0022-1767/91/1471-036802.00/0
THE JOURNAL OF IMMUNOLOGY
Copyright © 1991 by The American Association of Immunologists
Vol. 147, 369–374, No. 1, July 1, 1991
Printed in U.S.A.

CLONING OF THE MURINE LYMPHOCYTE FUNCTION-ASSOCIATED MOLECULE-1 α-SUBUNIT AND ITS EXPRESSION IN COS CELLS[1]

YAEL KAUFMANN,[2] ELAINE TSENG, AND TIMOTHY A. SPRINGER[3]

From the Center for Blood Research, and the Department of Pathology, Harvard Medical School, 800 Huntington Ave., Boston, MA 02115

The lymphocyte function-associated molecule 1 (LFA-1, CD11a/CD18) is an integrin that mediates adhesion of immune cells by interaction with two members of the Ig superfamily, ICAM-1 and ICAM-2. LFA-1 consists of an α subunit (M_r = 180,000) and a β subunit (M_r = 95,000). We report here the isolation and expression of the murine α subunit cDNA (GenBank accession no. M60778). The deduced sequence comprises a 1061 amino acid extracellular domain, a 29 amino acid transmembrane region, and a 50 amino acid cytoplasmic domain. It has a 72% amino acid identity with its human counterpart and 34% identity with the murine Mac-1 α subunit. The murine LFA-1 α subunit could be expressed on the cell surface of a fibroblastoid cell line, COS, by cotransfection with either the human or murine β subunit cDNA.

LFA-1[4], and the other leukocyte integrins, Mac-1 and p150,95, are involved in leukocyte interactions during inflammation and the immune response. LFA-1, expressed on virtually all leukocytes, is involved in a variety of immune phenomena including leukocyte-endothelial cell interaction, CTL-mediated killing, and antibody-dependent killing by granulocytes and monocytes (1). LFA-1 has two counter-receptors, ICAM-1 (2, 3) and ICAM-2 (4), which are both members of the Ig superfamily. Mac-1 and p150,95 are expressed primarily on myeloid cells. Both Mac-1 and p150,95 may be involved in iC3b binding (5, 6) and myeloid cell-endothelial cell interaction (7, 8). The three leukocyte integrins are αβ heterodimers having distinct α subunits (LFA-1α or CD11a, Mac-1α or CD11b, and p150,95α or CD11c) and a common β subunit, CD18 (9–11). These three molecules constitute a subfamily of the integrin family. The three leukocyte integrin α subunits are more homologous to each other than to other integrin receptors and possess an inserted or I domain of 200 amino acids, which is not found in other integrin α subunits, with the exception of VLA-1 and VLA-2 (12).

LFA-1 was originally identified in the mouse (13, 14) and later in the human system where it has been cloned and sequenced (12, 15). There are species-specific differences, because human LFA-1 will bind to both murine and human ICAM-1, whereas murine LFA-1 will bind to murine and not human ICAM-1. This species specificity resides in the murine α subunit (16). We have been interested in the structural basis for the function of LFA-1 in inflammation and the immune response and in identifying regions on the LFA-1 subunits that are important to their adhesive and signal transduction functions. As a first step toward this end, we have cloned the murine LFA-1 α subunit cDNA, determined its nucleotide and deduced amino acid sequence, and showed that it can be expressed in COS cells in association with either the murine or the human β subunit.

MATERIALS AND METHODS

Library screening and clone isolation and characterization. Procedures for hybridization of human LFA-1 α probe to the cDNA library and clone characterization were as described in Maniatis et al. (17). An *Eco*RI-*Bam*HI fragment corresponding to most of the coding region (nucleotides 1-2955) of the human LFA-1 α cDNA (12) was labeled with ^{32}P by nick translation, hybridized in 5x SSC, 5x Denhardt's solution, 0.1% SDS, 50% formamide, and 50 μg/ml denatured herring sperm DNA at 37°C to a murine cDNA library kindly provided by A. Turner and M. Davis, Stanford University, Stanford, CA. The library was constructed from cytoplasmic RNA of B cell lymphoma line BCL1 (American Type Culture Collection, CRL 1669, Bethesda, MD), which had been induced for 19 h with rIL-2 (1000 U/ml), and IL-5 (10% of a COS cell supernatant of a transient transfection with IL-5 clone). The library was constructed in lambda ZAP XHO-MID, a derivative of λ ZAP that had been modified to possess only one *Apa*I site in the SK^- polylinker. The cloning site was *Xho*I. Filters were washed twice with 2x SSC and 0.1% SDS at 20°C for 15 min and once at 37°C for 30 min and exposed to x-ray film overnight. Clones containing hybridizing sequences were purified and the plasmids containing inserts were excised with the helper phage R408 (Stratagene, La Jolla, CA).

Two clones denoted 23 and 28 were characterized by partial sequence and restriction enzyme mapping. The composite sequence of these clones contains the entire murine LFA-1 α coding region. Five restriction fragments were subcloned from clones 23 and 28 into pBluescript and the two DNA strands were sequenced by the dideoxy chain termination method (18). The ends of the fragments were sequenced with the pBluescript primers and internal regions were sequenced using synthetic primers complementary to the murine LFA-1 α sequence (Fig. 1).

DEAE-dextran transfection of COS cells. The entire coding region of the human LFA-1 α and β subunit cDNA (12, 15) and the murine β subunit cDNA (19) were subcloned into the expression vector Ap^rM8, an ampicillin resistant modification of CDM8 (L. B. Klickstein, unpublished observations). A cDNA clone containing the entire coding sequence of the murine LFA-1 α subunit was prepared by removing the *Eco*RI fragment of clone 28 corresponding to the 3′ end of its cDNA and replacing it with the *Eco*RI fragment of clone 23 containing the downstream cDNA portion of 3083 nucleotides (Fig. 1). The proper orientation of the 3′ insert was determined by

Received for publication February 8, 1991.
Accepted for publication April 12, 1991.
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] This work was supported by National Institute of Health Grant number 31798 and by an Eleanor Roosevelt International Cancer Research Fellowship from UICC to Y.K.
[2] On leave of absence from the Institute of Hematology, Chaim Sheba Medical Center, Tel Hashomer and Sackler School of Medicine, Tel Aviv University, Tel-Aviv, Israel.
[3] Address correspondence and reprint requests to Dr. Timothy Springer, Center for Blood Research, 800 Huntington Avenue, Boston, MA 02115.
[4] Abbreviations used in this paper: LFA-1, lymphocyte function-associated Ag 1; mLFA, murine LFA.

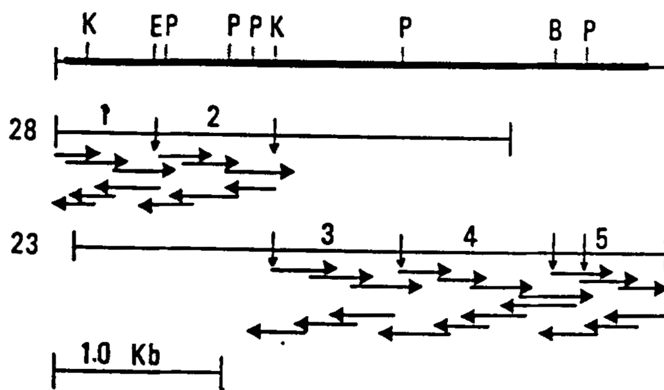

Figure 1. Restriction map of murine LFA-1 α subunit cDNA clones. The open reading frame and untranslated regions are indicated with a thick and thin line, respectively. *Numbers 1* to *5* show restriction fragments subcloned for sequencing. *Arrows* indicate the sequencing strategy. The relevant restriction sites are: *EcoR*1 (*E*), *Pst*1 (*P*), *Kpn*1 (*K*), and *BamH*1 (*B*).

complete digestion with *Hin*dIII + *Sal*I. The murine LFA-1 α cDNA (3662 nucleotides) and murine β cDNA (2856 nucleotides) were excised from pBluescript by digestion with *Xho*I and *Eco*RI, respectively, ligated with *Bst*XI linkers after blunting of the *Xho*I and *Eco*RI sites with Klenow fragment of DNA polymerase I and subcloned into the *Bst*XI site in Ap^{r}M8 (20). The human LFA-1 α and β subunits cDNA (4.5 and 2.7 kb, respectively) were isolated by complete digestion with *Hin*dIII + *Xba*I and ligated to Ap^{r}M8. The α and β subunit cDNA were cotransfected into COS cells, a monkey kidney fibroblastoid cell line, using DEAE-dextran (20, 21).

Indirect immunofluorescence and mAb. COS cells were harvested with 5 mM EDTA in HBSS and cell surface expression of LFA-1 α and β subunits were examined by indirect immunofluorescence as detailed previously (21). The mAb used in these experiments include TS1/22 and TS1/18 to the human LFA-1 α and β subunit, respectively (10), and M17/4.2 and M18/2a.8 to the murine LFA-1 α and β subunits, respectively (22, 23). An mAb to horseradish peroxidase, ZPOI (Zymed Laboratories, San Francisco, CA) was used as a negative control. FITC-conjugated goat-anti-mouse Ig (0.1 mg/ml) (Zymed Laboratories), was used as a second antibody.

RESULTS AND DISCUSSION

Identification and characterization of murine LFA-1 α cDNA clones. A lambda ZAP cDNA library constructed from the mouse B cell lymphoma line BCL1 was screened with a probe containing the majority of the coding region of human LFA-1 α subunit cDNA (12). Seven clones were isolated and two, denoted 28 and 23, were characterized in detail. Clone 28 is 2.75-kb long and contains the nucleotide sequence corresponding to the 5′ end of the human LFA-1 α subunit open reading frames, including a putative initiation codon (ATG). Clone 23 is 3.5-kb long, it overlaps most of clone 28 except for the 5′ 143 nucleotides and extends 0.9-kb further downstream to include the end of the coding sequence (Fig. 1). The composite sequence of clones 28 and 23 of 3662 nucleotides contains the 3489 nucleotide coding sequence, a 5′ untranslated region of 59 nucleotides and a 3′ untranslated region of 114 nucleotides (Fig. 2).

Nucleotide and deduced amino acid sequence of the mLFA-1 α chain. The sequence of the N-terminal 20 amino acids of the mLFA-1 α subunit previously determined by N-terminal sequencing (24) is in perfect agreement with that deduced from the DNA sequence (*underlined* in Fig. 2). The mature α subunit is preceded by a 23 residue signal peptide with a consensus peptidase cleavage sequence (Ala-X-Ser/Pro) (25). There is a single upstream translation initiation codon at nucleotide 60. The mature mLFA-1 α subunit has an extracellular domain of 1061 residues, a single hydrophobic transmembrane region of 29 residues and a cytoplasmic tail of 50 residues (Fig. 2). The mature protein is predicted to be M_r = 125,400. Fifteen N-linked glycosylation sites (Asn-X-Thr/Ser) are present in the extracellular domain (Figs. 2 and 4). These values agree with the M_r of the mLFA-1 subunit translated in vitro (M_r = 140,000) and the mLFA-1 α glycoprotein (M_r = 180,000) (26).

The mLFA-1 α subunit contains within the extracellular domain an inserted (I) domain of about 190 amino acids (residues 124-310) (Fig. 3). I domains are found in the three human leukocyte integrin α subunits sequenced to date, murine Mac-1 α, and in VLA-1 and 2 but not other VLA α subunits or the IIbIIIa and vitronectin receptor α subunits. These I domains are homologous with repeated domains in von Willebrand factor, cartilage matrix protein, and a single domain in complement components C2 and factor B (1, 27). Three repeats with a putative divalent cation binding site motif are found at amino acid residues 432-587. Other integrin α subunits contain either three or four putative divalent cation binding motifs (1, 27). Most of the cysteines and N-glycosylation sites are found outside the I region and the putative divalent cation binding motifs (Fig. 3). A paucity of cysteines in this region is found in other leukocyte integrin α subunits as well (12, 28–31), and is consistent with a previous suggestion that this region may undergo conformational changes, important in ligand binding (12, 32).

Comparison of sequence homology between the mLFA-1 α chain and its human counterpart (Figs. 3 and 4) shows overall 72% identity with the highest identity (82%) for the putative divalent cation binding repeats and the lowest identity (55%) for the cytoplasmic domain (Fig. 3). The high conservation of the putative cation binding motif is consistent with an involvement of this region in the functional activity of LFA-1 α. Divalent cations (1 mM Mg^{2+} or 0.1 mM Mg^{2+} and 1 mM Ca^{2+}) are required for cellular interactions that are dependent on LFA-1 (32), for LFA-1-dependent binding of cells to purified ICAM-1 (3), and for binding of purified LFA-1 protein micelles to purified ICAM-1 (33). The low conservation of the cytoplasmic tail is consistent with a recent finding (34) that the human LFA-1 α chain with its cytoplasmic domain truncated so that it contains only the 14 residues adjacent to the membrane, can associate with the β chain and fully supports binding to ICAM-1. Comparison of sequence identity between mouse LFA-1 α and mouse Mac-1 α chains reveals an overall identity of 34% with the highest identity between the first two putative divalent cation binding sites.

Expression of murine LFA-1 α subunit on surface of COS cells. We examined whether the murine α subunit could be expressed in the fibroblastoid COS cell line, and the requirement for coexpression with the murine or human β subunit. COS cells were transiently transfected with α or β subunit cDNA alone or together and surface expression was detected with mouse anti-human or rat anti-mouse first antibody, followed by a second antibody to mouse IgG and flow cytometry (Fig. 5). This second antibody stained rat IgG (somewhat more weakly than mouse IgG) by cross-reaction. Cotransfection of the α and β subunit cDNA yielded efficient expression on the sur-

```
   1 GCAAATCTCAAGAGCTCTCTGAGATCGAGTCCGGACCCACAGGTCCCTCCTAAGCGCAGATGAGTTTCCGGATTGCGGGCCCCAGACTTTTGCTACTGGGACTCCAGCTGTTTGCCAAGG
 -23                                                             M  S  F  R  I  A  G  P  R  L  L  L  L  G  L  Q  L  F  A  K  A
 121 CCTGGAGCTACAACCTGGACACACGGCCTACGCAGAGCTTCTTGGCACAAGCTGGAAGACATTTTGGGTACCAGGTCTTGCAGATTGAAGATGGGGTTGTCGTGGGAGCCCCAGGTGAGG
  -2  W  S  Y  N  L  D  T  R  P  T  Q  S  F  L  A  Q  A  G  R  H  F  G  Y  Q  V  L  Q  I  E  D  G  V  V  V  G  A  P  G  E  G
 241 GGGACAACACGGGAGGCCTCTATCACTGCCGAACAAGCAGCGAGTTCTGCCAGCCAGTCAGCCTACATGGTTCTAACCATACCTCCAAGTACTTGGGAATGACGCTGGCAACAGATGCCG
  39  D  N  T  G  G  L  Y  H  C  R  T  S  S  E  F  C  Q  P  V  S  L  H  G  S  N  H  T  S  K  Y  L  G  M  T  L  A  T  D  A  A
 361 CCAAGGGAAGCCTTTTGGCCTGTGACCCTGGACTGTCTCGGACATGCGATCAGAACACTTACCTCAGTGGCCTCTGCTACCTCTTCCCCCAGAGTCTGGAGGGACCTATGTTACAAAATC
  79  K  G  S  L  L  A  C  D  P  G  L  S  R  T  C  D  Q  N  T  Y  L  S  G  L  C  Y  L  F  P  Q  S  L  E  G  P  M  L  Q  N  R
 481 GTCCCGCCTATCAGGAATGTATGAAGGGCAAAGTCGACCTGGTGTTTCTGTTCGATGGCTCACAGAGCTTGGATAGAAAGGACTTTGAAAAAATCCTGGAATTCATGAAGGATGTGATGA
 119  P  A  Y  Q  E  C  M  K  G  K  V  D  L  V  F  L  F  D  G  S  Q  S  L  D  R  K  D  F  E  K  I  L  E  F  M  K  D  V  M  R
 601 GGAAGCTCAGCAATACTTCCTACCAGTTTGCTGCCGTCCAGTTCTCCACAGACTGCAGAACAGAATTTACTTTCTTGGACTACGTTAAGCAGAACAAGAACCCCGATGTTCTGCTAGGCA
 159  K  L  S  N  T  S  Y  Q  F  A  A  V  Q  F  S  T  D  C  R  T  E  F  T  F  L  D  Y  V  K  Q  N  K  N  P  D  V  L  L  G  S
 721 GCGTGCAGCCCATGTTCTTGCTGACCAATACCTTTCGTGCCATCAACTATGTGGTGGCACACGTGTTCAAAGAAGAGTCTGGTGCCAGGCCGGATGCTACCAAGGTGCTTGTCATCATTA
 199  V  Q  P  M  F  L  L  T  N  T  F  R  A  I  N  Y  V  V  A  H  V  F  K  E  E  S  G  A  R  P  D  A  T  K  V  L  V  I  I  T
 841 CAGACGGGGAGGCAAGTGATAAAGGCAACATCAGTGCGGCCCACGACATAACCCGCTACATCATCGGGATTGGCAAGCATTTTGTGAGCGTACAAAAGCAAAAGACGCTCCACATATTTG
 239  D  G  E  A  S  D  K  G  N  I  S  A  A  H  D  I  T  R  Y  I  I  G  I  G  K  H  F  V  S  V  Q  K  Q  K  T  L  H  I  F  A
 961 CCTCAGAACCTGTAGAGGAATTTGTGAAGATTCTGGACACCTTTGAGAAGCTGAAGGATCTTTTTACTGACCTGCAGAGGAGGATTTATGCTATTGAGGGCACAAACAGACAGGACCTGA
 279  S  E  P  V  E  E  F  V  K  I  L  D  T  F  E  K  L  K  D  L  F  T  D  L  Q  R  R  I  Y  A  I  E  G  T  N  R  Q  D  L  T
1081 CATCCTTTAACATGGAACTCTCCTCCAGCGGGATCAGCGCAGACCTCAGCAAGGGCCATGCAGTTGTGGGAGCTGTTGGGGCTAAGGATTGGGCCGGGGGCTTTCTGGACCTGCGTGAAG
 319  S  F  N  M  E  L  S  S  S  G  I  S  A  D  L  S  K  G  H  A  V  V  G  A  V  G  A  K  D  W  A  G  G  F  L  D  L  R  E  D
1201 ACCTGCAGGGTGCCACATTTGTTGGGCAGGAACCGCTGACCTCAGATGTGAGAGGGGGCTACCTGGGTTACACTGTGGCCTGGATGACCTCCCGGAGCTCCAGACCCCTGCTGGCAGCAG
 359  L  Q  G  A  T  F  V  G  Q  E  P  L  T  S  D  V  R  G  G  Y  L  G  Y  T  V  A  W  M  T  S  R  S  S  R  P  L  L  A  A  G
1321 GAGCCCCACGGTACCAGCATGTGGGACAAGTACTGCTTTTCCAAGCCCCAGAGGCTGGAGGACGTTGGAACCAAACCCAGAAGATAGAAGGGACTCAGATCGGATCTTACTTTGGTGGGG
 399  A  P  R  Y  Q  H  V  G  Q  V  L  L  F  Q  A  P  E  A  G  G  R  W  N  Q  T  Q  K  I  E  G  T  Q  I  G  S  Y  F  G  G  E
1441 AACTATGTAGTGTTGACCTGGACCAAGATGGCGAGGCAGAGCTGCTGCTGATTGGAGCACCCCTGTTCTTTGGGGAGCAGAGAGGAGGCCGAGTGTTCACTTACCAGAGAAGACAGTCGC
 439  L  C  S  V  D  L  D  Q  D  G  E  A  E  L  L  L  I  G  A  P  L  F  F  G  E  Q  R  G  G  R  V  F  T  Y  Q  R  R  Q  S  L
1561 TGTTTGAAATGGTCTCAGAGCTACAGGGTGACCCTGGCTACCCGCTTGGTCGGTTTGGAGCCGCCATAACTGCCCTGACGGACATCAATGGGGATAGGCTGACTGATGTGGCTGTGGGAG
 479  F  E  M  V  S  E  L  Q  G  D  P  G  Y  P  L  G  R  F  G  A  A  I  T  A  L  T  D  I  N  G  D  R  L  T  D  V  A  V  G  A
1681 CCCCTTTGGAGGAGCAGGGTGCTGTGTACATCTTCAATGGGAAGCCTGGTGGGCTCAGTCCCCAGCCAAGCCAGCGTATACAAGGAGCCCAGGTGTTCCCAGGAATCCGGTGGTTTGGCC
 519  P  L  E  E  Q  G  A  V  Y  I  F  N  G  K  P  G  G  L  S  P  Q  P  S  Q  R  I  Q  G  A  Q  V  F  P  G  I  R  W  F  G  R
1801 GCTCCATCCATGGGGTGAAGGACCTTGGAGGGGACAGGCTGGCAGATGTGGTTGTAGGAGCTGAGGGTCGGGTGGTTGTGCTGAGCTCCAGGCCGGTGGTGGATGTGGTCACTGAGCTGT
 559  S  I  H  G  V  K  D  L  G  G  D  R  L  A  D  V  V  V  G  A  E  G  R  V  V  V  L  S  S  R  P  V  V  D  V  V  T  E  L  S
1921 CGTTCTCCCCAGAGGAAATCCCAGTGCACGAGGTGGAGTGCTCCTACTCAGCCAGGGAGGAGCAGAAACACGGAGTCAAGCTCAAGGCATGCTTCCGGATCAAGCCCCTCACGCCACAGT
 599  F  S  P  E  E  I  P  V  H  E  V  E  C  S  Y  S  A  R  E  E  Q  K  H  G  V  K  L  K  A  C  F  R  I  K  P  L  T  P  Q  F
2041 TTCAAGGTCGCCTGCTTGCCAACCTCAGCTACACCCTGCAGCTGGATGGCCATCGGATGAGGAGCCGAGGGTTGTTCCCAGATGGAAGCCACGAGCTCAGTGGAAACACCTCCATCACCC
 639  Q  G  R  L  L  A  N  L  S  Y  T  L  Q  L  D  G  H  R  M  R  S  R  G  L  F  P  D  G  S  H  E  L  S  G  N  T  S  I  T  P
2161 CAGATAAATCCTGCTTGGACTTCCACTTCCACTTCCCGATCTGCATTCAAGACCTCATCTCCCCTATCAATGTCTCCCTGAATTTCTCTCTTTTGGAGGAAGAAGGAACACCAAGGGACC
 679  D  K  S  C  L  D  F  H  F  H  F  P  I  C  I  Q  D  L  I  S  P  I  N  V  S  L  N  F  S  L  L  E  E  E  G  T  P  R  D  Q
2281 AAAAGGGCAGGGCCATGCAGCCTATCCTGAGACCTTCAATCCACACAGTGACTAAGGAGATCCCTTTTGAGAAGAACTGTGGTGAAGATAAGAAGTGTGAGGCAAACCTGACCCTGTCAT
 719  K  G  R  A  M  Q  P  I  L  R  P  S  I  H  T  V  T  K  E  I  P  F  E  K  N  C  G  E  D  K  K  C  E  A  N  L  T  L  S  S
2401 CCCCTGCCAGATCTGGACCCCTGCGTCTGATGTCCTCTGCCAGCCTTGCTGTGGAGTGGACACTGAGCAACTCAGGGGAAGATGCCTACTGGGTGCGATTAGACCTGGACTTCCCTCGGG
 759  P  A  R  S  G  P  L  R  L  M  S  S  A  S  L  A  V  E  W  T  L  S  N  S  G  E  D  A  Y  W  V  R  L  D  L  D  F  P  R  G
2521 GACTCTCCTTCCGGAAAGTGGAGATGCTTCAGCCACACAGCCGAATGCCTGTGAGCTGCGAGGAGCTCACCGAGGGGTCAAGTCTCCTGACTAAGACACTGAAATGCAATGTAAGCTCTC
 799  L  S  F  R  K  V  E  M  L  Q  P  H  S  R  M  P  V  S  C  E  E  L  T  E  G  S  S  L  L  T  K  T  L  K  C  N  V  S  S  P

2641 CCATCTTCAAAGCAGGCCAGGAGGTGAGCCTCCAGGTGATGTTTAACACGCTACTCAACAGCTCCTGGGAAGACTTCGTCGAGCTGAATGGCACTGTGCACTGTGAGAATGAGAACTCAA
 839  I  F  K  A  G  Q  E  V  S  L  Q  V  M  F  N  T  L  L  N  S  S  W  E  D  F  V  E  L  N  G  T  V  H  C  E  N  E  N  S  S
2761 GCCTCCAGGAGGACAACTCAGCCGCCACCCACATTCCTGTCCTGTACCCTGTCAACATCCTTACTAAGGAGCAGGAGAACTCCACCCTCTATATCAGTTTCACCCCTAAAGGTCCCAAGA
 879  L  Q  E  D  N  S  A  A  T  H  I  P  V  L  Y  P  V  N  I  L  T  K  E  Q  E  N  S  T  L  Y  I  S  F  T  P  K  G  P  K  T
2881 CCCAACAAGTCCAGCATGTCTACCAGGTGAGGATTCAGCCATCTGCCTATGACCACAACATGCCCACACTAGAGGCCTTGGTTGGGGTGCCCCGGCCTCACAGTGAGGACCTCATCACAT
 919  Q  Q  V  Q  H  V  Y  Q  V  R  I  Q  P  S  A  Y  D  H  N  M  P  T  L  E  A  L  V  G  V  P  R  P  H  S  E  D  L  I  T  Y
3001 ACACATGGAGTGTACAGACGGATCCCCTTGTCACTTGCCACAGCGAGGACCTGAAGAGGCCGTCCAGCGAAGCTGAGCAGCCTTGTCTGCCTGGAGTCCAGTTCCGCTGTCCAATTGTCT
 959  T  W  S  V  Q  T  D  P  L  V  T  C  H  S  E  D  L  K  R  P  S  S  E  A  E  Q  P  C  L  P  G  V  Q  F  R  C  P  I  V  F
3121 TCAGGTGGGAGATCCTCATCCAAGTGACGGGGACCGTGGAACTCTCCAAGGAAATCAAGGCCTCCTCCACACTCAGCCTCTGCAGCTCACTCTCCGTCTCCTTCAACAGCAGCAAGCATT
 999  R  W  E  I  L  I  Q  V  T  G  T  V  E  L  S  K  E  I  K  A  S  S  T  L  S  L  C  S  S  L  S  V  S  F  N  S  S  K  H  F
3241 TCCATTTGTATGGCAGCAAAGCCTCTGAGGCCCAGGTCCTCGTGAAGGTTGACCTGATCCACGAGAAGGAGATGCTTCACGTGTACGTGCTCAGCGGCATTGGGGGCCTCGTGCTTCTGT
1039  H  L  Y  G  S  K  A  S  E  A  Q  V  L  V  K  V  D  L  I  H  E  K  E  M  L  H  V  Y  V  L  S  G  I  G  G  L  V  L  L  F
3361 TCCTGATTTTCCTGGCGCTCTACAAGGTTGGCTTCTTCAAACGGAACCTGAAGGAGAAGATGGAGGCTGATGGAGGTGTTCCAAATGGAAGCCCTCCAGAAGACACTGACCCTCTGGCAG
1079  L  I  F  L  A  L  Y  K  V  G  F  F  K  R  N  L  K  E  K  M  E  A  D  G  G  V  P  N  G  S  P  P  E  D  T  D  P  L  A  V
3481 TACCTGGGGAAGAGACCAAAGATATGGGCTGTCTAGAGCCCTCCGGGAGAGTGACAAGGACTAAGGCCTAGGTCTGATACACTGACAGCCCAGGAATAGACTTGAGAGCCCTGGCTCTGA
1119  P  G  E  E  T  K  D  M  G  C  L  E  P  S  G  R  V  T  R  T  K  A
3601 CCAGCTTCAGTCACATGCCACCGTGTATCCTGTCCTTCACTAGTCATTGCTGTTCCTAGTCC
```

Figure 2. Nucleotide sequence and derived protein sequence of the murine LFA-1 α subunit. The mature N-terminal amino acids previously sequenced (24) and the transmembrane region are *underlined*. Potential N-linked glycosylation sites are marked with a *diamond*.

face of COS cells of human and murine LFA-1 $\alpha\beta$ heterodimers (Fig. 5, *panels 1* and *2* and *panels 3* and *4*, respectively). After transfection with the α subunit cDNA, in the absence of the β subunit cDNA, or vice versa, the murine subunits were not expressed at all (Fig. 5, *panels 13* and *14*) and the human subunits were expressed only very weakly (Fig. 5, *panels 15* and *16*). This result indicates that efficient expression of each subunit on the cell surface is dependent on its association with the complementary subunit. The murine and human subunits were detected on a high percentage of COS cells after cotransfection with human α + murine β cDNA or murine α + human β cDNA (Fig. 5, *panels 5* and *6* and *panels 7* and *8*, respectively), suggesting that the murine and human

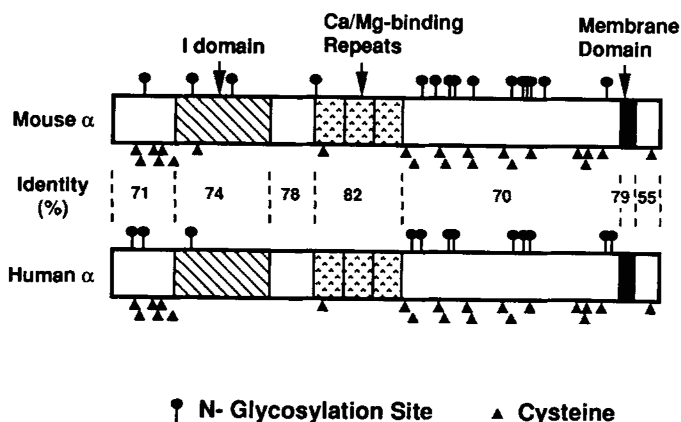

Figure 3. Schematic representation of murine and human LFA-1 α subunits. *Black lollipops* (above schematic) and *triangles* (below schematic) indicate potential glycosylation sites and cysteines, respectively. Sequences of the domains were compared using the ALIGN program; the percent identities are indicated.

LFA-1 subunits form interspecies hybrid αβ complexes. Formation of murine-human αβ complexes was previously detected in somatic cell hybrids of a murine T cell line with normal human lymphocytes or with β subunit-deficient (LAD patient) lymphocytes (35).

The sequence of the mLFA-1 α subunit determined in this study, which shows high homology with its human counterpart, explains the efficient association of mouse-human αβ hybrid complexes on the cell surface. The finding that the highest homology between murine and human LFA-1 α subunits (as well as between mLFA-1 and mMac-1 α subunits) is located in the putative cation binding region is compatible with the importance of this region in the function of leukocyte integrins. The relatively low homology (55%) of the murine and human LFA-1 α cytoplasmic domains is of interest. By contrast, the murine and human β subunit cytoplasmic domains are 96% identical (19, 36, 37). These findings suggest that association with the cytoskeleton or regulation of avidity for ICAM-1 by signals from the cytoplasm (33), may be mediated by the β subunit rather than the α subunit. This is in agreement with the observation that truncation of the LFA-1 α subunit cytoplasmic domain has no effect on binding to ICAM-1, whereas binding is markedly diminished by β subunit cytoplasmic domain truncation (34).

We have isolated and sequenced a murine LFA-1 α subunit cDNA and shown that it is competent for expression in COS cells in the presence of a human or murine β subunit cDNA. The regions of the LFA-1 α subunits that are most strongly conserved in evolution have been

Figure 4. Comparison of the murine and human α subunits. The amino acid sequences of the murine and human LFA-1 α subunits were aligned. Only the human LFA-1 α residues different from the mouse are shown. Gaps are marked with a *period*. Boundaries of domains are marked and are based on homologies to other integrins and the p150,95 α subunit exon structure (38).

```
mLFA-1    1  YNLDTRPTQSF.LAQAGRHFGYQVLQIEDGVVVGAPGEGDNTGGLYHCRTSSEFCQPVSLHGSNHTSKYLGMTLATDAAK
hLFA-1           V GAR  SPPR       R   VGN  I       NS  S  Q QSGTGH L  T R   Y            PTD

         81  GSLLACDPGLSRTCDQNTYLSGLCYLFPQSLEGPMLQNRPAYQECMKGKVDLVFLFDGSQSLDRKDFEKILEFMKDVMRK
               I                        R N Q     G  GF   I  N          M  QPDE Q   D      K
                                                         |I Domain -->

        161  LSNTSYQFAAVQFSTDCRTEFTFLDYVKQNKNPDVLLGSVQPMFLLTNTFRAINYVVAHVFKEESGARPDATKVLVIITD
                            SYK   D S    .W D  A  KH KH L      G     ATE  R  L          I

        241  GEASDKGNISAAHDITRYIIGIGKHFVSVQKQKTLHIFASEPVEEFVKILDTFEKLKDLFTDLQRRIYAIEGTNRQDLTS
                T S   D  K  I          QTKES E   K   K AS                 E  KK  V    SK
                                                                        <-- I Domain|

        321  FNMELSSSGISADLSKGHAVVGAVGAKDWAGGFLDLREDLQGATFVGQEPLTSDVRGGYLGYTVAWMTSRSSRPLLAAGA
                            R                    KA   DD  I N    PE  A       T LP  QKTS   S

        401  PRYQHVGQVLLFQAPEAGGRWNQTQKIEGTQIGSYFGGELCSVDLDQDGEAELLLIGAPLFFGEQRGGRVFTYQRRQSLF
                  M R     E QG  H S V T H             G  V     T          Y         I      LG
                                            |Metal Binding Domains -->

        481  EMVSELQGDPGYPLGRFGAAITALTDINGDRLTDVAVGAPLEEQGAVYIFNGKPGGLSPQPSQRIQGAQVFPGIRWFGRS
              E                E           G V                   RH           E T  LS  Q

        561  IHGVKDLGGDRLADVVVGAEGRVVVLSSRPVVDVVTELSFSPEEIPVHEVECSYSAREEQKHGVKLKACFRIKPLTPQFQ
                    E  G    A    SQMI         M  LM    A            TSNKM E  NITI  Q  S Y
              <-- Metal Binding Domains|

        641  GRLLANLSYTLQLDGHRMRSRGLFPDGSHELSGNTSITPDKSCLDFHFHFPICIQDLISPINVSLNFSLLEEEGTPRDQK
                V   T         T R     G R   RR IAV TSM  T  S    V V               W         R

        721  ..GRAMQPILRPSIHTVTKEIPFEKNCGEDKKCEANLTLS.SPARSGPLRLMSSASLAVEWTLSNSGEDAYWVRLDLDFP
             AQ KDIP      L SE W                  RV F     RA   TAF   S  LS   LE      Q   H

        801  RGLSFRKVEMLQPHSRMPVSCEELTEGSSLLTKTLKCNVSSPIFKAGQEVSLQVMFNTLLNSSWEDFVELNGTVHCENEN
             P          K   QI       P E R  SRA S           HS A  M     V    G S   HAN T N  D

        881  SSLQEDNSAATHIPVLYPVNILTKEQENSTLYISFTPKGPKTQQVQHVYQVRIQPSAYDHNMPTLEALVGVPRPHSEDLI
              D L     T I  I   I   IQD  D    V        IH  K M        IH   I     V    Q P   GP

        961  TYTWSVQTDPLVTCHSEDLKRPSSEAEQPCLPGVQFRCPIVFRWEILIQVTGTVELSKEIKASSTLSLCSSLSVSFNSSK
              HQ    ME P P  Y   E L.PD AE     AL    V   Q   V  I  L  VG  E   MF       I

       1041  HFHLYGSKASEAQVLVKVDLIHEKEMLHVYVLSGIGGLVLLFLIFLALYKVGFFKRNLKEKMEADGGVPNGSPPEDTDPL
                    N  L   VM   VVY  Q  YL         L  L   IV                 GR     I A  SEQ

       1121  AVPGEETKDMGCLEPSGRVTRTKA
              .S Q AG P   K LHEKDSESG
```

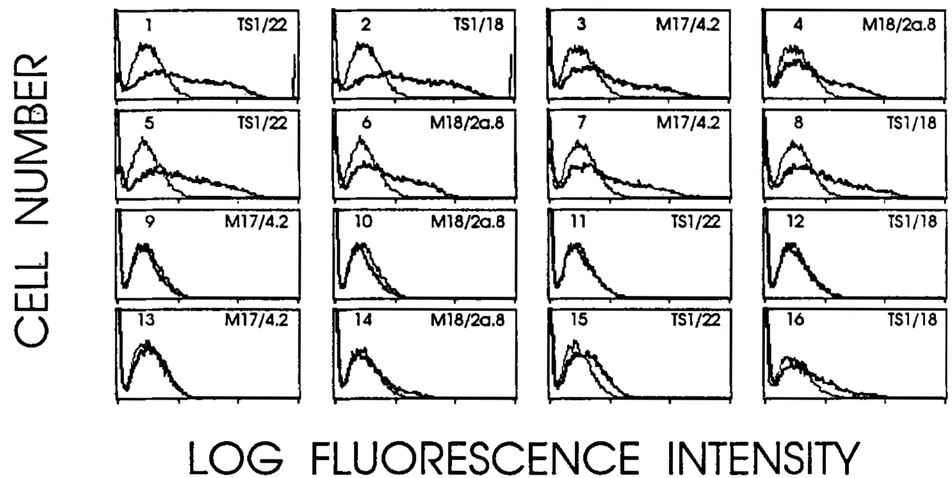

Figure 5. Immunofluorescence flow cytometry of COS cells after transfection with murine and human α and β subunit cDNA alone or together. COS cells were transfected with the following LFA-1 cDNA: *panels 1* and *2*, human (*h*) α + hβ; *panels 3* and *4*, murine (*m*) α + mβ; *panels 5* and *6*, hα + mβ; *panels 7* and *8*, mα + hβ; *panels 9–12*, expression vector with no insert; *panel 13*, mα; *panel 14*, mβ; *panel 15*, hα; and *panel 16*, hβ. The mAb used to detect surface proteins are indicated (anti-hα TS1/22, anti-hβ TS1/18, anti-mα M17/4.2, and anti-mβ M18/2a.8). In all panels the specific mAb staining profile is shown with a *dark line* and staining with the negative control mAb ZP01 is shown with a *light line*.

defined. The work reported here should facilitate further studies on the structure and function of the LFA-1 α subunit.

Acknowledgments. We thank Ed Luther for the fluorescence flow cytometer operation, Drs. A. Turner and M. Davis for the BCL1 cDNA library and Drs. R. Wilson and A. L. Beaudet for the murine LFA-1 β cDNA.

REFERENCES

1. **Springer, T. A.** 1990. Adhesion receptors of the immune system. *Nature 346:425.*
2. **Rothlein, R., M. L. Dustin, S. D. Marlin, and T. A. Springer.** 1986. A human intercellular adhesion molecule (ICAM-1) distinct from LFA-1. *J. Immunol. 137:1270.*
3. **Marlin, S. D., and T. A. Springer.** 1987. Purified intercellular adhesion molecule-1 (ICAM-1) is a ligand for lymphocyte function-associated antigen 1 (LFA-1). *Cell 51:813.*
4. **Staunton, D. E., M. L. Dustin, and T. A. Springer.** 1989. Functional cloning of ICAM-2, a cell adhesion ligand for LFA-1 homologous to ICAM-1. *Nature 339:61.*
5. **Beller, D. I., T. A. Springer, and R. D. Schreiber.** 1982. Anti-Mac-1 selectively inhibits the mouse and human type three complement receptor. *J. Exp. Med. 156:1000.*
6. **Myones, B. L., J. G. Dalzell, N. Hogg, and G. D. Ross.** 1988. Neutrophil and monocyte cell surface p150,95 has iC3b-receptor (CR4) activity resembling CR3. *J. Clin. Invest. 82:640.*
7. **Anderson, D. C., and T. A. Springer.** 1987. Leukocyte adhesion deficiency: an inherited defect in the Mac-1, LFA-1, and p150,95 glycoproteins. *Annu. Rev. Med. 38:175.*
8. **Smith, C. W., S. D. Marlin, R. Rothlein, M. B. Lawrence, L. V. McIntire, and D. C. Anderson.** 1989. The role of ICAM-1 in the adherence of human neutrophils to human endothelial cells in vitro. In *Structure and Function of Molecules Involved in Leukocyte Adhesion.* T. A. Springer, D. C. Anderson, R. Rothlein, and A. S. Rosenthal, eds. Springer-Verlag, New York, NY, p. 170.
9. **Kürzinger, K., and T. A. Springer.** 1982. Purification and structural characterization of LFA-1, a lymphocyte function-associated antigen, and Mac-1, a related macrophage differentiation antigen. *J. Biol. Chem. 257:12412.*
10. **Sanchez-Madrid, F., J. Nagy, E. Robbins, P. Simon, and T. A. Springer.** 1983. A human leukocyte differentiation antigen family with distinct alpha subunits and a common beta subunit: the lymphocyte function-associated antigen (LFA-1), the C3bi complement receptor (OKM1/Mac-1), and the p150,95 molecule. *J. Exp. Med. 158:1785.*
11. **Kishimoto, T. K., R. S. Larson, A. L. Corbi, M. L. Dustin, D. E. Staunton, and T. A. Springer.** 1989. The leukocyte integrins: LFA-1, Mac-1, and p150,95. *Adv. Immunol. 46:149.*
12. **Larson, R. S., A. L. Corbi, L. Berman, and T. A. Springer.** 1989. Primary structure of the LFA-1 alpha subunit: an integrin with an embedded domain defining a protein superfamily. *J. Cell Biol. 108:703.*
13. **Davignon, D., E. Martz, T. Reynolds, K. Kürzinger, and T. A. Springer.** 1981. Monoclonal antibody to a novel lymphocyte function-associated antigen (LFA-1): mechanism of blocking of T lymphocyte-mediated killing and effects on other T and B lymphocyte functions. *J. Immunol. 127:590.*
14. **Pierres, M., C. Goridis, and P. Golstein.** 1982. Inhibition of murine T cell-mediated cytolysis and T cell proliferation by a rat monoclonal antibody immunoprecipitating two lymphoid cell surface polypeptides of 94,000 and 180,000 molecular weight. *Eur. J. Immunol. 12:60.*
15. **Kishimoto, T. K., K. O'Connor, A. Lee, T. M. Roberts, and T. A. Springer.** 1987. Cloning of the beta subunit of the leukocyte adhesion proteins: homology to an extracellular matrix receptor defines a novel supergene family. *Cell 48:681.*
16. **Johnston, S. C., M. L. Dustin, M. L. Hibbs, and T. A. Springer.** 1990. On the species specificity of the interaction of LFA-1 with intercellular adhesion molecules. *J. Immunol. 145:1181.*
17. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
18. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain terminating inhibitors. *Proc. Natl. Acad. Sci. USA 74:5463.*
19. **Wilson, R., W. O'Brien, and A. Beaudet.** 1989. Nucleotide sequence of the cDNA from the mouse leukocyte adhesion protein CD18. *Nucleic Acids Res. 17:5397.*
20. **Seed, B., and A. Aruffo.** 1987. Molecular cloning of the CD2 antigen, the T-cell erythrocyte receptor, by a rapid immunoselection procedure. *Proc. Natl. Acad. Sci. USA 84:3365.*
21. **Larson, R. S., M. L. Hibbs, and T. A. Springer.** 1990. The leukocyte integrin LFA-1 reconstituted by cDNA transfection in a nonhematopoietic cell line is functionally active and not transiently regulated. *Cell Regul. 1:359.*
22. **Sanchez-Madrid, F., D. Davignon, E. Martz, and T. A. Springer.** 1982. Antigens involved in mouse cytolytic T-lymphocyte (CTL)-mediated killing: functional screening and topographic relationship. *Cell. Immunol. 73:1.*
23. **Sanchez-Madrid, F., P. Simon, S. Thompson, and T. A. Springer.** 1983. Mapping of antigenic and functional epitopes on the alpha and beta subunits of two related glycoproteins involved in cell interactions, LFA-1 and Mac-1. *J. Exp. Med. 158:586.*
24. **Springer, T. A., D. B. Teplow, and W. J. Dreyer.** 1985. Sequence homology of the LFA-1 and Mac-1 leukocyte adhesion glycoproteins and unexpected relation to leukocyte interferon. *Nature 314:540.*
25. **von Heijne, G.** 1986. A new method for predicting signal sequence cleaving sites. *Nucleic Acids Res. 14:4683.*
26. **Sastre, L., T. K. Kishimoto, C. Gee, T. Roberts, and T. A. Springer.** 1986. The mouse leukocyte adhesion proteins Mac-1 and LFA-1: studies on mRNA translation and protein glycosylation with emphasis on Mac-1. *J. Immunol. 137:1060.*
27. **Hemler, M. E.** 1990. VLA proteins in the integrin family: Structures, functions, and their role on leukocytes. *Annu. Rev. Immunol. 8:365.*
28. **Corbi, A. L., L. J. Miller, K. O'Connor, R. S. Larson, and T. A. Springer.** 1987. cDNA cloning and complete primary structure of the alpha subunit of a leukocyte adhesion glycoprotein, p150,95. *EMBO J. 6:4023.*
29. **Pytela, R.** 1988. Amino acid sequence of the murine Mac-1 alpha chain reveals homology with the integrin family and an additional domain related to von Willebrand factor. *EMBO J. 7:1371.*
30. **Corbi, A. L., T. K. Kishimoto, L. J. Miller, and T. A. Springer.** 1988. The human leukocyte adhesion glycoprotein Mac-1 (complement receptor type 3, CD11b) alpha subunit: cloning, primary structure, and relation to the integrins, von Willebrand factor and factor B. *J.*

Biol. Chem. 263:12403.
31. **Arnaout, M. A., S. K. Gupta, M. W. Pierce, and D. G. Tenen.** 1988. Amino acid sequence of the alpha subunit of human leukocyte adhesion receptor Mo1 (complement receptor type 3). *J. Cell Biol. 106:2153.*
32. **Rothlein, R., and T. A. Springer.** 1986. The requirement for lymphocyte function-associated antigen 1 in homotypic leukocyte adhesion stimulated by phorbol ester. *J. Exp. Med. 163:1132.*
33. **Dustin, M. L., and T. A. Springer.** 1989. T cell receptor cross-linking transiently stimulates adhesiveness through LFA-1. *Nature 341:619.*
34. **Hibbs, M. L., H. Xu, S. A. Stacker, and T. A. Springer.** 1991. Regulation of adhesion to ICAM-1 by the cytospasmic domain of LFA-1 integrin beta subunit. *Science 251:1611.*
35. **Marlin, S. D., C. C. Morton, D. C. Anderson, and T. A. Springer.** 1986. LFA-1 immunodeficiency disease: Definition of the genetic defect and chromosomal mapping of alpha and beta subunits of the lymphocyte function-associated antigen 1 (LFA-1) by complementation in hybrid cells. *J. Exp. Med. 164:855.*
36. **Kishimoto, T. K., N. Hollander, T. M. Roberts, D. C. Anderson, and T. A. Springer.** 1987. Heterogenous mutations in the beta subunit common to the LFA-1, Mac-1, and p150,95 glycoproteins cause leukocyte adhesion deficiency. *Cell 50:193.*
37. **Law, S. K. A., J. Gagnon, J. E. K. Hildreth, C. E. Wells, A. C. Willis, and A. J. Wong.** 1987. The primary structure of the beta subunit of the cell surface adhesion glycoproteins LFA-1, CR3 and p150,95 and its relationship to the fibronectin receptor. *EMBO J. 6:915.*
38. **Corbi, A. L., J. Garcia-Aguilar, and T. A. Springer.** 1990. Genomic structure of an integrin alpha subunit, the leukocyte p150,95 molecule. *J. Biol. Chem. 265:2782.*