**Molecular Basis of Cell and Developmental Biology:**
**A Mechanism for Antibody-mediated Outside-in Activation of LFA-1**

Roberto Carreño, Dan Li, Mehmet Sen, Iris Nira, Tatsuo Yamakawa, Qing Ma and Glen B. Legge

doi: 10.1074/jbc.M704699200 originally published online January 16, 2008

Access the most updated version of this article at doi: 10.1074/jbc.M704699200

Find articles, minireviews, Reflections and Classics on similar topics on the JBC Affinity Sites.

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 69 references, 32 of which can be accessed free at http://www.jbc.org/content/283/16/10642.full.html#ref-list-1
A Mechanism for Antibody-mediated Outside-in Activation of LFA-1*

Received for publication, June 7, 2007, and in revised form, January 11, 2008 Published, JBC Papers in Press, January 16, 2008 DOI 10.1074/jbc.M704699200

Roberto Carreño‡1, Dan Li‡, Mehmet Sen‡2, Iris Nira‡1, Tatsuo Yamakawa‡, Qing Ma‡, and Glen B. Legge‡3

From the ‡Department of Biology and Biochemistry, University of Houston, Houston, Texas 77204-5001 and the §Department of Stem Cell Transplantation and Cellular Therapy, University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030

MEM83 is an inserted domain (I-domain)-specific antibody that up-regulates the interaction of LFA-1 with ICAM-1 through an outside-in activation mechanism. We demonstrate here that there is no change in the affinity of the MEM83 antibody for the I-domain in either its low (wild-type) or high affinity form and that MEM83 does not enhance the binding of the wild-type I-domain to ICAM-1. Furthermore, we show that the antibody acts as an activating agent to induce LFA-1/I-domain-dependent homotypic cell aggregation only as an IgG, but not as a Fab fragment. On the basis of these data, we propose an avidity-based mechanism that requires no direct activation of the LFA-1 I-domain by the binding of the antibody; rather, activation is enhanced when there is an interaction with both arms of the IgG. A molecular model of the antibody interaction with LFA-1 illustrates the symmetry and accessibility of the two MEM83 epitopes across the LFA-1/I-domain heterotetramer. We hypothesize that MEM83 stabilizes adjacent LFA-1 molecules in their active form by the free energy that is gained from the binding of the I-domains to each arm of the IgG. This leads to stabilization of the open state of the integrin and outside-in signaling. Our model supports a mechanism in which both affinity and avidity regulation are required in the activation of LFA-1.

The interaction of leukocyte function-associated antigen-1 (LFA-1)*1 and intercellular adhesion molecules (ICAMs) plays a key role in the immune system and in the development of atherosclerosis, autoimmune diseases, and inflammation (1–5). Further understanding of the LFA-1 activation mechanism is necessary for the development of novel therapeutics that could modulate the immune system (6, 7). Inhibitors that stabilize the inactive form or block the active form of LFA-1 suppress immune responses and may prevent inflammatory and autoimmune disease (8–10). Similarly, up-regulation of LFA-1 can be used to enhance immune responses (11–13) and may have roles in restoring T cell function in human immunodeficiency virus-immunocompromised individuals (14) or in overcoming tumor-specific toleration (15).

Activation of the integrin LFA-1 results in a rapid and reversible increase in affinity for its ligands, ICAM-1–5 (16–19). ICAM-1–3 regulate T cell activation (13) and leukocyte homing (20, 21); ICAM-4 is a red blood cell-specific ligand that can interact with a several β2/CD18 integrins (22); and ICAM-5 plays a role in leukocyte recruitment to neurons in the central nervous system (19). In T cells, LFA-1 and ICAM-1 act as co-stimulatory molecules to the peptide/major histocompatibility complex (23, 24). The role of LFA-1 and ICAM-1 interaction is critical to the formation of the immunological synapse, and aberrant activation or expression of LFA-1 may lead to selectively disabled T cell receptor clustering (25) or the development of autoimmune diseases (26, 27).

Recognition and binding of its ICAM-1 ligand by LFA-1 are mediated through the inserted domain (I-domain) in its α-subunit; this forms a heterotetrameric complex through D1/D1-domain dimerization within ICAM-1 (28). Activation of this integrin entails both a quaternary rearrangement of the αβ2 heterodimer and a tertiary structural rearrangement within the I-domain (29). As a result, the affinity of the isolated I-domains for ICAMs can be modulated by locking the I-domain in its high affinity open conformation by the introduction of a disulfide bridge (28, 30). Yeast display selection also identified two activating I-domain mutations (F265S/F292G) that enhance ICAM affinity by 200,000-fold (31).

Integrins switch from the inactive to active form by either inside-out or outside-in activation (32, 33). Inside-out activation of LFA-1 is induced by chemokine activation of G-protein-coupled receptors (34, 35) and can be mediated by the use of phorbol esters (phorbol 12-myristate 13-acetate (PMA)) (36). Outside-in activation of β2 and other integrins is a method for ligand-induced “cross-talking” to modulate signaling pathways within a cell (37, 38). Outside-in activation of LFA-1 that leads to high affinity ICAM binding can be directly triggered by the addition of manganese (39). There are also several monoclonal antibodies known to outside-in activate LFA-1 and specifically bind to the αL-subunit (MEM83 (40–42) and NKI-L16 (43)) or the β2-subunit (KIM185 and MEM48 (44–46)). The activating antibody NKI-L16 was mapped to a region within the αL-subunit near the transmembrane segment of the molecule (43).

*This work was supported in part by American Heart Foundation Grant 0435123N (to G. B. L.). 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.
1 Recipients of research studentships from the Rice-Houston Alliance for Graduate Education and the Professoriate supported by National Science Foundation Grant 0450363.
2 Present address: CBR Institute for Biomedical Research, Inc., Boston, MA 02115.
3 To whom correspondence should be addressed: Dept. of Biology and Biochemistry, University of Houston, 4800 Calhoun Rd., Houston, TX 77204-5001. Fax: 713-743-8351; E-mail: glegge@uh.edu.
4 The abbreviations used are: LFA-1, leukocyte function-associated antigen-1; ICAM, intercellular adhesion molecule; I-domain, inserted domain; PMA, phorbol 12-myristate 13-acetate; WT, wild-type; HA, high affinity; IA, intermediate affinity; SPR, surface plasmon resonance.
The monoclonal antibody MEM83 binds the αL-subunit of LFA-1 (40) and was later identified as an activating antibody for the LFA-1/ICAM-1 interaction (40–42, 47–49). Interestingly, this antibody was also found to block LFA-1 binding to ICAM-3–4 (47, 50). The epitope of MEM83 maps to two regions within the I-domain (Asp153–Phe183 and Thr217–Ile248), with Asp182 being required in the first region and Glu218 being required combinatorially in the second (51). Here, we investigate potential mechanisms for MEM83 interaction with LFA-1.

EXPERIMENTAL PROCEDURES

LFA-1 I-domain Constructs—Wild-type (WT) and mutant (high affinity (HA) and intermediate (IA) affinity) I-domains (Gly123–Tyr304) were expressed in Escherichia coli BL21(DE3) cells using supplemented M9 minimal medium. The recombinant proteins were subsequently refolded and purified as described (52, 67). Proteins were concentrated using Centriprep C10 (Millipore Corp.), and sample concentrations were determined using BCA (Pierce) and at A$_{280}$ nm. Each LFA-1 I-domain was also 15N-labeled to allow acquisition of 1H/15N heteronuclear single quantum coherence nuclear magnetic resonance spectra for quality assurance prior to surface plasmon resonance (SPR) analysis (data not shown).

MEM83 Binding Assays—MEM83 was provided as a gift by Dr. Václav Hořejší (Institute of Molecular Genetics AS CR, Prague, Czech Republic) and was purified by size exclusion chromatography (Superdex™ 75, GE Healthcare). An aliquot of MEM83 was digested by papain, and the Fab fragments were purified on a protein A column as described (41). MEM83 fractions (either IgG or Fab) were concentrated, and sample concentrations were determined by both Bradford (Bio-Rad) and BCA assays. MEM83 IgG was coupled to a CM5 sensor chip (Biacore/GE Healthcare) by amine coupling following the manufacturer’s protocols to a response unit of 4630. LFA-1 I-domains (WT, IA, and HA) at concentrations of 20, 50, and 100 nM were subsequently injected over the chip at 10 μl/min with Running Buffer A (10 mM HEPES (pH 7.4), 150 mM NaCl, 0.5 mM MgSO$_4$, and 0.005% (v/v) Tween 20). For the Fab binding analysis, the HA I-domain was bound to a CM5 chip by amine coupling to a response unit of 743. Buffers and binding conditions were as described for the MEM83 binding experiments.

ICAM-1/Fc Binding Assays—ICAM-1/Fc (R&D Systems) was coupled to a CM5 sensor chip by amine coupling. LFA-1 I-domains (WT, IA, and HA) at concentrations of 100, 200, and 400 nM and LFA-1 I-domains complexed with excess (10-fold) MEM83 were injected over the chip at 10 μl/min with Running Buffer A. Negative binding controls with matching MEM83 concentrations were run as described above except that 3 mM EDTA was substituted for MgSO$_4$ (Running Buffer B).

Biacore Binding Analysis—All SPR (Biacore) sensorgrams were analyzed from triplicate runs using a 1:1 Langmuir model by Scrubber (Version 2.0, Center for Biomolecular Interaction Analysis, University of Utah).

Homotypic Aggregation Assay—The aggregation experiment was performed in flat-bottom 96-well plates at 100 μl cells/well as described (35). Human JY cells were washed twice and diluted in RPMI 1640 medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (1000 units/ml.

![FIGURE 1. Binding affinities of HA, IA, and WT LFA-1 I-domains for MEM83. Shown are the results for SPR binding assays of HA (A), IA (B), and WT (C) LFA-1 I-domain binding to immobilized MEM83 antibody. All experiments were performed using a Biacore 2000 instrument and CM5 chip. LFA-1 I-domains were diluted to various concentrations with Running Buffer A. Following each injection, the chip was regenerated with 10 mM glycine (pH 2.5). WT and HA LFA-1 I-domain structures were superimposed using VMD multiple alignment (68) and show Asp182 and Glu218 from the MEM83 epitope (D).](http://www.jbc.org/)

<table>
<thead>
<tr>
<th>LFA-1 I-domain</th>
<th>k$_{on}$</th>
<th>k$_{off}$</th>
<th>K$_D$</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA</td>
<td>230,000 ± 40,000</td>
<td>0.0009 ± 0.0001</td>
<td>4.0 ± 0.4</td>
</tr>
<tr>
<td>HA Fab</td>
<td>520,000 ± 300,000</td>
<td>0.0017 ± 0.0013</td>
<td>3.0 ± 0.8</td>
</tr>
<tr>
<td>IA</td>
<td>280,000 ± 90,000</td>
<td>0.0010 ± 0.0002</td>
<td>4.0 ± 1.1</td>
</tr>
<tr>
<td>WT</td>
<td>340,000 ± 80,000</td>
<td>0.00089 ± 0.0006</td>
<td>2.8 ± 0.7</td>
</tr>
</tbody>
</table>
penicillin and 100 μg/ml streptomycin; Invitrogen) to a final concentration of 10^7 cells/ml. Cells were treated with 10 ng/ml PMA, 0.5 μM Mn²⁺, 0.5 μM Ca²⁺, and MEM83 or MEM83 Fab at various concentrations and incubated at 37°C for 20 min. The cells were observed, and photographs were taken under a light microscope. SPR binding assays for the MEM83 Fab fragment were performed both before and after the cell assays to ensure there was no inactivation of LFA-1 binding activity.

RESULTS AND DISCUSSION

Binding of LFA-1 I-domains by MEM83 as Measured by SPR—A potential mode of MEM83 activation may be due to an enhanced affinity of the antibody for the I-domain in its open HA form relative to its closed low affinity (WT) form. Hence, activation is mediated by direct tertiary interactions within the I-domain. To test this, we expressed recombinant LFA-1 I-domains (Gly123–Tyr307) in E. coli and purified and refolded these protein samples from inclusion bodies. We used SPR (Biacore) to measure the affinity of MEM83 IgG and Fab for the WT, disulfide-locked IA (L161C/F299C), and HA (K287C/K294C) forms (52). No binding preference for the open form was observed (Fig. 1, A–C, and Table 1); thus, activation cannot be mediated by an induced tertiary switch directly upon antibody binding.

To determine whether ICAM-1 and the MEM83 antibody are both required to enhance LFA-1 I-domain activation (47), we bound ICAM-1/Fc to the Biacore CM5 chip surface and measured the affinity of ICAM-1 for the HA and WT LFA-1 I-domains in the presence and absence of the antibody (Fig. 2). Although there was an increased response unit upon premixing the antibody with the HA I-domain, no change in affinity was observed (Table 2). The presence of a divalent cation was required, as the addition of EDTA to the running buffer prevented the interaction with ICAM-1.

FIGURE 2. Effect of MEM83 on LFA-1 binding to ICAM-1/Fc. Shown are the results from SPR binding analysis of LFA-1 HA I-domain/MEM83 complex (A), LFA-1 HA I-domain (B), LFA-1 WT I-domain/MEM83 complex (C), and LFA-1 WT I-domain (D) binding to ICAM-1/Fc. LFA-1 I-domains and I-domain/MEM83 complexes were diluted to various concentrations with Running Buffer A. HA I-domain samples were used as positive controls, with MEM83 added in 10-fold excess to form the I-domain/MEM83 complex. The chip surface was regenerated with Running Buffer B.
Finally, no binding was observed for the WT I-domain in either the presence or absence of the antibody (Fig. 2).

Our data indicate that MEM83 neither directly enhanced nor blocked the binding of the LFA-1 I-domain to ICAM-1. The HA I-domain in its open conformation enhances stable adhesion with ICAM-1 (53, 54) and was not blocked by the addition of MEM83 (Table 2). This is consistent with the mapped MEM83 epitope, which indicates that the antibody binds to a region within the I-domain that does not significantly change its local structure upon activation (Fig. 1D) (51). Published work on glycosylphosphatidylinositol-anchored LFA-1 I-domains showed increased cell rolling upon binding of MEM83 (47). Based on our data, this increase in rolling efficiency in the presence of MEM83 may be due to an increased affinity that is below the threshold of detection for SPR. Alternatively, MEM83 binding may preferentially orient the glycosylphosphatidylinositol-anchored I-domain for ICAM-1 binding or be the result of shear flow conditions (55).

**Avidity and Steric Wedge Activation Mechanisms**—Rather than a directly induced change in the tertiary structure of the I-domain, our data suggest that the activation mechanism for MEM83 results from induced changes in the overall quaternary structure of LFA-1. We propose two models, avidity and steric wedge, for MEM83-mediated activation of LFA-1 that are based on the accessibility of the antibody to bind the I-domain in the full-length protein.

An avidity model is based on the binding of the LFA-1 I-domains to both arms of the IgG. In this model, MEM83 can bind only one I-domain in the bent/closed conformation because of the close proximity of the I-domain to the membrane, whereas the IgG is able to bind and cross-link I-domains from two separate LFA-1/ICAM-1 heterotetramers (28) in its extended/open conformation. Thus, an avidity model of outside-in activation of LFA-1 links both arms of an IgG molecule to two LFA-1/ICAM-1 heterotetramers, and vice versa, two I-domains within a single heterotetramer to two separate IgG molecules, to generate a one-dimensional array.

An alternative mode of activation could be that MEM83 acts as a steric wedge between the LFA-1 I-domain and the adjacent β-propeller domain or that the accessibility for the MEM83 epitope is blocked by the membrane when the integrin is in its closed form. Electron microscopy studies of the closed form of LFA-1 have estimated a distance of 8–74 Å between the I-domain and cell membrane; in the open form of LFA-1, this distance increases to 230–250 Å (56). Thus, as the integrin exchanges between both conformations, the MEM83 antibody would bind and stabilize the extended/open form of LFA-1.

The primary difference between these models is that the avidity model depends on the presence of two binding sites on the intact IgG portion of MEM83. Thus, to explore whether both binding sites are required, we used homotypic cell aggre-
FIGURE 4. Avidity model for MEM83-mediated activation of LFA-1. A, the LFA-1 IA I-domain/ICAM-1 D1/D2-domain heterotetrameric crystal structure (Protein Data Bank code 1mq8) (28), in ribbon format, is linked to MEM83 IgG at the LFA-1 MEM83 epitope. The side chains of the mapped MEM83 epitope residues (Asp182 and Glu218) are as green Corey-Pauling-Koltun representations, and the hypervariable regions of a template IgG structure (Protein Data Bank codes 2ig2 and 1fc2) (59) are oriented adjacent to the I-domain epitope. Two repeating units, each consisting of an MEM83 monoclonal antibody (mAb) and an LFA-1/ICAM-1 heterotetramer, are shown; the placement of Repeating Unit 2 was obtained by an x-axis translation relative to Repeating Unit 1. B, to illustrate the overall arrangement of LFA-1 and ICAM-1 across two cell membranes, A was rotated 90° along the x-axis. We expanded the illustration by including the transmembrane domain models of ICAM-1 and LFA-1 in its open conformation (adapted from Ref. 62). The LFA-1 I-domain ribbons are colored black; the ICAM-1 D1/D2-domain molecules are shown as Corey-Pauling-Koltun representations. In both A and B, one ICAM-1 D1/D2-domain molecule is colored white and the other orange; the light and heavy chains of the IgG are shown as white and yellow Corey-Pauling-Koltun representations, respectively. A and B were generated using the graphics program MOLMOL (69) and modified in GIMP.
Mechanism for Antibody-mediated Outside-in LFA-1 Activation

Our model can readily incorporate ICAM-1 D4/D4-domain dimerization to form a one-dimensional array of adjacent heterotetramers (66), together with MEM83-linked I-domain dimerization. This would link an increase in avidity to an increase in affinity of LFA-1. In this manner, clustering induced by the MEM83 antibody may be analogous to that induced by the T cell receptor in the immunological synapse of the T cell (23).

In light of our data indicating that the binding of MEM83 to isolated I-domains is activation-insensitive, we hypothesize that MEM83 can stabilize adjacent LFA-1 molecules only while they are in their active form. This stabilization occurs via I-domain binding to each arm of the IgG hypervariable regions. The resting state of LFA-1 is the closed/low affinity form, and cross-linking this form by a non-blocking antibody would not enhance integrin activation. Alternately, a non-blocking antibody such as MEM83, which can recognize two I-domain epoigms across two LFA-1/ICAM-1 heterotetramers, would provide the additional free energy necessary to stabilize the active form of LFA-1. Hence, this antibody-mediated stabilization of the open/high affinity conformations of the integrin leads to outside-in signaling.

Acknowledgments—We thank Professor Václav Hořejší for generously providing the MEM83 antibody; Professor Timothy A. Springer for advice and providing the LFA-1 I-domain clones; Andrea L. Creath and John W. Craft, Jr., for help with this manuscript; and Dr. Laura Gutierrez.

REFERENCES
