THE JOURNAL OF BIOLOGICAL CHEMISTRY
© 2002 by The American Society for Biochemistry and Molecular Biology, Inc.
Vol. 277, No. 12, Issue of March 22, pp. 10638–10641, 2002
Printed in U.S.A.

Activation-induced Conformational Changes in the I Domain Region of Lymphocyte Function-associated Antigen 1*

Received for publication, December 27, 2001, and in revised form, January 14, 2002
Published, JBC Papers in Press, January 15, 2002, DOI 10.1074/jbc.M112417200

Qing Ma‡, Motomu Shimaoka, Chafen Lu§, Hua Jing, Christopher V. Carman, and Timothy A. Springer¶

From the Department of Pathology, Center for Blood Research and Harvard Medical School, Boston, Massachusetts 02115

Conformational changes in integrins are important for efficient ligand binding during activation. We proposed that the I domain of the integrin lymphocyte function-associated antigen 1 (LFA-1) could exist in both open and closed conformations and generated constitutively activated LFA-1 by locking the I domain in the open conformation. Here we provide structural and biochemical evidence to validate conformational change in the I domain of LFA-1 upon activation. Two monoclonal antibodies to α_L, HI111 and CBR LFA-1/1, bind wild-type LFA-1 well, but their binding is significantly reduced when LFA-1 is locked in the open conformation. Furthermore, this reduction in monoclonal antibody binding also occurs when LFA-1 is activated by divalent cations. HI111 maps to the top region of the I domain that is close to the putative ligand-binding site surrounding the MIDAS (metal ion-dependent adhesion site). The epitope of CBR LFA-1/1 is at the C-terminal segment of the I domain that links to the β-propeller, and undergoes a large movement between the open and closed conformations. Our data demonstrate that these two regions undergo significant conformational changes during LFA-1 activation and that the I domain of activated LFA-1 adopts a similar tertiary structure as the predicted locked open form.

Lymphocyte function-associated antigen 1 (LFA-1)[1] is a leukocyte integrin that contains the α_L (CD11a) and β_2 (CD18) chains. LFA-1 plays an important role in inflammatory and immune responses by regulating cell adhesion and leukocyte trafficking. The cell surface ligands for LFA-1 are members of the Ig superfamily, including intercellular adhesion molecule 1 (ICAM-1), ICAM-2, and ICAM-3 (1–3). LFA-1 mediates signals transduced bidirectionally across the plasma membrane. Signals from the cytoplasm (inside-out) activate LFA-1 enabling it to bind ligands. Subsequent binding to ligands on the cell surface results in signal transduction to the cytoplasm (outside-in) (4–6). It has been proposed that the activation of LFA-1 is regulated through both increased avidity and affinity (7–10).

Similar to other integrin α subunits, α_L has a complex domain structure that includes a large extracellular domain, a single transmembrane region, and a short cytoplasmic tail (11, 12). The N-terminal region of α_L folds into a seven-bladed β-propeller domain (13). The I domain of about 200 amino acids is inserted between β-sheets 2 and 3 of the β-propeller domain. The I domain of α_L is sufficient for maximal ligand binding affinity and adhesiveness (14, 15). Three-dimensional crystal structures of the I domain demonstrate the dinucleotide-binding fold similar to that of small G proteins with a metal ion-dependent adhesion site (MIDAS) (16–19).

Although both receptor clustering in the cell membrane (avidity) and receptor conformational change (affinity) are proposed to contribute to integrin activation, there is increasing evidence that conformational change in the I domain represents a key step in the activation of integrins (14, 15, 20–24). Indeed, the I domains of both the α_2 and α_M subunits have been crystallized in both "open" and "closed" conformations (16, 17, 19). The open conformations crystallized with a ligand-like lattice contact or a ligand bound at the MIDAS. However, structures have been determined for the I domain of α_L only in the closed conformation (25–27). Moreover, correlation of the observed conformational states in isolated crystallized LFA-1 I domains with cell surface expressed LFA-1 is lacking.

Recently, we have designed mutants of the α_L I domain stabilized in the open or closed conformation with disulfide bonds. Locking the I domain open resulted in a dramatic increase in affinity for ICAM-1, which can be reversed by disulfide reduction. In contrast, the closed form abolished adhesion (14, 15, 21, 24). These data suggest that conformational changes in the I domain are important for adhesive function.

Here, we provide structural and biochemical evidence to validate the conformational changes predicted by the locked open model. Two α_L mAbs, CBR LFA-1/1 and HI111, were identified as activation-sensitive. Both of them favor the closed or inactivated conformation of α_L. When LFA-1 is locked in the open conformation or activated by divalent cations, binding of both antibodies is reduced. The epitopes of HI111 and CBRLFA-1/1 are mapped on top of the I domain and within the C-terminal region of the I domain, respectively. These two regions shift significantly when the locked-open model is compared with the structure of the closed I domain structure. The data not only support our predicted open conformation model, but also provide direct evidence that conformational change is critical during LFA-1 activation.

* This work was supported by National Institutes of Health Grant CA317988. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Present address: Clinical Research Division, Fred Hutchinson Cancer Research Center, D1–100, 1100 Fairview Ave. North, Seattle, WA 98109.

§ Present address: Millennium Pharmaceuticals, 75 Sidney St., Cambridge, MA 02139.

¶ To whom correspondence should be addressed: Dept. of Pathology, Center for Blood Research and Harvard Medical School, 200 Longwood Ave., Boston, MA 02115. Tel.: 617-278-3200; Fax: 617-278-3232; E-mail: springeroffice@cbr.med.harvard.edu.

[1] The abbreviations used are: LFA-1, lymphocyte function-associated antigen 1; ICAM, intercellular adhesion molecule; MIDAS, metal ion-dependent adhesion site; mAb, monoclonal antibody; HBSS, Hanks' balanced salt solution.

This paper is available on line at http://www.jbc.org

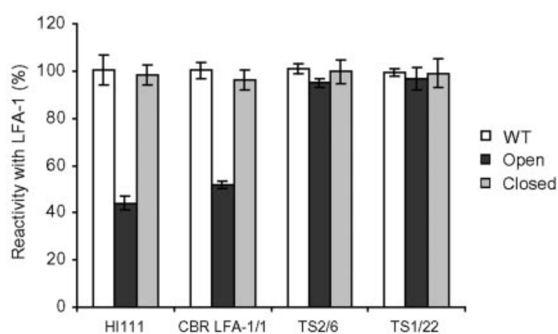

FIG. 1. **Reduced binding of anti-α_L antibodies HI111 and CBR LFA-1/1 to locked open LFA-1.** Wild-type, locked open, and locked closed α_L were coexpressed with wild-type β_2 at similar levels on K562 cells. Reactivity of antibodies with LFA-1 was determined by immunofluorescence flow cytometry. The mean specific fluorescence of each antibody was normalized to that of mAb TS1/18, to the β_2 subunit on the same cells, and expressed as the percentage of the normalized binding to cells with wild-type LFA-1. Results are mean ± S.D. of three independent experiments in duplicate.

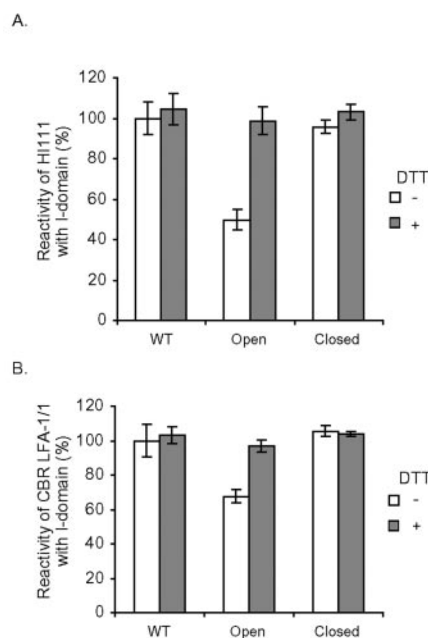

FIG. 2. **Binding of mAb to isolated I domains with and without dithiothreitol treatment.** *A*, HI111; *B*, CBR LFA-1/1. Binding of mAbs to isolated I domains expressed on the surface of K562 cells was determined by immunofluorescence flow cytometry. The mean fluorescence of each antibody was normalized to that of mAb TS1/22 to the α_L I domain. Binding of mAbs to K562 cells was performed in L15/FBS in the absence or presence of 10 mM dithiothreitol. Results are presented as the percentage of binding to wild-type I domain. Data are mean ± S.D. of three independent experiments in duplicate.

MATERIALS AND METHODS

The human and mouse chimeric α_L mutants were named as described previously (28). The K562 stable cell lines were characterized previously (14, 24). The mouse anti-human α_L mAbs TS2/4, TS1/22, CBR LFA-1/1, and IgG X63 have been described before. HI111 is a mouse anti-human α_L mAb (PharMingen) and was characterized in the Fourth International Leukocyte Workshop as IV N231 (29).

Flow cytometry analysis was done as described previously (30). Purified mAbs, TS1/18, TS2/4, TS2/6, TS1/22, CBR LFA-1/1, and HI111, were used at 10 μg/ml.

Surface plasmon resonance with a BIAcore instrument was used to measure the affinity of mAbs to the isolated I domain. HI111 or activation-insensitive mAb MEM83 were covalently immobilized to the dextran surface of CM5 sensor chips by an amine-coupling kit (BIAcore, Piscataway, NJ), and assays were performed as described previously (15). Soluble forms of wild-type or locked open α_L I domains was flowed over the sensor chip at 10 μl/ml. HEPES-buffered saline (pH 7.4) containing 0.005% surfactant P20 (BIAcore) was used as running buffer. Kinetic constants k_{on} and k_{off} were obtained by curve fitting using a 1:1 binding model and BIA evaluation 3.0 software. K_D was calculated as k_{off}/k_{on}.

The model of the locked open LFA-1 I domain was built as described previously (15). Superposition of the model onto the crystal structure of the closed I domain (Protein Data Bank number 1zon) was done with 166 Cα atoms with a root mean square deviation of 0.93 Å.

RESULTS

We have previously introduced disulfide bonds into the I domain of α_L to generate constitutively active (open) or inactive (closed) conformations (14). A panel of antibodies mapped to various regions of α_L, including 10 mAbs to the I domain, was previously tested by our laboratory for binding to these mutants (24). All of the antibodies tested bound equally well to wild-type and mutant LFA-1, with the exception of mAb CBR LFA-1/1. The binding of mAb CBR LFA-1/1 to the open conformation was reduced compared with wild-type or locked closed LFA-1. We confirmed these results with K562 cells expressing wild-type, locked open, and locked closed LFA-1 (Fig. 1). Furthermore, we identified another anti-α_L antibody, HI111, that behaved similarly to CBR LFA-1/1. Both HI111 and CBR LFA-1/1 mAbs bound equally well to wild-type and locked closed LFA-1, but the binding of HI111 and CBR LFA-1/1 to the locked open LFA-1 was reduced to about 40–50% of the wild-type. The difference was not attributable to lower expression of open LFA-1, because all other α_L antibodies, including TS2/6 and TS1/22, demonstrated similar reactivity to wild-type and the locked open and closed mutants (Fig. 1).

To further investigate whether reduced binding of the mAbs is due to conformational change within the open I domain, the mAbs were tested for binding to the α_L I domain (residues 130–338) expressed in isolation from other integrin domains on the cell surface using a platelet-derived growth factor receptor transmembrane domain (24) (Fig. 2). mAb HI111 bound only 50% as well to the isolated locked open I domain as to the isolated wild-type and locked closed I domains (Fig. 2*A*). By contrast, when cells were treated with dithiothreitol to reduce disulfide bonds (14), the binding of HI111 mAb was restored to the same level as to wild-type and closed I domains (Fig. 2*A*). Similar results were obtained with mAb CBR LFA-1/1 (Fig. 2*B*). Disulfide bond reduction has been shown to abolish the increased adhesiveness and affinity of the isolated, locked open mutant I domains for ICAMs and appears to result in conversion to the closed conformation (14, 15). Therefore, both HI111 and CBR LFA-1/1 mAbs preferentially bind to the conformation of the closed I domain, and the binding is reduced when LFA-1 is locked in the open conformation.

Although mutations can be used to stabilize the open conformation of the I domain, it is important to demonstrate that a similar open conformation can be accessed by wild-type LFA-1 on the cell surface. LFA-1 can be activated by Mn^{2+}, or by the combined presence of Mg^{2+} and absence of Ca^{2+} (31). Therefore, we examined the effect of divalent cations on expression of the HI111 and CBR LFA-1/1 epitopes on wild-type LFA-1. The binding of TS2/4 mAb was unaffected by activation, suggesting that the expression level of LFA-1 did not change (Fig. 3). Similar results were obtained with other mAb to LFA-1. However, the binding of HI111 and CBR LFA-1/1 mAbs to LFA-1 on activated cells was about 60–80% of the binding to LFA-1 on resting cells (Fig. 3). The decrease in binding was consistently seen in multiple experiments and was statistically significant. Thus, activation of LFA-1 either by locked open mutations or divalent cations can reduce the binding of HI111 and CBR LFA-1/1 mAbs, showing that the locked open conformation is similar to the native structure of activated LFA-1. Although the reduction in binding of the two mAbs was lesser than seen

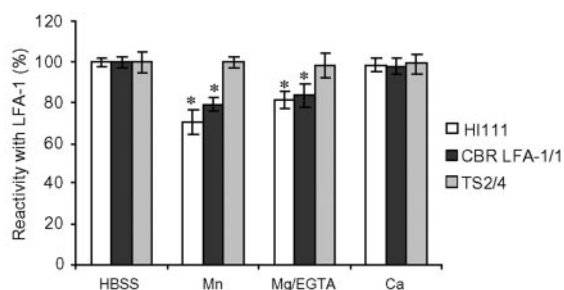

FIG. 3. **Binding of HI111 and CBR LFA-1/1 mAbs to LFA-1 after activation by divalent cations.** Staining by antibodies of wild-type LFA-1 expressed on K562 cells was performed in HBSS/bovine serum albumin medium with or without supplementation with 2 mM Mn^{2+}, 2 mM Ca^{2+}, or 2 mM Mg^{2+}, and 2 mM EGTA. Reactivity of HI111, CBR LFA-1/1, and TS2/4 mAbs was determined by flow cytometry. The mean fluorescence of each reaction was normalized to the intensity of TS1/18 staining. Results are shown as the percentage of staining in HBSS medium in the absence of the supplements indicated above. Data are mean ± S.D. of three independent experiments in duplicates. *Asterisks*, data with p values less than 0.05 in t test comparisons to data in HBSS.

with locked open LFA-1, this may reflect incomplete conversion to the open conformation.

To examine the structural features shared between the conformations of mutant, locked open α_L and of activated, wild-type α_L, we defined the epitopes of mAbs CBR LFA-1/1 and HI111. CBR LFA-1/1 was previously mapped to residues 301–359 (28) and reacts with the isolated I domain containing residues 130–338 (24). Therefore, its epitope comprises the C-terminal α-helix (residues 299–305) of the I domain and the following linker to the β-propeller domain. We used human-mouse α_L chimeras to map the epitope recognized by HI111. Eight α_L chimeras containing segments of the mouse α_L I domain swapped into human α_L or vice versa mapped the epitope to residues 249–300 (Table I, *left*). Individual amino acid substitutions of human with mouse sequence in this region showed that the HI111 mAb is specific for Lys-268, which is substituted to Val in the mouse sequence (Table I, *right*). This amino acid is located on the top face of the I domain not far from the MIDAS (Fig. 4).

To understand the basis of the reduced binding to the open conformation of the LFA-1 I domain, we measured the kinetics and affinity of HI111 binding to purified I domains. The HI111 mAb bound to the locked open I domain with a k_{on} value 5-fold lower than to the wild-type I domain (Table II). The k_{off} values were similar. This resulted in a 5-fold lower affinity of HI111 for the locked open I domain than for the wild-type I domain.

The affinity measurements are in excellent agreement with the differences seen in immunofluorescent flow cytometry. The antibody concentration was 1.3×10^{-7} M for staining cells; assuming monovalent binding, the K_D values predict 63% saturation of binding to wild-type and 24% saturation of binding to open mutant I domains. This 2.6-fold difference is in agreement with the ratio of 2- to 2.5-fold found in flow cytometry experiments. At a concentration of 2.7×10^{-7} M, HI111 mAb almost completely inhibited adhesion to ICAM-1 of transfectants expressing wild-type LFA-1 (99%) and only partially inhibited binding of transfectants expressing locked open LFA-1 (18%) (data not shown). This is consistent with the predicted 78% saturation of cell surface binding to wild-type LFA-1, the 39% saturation of cell surface binding to LFA-1 containing a locked open I domain, and the greater affinity of the open conformation for ICAM-1.

DISCUSSION

We demonstrate here that two mAbs that bind to distinct epitopes, HI111 and CBR LFA-1/1, report conformational change in LFA-1 I domains on the cell surface. Both mAbs bind better to wild-type and locked closed I domains than to the locked open I domains. When the I domain is locked in the open conformation, there is nearly a 50% reduction of their binding. Most importantly, a similar trend is also observed when wild-type LFA-1 is activated by cations, suggesting that change toward the open conformation occurs when LFA-1 is activated on the cell surface. The native LFA-1 activation process is dynamic and may involve an equilibrium between closed and open conformers, or intermediate conformational states. This could explain the observation that binding of HI111 and CBR LFA-1/1 to LFA-1 activated by cations was decreased less than when the I domain was locked open by mutational introduction of a disulfide bond. Antibodies have previously been used with $\alpha_L\beta_2$ to demonstrate change in conformation or accessibility in the I-like domain (24, 31) and the integrin epidermal growth

TABLE I
Mapping of the HI111 epitope to residue Lys-268 in the human α_L subunit

The human and mouse chimeric α_L mutants are named as described previously (28), *e.g.* h153m359h has human α_L sequence except for a murine segment from residues 154 to 359. The indicated human/mouse α_L chimeras and mutants were cotransfected with β_2 into 293T cells. The fluorescent intensity of staining with mAb HI111 was normalized to staining with β_2 antibody TS1/18. The ratio of fluorescence intensity was compared to that of human wild-type α_L: +, indistinguishable from wild-type α_L; −, indistinguishable from mock transfectant.

α_L chimera	HI111 binding	α_L mutant	HI111 binding
h	+	D249S/K252H	+
m	−	I255H	+
h153m359h	−	Q266V	+
m153h359m	+	T267S	+
h118m153h	+	K268V	−
h153m183h	+	E269Q	+
h184m215h	+	S270K	+
h217m248h	+	E272K	+
h249m303h	−	K276I	+
h300m359h	+	K280E/A282V/S283E	+
Epitope	249–300	Epitope	268

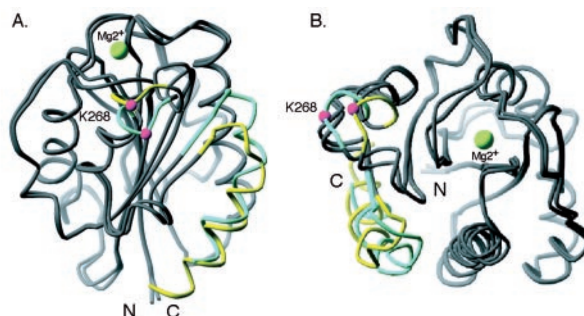

FIG. 4. **Structural comparison of the locked open mutant with the wild-type I domain.** These two conformers were superimposed and are shown as *backbone traces* viewed from the side (*A*) or top (*B*). The regions undergoing the largest backbone movements (residues 267–270 and 288–291) are highlighted in *cyan* (closed structure 1ZON (25)) and *yellow* (open model (15)). The Cα atom of Lys-268 is shown as a *magenta sphere*, and the Mg^{2+} ion in the open model is shown as a *green sphere*. Prepared with Ribbons (41).

TABLE II
Binding kinetics of HI111 to the I domain of α_L

The kinetics of binding of wild-type and locked open I domains to HI111 mAb immobilized on sensorchips was measured with surface plasmon resonance.

I domain	k_{on}	k_{off}	K_D
	$M^{-1} \cdot s^{-1}$	s^{-1}	M
WT	1.25×10^5	9.61×10^{-3}	7.68×10^{-8}
Open	2.32×10^4	9.72×10^{-3}	4.19×10^{-7}

factor modules of the β_2 subunit (32–37); however, this is the first time that mAbs to the LFA-1 I domain have been found to report conformational change. It is important that decreased binding to the open conformation was seen with both isolated I domains and $\alpha_L\beta_2$, because this demonstrates that changes within the I domain itself, rather than inter-domain rearrangements that affect accessibility, are recognized by the HI111 and CBR LFA-1/1 mAbs.

The CBR LFA-1/1 and HI111 mAbs map to distinct, functionally important sites in the I domain. CBR LFA-1/1 maps to the residues at the lower part of the C-terminal α-helix of the I domain and the subsequent segment that connects to the β-propeller domain. This region is predicted to move downward in the transition to the open conformation (Fig. 4) and is part of the I domain allosteric site that regulates the activation of LFA-1 as shown by mutations and chemical shift perturbations when ICAM-1 is bound (22, 38). Although the C-terminal α helix undergoes significant conformational changes upon ligand binding, this region plays only an indirect or regulatory role in ligand binding. Multiple classes of small molecules have been identified that bind between the C-terminal α-helix and the body of the I domain and inhibit binding to ICAM-1 by stabilizing the closed conformation (26, 39, 40) as confirmed by resistance to these inhibitors of locked open LFA-1 (14).

The conformation-sensitive HI111 mAb is specific for residue Lys-268 of the I domain. Lys-268 is the first residue of α-helix 5, at the junction of the loop from the preceding β-strand 5, and is located near the MIDAS on the ligand-binding, "top" face of the I domain (Fig. 4). Superposition of the closed α_L I domain structure and the open α_L I domain model reveals a number of regions that are predicted to undergo significant backbone displacements in the closed to open transition. Those regions that shift the most are shown in *yellow* (open) and *cyan* (closed) and include the segment containing Lys-268 (Fig. 4).

In conclusion, we have mapped mAb to the two distinct regions in the I domain of α_L, which are predicted to undergo the largest backbone movements in the transition between the open and closed conformation. HI111 binds to the edge of the top face bearing the MIDAS, in or near the ligand binding site. By contrast, CBR LFA-1/1 binds to the lower part of the C-terminal α-helix or the linker, far from the MIDAS, to an allosteric site that is conformationally linked to movements at the MIDAS. The conformational movements detected by these mAb occur in comparisons between mutants stabilized in the open and closed conformations, upon reduction of the disulfide stabilizing the open mutant, and upon activation of wild-type LFA-1 on the cell surface. These studies add further support to the accumulating evidence that conformational change in integrin I domains regulates ligand binding. We conclude that LFA-1 undergoes conformational changes during activation, and the changes involve at least the two regions recognized by mAbs HI111 and CBR LFA-1/1.

REFERENCES

1. Larson, R. S., and Springer, T. A. (1990) *Immunol. Rev.* **114,** 181–217
2. Springer, T. A. (1994) *Cell* **76,** 301–314
3. Gahmberg, C. G., Tolvanen, M., and Kotovuori, P. (1997) *Eur. J. Biochem.* **245,** 215–232
4. Schwartz, M. A., Schaller, M. D., and Ginsberg, M. H. (1995) *Ann. Rev. Cell Dev. Biol.* **11,** 549–599
5. Diamond, M. S., and Springer, T. A. (1994) *Curr. Biol.* **4,** 506–517
6. Hughes, P. E., and Pfaff, M. (1998) *Trends Cell Biol.* **8,** 359–364
7. Dustin, M. L., and Springer, T. A. (1989) *Nature* **341,** 619–624
8. Lollo, B. A., Chan, K. W. H., Hanson, E. M., Moy, V. T., and Brian, A. A. (1993) *J. Biol. Chem.* **268,** 21693–21700
9. Stewart, M., and Hogg, N. (1996) *J. Cell. Biochem.* **61,** 554–561
10. McDowall, A., Leitinger, B., Stanley, P., Bates, P. A., Randi, A. M., and Hogg, N. (1998) *J. Biol. Chem.* **273,** 27396–27403
11. Humphries, M. J. (2000) *Biochem. Soc. Trans.* **28,** 311–339
12. Xiong, J.-P., Stehle, T., Diefenbach, B., Zhang, R., Dunker, R., Scott, D. L., Joachimiak, A., Goodman, S. L., and Arnaout, M. A. (2001) *Science* **294,** 339–345
13. Springer, T. A. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94,** 65–72
14. Lu, C., Shimaoka, M., Ferzly, M., Oxvig, C., Takagi, J., and Springer, T. A. (2001) *Proc. Natl. Acad. Sci. U. S. A.* **98,** 2387–2392
15. Shimaoka, M., Lu, C., Palframan, R., von Andrian, U. H., Takagi, J., and Springer, T. A. (2001) *Proc. Natl. Acad. Sci. U. S. A.* **98,** 6009–6014
16. Lee, J.-O., Rieu, P., Arnaout, M. A., and Liddington, R. (1995) *Cell* **80,** 631–638
17. Lee, J.-O., Bankston, L. A., Arnaout, M. A., and Liddington, R. C. (1995) *Structure* **3,** 1333–1340
18. Qu, A., and Leahy, D. J. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92,** 10277–10281
19. Emsley, J., Knight, C. G., Farndale, R. W., Barnes, M. J., and Liddington, R. C. (2000) *Cell* **101,** 47–56
20. Oxvig, C., Lu, C., and Springer, T. A. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96,** 2215–2220
21. Shimaoka, M., Shifman, J. M., Jing, H., Takagi, J., Mayo, S. L., and Springer, T. A. (2000) *Nature Struct. Biol.* **7,** 674–678
22. Huth, J. R., Olejniczak, E. T., Mendoza, R., Liang, H., Harris, E. A., Lupher, M. L., Jr., Wilson, A. E., Fesik, S. W., and Staunton, D. E. (2000) *Proc. Natl. Acad. Sci. U. S. A.* **97,** 5231–5236
23. Xiong, J.-P., Li, R., Essafi, M., Stehle, T., and Arnaout, M. A. (2000) *J. Biol. Chem.* **275,** 38762–38767
24. Lu, C., Shimaoka, M., Zang, Q., Takagi, J., and Springer, T. A. (2001) *Proc. Natl. Acad. Sci. U. S. A.* **98,** 2393–2398
25. Qu, A., and Leahy, D. J. (1996) *Structure* **4,** 931–942
26. Kallen, J., Welzenbach, K., Ramage, P., Geyl, D., Kriwacki, R., Legge, G., Cottens, S., Weitz-Schmidt, G., and Hommel, U. (1999) *J. Mol. Biol.* **292,** 1–9
27. Legge, G. B., Kriwacki, R. W., Chung, J., Hommel, U., Ramage, P., Case, D. A., Dyson, H. J., and Wright, P. E. (2000) *J. Mol. Biol.* **295,** 1251–1264
28. Huang, C., and Springer, T. A. (1995) *J. Biol. Chem.* **270,** 19008–19016
29. Knapp, W., Dörken, B., Gilks, W. R., Rieber, E. P., Schmidt, R. E., Stein, H., and von dem Borne, A. E. G. K. (1989) *Leucocyte Typing IV: White Cell Differentiation Antigens*, Oxford University Press, Oxford
30. Lu, C., and Springer, T. A. (1997) *J. Immunol.* **159,** 268–278
31. Dransfield, I., Cabañas, C., Craig, A., and Hogg, N. (1992) *J. Cell Biol.* **116,** 219–226
32. Robinson, M. K., Andrew, D., Rosen, H., Brown, D., Ortlepp, S., Stephens, P., and Butcher, E. C. (1992) *J. Immunol.* **148,** 1080–1085
33. Andrew, D., Shock, A., Ball, E., Ortlepp, S., Bell, J., and Robinson, M. (1993) *Eur. J. Immunol.* **23,** 2217–2222
34. Binnerts, M. E., van Kooyk, Y., Simmons, D. L., and Figdor, C. G. (1994) *Eur. J. Immunol.* **24,** 2155–2160
35. Petruzzelli, L., Maduzia, L., and Springer, T. A. (1995) *J. Immunol.* **155,** 854–866
36. Stephens, P., Romer, J. T., Spitali, M., Shock, A., Ortlepp, S., Figdor, C., and Robinson, M. K. (1995) *Cell Adhes. Commun.* **3,** 375–384
37. Zang, Q., Lu, C., Huang, C., Takagi, J., and Springer, T. A. (2000) *J. Biol. Chem.* **275,** 22202–22212
38. Lupher, M. L., Jr., Harris, E. A., Beals, C. R., Sui, L., Liddington, R. C., and Staunton, D. E. (2001) *J. Immunol.* **167,** 1431–1439
39. Liu, G., Huth, J. R., Olejniczak, E. T., Mendoza, R., DeVries, P., Leitza, S., Reilly, E. B., Okasinski, G. F., Fesik, S. W., and von Geldern, T. W. (2001) *J. Med. Chem.* **44,** 1202–1210
40. Last-Barney, K., Davidson, W., Cardozo, M., Frye, L. L., Grygon, C. A., Hopkins, J. L., Jeanfavre, D. D., Pav, S., Qian, C., Stevenson, J. M., Tong, L., Zindell, R., and Kelly, T. A. (2001) *J. Am. Chem. Soc.* **123,** 5643–5650
41. Carson, M. (1997) *Methods Enzymol.* **277,** 493–505