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A Binding Interface on the I Domain of Lymphocyte Function-associated Antigen-1 (LFA-1) Required for Specific Interaction with Intercellular Adhesion Molecule 1 (ICAM-1)*

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Previous studies have shown that lymphocyte function-associated antigen-1 (LFA-1) molecules containing the human α (CD11a) and human β (CD18) subunits but not the murine α and human β subunits can bind to human intercellular adhesion molecule 1 (ICAM-1). Using human/mouse LFA-1 α subunit chimeras, we mapped regions required for binding to ICAM-1 N-terminal to amino acid (aa) residue 359. Ligand binding sites were mapped in greater detail by scanning this region with murine sequences from 56 down to 17 aa in length and finally by introducing single or few murine aa residue replacements into the human sequence. Replacement of two non-contiguous regions of aa residues 119–153 and 218–248 in the I domain with the corresponding mouse sequences abolished most binding to human ICAM-1, without affecting αβ subunit association or expression on the surface of transfected COS cells. Specific residues within the I domain found to be important were Met-140, Glu-146, Thr-243, and Ser-245. Using the recently solved structure of the Mac-1 (CD11b) I domain as a model (Lee, J.-O., Rieu, P., Arnaout, M. A., and Liddington, R. (1995) *Cell* 80, 631–638), these residues are shown to be located on the surface of the I domain surrounding the site to which Mg^{2+} is chelated, and define a ligand binding interface. Mapping of the epitopes of a panel of mouse anti-human and rat anti-mouse monoclonal antibodies gave concordant results. Epitopes were mapped to two different regions in the N-terminal domain, four regions within the I domain, and two regions between the I domain and the EF hand-like repeats. Monoclonal antibodies to epitopes within the mid- to C-terminal portion of the I domain and the N-terminal portion of the region between the I domain and the EF hand-like repeats gave good inhibition of LFA-1-dependent homotypic aggregation with cells that express either ICAM-1 or ICAM-3 as the major LFA-1 ligand.

The lymphocyte function-associated antigen-1 (LFA-1,[1] CD11a/CD18) was initially identified with mAb because of its importance in antigen-specific T lymphocyte interactions (Larson *et al.*, 1989). Subsequently, LFA-1 was shown to be important in a wide variety of lymphocyte functions and to be required for interactions with vascular endothelium in immigration of lymphocytes, monocytes, and neutrophils in inflammation and homing and recirculation of lymphocytes (Springer, 1990, 1994, 1995). LFA-1 is an integrin αβ heterodimer. Two other integrins restricted in expression to leukocytes, Mac-1 (CD11b/CD18) and p150,95 (CD11c/CD18) (Sanchez-Madrid *et al.*, 1983), share the same CD18 or β2 integrin subunit and have homologous α subunits. LFA-1 binds to three counter-receptors, ICAM-1, ICAM-2, and ICAM-3, that are members of the Ig superfamily (IgSF) and have distinctive patterns of surface expression and in the aggregate are constitutively or inducibly expressed on almost all cells in the body (Springer, 1995). Binding of LFA-1 to ICAMs is Mg^{2+}-dependent (Marlin and Springer, 1987). Although LFA-1 is constitutively expressed on most leukocytes, binding to its counter-receptors requires cellular activation, which is thought to induce conformational changes in LFA-1 that affect its affinity for ligand and interaction with the cytoskeleton (Diamond and Springer, 1994).

Structure-function studies on LFA-1 are crucial for mapping ligand binding sites and determining the molecular basis for the interaction with ICAMs and the activation of adhesiveness of LFA-1. The LFA-1 α subunit, αL, has two prominent structural features, an inserted or I domain of about 200 aa residues present in some but not most integrins, and three EF hand-like putative divalent cation binding repeats that are shared with all other integrin α subunits (Hynes, 1992). The I domain is homologous to motifs in other proteins including the three A domains of von Willebrand factor, which have been implicated in ligand binding, multiple repeats in cartilage matrix protein and collagen type VI, and single repeats in complement component C2 and factor B (Colombatti and Bonaldo, 1991). Mac-1 and p150,95 α subunit chimeras were previously used to map epitopes of function-blocking mAb to Mac-1 and thus to map a site important for binding of the ligands iC3b, fibrinogen, and ICAM-1 to the I domain of Mac-1 (Diamond *et al.*, 1993). Subsequently, multiple studies on Mac-1 (Lee *et al.*, 1995; Michishita *et al.*, 1993; Muchowski *et al.*, 1994; Rieu *et al.*, 1994; Zhou *et al.*, 1994), LFA-1 (Champe *et al.*, 1995; Landis *et al.*, 1993, 1994; Randi and Hogg, 1994), VLA-1 (Kern *et al.*, 1994), and VLA-2 (Kamata *et al.*, 1994; Kamata and Takada, 1994), have implicated the I domain in ligand binding.

Very recently, the three-dimensional structure of the Mac-1 I domain has been determined (Lee *et al.*, 1995). It has a double-twisted fold, with a central hydrophobic β-sheet surrounded by amphipathic α-helices. A single Mg^{2+} ion is bound to residues in three connecting loops at what will be referred to as the top of the I domain, above one end of the central β-sheet. The Mg^{2+} is coordinated by the hydroxyls of Thr-209, Ser-142,

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[1] The abbreviations used are: LFA, lymphocyte function-associated antigen-1; ICAM, intercellular adhesion molecule; IgSF, Ig superfamily; MIDAS, metal ion-dependent adhesion site; VLA, very late antigen; mAb, monoclonal antibody; kb, kilobase pair(s); PCR, polymerase chain reaction.

and Ser-144, and by 2 water molecules. Asp-140 and Asp-242 are in an outer sphere of coordination and coordinate with a water molecule and Ser-142 and Ser-144 that directly coordinate Mg^{2+}. Asp-140 and Asp-242 are buried beneath the Mg^{2+}, and unavailable for contact with ligand. This structural motif has been called a metal ion-dependent adhesion site (MIDAS). In the crystal structure, the Mg^{2+} forms an adventitious coordination with a Glu residue from a neighboring I domain. In ICAM-1, Glu-34 in IgSF domain 1 is the most important residue for binding to LFA-1 (Staunton *et al.*, 1990) and might form an analogous coordination with Mg^{2+}. Mutation of residues that form the primary or secondary coordination shell with the Mg^{2+} ion, including Asp-140 and Asp-242 in the I domain of Mac-1 (Lee *et al.*, 1995; Michishita *et al.*, 1993), their homologues in the I domain of VLA-2 (Kamata *et al.*, 1994) and VLA-1 (Kern *et al.*, 1994), and the homologue of Thr-209 in the I domain of VLA-2 (Kamata and Takada, 1994), disrupt ligand binding. Such mutations abolish divalent cation binding in Mac-1 (Michishita *et al.*, 1993) and are predicted to do the same in the α1 and α2 subunits, suggesting that the divalent cation may indirectly stabilize a ligand binding site, or that the cation may directly coordinate with ligand. Other residues required for ligand binding and that are likely to form direct contacts with ligand have yet to be identified in any integrin I domain.

In this study, we have utilized chimeric LFA-1 α subunits in intact heterodimers expressed on the cell surface to define in detail structural regions of the LFA-1 α subunit that are important for binding to ICAM-1. Our studies were made possible by a previous observation that human but not mouse LFA-1 would bind to human ICAM-1, and that this species specificity mapped to the LFA-1 α subunit (Johnston *et al.*, 1990). The human and murine LFA-1 α subunits have 72% amino acid sequence identity (Kaufman *et al.*, 1991), allowing interspecies chimeric subunits to be constructed with little disruption of conformation. The chimeras have been used to map residues required for binding of LFA-1 to ICAM-1, and also to map epitopes for a panel of 20 mAb and to correlate epitope location with inhibition of binding to ICAMs. We demonstrate that four specific amino acid residues in two noncontiguous regions of the I domain, aa residues 119–153 and 218–248, are crucial for binding to ICAM-1. These residues surround the Mg^{2+} ion on the top of the I domain, and define a ligand-binding interface. mAbs that block binding to ICAM-1 and ICAM-3 map to epitopes within or adjacent to these regions.

MATERIALS AND METHODS

Cell Lines and Monoclonal Antibodies—COS-7 cells, Epstein-Barr virus-transformed B-lymphoblastoid cell line, JY, and the T cell line, SKW3, were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum.

The mouse anti-human CD11a mAbs TS1/11, TS1/12, TS2/4, TS2/14, TS1/22, and TS2/6 and CD18 mAb TS1/18 (Sanchez-Madrid *et al.*, 1982b) and the rat anti-mouse CD11a mAbs M17/7 (Sanchez-Madrid *et al.*, 1982a) and M7/14 (Davignon *et al.*, 1981) have been described previously. The rat anti-mouse CD11a mAb FD441.8 (Sarmiento *et al.*, 1982) was obtained from ATCC. The mAb S6F1 (Morimoto *et al.*, 1987), BL5, F8.8, MAY.035 (Ohashi *et al.*, 1992), 25-3-1 (Fischer *et al.*, 1986), YTA-1 (Nakamura *et al.*, 1989), G-25.2, NKI-L16 (Keizer *et al.*, 1988), CBR LFA-1/10, CBR LFA-1/9, CBR LFA-1/3, and CBR LFA-1/1, were obtained through the 5th International Leukocyte Workshop.

Wild-type and Chimeric Integrin Subunit Constructs—The human LFA-1α cDNA in pSP65 (Larson *et al.*, 1990) was excised with *Hin*dIII and *Sph*I and cloned into the polylinker of pUC19. It was excised with *Xba*I and cloned into the *Xba*I site of Ap^{r}M8, a derivative of CDM8 containing the β-lactamase gene from pBluescript.[2] The murine LFA-1α cDNA (Kaufman *et al.*, 1991) and human β cDNA (Kishimoto *et al.*, 1987) were in Ap^{r}M8.

Chimeric α subunits with exchanges at *Bsp*HI (aa residue 153), *Sse*8387I (aa residue 359), and *Msc*I (aa residue 654) sites occurring in both the human and murine cDNA were prepared by restriction enzyme digestion, purification of fragments by agarose gel electrophoresis, and ligation (see Fig. 1A). Chimeras or scanning mutants were named according to the species origin of their segments. For example, h153m359h indicates that residues 1–153 are from hαL, residues 154–359 are from mαL, and residues 360 to the C terminus are from hαL. Amino acid sequence numbering was according to the human sequence (Larson *et al.*, 1990). Chimeras h654m and m654h were constructed by ligation of 7.3- or 6.8-kb fragments from partial *Msc*I and complete *Hin*dIII digestion of hαL or mαL, respectively, and 2.1-kb fragments from complete *Msc*I and *Hin*dIII digestion of mαL or hαL cDNA, respectively. Chimera h359m was constructed by ligation of a 8.2-kb fragment from complete *Hin*dIII and partial *Sse*8387I digestion of hαL cDNA and a 1.2-kb fragment from complete *Hin*dIII and *Sse*8387I digestion of mαL cDNA. m153h359m was constructed with a 0.6-kb fragment from complete *Hin*dIII and *Bsp*HI digestion of mαL and a 8.3-kb fragment from complete *Hin*dIII and partial digestion with *Bsp*HI of h359m. h153m359h was constructed in two steps. A 0.6-kb fragment from complete digestion of hαL with *Hin*dIII and *Bsp*HI was ligated to a 8.3-kb fragment from complete *Hin*dIII and partial *Bsp*HI digestion of mαL to generate h153m. Then a 1.2-kb fragment from complete *Hin*dIII and *Sse*8387I digestion of h153m was ligated with a 8.2-kb fragment from complete *Hin*dIII and partial *Sse*8387I digestion of hαL to make h153m359h.

Scanning Mutant Constructs—The region from the N terminus to the N-terminal end of the EF hand-like repeats was scanned by insertion of short segments of mouse sequence into the human αL subunit (Fig. 2A). To make scanning mutants h217m248h, h249m303h, and h300m442h, we took advantage of unique *Nru*I, *Cla*I, and *Sal*I restriction sites and two adjacent *Bgl*II sites at residues 300 and 303 in hαL. None of these sites were present at the same position in mαL; therefore, oligonucleotides containing these sites were used to amplify with PCR the corresponding mouse sequences. PCR fragments were restriction digested and *Nru*I-*Cla*I, *Cla*I-*Bgl*II, or *Bgl*II-*Sal*I fragments were ligated to hαL cut with the same enzymes to construct h217m248h, h249m303h, and h300m442h, respectively. A PCR fragment amplified from m359h was digested with *Bgl*II-*Sal*I and ligated into hαL cut with the same enzymes to construct h300m359h (Fig. 2A).

Other scanning mutants were constructed with the PCR overlap extension technique (Ho *et al.*, 1989). Two successive PCR were used to generate a chimeric fragment, which was then restriction digested and inserted in hαL. A silent substitution in the sequence of the overlap oligonucleotide was frequently used to introduce a restriction site at or nearby the mouse-human junction. These sites were used diagnostically and in some cases for construction of subsequent scanning mutants. In the first PCR, two separate reactions were performed to generate one fragment from hαL and a neighboring fragment from mαL. The two oligonucleotide primers at the overlap region were complementary for at least 18 bases (Table I). The two PCR reactions used 5′ upstream and 3′ complementary primers, and 5′ complementary and 3′ downstream primers, respectively. Restriction sites that were unique in αL or the vector were included in the 5′ upstream and 3′ downstream primers. The products of each PCR reaction were separated on an agarose gel, and bands of correct size were purified using Promega's PCR DNA preparation system (Promega). One tenth of the purified DNA samples were mixed and served as templates for the second PCR reaction, with the 5′ upstream and 3′ downstream primers of the previous two PCR reactions. The PCR products were digested with the proper restriction enzymes and ligated into the hαL cDNA fragment in Ap^{r}M8 produced by digestion with the same enzymes. Scanning mutants m57h, h57m74h, h74m93h, and h93m117h (Fig. 2A) were transferred into αL using *Hin*dIII and *Kas*I sites. The h57m117h construct was used as an intermediate in construction of h93m117h and h57m93h. The h57m93h construct was used as an intermediate in construction of h57m74h and h74m93h. The sequence of all these mutants was confirmed from the *Hin*dIII to *Kas*I site by dideoxysequencing using Sequenase (U. S. Biochemical Corp.) according to manufacturer's instructions. Scanning mutants h118m153h, h153m183h, and h185m215h (Fig. 2A) were transferred into αL using unique *Kas*I and *Cla*I sites. The h118m215h construct was used as an intermediate in construction of h153m215h, which in turn was used in construction of h153m183h and h184m215h. The sequence of all these mutants was confirmed from the *Kas*I to *Cla*I sites.

Construction of Multiple and Single Point Mutants—PCR amplification with primers that encompassed the *Kas*I or *Cla*I sites and encoded mutations near these sites was used to generate the F122Y, I126M, L224S, T243S, and S245K mutants (Table I). The PCR products were

[2] L. B. Klickstein, unpublished data.

TABLE I
Nucleotide sequences used for constructing the scanning or point mutants

Mutants	Overlap sequences[a]	Restriction site[b]
m57h	CTGCCAACCGGTCACCCT	*Age*I
h57m74h	CTGCCTACCGGTCAGCCT	*Age*I
h57m74h, h74m93h	ACTTGGGAATGACGCTAGCAACAGA	*Nhe*I
h74m93h, h93m117h	CTGTCTCGCACGTGCGACCAGAACAC	*Pml*I
h153m183h	TTTTCTAGATTATGTTAA	*Xba*I
h184m215h	TTTTCTAGACTACGTTAA	*Xba*I
E218/R221K/L224S	GCCCGGCCGGATGCCACCAA	*Eag*I
I235V/T243S/S245K	GCCCGGCCGGATGCCACCAA	*Eag*I
N129K	CAAGGGCAAGGTCGACTGGTA	*Sal*I
M140Q	TTTCTGTTTGATGGTTCGCAGAGCTTG	
Q143D	ATGAGCTTGGATCCAGATGAATTCCAGAAA	*Eco*RI
P144R	TTGCAGCGAGATGAATTCCAGAAA	*Eco*RI
D145K	AGCTTGCAGCCAAAAGAATTCCAGAAA	*Eco*RI
E146D	AGATGACTTTCAGAAAATTCTAGAC	*Xba*I
Q148E	CCAGATGAATTCGAAAAAATTCTGG	*Bst*BI
D152E	AAAATTCTGGAGTTTATGAAGGATG	No *Bsp*HI
I235V	GGGGCCCGGCCGGATGCCACCAAAGTACTTGTCATC	*Sca*I

Mutants	PCR primer[c]	Restriction site
F122Y	CTGCAGGGGCGCCCCGGGATTCAGGAATG	*Sma*I
I126M	CATGCTGCAGGGGCGCCCCGGGTTTCAGGAATGTATGAAGGG	*Sma*I
L224S	AATTATGTCGCGACAGAGGTGTTCAGAGAAG	No *Pml*I
T243S	GCCGCATCGATGTTGCCACTGTCGCTAGCCTC	*Nhe*I
S245K	GGCCGCATCGATGTTGCCTTTGTCAGTGG	

[a] The complementary overlap region of primers for overlap extension PCR. Restriction sites introduced or deleted by silent mutation are underlined.
[b] Restriction sites that were introduced or deleted for identifying correct products. Wild-type restriction sites were also present in primers for F122Y and I126M (*Kas*I), L224S (*Nru*I), and T243S and S245K (*Cla*I).
[c] Primers used to introduce mutations at the 5′ end (F122Y, I126M, and L224S) or at the 3′ end (T243S and S245K) in PCR reactions.

digested with *Kas*I and *Cla*I and ligated into hαL digested with the same enzymes. PCR overlap extension was used to produce mutants N129K, M140Q, Q143D, P144R, D145K, E146K, Q148E, D152E, I231V, E218H/R221K/L224S, and I235V/T243S/S245K. The 5′ upstream and 3′ downstream primers encompassed the *Kas*I and *Cla*I sites, respectively, and these enzymes were used to move the PCR fragments into hαL as described above for scanning mutants. The entire *Kas*I-*Cla*I fragment was sequenced for each mutant.

COS Cell Transfection and Flow Cytometry—Plasmids were purified with a Wizard Maxi preparation kit (Promega) and ethanol precipitation. COS cells were transiently cotransfected with wild type, mutant, or chimeric LFA-1 α subunits and human β2 subunit constructs using DEAE-Dextran (Aruffo and Seed, 1987). Transfected cells were treated with trypsin-EDTA on day 2 and replated. On day 3, cells were harvested in 5 mM EDTA/phosphate-buffered saline and washed with L15 medium (Sigma) supplemented with 2.5% fetal bovine serum.

Immunofluorescence flow cytometry was as described previously (Hibbs *et al.*, 1990), with a first incubation of 10 μg/ml purified mAb or 1:200 dilution of ascites and staining with 1:20 dilution of fluorescein isothiocyanate-conjugated second antibody.

Adhesion Assay—A soluble form of human ICAM-1 (sICAM-1) truncated before the transmembrane domain (Y452E/F*) was expressed in SF9 insect cells and purified by immunoaffinity chromatography (Casasnovas *et al.*, 1994). Transfected COS cells were labeled with 2′,7′-bis-(2-carboxyethyl)-5-(and -6)-carboxyfluorescein acetoxymethyl ester and assayed for binding to purified ICAM-1 absorbed to 96-well plates with a fluorimeter as described (Bilsland and Springer, 1994), except binding was for 25 min at 37 °C and washing was with a 26-gauge 5/8-inch needle. Binding of transfected cells to ICAM-1 was expressed as a percent of wild type = 100 × (mutant − mock binding)/(wild type − mock binding). Triplicates in each experiment were averaged and considered a single data point for calculation of S.D. among different experiments.

Aggregation Assay—JY or SKW3 cells were harvested near confluence (about 6×10^5 cells/ml). Cells (2×10^6/ml) preincubated with 1:200 ascites or 10 μg/ml purified protein for 20 min were stimulated with phorbol 12-myristate 13-acetate at a final concentration of 50 ng/ml in 100 μl in microtiter plates gently shaken for 30 min for JY cells or 2 h for SKW3 cells at 37 °C, as described previously (Rothlein *et al.*, 1986), except with 5% fetal bovine serum.

RESULTS

Construction and Expression of Human/Mouse CD11a Chimeras—To localize sites on the human LFA-1 α subunit cognate for human ICAM-1, a set of five human/mouse α subunit chimeras were constructed in the expression vector Ap^{r}M8 (Fig. 1*A*). These chimeras swapped the N-terminal region, I domain, metal binding domain, and the remaining C-terminal portion between the human and mouse α chains. Chimeras and scanning mutants (see below) were named according to the species origin of their segments, *e.g.* h153m359h is an α subunit with human aa residues 1–152, mouse residues 153–358, and human residues 359 to C terminus.

COS cells were cotransfected with the chimeric α subunits and the wild-type human β2 subunit. To test for association of the α subunit chimeras with the β2 subunit and expression on the cell surface, the transfected COS cells were stained with anti-αL mAb and anti-β2 mAb TS1/18 and subjected to flow cytometry. Association between α and β subunits is required for efficient surface expression of the LFA-1 α and β subunits (Hibbs *et al.*, 1990; Larson *et al.*, 1990); as shown by stimulation of β subunit expression, all five α subunit chimeras associated with the β2 subunit and were expressed on the COS cell surface comparably to wild type human and murine α subunits (Fig. 1*B*). The overall conformation of the chimeric α subunits on the cell surface was intact, because they reacted with a variety of antibodies to different epitopes on the α subunits (see below, Fig. 5*A*). The molecular size of the chimeric α subunits was the same as human or mouse α as shown by ^{125}I labeling of COS cell transfectants and immunoprecipitation with both anti-α and anti-β mAbs (data not shown).

Binding of LFA-1 with Human/Mouse Chimeric α Subunits to ICAM-1—The functional activity of LFA-1 was tested by measuring binding of transfected COS cells to soluble ICAM-1 absorbed on plastic microtiter wells (Fig. 1*B*). COS cells cotransfected with hαL and β2 subunit cDNAs bound to human ICAM-1, whereas COS cells that were mock-transfected or transfected with the β2 subunit alone did not bind. By contrast, COS cells cotransfected with the mouse αL and human β2 subunit cDNAs did not bind human ICAM-1, confirming previous results (Johnston *et al.*, 1990; Kaufman *et al.*, 1991). The h654m chimera bound to ICAM-1 as well as human LFA-1 (Fig.

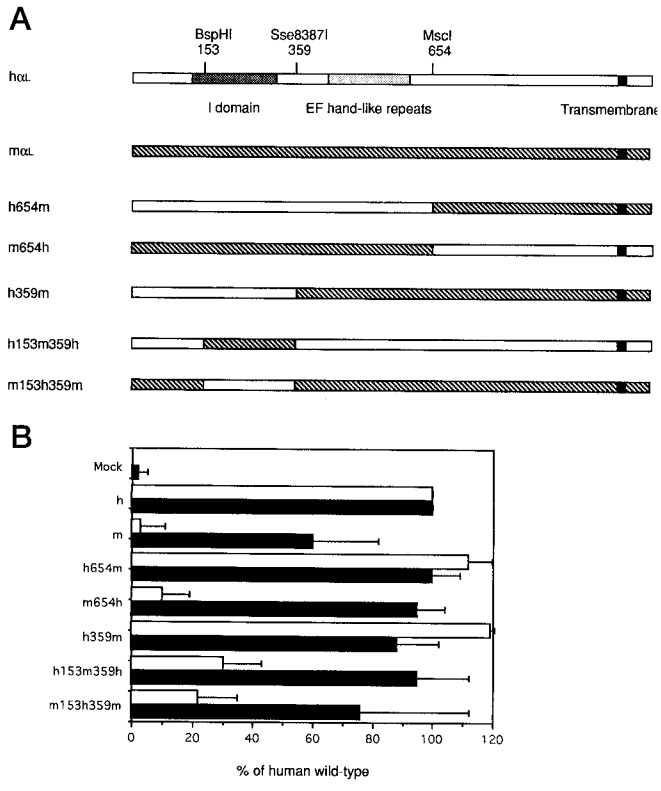

FIG. 1. **Structure-function studies with α subunit chimeras.** *A*, three restriction sites were used to generate α subunit chimeras (*open bars*, human sequence; *hatched bars*, mouse sequence). Sites and the aa residue at which they occur are indicated. *B*, human αL (*h*), mouse αL (*m*), and human/mouse αL subunit chimeras or vector alone were coexpressed with the human β subunit in transfected COS cells. β2 subunit expression was detected with anti-CD18 monoclonal antibody TS1/18 and flow cytometry. Binding to ICAM-1 was measured with carboxyfluorescein-labeled transfected COS cells in microtiter wells coated with purified ICAM-1. □, binding to ICAM-1; ■, β2 subunit expression. Data are mean and S.D. of at least three experiments and are normalized to the percent of human wild-type transfectants that expressed the β2 subunit or bound to ICAM-1.

1*B*), whereas the reciprocal chimera m654h did not bind to ICAM-1, suggesting that the region controlling the species specificity is located N-terminal to the *Msc*I site. The *Sse*I site at residue 359 is intermediate between the I domain and the EF hand-like repeats (Fig. 1*A*). The chimera h359m bound to ICAM-1 as well as human LFA-1, further mapping the species specificity of binding N-terminal to the *Sse*I site. The reciprocal chimera m359h was not expressed on the cell surface (data not shown). The *Bsp*HI site at residue 153 is in the I domain (Fig. 1*A*). Chimeras in which the segment between aa residues 153 and 359 was exchanged, h153m359h and m153h359m, showed binding to ICAM-1 that was less than for human αL, but greater than for mouse αL or m654h. Taken together with the finding that h359m had full activity, these results suggested that both the aa residue 1–153 and 154–359 segments contributed to binding to ICAM-1.

Two Separate Regions, Residues 119–153 and 218–248, Crucial for ICAM-1/LFA-1 Recognition—To map binding sites in more detail, the region from residues 1 to 442 was scanned by replacing short segments of human sequence with the corresponding mouse sequence (Fig. 2*A*). The expression of each mutant in COS cells was determined by flow cytometry using mAb to the β2 subunit (Fig. 2*B*) and to the αL subunit (see Fig. 5*B* below), and compared to binding of each mutant to purified ICAM-1 (Fig. 2*B*).

The region from the N terminus to the beginning of the I domain (residues 1–117) was scanned with four different mu-

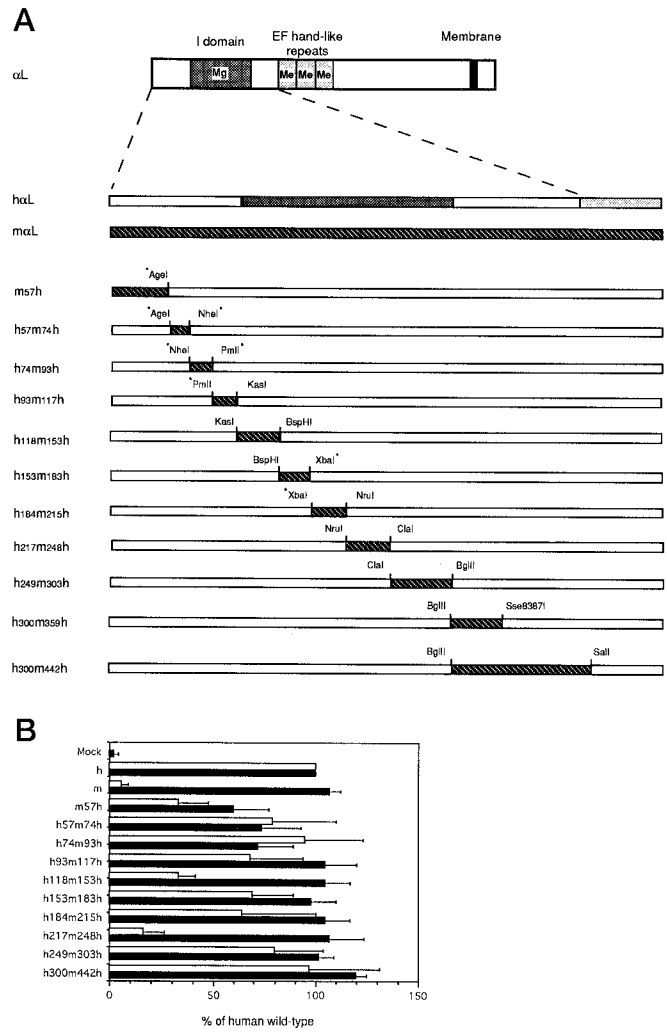

FIG. 2. **Structure-function studies with scanning mutants.** *A*, schematic structure. Restriction sites with *asterisks* were introduced with silent mutations. h300m359h was used only in epitope mapping (see Fig. 5 below). *B*, binding to ICAM-1 and β2 subunit expression of COS cells cotransfected with wild-type or scanning mutant α subunits and the human β2 subunit was measured as described in Fig. 1. □, binding to ICAM-1; ■, β2 subunit expression. Data are mean and S.D. of at least three experiments.

tants. Replacements with larger segments of mouse sequence in this region (1–117, 57–117, 57–93), which is 71% identical with human, resulted in chimeras that were not expressed (not shown). Replacements with the four smaller segments resulted in chimeras that were expressed, but not always as well as wild-type. Scanning mutant m57h was consistently expressed at about 60% the level of human αL, and bound about 35% as well, consistent either with a contribution to binding or an altered conformation. Scanning mutants h57m74h and h74m93h gave expression and binding to ICAM-1 that were concordant and were slightly depressed relative to wild-type. Binding and expression of h93m117h was not significantly different from human LFA-1.

The I domain was scanned with five different mutants, from residues 118–303. All five scanning mutants were expressed in COS cells as well as wild-type mouse and human LFA-1 (Fig. 2*B*). Scanning mutant h118m153h bound only 30% as well as human LFA-1 to ICAM-1, despite a difference of only 11 amino acids between mouse and human over the 28-aa residue segment that was exchanged. Mutants h153m183h and h184m215h showed only a slight and nonsignificant reduction in binding activity. By contrast, scanning mutant h217m248h

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                          140 144 146
                           |   | |
hLFA-1  125 CIKGNVDLVFLFDGSMSLQPDEFQKILDFMKDVMKKLSNTSYQFAAVQFSTSYKTEFDFS
mLFA-1  124 CmKGkVDLVFLFDGSgSLdrkdFeKILeFMKDVMrKLSNTSYQFAAVQFSTdcrTEFtFl
hMac-1  128 CpqedsDiaFLiDGSgSiiPhdFrrmkeFvstVMeqLkkssktlFslmQySeefrihFtFk
                βA  * * *          α1                βB       βC

                                                                       243
                                                                         |
hLFA-1  185 DYVK.WKDPDALLKHVKHMLLLTNTFGAINYVATEVFREELGARPDATKVLIIITDGEAT
mLFA-1  184 DYVKqnKnPDvLLgsVqpMfLLTNTFrAINYVvahVFkEEsGARPDATKVLvIITDGEAs
hMac-1  188 ef.qnnpnPrsLvKpitqlLgrThTatqIrkVvrElFnitnGARknAfKiLvvITDGEkf
             α2      α3          *    α4                     βD    *

             245
              |
hLFA-1  244 DSGN.....IDAA..KDIIRYIIGIGKHFQTKESQETLHKFASKPASEFVKILDTFEKLK
mLFA-1  244 DkGN.....IsAA..hDItRYIIGIGKHFvsvqkQkTLHiFASePveEFVKILDTFEKLK
hMac-1  247 gdplGyedvIpeAdregvIRYvIGvGdaFrsekSrqeLntiASKPprdhVfqvnnFEaLK
                   α5       βE          α6              βF     α7

hLFA-1  297 DLFTELQKKIYVIEG
mLFA-1  297 DLFTdLQrrIYaIEG
hMac-1  307 tiqnqLreKIfaIEG
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FIG. 3. **Alignments of the human and mouse LFA-1 I domains, and the human Mac-1 I domain.** The β strands and α helices of the Mac-1 I domain are *underlined*, and residues in the primary or secondary Mg^{2+} coordination shell are indicated by *asterisks*. Species-specific differences studied by mutation of individual or several amino acids in regions 119–153 and 218–248 are shown in *reverse type*. Residues are numbered that were found to be important for binding of human ICAM-1 (Met-140, Glu-146, Thr-243, Ser-245), or binding of mAb CBR LFA-1/9 and F8.8 (Pro-144).

was dramatically deficient in binding to ICAM-1. The 30 amino acid residues exchanged in this region contained only six differences between mouse and human. Exchange of the final segment of the I domain in chimera h249m303h had no effect on binding to ICAM-1.

The region between the I domain and the first EF hand-like putative divalent cation binding repeat was scanned in h300m442h. Substitution of the 142 residues in this region with murine sequence had no effect on surface expression or binding of LFA-1 to human ICAM-1.

Amino Acid Substitutions in Two Subregions of the I Domain—The two subregions of the I domain important in species-specific recognition of ICAM-1 (Fig. 3) contained 11 and 6 amino acid differences, respectively. To identify the critical residues in these regions, we replaced single or multiple aa residues of the human α chain with corresponding mouse α chain residues. The differences in residues 119 to 153 were scanned with 10 single substitutions (Fig. 4*A*). All mutants were well expressed. Two mutants, M140Q and E146D, retained only 35% and 50% of ICAM-1 binding activity, respectively. Binding by these mutants was almost as depressed as h118m153h, suggesting that residues Met-140 and Glu-146 make the major contributions to species-specific binding to ICAM-1 in this region. The other mutants in this region retained most or all binding activity.

The six species-specific residues from 218 to 248 were divided in two groups by constructing mutant E218H/R221K/L224S and mutant I235V/T254S/S245K. Point mutants in this region were also constructed. All mutants were well expressed (Fig. 4*B*). Mutant E218H/R221K/L224S and a point mutant with its least conservative substitution, L224S, bound to ICAM-1 with nearly wild-type activity. By contrast, binding to ICAM-1 of I235V/T245S/S245K was almost as depressed as for h217m248h. The point mutants T243S and S245K bound to ICAM-1 only 40–30% as well as human LFA-1, suggesting T243S and S245K contribute to species-specific binding.

Epitope Mapping of CD11a Antibodies—An alternative approach to map the binding site for ICAMs is to use chimeras and mutants to map the epitopes of mAbs, and test the mAbs for inhibition of LFA-1 function, to establish an epitope and function relationship map. Eighteen mouse mAb to human LFA-1 α, and three rat mAb to mouse LFA-1 α, were tested for immunofluorescent staining of α subunit chimeras, scanning mutants, and point mutants cotransfected with the human β subunit in COS cells. All but two groups of mAb could be assigned to one of three segments of the LFA-1 α subunit defined by the chimeras: residues 1–153, 154–359, and 360–654 (Fig. 5*A*). The mAbs CBR LFA-1/9, BL5, and F8.8 reacted with h359m, but not with h153m359h or m153h359m, suggesting that they recognize residues both in regions 1–153 and 154–359. The mAb G-25.2, NKI-L16, and CBR LFA-1/3 reacted with an epitope C-terminal to aa residue 359 as shown by staining of h153m359h and not m153h359m or h359m. However, the three mAb were negative on both h654m and m654h, suggesting the epitope(s) included residues in regions on both sides of residue 654.

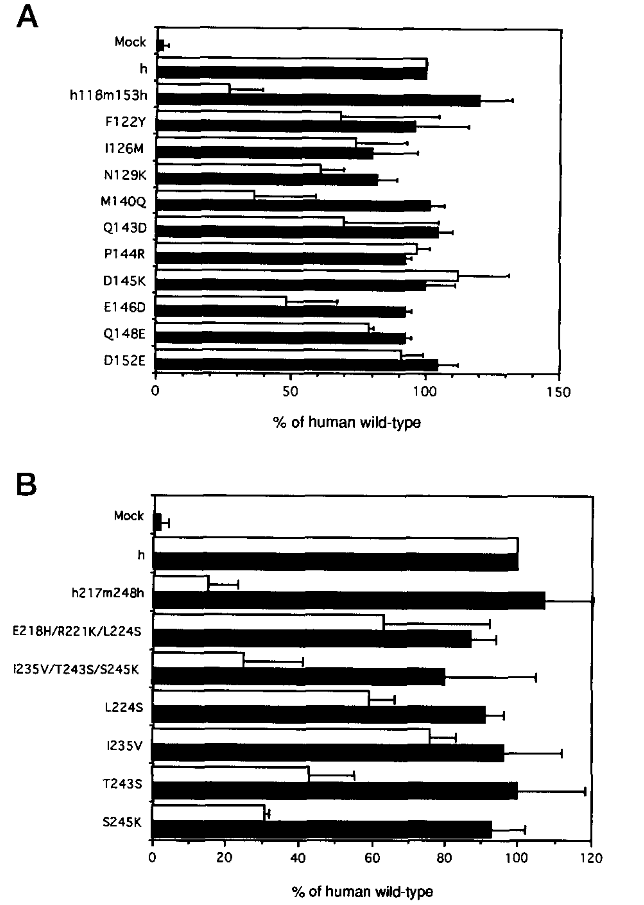

FIG. 4. **Mapping of species-specific recognition of ICAM-1 to specific aa residues.** *A*, mutation of individual human residues to mouse residues in region 119–153. *B*, mutation of residues in region 218–248. Binding to ICAM-1 and $\beta 2$ subunit expression of COS cells cotransfected with wild-type or scanning mutant α subunits and the human $\beta 2$ subunit was measured as described in Fig. 1. Data are mean and S.E. of at least three experiments. □, binding to ICAM-1; ■, $\beta 2$ subunit expression.

The epitopes were more precisely mapped with the scanning mutants (Fig. 5*B*). The S6F1 and TS2/4 mAb were negative on m57h and positive on all the other scanning mutants and thus mapped to residues 1–57. mAb CBR LFA-1/10 was positive on all mutants except for h74m93h and h93m117h and thus appears to recognize an epitope with contributions from both residues 75–93 and 93–117, and thus maps to residues 75–117. Lack of reaction with two different scanning mutants mapped mAb CBR LFA-1/9, BL5, and F8.8 to two separate regions, residues 119–153 and 185–215, confirming results with chimeras. Lack of reaction with individual scanning mutants mapped mAb TS2/6 to residues 154–183; mAb MAY.035, TS1/11, and TS1/12 to residues 185–215; mAb TS1/22, TS2/14, and 25-3-1 to

A

mAb	H	M	h359m	m354m	m354h	h153m359h	m153h359m	Epitope
S6F1	+	-	+	+	-	+	-	1-153
TS2/4	+	-	+	+	-	+	-	1-153
CBR LFA-1/10	+	-	+	+	-	+	-	1-153
CBR LFA-1/9	+	-	+	+	-	-	-	1-359
BL5	+	-	+	+	-	-	-	1-359
F8.8	+	-	+	+	-	-	-	1-359
MAY.035	+	-	+	+	-	-	+	154-359
TS1/11	+	-	+	+	-	-	+	154-359
TS1/12	+	-	+	+	-	-	+	154-359
TS2/6	+	-	+	+	-	-	+	154-359
TS1/22	+	-	+	+	-	-	+	154-359
TS2/14	+	-	+	+	-	-	+	154-359
25-3.1	+	-	+	+	-	-	+	154-359
CBR LFA-1/1	+	-	+	+	-	-	+	154-359
YTA-1	+	-	-	+	-	+	-	360-654
G-25.2	+	-	-	-	-	+	-	360-1063
NKI-L16	+	-	-	-	-	+	-	360-1063
CBR LFA-1/3	+	-	-	-	-	+	-	360-1063
M17/7	-	+	-	-	+	+	-	154-359
M7/14	-	+	-	-	+	+	-	154-359

B

mAb	m57h	h57m74h	h74m89h	h89m117h	h119m153h	h153m183h	h184m216h	h217m248h	h249m303h	h300m359h	h300m442h	Epitope
S6F1	-	+	+	+	+	+	+	+	+	nd	+	1-57
TS2/4	-	+	+	+	+	+	+	+	+	nd	+	1-57
CBR LFA-1/10	+	+	-	-	+	+	+	+	+	nd	+	75-117
CBR LFA-1/9	+	+	+	+	-	+	-	+	+	nd	+	119-153,185-215
BL5	+	+	+	+	-	+	-	+	+	nd	+	119-153,185-215
F8.8	+	+	+	+	-	+	-	+	+	nd	+	119-153,185-215
TS2/6	+	+	+	+	+	-	+	+	+	nd	+	154-183
MAY.035	+	+	+	+	+	+	-	+	+	nd	+	185-215
TS1/11	+	+	+	+	+	+	-	+	+	nd	+	185-215
TS1/12	+	+	+	+	+	+	-	+	+	nd	+	185-215
TS1/22	+	+	+	+	+	+	+	+	-	nd	+	250-303
TS2/14	+	+	+	+	+	+	+	+	-	nd	+	250-303
25-3.1	+	+	+	+	+	+	+	+	-	nd	+	250-303
CBR LFA-1/1	+	+	+	+	+	+	+	+	+	-	-	301-359
YTA-1	+	+	+	+	+	+	+	+	+	+	-	360-442
G-25.2	+	+	+	+	+	+	+	+	+	nd	+	443-1063
NKI-L16	+	+	+	+	+	+	+	+	+	nd	+	443-1063
CBR LFA-1/3	+	+	+	+	+	+	+	+	+	nd	+	443-1063
FD441.8	nd	nd	nd	nd	-	-	-	-	+	nd	-	250-303
M17/7	nd	nd	nd	nd	-	-	-	-	+	nd	-	250-303
M7/14	nd	nd	nd	nd	-	-	-	-	+	nd	-	250-303

FIG. 5. **Mapping of mAb epitopes on the α subunit.** *A*, chimeras. *B*, scanning mutants. COS cells cotransfected with wild type, chimera, or scanning mutant α subunits and the human β subunit- or mock-transfected cells were stained with mAb to the α subunits and subjected to immunofluorescence flow cytometry. +, percent of positive cells was comparable to COS cells transfected with wild-type LFA-1. −, mAb staining was not significantly different from staining with the negative control X63 IgG1 myeloma. *nd*, not done.

residues 250–303; and mAb CBR LFA-1/1 to residues 301–359. mAb YTA-1 lost reactivity with h300m442h but reacted with h300m359h; thus, at least a portion of its epitope localizes to residues 360–442. mAb G-25.2, NKI-L16, and CBR LFA-1/3 reacted with h300m442 and could be localized C-terminal to residue 442.

Three rat mAb to mouse LFA-1, all of which block function, were localized. mAb M17/7 and M7/14 were localized to residues 154–359 with the chimeras (Fig. 5*A*). M17/7, M7/14, and FD441.8 reacted with h249m303h and were negative on all other scanning mutants on which they were tested (Fig. 5*B*). Thus, murine residues 250–303 in a completely human background are sufficient for expression of the epitope(s) recognized by these mAb.

The point mutants in the segment from residues 119–153 were tested for reactivity with mAb to this region, CBR LFA-1/9, BL5, and F8.8. The P144R substitution, but none of the other point mutations, abolished binding of CBR LFA-1/9 and F8.8 (data not shown).

Inhibition of Lymphoid Cell Homotypic Aggregation with mAb to LFA-1—mAb were tested for inhibition of homotypic aggregation by two cell lines that utilize different ICAMs. JY cells express ICAM-1, less ICAM-2, and little ICAM-3 (de Fougerolles and Springer, 1992). Phorbol 12-myristate 13-acetate-stimulated JY cell homotypic aggregation is largely blocked by mAb to ICAM-1, unaffected by mAb to ICAM-2, and completely blocked by a combination of mAb to ICAM-1 and ICAM-2 (de Fougerolles *et al.*, 1991). SKW3 cells express ICAM-3, less ICAM-2, and no ICAM-1 (de Fougerolles and Springer, 1992). Phorbol 12-myristate 13-acetate-stimulated SKW3 cell homotypic aggregation or binding to purified LFA-1 is partially inhibited by mAb to ICAM-3 and completely inhibited by a combination of mAb to ICAM-2 and ICAM-3 (de Fougerolles *et al.*, 1994; de Fougerolles and Springer, 1992). Inhibition of SKW3 and JY cell aggregation by LFA-1 mAb was concordant (Table II). Inhibition by mAb of aggregation was a more stringent assay than inhibition of binding of lymphoid cells to purified ICAM-1 on a substrate, *i.e.* the same trends were seen, but mAb that were partial blockers of aggregation were more complete blockers of binding to ICAM-1 (data not shown). Of 18 mAb, six completely inhibited homotypic aggregation of JY and SKW3 cells. These mAb mapped to residues 154–183, 185–215, and 250–303 within the I domain, and residues 301–359 just C-terminal to the I domain. mAb to adjacent residues 119–153 gave partial inhibition, whereas mAb to residues 1–117 and 360-1063 gave little or no inhibition.

DISCUSSION

We have used two approaches to define segments of the LFA-1 α subunit important for binding to ICAMs. The first approach relied on the observation that the human but not mouse α subunit enabled binding of the LFA-1 αβ complex to human ICAM-1 (Johnston *et al.*, 1990). The human and murine ICAM-1 molecules are 55% identical in overall amino acid sequence and 52% identical in the first IgSF domain, where the binding site for LFA-1 has been mapped. The inability of murine LFA-1 to bind human ICAM-1 no doubt reflects the divergence of at least some of the ICAM-1 residues that are present at the binding interface with LFA-1.

By dividing the αL subunit into four portions with chimeras, we mapped a region important for species-specific interaction with ICAM-1 to aa residues 1–359, which contains the I domain. The chimeras also provided evidence for multiple subregions within the I domain required for specific interactions, since substitution of human LFA-1 with either of two different murine segments (aa residues 1–153 or residues 154–359) greatly reduced binding to human ICAM-1. Working with scanning mutants provided further evidence for two subregions. The major differences between human and mouse ICAM-1 that restrict recognition of ICAM-1 were localized to residues 119–153 and 218–248. These subregions correspond to the first and third segments, respectively, of five I domain segments that we studied.

The human LFA-1 α subunit was substituted with single or several murine aa residues for fine localization of residues required for specific binding to ICAM-1. Two of 10 point mutants in region 119–153, M140Q and E146D, lost a large portion of ICAM-1 binding activity. In the region of 218–248, the multiple substitution mutant I235V/T243S/S245K lost most of the ICAM-1 binding activity, and the individual point mutations T243S and S245K lost a large portion of the binding activity.

After this work was completed, the three-dimensional structure of the I domain of Mac-1 was reported (Lee *et al.*, 1995). We have used this structure as a model for the I domain of LFA-1; the only significant difference between the structures is predicted to be a shortening of the α5 helix of LFA-1 (Fig. 3). The four residues in LFA-1 important in species-specific binding to ICAM-1 are superimposed on the position of the homologous residues in Mac-1 in Fig. 6. Residue Met-140 is in between Ser-139 and Ser-141, which coordinate with the Mg^{2+} ion; the Met-140 side chain is a prominent feature of the I domain surface. Glu-146 forms the base of a depression near the Mg^{2+}. The Thr-243 and Ser-245 residues are located on the other side of the Mg^{2+} from Met-140 and Glu-146. Previous mutagenesis

TABLE II
Inhibition of SKW3 and JY cell homotypic aggregation with mAbs

mAb	Epitope	Aggregation score[a]		Aggregation ± S.D.[b]	
		SKW3	JY	SKW3	JY
				%	
X63		4+	5+	85 ± 3	96 ± 1
S6F1	1–57	4+	5+	88 ± 5	96 ± 2
TS2/4	1–57	3+	4+	65 ± 5	93 ± 4
CBR LFA-1/10	75–117	4+	4+	81 ± 2	92 ± 2
CBR LFA-1/9[c]	119–153, 185–215	4+	4+	56 ± 6	64 ± 8
BL5	119–153, 185–215	2+	3+	35 ± 16	50 ± 14
F8.8	119–153, 185–215	2+	2+	18 ± 15	12 ± 6
TS2/6	154–183	1+	1+	5 ± 1	10 ± 4
MAY.035	185–215	1+	1+	4 ± 5	2 ± 1
TS1/11	185–215	2+	2+	11 ± 1	11 ± 2
TS1/12	185–215	2+	2+	12 ± 4	12 ± 6
TS1/22	250–303	1+	1+	7 ± 1	2 ± 2
TS2/14	250–303	1+	1+	4 ± 2	4 ± 4
25-3-1	250–303	1+	1+	10 ± 4	10 ± 6
CBR LFA-1/1	301–359	1+	1+	6 ± 6	10 ± 7
YTA-1	360–442	3+	4+	53 ± 16	82 ± 13
G-25.2	443–1063	4+	4+	79 ± 11	93 ± 1
NKI-L16	443–1063	4+	5+	92 ± 7	96 ± 1
CBR LFA-1/3	443–1063	4+	5+	79 ± 2	95 ± 1

[a] Aggregation was scored as described (41), where 1+ indicates less than 10% of the cells were in aggregates, 2+ indicates that 10 to 50% of the cells were in aggregates, 3+ indicates that 50 to 100% of the cells were in small loose clusters, 4+ indicates that up to 100% of the cells were in large clusters, and 5+ indicates that all cells were in large, very compact aggregates.

[b] After the aggregation assay, 1/10 of the cells were transferred to a fresh microtiter well and free cells were counted in four different microscope grids. Aggregation = 100 × (1 − free cells with mAb/free cells without mAb). Data are mean and S.D. of three independent experiments.

[c] CBR LFA-1/9 was the only mAb tested of the IgM class; this may be related to lower inhibition of adhesion by this mAb.

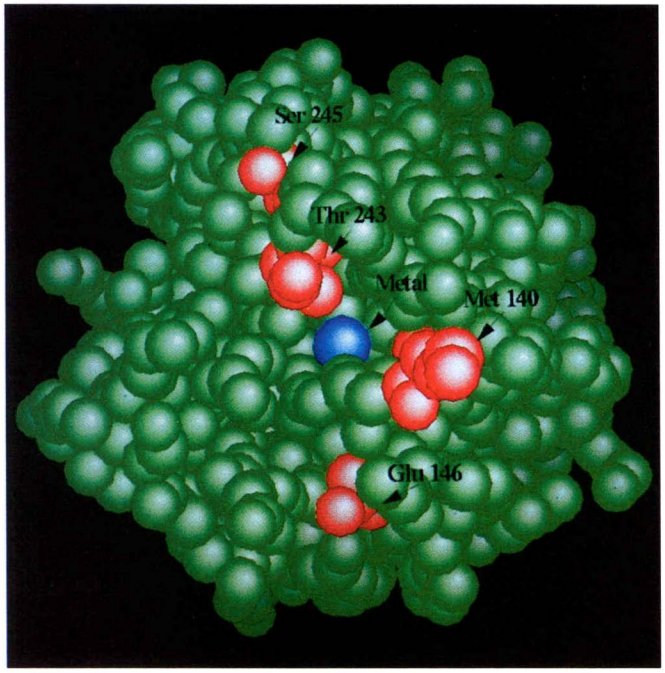

FIG. 6. **The Mac-1 I domain, with residues in LFA-1 shown to be important in specific interaction with ICAM-1 superimposed on the position of the homologous residues in Mac-1.** Alignment was as shown in Fig. 3.

studies have identified residues in integrin I domains that form the primary or secondary coordination shell with Mg^{2+} and are required for ligand recognition (Kamata *et al.*, 1994; Kamata and Takada, 1994; Kern *et al.*, 1994; Lee *et al.*, 1995; Michishita *et al.*, 1993). These mutations are known (Michishita *et al.*, 1993) or predicted (Lee *et al.*, 1995) to prevent divalent cation binding. The Mg^{2+} in the MIDAS motif is predicted to form one coordination with ligand (Lee *et al.*, 1995); divalent cations have for some time been predicted to bridge integrins and their ligands (Corbi *et al.*, 1987). Thus, these mutations suggest the importance of the Mg^{2+}, rather than particular amino acid residues, in ligand binding. By contrast, none of the four residues we have identified are predicted to coordinate Mg^{2+}. Rather, the striking feature of the residues we have identified is that they surround the Mg^{2+} binding site, and for the first time define a ligand binding face for an integrin I domain. We hypothesize that the Mg^{2+} in the MIDAS motif coordinates with Glu-34 in ICAM-1, by far the most important residue yet identified for binding to LFA-1 (Staunton *et al.*, 1988). Glu-34 is completely conserved in ICAM-1 in the mouse, human, and three other species, and in murine and human ICAM-2 and in human ICAM-3 (Vonderheide *et al.*, 1994). We hypothesize that residues surrounding Glu-34 in the binding interface on ICAM-1 that differ between mouse and human are responsible for the species-specific differences we have mapped on the I domain of LFA-1. A residue that may play an analogous role to Glu-34 in ICAM-1 is Asp-40 in VCAM-1, although the integrin to which VCAM-1 binds, VLA-4, lacks an I domain (Osborn *et al.*, 1994; Renz *et al.*, 1994; Vonderheide *et al.*, 1994). Asp-40 in VCAM-1 is in a prominent loop between the C and D β strands of the first IgSF domain (Jones *et al.*, 1995), as predicted previously for Glu-34 in ICAM-1 (Staunton *et al.*, 1988).

Our second approach to define segments of the LFA-1 α subunit important for binding ICAM-1 was to construct a structure-function map with mAb. We mapped epitopes of a panel of eighteen mouse anti-human CD11a and three rat anti-mouse CD11a antibodies. These mAbs were mapped to nine different segments of the α subunit (Fig. 7). Furthermore, we examined the ability of these mAbs to block LFA-1-dependent homotypic aggregation that was primarily dependent on ICAM-1 for JY cells and ICAM-3 for SKW3 cells. These results extended previous studies that have mapped mAb to one to four different segments of the LFA-1 α subunit (Champe *et al.*, 1995; Landis *et al.*, 1993, 1994; Randi and Hogg, 1994). Our epitope-function map (Fig. 7) showed that function-blocking mAb localized to all four subregions to which epitopes were mapped in the I domain and to a segment C-terminal to the I domain, but not to two N-terminal or two C-terminal subregions. Strongest blocking was obtained with mAb that bound to epitopes in segments from aa residues 154–359. However, a group of three mAb that

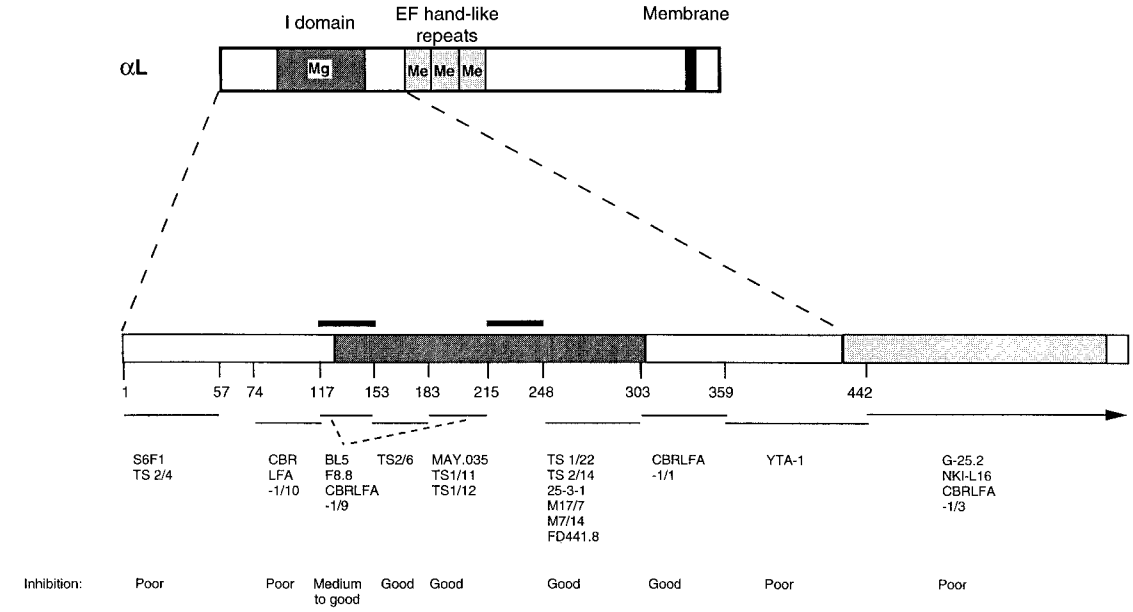

FIG. 7. **Schematic map of functionally important regions and epitopes of the LFA-1 α subunit.** Amino acid residue numbers are for the human LFA-1 α subunit. The *bold lines* over the α subunit indicate the regions that restrict species-specific binding to ICAM-1. The *thin lines* below the α subunit indicate mAb epitope localization. Inhibition refers to potency of mouse anti-human mAb in inhibition of LFA-1-dependent homotypic aggregation. All three rat anti-mouse mAb were previously selected for strong inhibition of LFA-1-dependent T cell-mediated killing.

recognized epitopes in both segments 119–153 and 185–215 gave intermediate inhibition; the epitopes of two of these mAb, CBR LFA-1/9 and F8.8, included Pro-144, which is located just prior to the beginning of α helix 1. The studies with mAb suggest that multiple segments of the I domain contribute to the interaction with ICAM-1 and further suggest that the segment between the I domain and EF hand-like repeats is important for binding to ICAM-1. Our epitope mapping results are in agreement with previous cross-blocking and functional studies on the TS (Ware *et al.*, 1983) and M series (Sanchez-Madrid *et al.*, 1982a) of mAb. Thus TS1/11 and TS1/12 that map to residues 183–215 cross-blocked one another and were distinct from all other TS series mAb; TS1/22 and TS2/14 that map to residues 248–303 also showed cross-blocking. Studies on T cell-mediated killing showed that TS mAb that map to the I domain were inhibitors, whereas TS2/4 that maps to the N terminus was not. The mAb M7/4 and M17/7, which block T cell-mediated killing by 80%, cross-blocked one another and all other tested function-blocking rat anti-mouse mAb (Sanchez-Madrid *et al.*, 1982a); less sequence divergence between mouse and rat than between mouse and human may have led to a more focused response to epitopes in the residue 249–303 segment, where all three studied rat anti-mouse mAb map.

There are agreements and also contradictions between our study and a recent study in which eight mAb shown in the literature to inhibit LFA-1 function were mapped to three regions within the I domain (Champe *et al.*, 1995). Three mAb were mapped to segments 143–148 and 197–203, and one was mapped to 197–203 only. The three mAb thus recognize an epitope similar to CBR LFA-1/9, BL5, and F8.8, which were shown here to recognize regions 119–153 and 185–215. Both Champe *et al.* (1995) and our group found that reactivity of these mAb was abolished by the P144R human to mouse substitution. These residues are located on the first turn of the α1 helix and the long loop between the α3 and α4 helices, and are on the same face of the I domain as the residues involved in specific interactions. This localization is consistent with inhibition of function that we found with the mAb to this epitope. Champe *et al.* (Champe *et al.*, 1995) found that the anti-human mAb TS1/22 and 25-3-1, as well as the anti-mouse mAb M17/4 and I21/7, mapped to residues Ile-126 and Asn-129. In contrast, we mapped TS1/22, 25-3-1, and three anti-mouse mAb, including M17/7 and M7/14 (which are known to cross-block M17/4; Sanchez-Madrid *et al.*, 1982a), to residues 250–303. Each study grouped the same antibodies as reacting with a common epitope, but mapped them to completely different sites. Champe *et al.* used immunoprecipitation of biosynthetically-labeled transfected α subunits expressed in the absence of β subunit, whereas we used immunofluorescent labeling of αβ complexes on the cell surface, but this cannot explain the discrepancy. The mapping to Ile-126 and Asn-129 was supported by a single mutant construct, and no effect of individual mutations at residues 126 and 129 was found, including an I126D substitution more radical than the I126M human-mouse substitution. Champe *et al.* (1995) made no substitutions C-terminal to residue 218, and thus did not study the C-terminal half of the I domain where we localized this group of mAb. Our localization to 250–303 as opposed to 126–129 was supported by four constructs: h153m359h, m153h359m, h118m153h, and h249m303h. The group of mAb we localized to residues 250–303 all gave maximal inhibition of LFA-1 function, implying the epitope is localized close to the MIDAS motif. By contrast, residues 126 and 129 are at the very beginning of the I domain, prior to β strand A, and are on the opposite side of the I domain from the MIDAS motif.

Our study has identified residues that surround the Mg^{2+}-binding site on the LFA-1 I domain that are required for specific binding to ICAM-1. Other studies on multiple integrin I domains have shown that residues that directly or indirectly coordinate Mg^{2+} in the MIDAS motif are required for ligand binding. Together, this work defines a ligand binding interface on integrin I domains. The epitope structure-function mapping experiments confirm the importance of multiple subregions of the I domain in ligand binding, and also suggest that the region C-terminal to the I domain residues 301–359, is important. Monoclonal antibodies that bind to the β subunit (Sanchez-Madrid *et al.*, 1983) and mutation of a MIDAS-like site in the conserved region of the β subunit (Bajt and Loftus, 1994; Lee *et al.*, 1995) also inhibit ligand binding. How both the α and β subunit contribute to ligand binding and work together to regulate integrin adhesiveness remains to be established.

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