The I Domain of Integrin LFA-1 Interacts with ICAM-1 Domain 1 at Residue Glu-34 but Not Gln-73*

(Received for publication, October 1, 1997, and in revised form, November 8, 1997)

Paula Stanley and Nancy Hogg‡
From the Leukocyte Adhesion Laboratory, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, United Kingdom

Using a solid phase assay, we show that isolated LFA-1 I domain binds ICAM-1 in a Mg²⁺-dependent manner and is blocked by anti-I domain monoclonal antibodies. This activity mirrors that of the intact receptor (Dransfield, I., Cabanillas, C., Craig, A., and Hogg, N. (1992) J. Cell Biol. 116, 219–226) and suggests that the I domain controls divalent cation-dependent receptor function. In ICAM-1, domain 1 residues Glu-34 and Gln-73 have been identified as critical for binding of LFA-1 as an intact receptor (Staunton, D. E., Dustin, M. L., Erickson, H. P., and Springer, T. A. (1990) J. Cell Biol. 116, 219–226) and suggests that the I domain controls divalent cation-dependent receptor function. In ICAM-1, domain 1 residues Glu-34 and Gln-73 have been identified as critical for binding of LFA-1 as an intact receptor (Staunton, D. E., Dustin, M. L., Erickson, H. P., and Springer, T. A. (1990) J. Cell Biol. 116, 219–226). For the first time, we show that isolated I domain binds to domain 1 of ICAM-1 and that this interaction is inhibited partially by mutation of Glu-34 but not by Gln-73. The anti-ICAM-1 monoclonal antibody RR1/1, which maps to Gln-73 (Staunton, D. E., Dustin, M. L., Erickson, H. P., and Springer, T. A. (1990) J. Cell Biol. 116, 243–254), enhances I domain binding, suggesting potential allosteric control or coordinate binding by this region. Finally, I domain binding inhibited by Glu-34 ICAM-1 mutation correlates with divalent cation dependence, indicating that this residue might be in direct contact with the metal ion-dependent adhesion site. Thus, we describe the interaction between the LFA-1 I domain and ICAM-1, an event that controls the function of the intact receptor but includes only part of the complete ligand binding site.

Cell-cell interactions have an essential role in maintaining an immune response and are a requirement for effective functioning of leukocytes (3, 4). For example, adhesion of leukocytes to endothelium is critical for their transmigration from the circulation to sites of inflammation, and firm contact with antigen-presenting cells is required for T lymphocyte stimulation and proliferation. The leukocyte integrin LFA-1 (CD11a/CD18) is an important receptor for these adhesive interactions and has three major ligands: ICAM-1, ICAM-2, and ICAM-3 (CD54, CD102, CD50), which are members of the immunoglobulin superfamily (IgSF). ICAM-1 is the principle ligand for LFA-1 in many of its interactions. Mutagenesis studies of ICAM-1 have shown the residues Glu-34 and Gln-73 in domain 1 to be essential for binding of purified LFA-1 to ICAM-1 (2, 5, 6). The importance of these residues in adhesion has also been established for other IgSF adhesion ligands, where residues in homologous positions are both conserved and essential for integrin function; for example, ICAM-3 contains Gln-75 and Glu-37 (7–9), and VCAM-1, the ligand for α₅β₇, contains Asp-40 (10–12). However, it has not been shown which region of integrin interacts with these residues.

Eight integrins have an I domain in the α subunit to which ligand binding sites have been mapped (13). In LFA-1, the I domain contains a major ICAM-1 binding site (14). The I domains of the two β₂ integrins LFA-1 and Mac-1 have recently been crystallized and an important part of the structure shown to be a Mg²⁺/Mn²⁺ binding site termed the MIDAS motif (15–17). Since divalent cations are required by integrins for activity this suggests an important role for the I domain in activation as well as ligand binding. Other regions of LFA-1 that have been implicated in binding to ICAM-1 and also potentially bind to divalent cation are domains V/VI of the integrin αI subunit (18) and the I domain-like region of the β₂ subunit (19). Whether these different domains form one large ligand binding pocket or bind ICAM-1 separately remains to be determined (13). In this study, we use an isolated LFA-1 I domain to locate residues of ICAM-1 that interact with this domain.

EXPERIMENTAL PROCEDURES

Monoclonal Antibodies—The following monoclonal antibodies (mAbs) were used in this study. Anti-LFA-1 (CD11a) mAbs mapping to the I domain (14) were mAb 38 (this laboratory), MEM 25, MEM 30, MEM 83 (gifts from Dr. V. Horesji, Institute of Molecular Genetics, Prague), and F110.22 (gift from Dr. T. Plesner, Herlev Hospital, Denmark). Anti-ICAM-1 (CD54) mAbs were 15.2 (this laboratory), RR1/1 (gift from Dr. R. Rothlein, Boehringer Ingleheim, Inc., Ridgefield), S.4A6 (gift from Dr. D. Haskard, RPMS, Hammersmith Hospital, London) and WEHI-CAM-1 (gift from Dr. A. Boyd, Queensland Institute for Medical Research, Australia). The anti-CD64 mAb 10.1 (this laboratory) was used most frequently as a control.

Synthesis of ICAM-1Fc Proteins—Chimeric wild type (wt) ICAM-1Fc protein consists of the five extracellular domains of ICAM-1 plus the hinge, CH2, and CH3 domains of IgG1 (20). The cDNA construct was ligated into the vector pEE14 and stably transfected into CHO-K1 cells by calcium phosphate precipitation (21). Protein was isolated from high expressing transfectants after 10 days in serum-free medium. ICAM-1Fc mutants E34A and Q73N were made by polymerase chain reaction using site-directed mutagenesis by standard protocols and were fully sequenced by dyeoxy sequencing. Proteins were synthesized by electroporated COS cells and collected in serum-free medium for up to 4 days. All proteins were purified on a Protein A-Sepharose column and eluted with 0.1 M citrate, pH 3.0, using standard methods.

The VCAM-1Fc chimeric protein contains VCAM-1 domains 1 and 2 and the same Fc domain as ICAM-1Fc (22) (a kind gift from Dr. R. Lobb, Biogen). Both ICAM-1Fc and VCAM-1Fc were equally able to bind stimulated T cells at equivalent molarity.2

Synthesis of I-GST Protein—The LFA-1 I domain (Leu-111 to Ser-327) including the predicted sequence and a 17-amino acid N-terminal extension, was made by polymerase chain reaction from an LFA-1 α subunit.

2 J. Porter, personal communication.

* This work was supported by the Imperial Cancer Research Fund. 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.

‡ To whom correspondence should be addressed. Tel.: 44-171-269-3255; Fax: 44-171-269-3093; E-mail: hogg@europa.lif.icnet.uk.

1 The abbreviations used are: LFA-1, leukocyte function-associated antigen-1; ICAM, intercellular adhesion molecule; VCAM, vascular cell adhesion molecule; MIDAS, metal ion-dependent adhesion site; GST, glutathione S-transferase; ELISA, enzyme-linked immunosorbent assay; IgSF, Ig superfamily; mAb, monoclonal antibody; TBS, Tris-buffered saline; wt, wild type; BSA, bovine serum albumin.
subunit cDNA by a standard protocol and ligated into the vector pGE2T (Pharmacia Biotech) to produce an I-GST coding sequence. The sequenced DNA was transformed into Escherichia coli Topp 2 (Stratagene) and grown to log phase before inducing with 0.1 mM isopropyl-1-thio-β-D-galactopyranoside for 4 h. I-GST was extracted from bacterial pellets using the GST purification module kit (Pharmacia) essentially following the manufacturer’s instructions. Isolated I domain was made by cleaving directly from glutathione-Sepharose with thrombin. The I-GST and I domain proteins ran as single bands of domain was made by cleaving directly from glutathione-Sepharose with thrombin. The I-GST and I domain proteins ran as single bands of

Solid Phase ELISA Assay—The wells of Maxisorp 96-well plates (Nunc) were coated for 16 h with 500–750 ng/well ICAM-1Fc in phosphate-buffered saline at 4 °C, then blocked with 3% BSA (Calbiochem) in 20 mM Tris-HCl, pH 7.5, 150 mM NaCl (TBS) for 1 h at room temperature. The wells were washed three times with TBS, and 50 μl/well I-GST diluted in TBS, 1 mM MgCl2, 1 mM EDTA was added and incubated at 37 °C for 3 h. Bound I-GST and GST were detected with 50 μl/well rabbit anti-GST antibody at 5 μg/ml (Sigma) in TBS, 1 mM MgCl2, 0.5 mg/ml BSA for 30 min at room temperature followed by 50 μl of peroxidase-conjugated goat anti-rabbit IgG (Dako) at 1:1000 diluted in TBS, 1 mM MgCl2, 0.5 mg/ml BSA added for 30 min at room temperature. The wells were washed three times with TBS between each layer. Bound I-GST was detected with 75 μl/well 0.5 mg/ml o-phenylenediamine dihydrochloride (Sigma, made up as recommended and added for 10 min, stopped by addition of 50 μl of 3 M H2SO4 and plates read in an ELISA reader at 492 nm. In experiments where isolated domain was isolated, it was used, it was detected using 5 μg/ml mAb F110.22 followed by a 1:1000 dilution of peroxidase-conjugated goat anti-mouse IgG (Dako). When divalent cations or blocking antibodies were added, these and the I-GST were made up to 2-fold concentrations and 30 μl of each added to the wells. I-GST or GST was always added last. Comparison of binding between VCAM-1Fc and ICAM-1Fc or I-GST and GST used equimolar concentrations as determined by protein estimation and SDS-polyacrylamide gel electrophoresis.

RESULTS

ICAM-1 Mutants E34A and Q73N Are Not Recognized by T Cell LFA-1—The location of the LFA-1 I domain binding site on ICAM-1 has not been identified, but residues Glu-34 and Gln-73 in domain 1 of ICAM-1 have been demonstrated to be critical for binding of the intact integrin LFA-1 (2, 5, 6). Previous studies have shown the sensitivity of LFA-1 to these residues in an ICAM-1Fc chimeric protein and showing that neither mutant E34A nor Q73N ICAM-1Fc immobilized on plastic support any T cell adhesion compared with wt ICAM-1Fc (Fig. 1). Nine mAbs specific for epitopes in ICAM-1 domains 1 and 2 (20) bound wt ICAM-1Fc and the two mutants to an equivalent extent, showing that the mutant proteins are correctly folded (data not shown). The epitope recognized by mAb RR1/1 is eliminated by Q73N mutation as shown previously (2).

Characterization of Isolated LFA-1 I Domain Binding to ICAM-1 Using an ELISA Assay—To investigate the specificity of LFA-1 I domain binding to ligand, we tested the ability of isolated recombinant I domain to bind to ICAM-1Fc in a solid phase assay. The specificity of adhesion is demonstrated by the ability of LFA-1 I domain, as a GST fusion or cleaved protein, to bind immobilized ICAM-1Fc in a saturable fashion compared with a lack of binding to either BSA (Fig. 2A) or a second IgSF, protein VCAM-1Fc (Fig. 2B). The GST fusion partner protein expressed alone did not interact with ICAM-1Fc or VCAM-1Fc (Fig. 2B), showing that adhesion to ICAM-1Fc is mediated solely by the I domain in the fusion protein.

The LFA-1 and Mac-1 I domain structures have recently been solved, and a key feature is a Mg2+/Mn2+ binding site, the MIDAS motif (15–17), which suggests an important role for divalent cation in I domain interactions. However, although divalent cations are essential for adhesion of intact integrins, solid phase binding assays using isolated I domains have provided conflicting evidence for their requirement (23, 24). Here, we show that isolated LFA-1 I domain requires the metal ion Mg2+ for full activity (Fig. 3). Furthermore, binding to ICAM-1Fc is diminished by EDTA or Ca2+, while Mg2+ binding is enhanced when Ca2+ is chelated using EGTA. In the absence of Mg2+ or with Ca2+, a limited amount of binding is seen (Fig. 3). Therefore, the ligand binding behavior of the isolated I domain under different cation conditions resembles that of intact receptor, further validating the assay and suggesting that the I domain may dominate LFA-1 function. The GST protein serves as a control for all of the divalent cation conditions.

Anti-LFA-1 (CD11a) mAbs, which map to epitopes in the I domain (14), are able to interfere with adhesion between cell surface-expressed LFA-1 and ICAM-1. We tested the effect of several of these mAbs on binding of isolated I domain to ICAM-1Fc and found that the blocking pattern exactly reflected that observed for LFA-1 on T cells (14, 25); mAbs 38, MEM 25, and MEM 30 blocked well, mAb F110.22 blocked to a lesser degree, and activating mAb MEM 83 and control mAbs were non-blocking (Fig. 4A). This concordance in mAb blocking activity between T cell LFA-1 and isolated I domain, together with binding specificity and cation dependence, confirms the native function of the recombinant I domain.

Identifying the ICAM-1 Binding Site for LFA-1 I Domain—To locate the I domain binding site on ICAM-1, we first tested a panel of anti-ICAM-1 (CD54) mAbs that interfere with LFA-1 binding and map to distinct epitopes on the CFG face of domain 1 and to the top of domain 2 (20). Four of the five mAbs tested had no effect on I domain/ICAM-1Fc binding compared with control isotype-matched mAbs (Fig. 4B), which suggests that the I domain does not bind to ICAM-1 domain 1 at the sites
This result, together with the enhanced binding shown with domain to the Q73N mutant resembled wt ICAM-1Fc (Fig. 5).

FIG. 3. Binding of I domain to wt ICAM-1Fc in the presence and absence of divalent cations. ICAM-1Fc was plated on 96-well plates. Equimolar amounts of I-GST and GST were made up to 2-fold concentrations in TBS containing 1 mM MgCl₂, 1 mM CaCl₂, and 4 mM MgCl₂/4 mM EGTA, and 4 mM EDTA. Data are from one representative experiment ± S.D. (n = 4). ○, I GST + Mg/EGTA; □, I GST + Mg; ■, GST + Mg; △, I GST + Ca; Δ, GST + Ca; ●, I GST + EDTA; ○, GST + EDTA.

mAb RR1/1 (Fig. 4B), which maps to Gln-73 on the G strand, suggests that this region of ICAM-1 influences binding of the I domain but does not directly interact with it.

A Role for the Glu-34 Residue of ICAM-1 in Divalent Cation-dependent Binding—The presence or absence of divalent cation has been shown to result in little alteration to the structure of the LFA-1 I domain (17). It has therefore been suggested that, during activation, Mg²⁺ may be involved either in conformational change between integrin domains or binds directly to ligand. As demonstrated in this study, Mg²⁺ produces a marked increase in I domain binding, showing that it has a direct role in the function of the isolated I domain and suggesting involvement in ligand binding. If, as has been predicted (26, 27), the Glu-34 residue is involved directly in cation-dependent ligand binding, then the limited binding of I domain to the Glu-34 mutant ICAM-1Fc should be insensitive to divalent cation. We tested the effect of Mg²⁺ and EDTA on the level of I domain binding to the E34A mutant and found that it was equivalent (Fig. 6). In addition, EDTA-treated wt ICAM-1Fc binding was comparable to the level of G34A ICAM-1Fc binding in Mg²⁺, suggesting that the Glu-34 residue has an essential role in divalent cation-dependent binding. Addition of anti-LFA-1 blocking mAb 38 results in binding equivalent to the GST control, showing that the binding not blocked by EDTA is specific. This result is consistent with the interpretation that residue Glu-34 does interact with Mg²⁺ at the MIDAS site. The mutant Q73N ICAM-1Fc showed equivalent binding to wt ICAM-1Fc in Mg²⁺ (Fig. 6) and was blocked similarly by EDTA (data not shown).

DISCUSSION

An ELISA assay showing isolated LFA-1 I domain binding to ICAM-1 was characterized with the aim of identifying the residues of ICAM-1 that are critical for this interaction. The main findings of the study are that: 1) I domain binding to ICAM-1 is Mg²⁺-dependent and mirrors the cation-dependent behavior of cell surface LFA-1; 2) intact LFA-1 receptor binding is inhibited by ICAM-1 domain 1 mutations at Glu-34 and Gln-73, in confirmation of previous studies; 3) the I domain is sensitive to mutation of residue Glu-34 but not Gln-73; 4) anti-
ICAM-1 mAb RR1/1, which maps to Gln-73, enhances binding of the I domain to ICAM-1; and 5) the limited binding of I domain to the Glu-34 mutant is cation-independent, which adds evidence to previous suggestions that this residue may form the point of ligand contact with the MIDAS motif in the I domain.

The ELISA assay detecting LFA-1 I domain binding to ICAM-1 reflected several characteristics of the interaction of intact receptor with ligand. The binding was specific for ICAM-1, with no recognition of the closely related IgSF adhesion protein VCAM-1. Additionally, monoclonal antibodies previously mapped to the I domain (14) and shown to block the interaction between intact LFA-1 and ICAM-1 by varying amounts (25) had a comparable blocking pattern in the isolated I domain assay.

The divalent cation binding characteristics of the isolated I domain in our assay mirrored the behavior of intact LFA-1 (1), with Mg$^{2+}$ promoting maximal binding to ICAM-1 and Ca$^{2+}$ failing to support binding. This suggests that the I domain controls the divalent cation-dependent function of the native receptor. Although the majority of LFA-1 I domain binding is dependent upon the presence of Mg$^{2+}$, there is a low level of binding in the assay, which occurs in the absence of divalent cation and is specific as it is blocked by anti-LFA-1 mAb. The specific binding of LFA-1 I domain with and without divalent cation supports both previous conflicting reports about divalent cation requirement of a$\alpha$ b$\beta$ I domain (23, 24). The LFA-1 I domain structure solved in the presence of Mg$^{2+}$ or absence of cation showed little difference in tertiary structure (17) and therefore does not exclude the possibility of low level cation-
independent binding as observed here. In the intact LFA-1 receptor, this may not be possible due to insufficient binding strength or masking of the binding face between α and β subunits, which are believed to undergo conformational change during activation (13, 26).

A main objective of this study was to determine the regions of ligand ICAM-1 that are important for recognition by the LFA-1 I domain to understand how integrin and ligand interact. As the anti-I domain mAbs blocked similarly in ELISA and whole cell assays, anti-ICAM-1 mAbs were also tested. Monoclonal antibodies that interfere with ICAM-1 binding of cell surface-expressed LFA-1 (20) were unable to block the ELISA assay. The explanation for this may be that, in intact LFA-1, the anti-ICAM-1 mAbs block function by interfering with a binding site on ICAM-1 not involving the I domain or that they block sterically rather than directly.

The domain 1 residues Glu-34 and Gln-73 of ICAM-1 have been shown to be essential for binding of intact LFA-1 receptor to ICAM-1 (2, 5, 6), as we have confirmed. However, the demonstration here that LFA-1 I domain binding is sensitive to Glu-34 mutated ICAM-1 shows limited binding, which is not enhanced by ad- preis-117. The distribution of equivalent residues Glu-37 in ICAM-3 (7–9) and Asp-40 in VCAM-1 (10–12) are also inhibitory in whole cell assays. The I domain may be expected to interact with Glu-37 of ICAM-3, but VCAM-1 is the ligand of αLβ2, an I domain-negative integrin. If residue Asp-40 in VCAM-1 has a similar function to Glu-34 in ICAM-1, then it must be recognized by a different integrin domain, potentially the β subunit I domain.

In contrast, binding of I domain to the Gln-73 ICAM-1 mutant resembled wt ICAM-1. Since there is a definite requirement for Gln-73 not only for cell-surface LFA-1 but also for LFA-1 in cell-free systems (2, 5, 6), this result implies that Gln-73 interacts with a region of LFA-1 other than the I domain either on the α or β subunit. However, the enhanced binding caused by anti-ICAM-1 mAb RR1/1, which maps to Gln-73 on the G strand, suggests that this region of ICAM-1 influences binding of the I domain. The mAb might mimic an allosteric alteration of ICAM-1 following LFA-1 binding or replace the cooperative binding of another LFA-1 domain by altering the orientation of the G strand to facilitate I domain binding. In ICAM-3, the comparable Glu-75 residue is also critical for intact LFA-1 binding and may have a similar role in I domain interaction. This residue is not conserved in VCAM-1.

In the solved Mac-1 I domain crystal structure, Mg²⁺ bound to the MIDAS motif coordinated a glutamic acid (Glu) residue in an adjacent domain (15). This was suggested to mimic the ligand bound state and led to the prediction that the function of critical acidic residues found in ligands might be to coordinate Mg²⁺ (26, 27). As demonstrated in this study, Mg²⁺ produces a marked increase in I domain binding, showing that it has a direct role in the function of the isolated I domain consistent with a critical role in ligand binding. However, Glu-34 mutated ICAM-1 shows limited binding, which is not enhanced by ad- dition of Mg²⁺. The equivalence in level of cation-independent I domain binding to wt ICAM-1 in the presence of EDTA and the binding of Glu-34 mutant supports the suggestion that the Glu-34 residue has an essential role in cation-dependent binding. This result is consistent with the interpretation that res-idue Glu-34 interacts with Mg²⁺, but this issue will be finally the main issue of this study.

The LFA-1 I Domain Binds ICAM-1 Domain 1

...
The I Domain of Integrin LFA-1 Interacts with ICAM-1 Domain 1 at Residue Glu-34 but Not Gln-73
Paula Stanley and Nancy Hogg

J. Biol. Chem. 1998, 273:3358-3362.
doi: 10.1074/jbc.273.6.3358

Access the most updated version of this article at http://www.jbc.org/content/273/6/3358

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 30 references, 14 of which can be accessed free at http://www.jbc.org/content/273/6/3358.full.html#ref-list-1