moved less than one cell diameter in 3 s and remained in contact with the substrate for greater than 5 s was considered firmly adhered.

**Coating of Cover Glass for the SPT Assay**

Each coverslip (22 × 22 mm; Fisher Scientific) was washed by immersing in 100% Ethyl Alcohol (Fisher Scientific) once and air dried in a 35-mm dish under a tissue-culture hood, then washed with 1 ml PBS three times. After the wash, the coverslips were incubated with 2 ml of 1 μg/ml poly-l-lysine (PLL; Sigma Chemical) at room temperature for 30 min. PLL-coated coverslips were then washed twice with 1 ml PBS and once with 1 ml staging buffer (HBSS supplemented with 2 mM CaCl₂ and 4 mM MgCl₂) and stored in 2 ml staging buffer at 4°C to be used within 2 days.

**Cell Preparation**

The 5 × 10⁴ cultured mouse primary T cells/condition were washed by 3 ml PBS once and suspended in 1 ml staging buffer. An aliquot was then added to each dish, and the cells were allowed to settle on PLL-coated coverslips at 37°C for 15–20 min.

**Conjugation of Antibodies to Beads**

The 0.2 μm carboxylate-modified green or red fluorescent fluorospheres (Molecular Probes, Carlsbad, CA) were incubated with 200 μl of 100 mM 2-(N-morpholino)ethanesulfonic acid and 50 μg of protein A (1 mg/ml; both from Sigma) in water and gently shaken at room temperature for 15 min. Then, 2 mg of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (Sigma) in 200 μl were added and the tubes were shaken for 2 h at room temperature. At the end of 2 h, 450 μl of glycine (1 M in H₂O, pH 6.25) were added to terminate the reaction, and the tubes were shaken for an additional 30 min. The beads were centrifuged at high speed in a microcentrifuge for 25 min, and the supernatant was discarded. The beads were then washed twice by centrifugation with 800 μl PBS (pH 8). The protein A-coated beads were stored for up to 2 mo at 4°C in 1 ml of PBS (pH 8) containing 1% BSA and 0.02% sodium azide (both from Sigma).

**Specific Antibody Coating**

Ten micrograms of rabbit anti-rat IgG (312-001-003, Jackson Immuno-Research Laboratories, West Grove, PA) were added to a 1.5-ml tube with 100 μl protein A-coated beads in 100 μl PBS (pH 8) and gently shaken at 4°C for 1 h. Unconjugated IgG was removed by
centrifugation and beads were suspended in 200 μl PBS (pH 8). Five micrograms rat anti-mouse αL-integrin antibody (CD11a, Clone M17/4; BD Pharmingen, San Jose, CA) were then added to 200 μl rabbit anti-rat IgG-conjugated green beads and gently shaken at 4°C for 1 h. For the negative control, 5 μg rat IgG (Jackson ImmunoResearch, West Grove, PA) were added to 200 μl rabbit anti-mouse IgG-conjugated red beads. Beads were separated from unconjugated antibodies by wash and centrifugation. Beads were suspended in 50 μl PBS (pH 8) and kept in the dark at 4°C until they were used. Fresh antibody coated beads were prepared on the same day for each SPT experiment.

SPT Experiments

The PLL-coated coverslips with attached mouse T cells were assembled into an SPT chamber (designed by the Kucik Laboratory), which allows both high-resolution microscopy and perfusion. Mixed anti-αL-antibody-coated 0.2-μm green fluorescent beads and control antibody coated 0.2-μm red beads in a 50/50 mixture were perfused into the chamber in HBSS at a concentration empirically determined to result in binding of one to two beads per cell (to avoid extensive crosslinking of integrins, which can be an activating signal). Experiments were then performed at room temperature.

Analysis of SPT Data

After cell surface binding of the green beads but not the red control beads was confirmed, cells to analyze were chosen at random. The cells were viewed on a Zeiss Axiovert 100 inverted microscope equipped with fluorescence optics. Video sequences were collected with a charged-coupled device camera (JVI S-3200; Edmund Scientific, Burlington, NJ) and digitized onto a computer hard drive for later analysis. Particle positions were later determined using Metamorph software (Molecular Devices, Sunnyvale, CA) and converted from pixel to nanometer coordinates by comparison with a known standard. Beads were tracked for 15 s, resulting in a particle track containing 450 data points (at 30 video frames/s). Particle tracks were then analyzed using software developed for this purpose (12) to determine a diffusion coefficient, D.

Statistics

All graphs were analyzed by one-way ANOVA followed by a Dunnett posttest except where indicated (see Fig. 5).

RESULTS

LFA-1 Constructs Expressed in T Cells From αL (CD11a)−/− Mice Mimics That of Native LFA-1 in Wild-Type T Cells

Interpretation of studies of integrin activation has often been limited by the failure of the experimental system to faithfully mimic physiological activation of naturally occurring integrins in primary cells. To confirm that activation of LFA-1 is normal when expressed in αL(CD11a)−/− T cells, transfected wild-type LFA-1 was compared with natively expressed wild-type LFA-1 (in T cells from a wild-type mouse) (Fig. 2). Adhesion was to a mixed substrate of P-selectin (to mediate capture and rolling adhesion) and ICAM-1 (a ligand for LFA-1 that mediates firm adhesion). Firm adhesion of both native and transfected LFA-1 was increased by low-dose cytochalasin D, which activates adhesion by integrin rearrangement without conformational changes (22), and by SDF-1α, a physiological activator of LFA-1.

SDF-1α Increases Lateral Mobility of LFA-1 in T Cells

Both affinity modulation and lateral mobility can activate LFA-1-mediated adhesion. Although transient modulation of LFA-1 affinity by SDF-1α has been demonstrated (3), the effects on LFA-1 lateral mobility have not been measured directly in previous studies. Therefore, SPT measurements (see MATERIALS AND METHODS) of lateral mobility changes were necessary to determine whether LFA-1 diffusion is increased by SDF-1α. Figure 3 demonstrates that, in both T cells from wild-type mice and in T cells from αL(CD11a)−/− mice expressing wild-type LFA-1, SDF-1α increases LFA-1 lateral mobility to the same extent as cytochalasin D, consistent with a release of cytoskeletal constraints on LFA-1 motion (11–13, 16).

The GFFKR Sequence Is Sufficient for Regulation of LFA-1 Lateral Mobility and Rearrangement-Based Adhesion

The highly conserved GFFKR sequence near the membrane in the αL-cytoplasmic domain has long been implicated in regulation of LFA-1-mediated adhesion, but whether it is involved in regulation of LFA-1 lateral mobility has not been previously determined. To test the role of GFFKR in regulation of lateral mobility and its consequences for LFA-1-mediated T-cell adhesion, both diffusion and adhesion were measured (Fig. 4) for the “GFFKR” construct, in which the entire αL-cytoplasmic domain is deleted except for the GFFKR (see Fig. 1). Both diffusion and adhesion increased in response to either cytochalasin D or SDF-1α, similar to wild-type LFA-1 (compare Figs. 3 and 4). This increase in the diffusion coefficient, D, is consistent with a release from cytoskeletal constraints.
SDF-1α INCREASES LFA-1 LATERAL MOBILITY

**Fig. 3.** SDF-1α releases LFA-1 from the cytoskeleton. Lateral mobility of wild-type LFA-1 in primary wild-type mouse T cells (A) and wild-type LFA-1 expressed in primary T cells from αL−/− mice (B) was measured following SDF-1α (3 nM × 1 min at 37°C), cytochalasin D (1 μg/ml × 30 min at 37°C, used as a positive control) or with no treatment. Both the native wild-type LFA-1 and the transfected wild-type LFA-1 were constrained by the cytoskeleton at baseline but released by both cytochalasin D and SDF-1α (**P < 0.01). D, diffusion coefficient.

The GFFKR Sequence Is Necessary for Regulation of LFA-1 Lateral Mobility and Rearrangement-Based Adhesion

To test whether there is an absolute requirement for GFFKR sequence in control of LFA-1 lateral mobility, or whether other portions of the αL-cytoplasmic domain might substitute, lateral mobility and adhesion of cells expressing the ΔGFFKR construct were tested. The ΔGFFKR contains the entire αL-cytoplasmic domain except for the GFFKR (see Fig. 1). Lateral mobility of the ΔGFFKR construct, as measured by SPT, was constitutively high, similar to the TM construct, which lacks the entire cytoplasmic domain. For both ΔGFFKR and TM, the rate of diffusion was not changed by either cytochalasin D or SDF-1α (Fig. 5A), as expected for a protein that is already freely diffusing. Consistent with this, adhesion to ICAM-1/P-selectin under shear stress was also constitutively high and not modified by cytochalasin D or SDF-1α (Fig. 5B).

DISCUSSION

Regulation of inflammation by SDF-1α and other chemokines is crucial to host defense against invading microbes. Without regulated leukocyte-endothelial adhesion, an immune response cannot be effective. An inflammatory response that is triggered inappropriately, however, or that is too robust, can also lead to disease. Thus chemokine-regulated lymphocyte adhesion is also involved in a number of autoimmune diseases, such as multiple sclerosis, rheumatoid arthritis, dermatitis, asthma, and organ transplant rejection, and plays a role in atherosclerosis as well. Therefore, a tightly regulated adhesion cascade is critical to good health. A fuller understanding of how this regulation is accomplished will contribute to our ability to impact a number of health problems.

SDF-1α is a physiological adhesion activator, the effects of which on leukocyte adhesion have largely been discussed in terms of affinity changes. Although both lateral mobility and clustering have been shown to contribute to leukocyte adhesion activation (22), these mechanisms are often ignored when constructing models of LFA-1 function, perhaps because they are less straightforward than affinity regulation. In this study, SDF-1α was directly shown to regulate LFA-1 lateral mobility in primary mouse lymphocytes. Increased LFA-1 lateral mobility in response to SDF-1α was nearly identical to that caused by cytochalasin D, consistent with a release from cytoskeletal constraints on motion. SDF-1α also led to nearly identical adhesion activation as did cytochalasin D (which does not induce high affinity; Ref. 22), suggesting that in this case the increased adhesion might be primarily due to LFA-1 movements, with a lesser contribution from conformational changes. This may be due to the fact that chemokine-induced integrin affinity increases tend to be transient (3), while integrin rearrangement produces more lasting effects (5, 22).

The fact that SDF-1α releases LFA-1 from cytoskeletal connections has implications for models of adhesion activation. For example, it has long been known that physiological
activators of LFA-1 are not as nearly as effective for binding of soluble ligand as for immobilized ligand (9). At least two mechanisms have been proposed to explain this observation: the traction force model (23) and the diffusion-limited adhesion model (1).

In the traction force model (23), it is proposed that cytoskeletonally driven translational motion of integrins on the cell surface exerts forces on the integrin to drive it into a higher-affinity conformation. It is proposed that this is effective only when the ligand is bound to a surface (or a cell) because soluble ligand cannot provide an anchor to resist cytoskeletonally-generated forces. While this mechanism may operate under some conditions (during cell motility, for example), it clearly does not explain SDF-1α-induced adhesiveness, because LFA-1 is released from the cytoskeleton by SDF-1α treatment. Thus this and other models that require cytoskeletal connections for adhesion activation by SDF-1α are inconsistent with the data presented here.

An alternative model, the diffusion-limited adhesion model (1), proposes that integrin movements are responsible for activation of adhesion to immobilized ligand independent of affinity changes. In this model, translational diffusion (even over minute distances) and/or rotational diffusion can improve alignment between integrin and ligand, increasing the kinetics of productive bond formation. This is only effective for anchored ligand, however. This is because, while the increased motion of diffusing LFA-1 (as compared with anchored LFA-1) can greatly increase the frequency of encounters with anchored ICAM-1, the diffusive motion of ICAM-1 in solution dominates the kinetics of integrin-ligand encounters, as it is orders of magnitude faster than diffusion of LFA-1 in the cell membrane.

The GFFKR sequence in the LFA-1 αL-subunit cytoplasmic domain is known to be important for regulation of adhesion, but its effects on LFA-1 mobility have been largely unknown, due to the lack of direct measurements. We demonstrated here that the GFFKR sequence is necessary and sufficient for cytoskeletal regulation of LFA-1 lateral mobility in primary T cells. That the GFFKR sequence is necessary is demonstrated by the fact that a construct retaining the entire αL-cytoplasmic domain except the GFFKR is unable to form the cytoskeletal interactions that would normally constrain LFA-1 motion at baseline. Since this construct is already constitutively free of cytoskeletal constraints on its motion, it is not affected by cytochalasin D or SDF-1α. The GFFKR alone is also sufficient for these interactions. That is, a truncation construct lacking the entire αL-cytoplasmic domain except the membrane-proximal GFFKR is immobile at baseline, as is wild type, and it is released by both cytochalasin D and SDF-1α in a manner indistinguishable from the wild type. Moreover, increased LFA-1 lateral mobility, whether in response to either cytochalasin D or SDF-1α, is associated with increased LFA-1-mediated adhesion.

This does not, of course, discount the general importance of affinity modulation, which is well established. LFA-1 clearly has multiple affinity states (15). Abundant information about the effect of affinity modulation on cell adhesion has been gained through studies (22) in which LFA-1 constructs are genetically modified to lock them into particular conformations. Information about the mechanism of LFA-1 activation in primary leukocytes in response to physiological stimuli in general and chemokines in particular, however, has been comparatively sparse. This study demonstrates that LFA-1 lateral mobility is increased by SDF-1α, consistent with a release from the cytoskeleton. This change in lateral mobility closely mimics that induced by cytochalasin D, which has been shown to increase integrin motion without inducing the high affinity state (22). As with lateral mobility, adhesion increases in response to SDF-1α and cytochalasin D are also nearly identical. It would be desirable to directly measure the contributions of LFA-1 movements and affinity changes in the same study. Unfortunately, however, while an array of conformation-specific antibodies exists to distinguish multiple LFA-1 conformations of human LFA-1, an analogous suite of antibodies does not exist for mouse integrins. Due to the unavailability of LFA-1-negative primary human leukocytes, however, it was possible only in the mouse system to study LFA-1 activation in primary T cells, rather than a cell line. Therefore, the contribution of affinity change in response to SDF-1α under our experimental conditions cannot be quantified. While this is somewhat unsatisfying, it is analogous to numerous
previous studies in which conformational states have been defined but lateral mobility has not been measured. We felt that the ability to express these constructs in a physiological, primary cell in which activation could be assumed to operate normally outweighed the inability to measure conformation directly. Future studies may address this question using human or chimeric LFA-1 in αL (CD11a)−/− mouse cells, provided that such constructs retain normal regulation of integrin-cytoskeleton interactions.

In any case, affinity modulation and avidity effects from integrin mobility and rearrangement are both likely to be important in activation of LFA-1. Therefore, a complete understanding of LFA-1 activation, whether in response to chemokines or other physiological means of activation, clearly must to take into account the proadhesive effects of integrin.

REFERENCES