Molecular cloning of the α subunit of human and guinea pig leukocyte adhesion glycoprotein Mo1: Chromosomal localization and homology to the α subunits of integrins

(complement receptors/inflammatory mediators/human leukocyte differentiation antigens)

M. AMIN ARNAOUT*, EILEEN REMOLD-O'DONNELL†, MARK W. PIERCE‡, PETER HARRIS*,
AND DANIEL G. TENEN§

*Renal and Genetics Divisions, The Children's Hospital, †Center for Blood Research, ‡Endocrine Division and Howard Hughes Medical Institute, Massachusetts General Hospital, §Hematology Division, Beth Israel Hospital, and Harvard Medical School, Boston, MA 02115

Communicated by K. Frank Austen, December 11, 1987

ABSTRACT The cell-surface glycoprotein Mo1 is a member of the family of leukocyte cell adhesion molecules (Leu-CAMs) that includes lymphocyte function-associated antigen 1 (LFA-1) and p150,95. Each Leu-CAM is a heterodimer with a distinct α subunit noncovalently associated with a common β subunit. Leu-CAMs play crucial roles in cell-cell and cell-matrix interactions. We describe the isolation and analysis of two partial cDNA clones encoding the α subunit of the Leu-CAM Mo1 in humans and guinea pigs. A monoclonal antibody directed against an epitope in the carboxyl-terminal portion of the guinea pig α chain was used for immunoscreening a λ gt11 expression library. The sequence of a 378-base-pair insert from one immunoreactive clone revealed a single continuous open reading frame encoding 126 amino acids including a 26-amino acid tryptic peptide isolated from the purified guinea pig α subunit. A cDNA clone of identical size was isolated from a human monocyte/lymphocyte cDNA library by using the guinea pig clone as a probe. The human clone also encoded a 126-amino acid peptide including the sequence of an additional tryptic peptide present in purified human Mo1 α chain. RNA gel blots revealed that mature Mo1 α chain mRNA is $\approx$ 5 kilobases in guinea pigs and humans. Southern analysis of DNA from hamster-human hybrids localized the human Mo1 α chain to chromosome 16, which has been shown to contain the gene for the α chain of lymphocyte function-associated antigen 1. A comparison of the Mo1 α chain coding region revealed significant homologies with carboxyl-terminal portions of the α subunits of fibronectin, vitronectin, and platelet IIb/IIIa receptors. These data suggest that the α subunits of Leu-CAMs evolved by gene duplication from a common ancestral gene and strengthen the hypothesis that the α subunits of these heterodimeric cell adhesion molecules on myeloid and lymphoid cells, platelets, and fibroblasts are evolutionary related.

The leukocyte differentiation antigen Mo1 (also known as CR3, CD11b/CD18, Mac-1, and MAM) is a two-chain surface glycoprotein with an α subunit (Mo1 α) of 155–165 kDa noncovalently associated with a β subunit of 94 kDa (1–4). In humans, Mo1 is present on circulating granulocytes, monocytes, and natural killer cells (1). It contains a binding site for the complement component C3 fragment iC3b and also promotes granulocyte cell-cell adhesion as well as adhesion of granulocytes and monocytes to endothelium (5–7). The β subunit of human Mo1 is shared by two additional leukocyte adhesion-promoting heterodimers, lymphocyte function-associated antigen 1 (LFA-1) and p150,95 (8–10) with α subunits of 180 kDa and 140 kDa, respectively. Limited amino acid sequencing revealed homologies among the N

termini of the three α subunits (11, 12). The biologic significance of the leukocyte cell adhesion molecules (Leu-CAMs) was underlined by the finding that inherited deficiency in the surface expression of all three molecules results in life-threatening bacterial infections. This is secondary to severe impairment in leukocyte adhesion-dependent functions such as phagocytosis, chemotaxis, and natural killing, which are mediated by Leu-CAMs (13–16). On the other hand, increased leukocyte surface expression of these adhesion molecules in clinical situations characterized by uncontrolled inflammatory responses, such as active lupus erythematosus, and in hemodialysis with a first-use dialysis membrane (17, 18) could have deleterious effects on the host by contributing to the tissue injury.

Leu-CAMs, in turn, appear to be members of a larger family of adhesion receptors (integrins), which include platelet glycoprotein (gp) IIb/IIIa, fibronectin receptor (FNR), vitronectin receptor (VNR), the very late antigens (VLA) protein family, and position-specific antigens in *Drosophila* (19). The α and β subunits in these heterodimeric glycoproteins are translation products of separate mRNA species and undergo an assembly process to form the heterodimers. The amino acid sequence of the common β subunit of Leu-CAMs, determined by cDNA cloning, is highly homologous to a subunit of the chicken integrin receptor with an overall amino acid identity of 47% (20–22). The Leu-CAM β subunit, like chicken integrin and gpIIIa, has a cohesive structure with extensive disulfide bonding (a total of 57 cysteine residues including 42 found within a stretch of 256 residues), and the positions of the cysteine residues are nearly identical in the β subunits (20–22). The structural relatedness of Leu-CAM α subunits to other integrins has been less well characterized. Available data are limited to the N-terminal amino acid sequence of Leu-CAM α subunits (11, 12, 23), which also shows significant homology to other proteins, such as interferon α (24).

The structural basis for the adhesion-promoting and complement receptor functions of Mo1 and for the formation of the $\alpha\beta$ heterodimers is unknown. The current study reports isolation and sequences of cDNA clones that encode a region in the carboxyl-terminal portion of Mo1 α in guinea pigs and humans.[†] The monoclonal antibody (mAb) M26 (3, 25), which defines this region, reacts with the monomeric α chain, before but not after, its association with the β chain, sug-

Abbreviations: Leu-CAM, leukocyte cell adhesion molecule; mAb, monoclonal antibody; LFA-1, lymphocyte function-associated antigen 1; FNR, fibronectin receptor; VNR, vitronectin receptor.

[†]These sequences are being deposited in the EMBL/GenBank data base (Intelligenetics, Mountain View, CA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J03270, for the human sequence, and accession no. J03271, for the guinea pig sequence).

gesting that the cloned region defined by the M26 epitope may be involved in formation of the Mo1 heterodimer.

MATERIALS AND METHODS

Identification of Mo1 α -Specific cDNA Clones. Guinea pig peritoneal macrophages and neutrophils and human monocytes and peripheral blood lymphocytes were obtained as described (1, 3, 13, 26). Total cellular RNA and poly(A)⁺ RNA were prepared as described (27, 28). A human monocyte/lymphocyte cDNA library and a guinea pig macrophage cDNA library (Clontech) were constructed in λ gt11 (28). A mAb, M26, directed against an 85-kDa C-terminal fragment (Fig. 1) was used to immunoscreen the expression library for Mo1 α clones (29). Human cDNA clones were isolated by screening the human library with a ³²P-labeled guinea pig Mo1 α cDNA as described (30). cDNA sequencing in M13 was performed as described by Sanger *et al.* (31) with the universal M13 primer (Bethesda Research Laboratories) and specific oligonucleotides synthesized with an Applied Biosystems synthesizer. Both strands of the cDNA clones were sequenced.

Immunoblotting of the Fusion Protein. The isopropyl β -D-thiogalactoside-induced hybrid proteins expressed by the transfected *Escherichia coli* containing λ gt11 were immunoblotted by using M26 mAb (32).

Protein Purification and Generation of Tryptic Peptides. The α subunits of guinea pig and human Mo1 were purified from peritoneal or circulating neutrophils, respectively (11, 33), by using lentil lectin and affinity chromatography with mAb M2 and mAb 44a that are specific for guinea pig and human Mo1 α , respectively (5, 11, 26, 33). N-terminal sequences of the purified antigens have been reported (11) and are clearly distinct from LFA-1 α chain or p150 (12, 24). The purified proteins (137 μ g/775 μ l of 70 mM *N*-ethylmorpholine acetate, pH 8.0) were incubated with 1.4 μ g of L-1-tosylamido-2-phenylethyl chloromethyl ketone-trypsin (Worthington) for 22 hr at 37°C with 1.4 μ g of trypsin added at 16 hr. The reaction was brought to pH 2.0 with 3 μ l of trifluoroacetic acid, and samples were analyzed on a Beckman Ultrasphere ODS C₁₈ column (0.46 $\times$ 15 cm), which was eluted with a linear gradient 20–60% buffer B per 40 min [buffer A = 5% (vol/vol) acetonitrile and 0.1% trifluoroacetic

FIG. 1. Autoradiograph of an immunoblot showing the reactivity of mouse mAb M26 with purified guinea pig Mo1 α before (–) or after (+) treatment with L-1-tosylamido-2-phenylethyl chloromethyl ketone-trypsin (6 μ g/ml; 20 min; room temperature). In addition to the 85-kDa fragment, another Coomassie-stained fragment of 71 kDa is generated that contains the N terminus of the intact α subunit (unpublished data). Arrowheads show the position of ¹⁴C-labeled molecular mass markers myosin (200 kDa), phosphorylase *b* (92.5 kDa), and bovine serum albumin (69 kDa).

FIG. 2. Immunoblotting of the fusion protein from *E. coli* transfected with clone 5A. Bacterial lysates from uninduced or isopropyl β -D-thiogalactoside (IPTG)-induced Y1089 *E. coli* cells were electroblotted onto nitrocellulose membrane following NaDodSO₄/polyacrylamide (6%) gel electrophoresis and immunostained with mAb M26 or anti- β -galactosidase mAb. M26 reacted with an inducible fusion protein but not with the wild-type protein. Anti- β -galactosidase (α - β -gal) reacted with the wild-type and fusion proteins.

acid; buffer B = 90% (vol/vol) acetonitrile and 0.1% trifluoroacetic acid]. Eluted UV peaks were concentrated in an evacuated centrifuge and sequenced (11). Nine peptides from the guinea pig and seven from the human with distinct N-terminal sequences were characterized. A guinea pig and a human peptide contained the following sequences (in the single-letter amino acid code):

SPPHSDFLAELEKTHVLXXSIAVXQR and VMQHQQVSNLQ

RNA Gel and Southern Blot Analyses and Chromosomal Mapping. RNA gel blots were performed as described (34). For Southern blots, 5 μ g of *Eco*RI- or *Hind*III-digested normal human male genomic DNA or DNA derived from human-hamster somatic cell hybrids was hybridized with a ³²P-labeled human Mo1 α insert (35). The somatic cell hybrids G35F1, G35A2, G35F3, and G35A4 were used. The human chromosomes contained in this panel of cells were F1:4,5,8,10,12,14,der(19),20,21; A2:3,4,6,11,14,der(19),20,21,22,der(X); F3:2,3,5,7,8,10,11,12,16,18,der(19),20; and A4:1,3,4,6,8,9,14,16,der(19),20,21,X (36).

Analysis of Data. A computer search of homology was performed by using Bionet.¹¹ Amino acid sequences of Mo1 α , FNRA, VNRA, and platelet IIB were initially aligned by using GENALIGN (Bionet), and alignment was maximized by using the Needleman–Wunsch algorithm (37) and the data matrix of Dayhoff *et al.* (38). The percent probability (%P) that homology is due to chance was determined by the sum of the values for each amino acid pair from the log-odd matrix of Dayhoff. The score for the amino acid matching the gap position is zero.

RESULTS

Isolation and Characterization of cDNA Clones. Immunoscreening of the guinea pig expression library with M26 mAb identified 50 clones among 1.5×10^5 clones screened. One of these clones, 5A, was further characterized. The 130-kDa β -galactosidase fusion protein, synthesized by lysogenic bacteria carrying the recombinant phage, reacted specifically with M26 mAb (Fig. 2). The insert size was ≈ 0.4 kilobases (kb) based on *Eco*RI digests or *Kpn* I/*Pvu* II double digests of phage DNA. Nucleotide sequencing of the 378-base-pair 5A insert revealed that it contains one open reading frame that includes a 26-amino acid tryptic peptide isolated from the

¹¹EMBL/GenBank Genetic Sequence Database (1987) (Intelligence, Mountain View, CA), Tape Release 54.

```

CAGCTGGAGCTGCCTGTGAAATATGCTGTCTACTTAATAGTCACAAGTGGTGAGGCCTCTACCACATAT 69
Q L E L P V K Y A V Y L I V T S G E A S T T Y 23

CTGAACCTCACAACCTTCAGAGAAAACCATTCAGACCATGAAGCACCAATATAAGTTCCACCAACCTGGGC 138
L N F T T S E K T I Q T M K H Q Y K F T N L G 46
-----
AAGAGGAGCCTCCCATCAGCGTGGTCTTCTGGGTCTGCGGTCTGAACAATGAGATTGTATGGGAC 207
S G L P I S V V F W V P V R L N N E I V W D 69

CGCCCTCAGGTCACCTTCTCCCTAATCTCTCGAGTGCCTGCAACACTGAGGAAAGATCACCTCCTCAC 276
R P Q V T F S P N L S S A C N T E E R S P P H 92
-----
TCTGATTCTCTGGCTGAGCTTGAGAAGACCCACGTGTTGAACTGCTCCATTGCTGTCTGCCAGAGAATT 345
S D F L A E L E K T H V L N C S I A V C Q R I 115
-----
GCATGTGACATCCCATACTTCAACATCCAGGAA 378
A C D I P Y F N I Q E 126

```

FIG. 3. Partial cDNA and the deduced protein sequence for guinea pig Mo1 α . The 26-amino acid tryptic peptide derived from purified guinea pig Mo1 α and coded by the cDNA is overlined. Three potential N-linked glycosylation sites are underlined. The single-letter amino acid code is used.

purified guinea pig Mo1 α antigen (Fig. 3) and also had three potential N-linked glycosylation sites suggesting that this portion of the protein is extracellular.

Screening of 5×10^4 recombinant plaques in the human library with 32 P-labeled 5A insert identified another clone, 5B, 378 base pairs long that has a single open reading frame containing an additional tryptic peptide derived from purified human Mo1 α (Fig. 4B). Identity of the human and guinea pig clones at the DNA level was 78.3% (Fig. 4A). The identical size of the cDNA clones isolated in two species suggests the presence of internal *Eco*RI sites flanking this region. In the two species, 71.4% of the amino acid residues were identical and 23% were conserved substitutions (Fig. 4B).

RNA Gel Blot Analysis. A band of ≈ 5.0 kb was observed in total cellular RNA derived from guinea pig macrophages or human monocytes (Fig. 5). Despite the extensive homology at the DNA level between the human and guinea pig probes, no cross hybridization signals with RNA from the opposite species were obtained (data not shown).

Chromosomal Localization of Mo1 α Gene. Southern blot analysis of *Eco*RI- or *Hind*III-digested normal male genomic DNA probed with the human insert revealed a single band of ≈ 2.2 kb or 22 kb, respectively (Fig. 6). Hybridization of this probe to a mapping panel containing DNA from four somatic cell hybrids localized the Mo1 α -derived genomic band to chromosome 16 (Fig. 6, lanes 3 and 4).

Homology of the Mo1 α Sequence with Other Adhesion Receptors. A search for homology of the Mo1 α sequence with that of other genes and proteins was initially made by using

the IFIND program and the GenBank and EMBL libraries available on Bionet.¹¹ To increase alignment, adjustments in sequence comparisons were made guided by the mutation data matrix of Dayhoff *et al.* (38). Significant homology at the protein level was observed between Mo1 α and an extracellular domain present in platelet IIb protein and the α chains of FNR and VNR (32, 39, 40) (Fig. 7). A stretch of three cysteine residues separated by two 4-amino acid groups (Cys-Xaa-Xaa-Xaa-Xaa-Cys-Xaa-Xaa-Xaa-Cys) was present in similar positions in all four molecules. In platelet IIb and the α chains of VNR and FNR, this region contains the junction between the larger N-terminal and the smaller carboxyl-terminal fragments of the α subunits (32, 39, 40). The probabilities that homology in this Mo1 α region (outlined in Fig. 7) with gpIIb and the α chains of VNR and FNR was due to chance were 0.00063%, 0.00032%, and 0.0126%, respectively. Another region, residues 1–65, was more homologous to a corresponding region in the α chain of VNR ($P = 0.1\%$) than to platelet IIb ($P = 2.5\%$) or the α chain of FNR ($P = 3.98\%$).

DISCUSSION

The predicted amino acid sequence for the cloned region of Mo1 α has several interesting properties. This region is highly homologous in man and guinea pigs with 94.4% of the amino acid residues identical or conserved (Fig. 4B). It appears to be extracellular based on the presence of three or four potential N-glycosylation sites in guinea pig and human antigens, respectively. It is located in an 85-kDa fragment

A

```

Gp CAGCTGGAGCTGCCTGTGAAATATGCTGTCTACTTAATAGTCACAAGTGGTGAGGCCTCTACCACATATC 70
Hu CAACCTGGAGCTGCCGGTGAATATGCTGTCTACATGGTGGTCACCAAGCCATGGGGTCTCCACTAAATATC
Gp TGAACCTCACAACCTTCAGAGAAAACCATTCAGACCATGAAGCACCAATATAAGTTCCACCAACCTGGGC 140
Hu TCAACTTCAGCGCTCAGAGAAATACCAAGTCCGGTCAAGCATCAATATCAGGTGAGCAACCTGGGGCA
Gp GAGGAGCCTCCCATCAGCGTGGTCTTCTGGGTCTGTCGCTGTAACAATGAGATTGTATGGGACCGC 210
Hu GAGGAGCCTCCCATCAGCGTGGTCTTCTGGGTCCCGTCCGGTGAACCAAGTGTATGAGGACCGC
Gp CCTCAGGTACCTTCTCCCTAATCTCTCGAGTGCCTGCAACACTGAGGAAAGATCACCTCCTCACTCTG 280
Hu CCCAGGTACCTTCTCCGAGAACTCTCGAGTACGTGCCACCAAGGAGCGCTTGCCTCTCACTCCG
Gp ATTTCCTGGCTGAGCTTGAGAAGACCCACGTGTTGAACTGCTCCATTGCTGTCTGCCAGAGAATTGCATG 350
Hu ACTTCTGGCTGAGCTTCGGAAGGCCCGTGGTGAAGTCTCCATCGCTGTCTGCCAGAGAATCCAGTG
Gp TGACATCCCATCTTCAACATCCAGGAA 378
Hu TGACATCCCGTTCTTGGCATCCAGGAA

```

B

```

Gp QLELPVKYAVYLVTSGEASTTYLNFTTSEKTIQTMKHQYKFTNLGKRSIPISVVFWVPVRLNNEIVWDR 126
Hu QLELPVKYAVYVMVTVSHGVSTKYNFTASENTRVQHQQVSNLQKRSIPISLVFLVPVRLNQTIVWDR
Gp PQVTFSPNLSACNTEERSPPHSDFLAELEKTHVLNCSIAVCQRIACDIPYFNIQE
Hu PQVTFSENLSSTCHTKERLPSSHDFLAELRKAPVNVCSIAVCQRIQCDIPFFGIQE

```

FIG. 4. Comparison of the isolated human Mo1 α clone, 5B (Hu), with that of the guinea pig (Gp) at the DNA (A) and protein (B) levels. Identical nucleotides or amino acids (in the single-letter amino acid code) are indicated by double dots, and conserved amino acid substitutions are indicated by single dots. The predicted amino acid sequences that correspond to the sequence of the tryptic peptides are overlined. The positions of the cysteine residues are indicated by stars. Positions for potential N-glycosylation sites are indicated by dashed lines.

FIG. 5. RNA gel blot analysis. Ten micrograms of guinea pig peritoneal macrophage RNA (left lane) or human monocyte mRNA (right lane) were electrophoresed on formaldehyde/agarose gels, blotted on nitrocellulose, and probed with the respective guinea pig (left lane) or human (right lane) cDNA inserts under high-stringency conditions. Autoradiography revealed a hybridizing band of ≈ 5 kb in both the human and guinea pig species. Arrows point to the positions of the 28S and 18S RNA markers.

that is the carboxyl terminus in Mo1 α based on the reactivity of mAb M26 (Fig. 1 and data not shown). It also defines a region in the molecule that is made inaccessible to mAb binding upon association of the $\alpha\beta$ complex and may contribute to its formation. This cloned region of Mo1 α also contains the sequence Cys-Xaa-Xaa-Xaa-Xaa-Cys-Xaa-Xaa-Xaa-Xaa-Cys that is precisely conserved in a homologous carboxyl-terminal region present in the α subunits of platelet IIb, FNR, and VNR (Fig. 7) (32, 39, 40). The overall homology in this region (residues 89–126 in Mo1 α) with other integrins is highly significant. This region contains a cleavage site between the large N-terminal fragment and the small C-terminal transmembrane fragment in each of the α subunits of IIb/IIIa, VNR, and FNR (32, 39, 40). Interestingly, no such cleavage site has been shown in Mo1 α or other Leu-CAM α chains. The large deletion proposed to precede this region (Fig. 7) may alter susceptibility of Mo1 α to such a proteolytic cleavage.

Mo1	1	QLELPVK--YA-VYVVVTS	SHGVSTKYLNF	TASENTSRVMQH	QYQVSNL	QQRSLPISL	VFLVP	*
IIb	721	QLQIRSKNSQNPNSKIVLLDVPVRAEAQVELRGNSFPA-SLVVAAEE-GEREQ-NSLDSWGP						
VNR	744	DLQIQSSNLFDPKSPVVS	SHKVDLAVLA	AVEIRGVSSPD-HIFLPIP	NWEHKNP	PETEEDVGP		
FNR	764	DFQILSKNLNNSQSDVVS	FRLSVEAQAQVT	LVGVSKPEAVLF-PVSDWHPRDQ	PQKEEDLGP			
Mo1	60	-VR--L-NQ-----	TVIWRDPQ-----	VTFSENLSSTCHTKE-----				*
IIb	780	KVEHTYELHNNPGT	VNGLHLSIHLPGQ	SPSLLYLDIQPQGG	GLQCFPPQ	PPVNP	PLKVDWG	
VNR	805	VVQHLYELRNNGP	SFSKAMHLQW	PKYNNNTLLYILHYDIDG	PMNCTSDMEIN	PLRIK--		
FNR	825	AVHHVYELINQGPSSISQGVLELSCPQALEGQQLLYVTRVT--GLNCTTNH-----						
Mo1	89	-----	RLPSHSDFLAELRKAPV	VVNC	SIACQRIQCDIP	FFGIQE		
IIb	842	LPIPSPSPIHFAHHRDRRQIFLPEPEQPSRLQDPVLVSCDSAPCTVVQCDLQEMARGQ						
VNR	865	ISSLQTTEKNDTVAGQGERDHLITKRDLALSEGDIHTLGC	GVAQCLKIVCQVGR	LDRLGK				
FNR	874	-PI-NPKGLELDPEGSLHHQKREAPSRSSASSGPQILKCPEAECFRLRCELGPLHQQE

FIG. 6. Southern blot analysis and chromosomal mapping of Mo1 α . Hybridization of 32 P-labeled human Mo1 α probe with *Eco*RI (A)- or *Hind*III (B)-digested normal genomic DNA (lanes 5) and DNA from the human-hamster somatic cell hybrids G35 F1 (lanes 1), G35 A2 (lanes 2), G35 F3 (lanes 3), and G35 A4 (lanes 4). The *Eco*RI- or *Hind*III-digested Mo1 α genomic components (arrows) segregated with the chromosome 16-positive hybrids (G35 F3 and G35 A4, lanes 3 and 4, respectively) but not the with chromosome 16-negative hybrids G35 F1 and G35 A2. Arrowheads show the size marker positions at 23 kb, 9.8 kb, 6.6 kb, 4.3 kb, 2.3 kb, 2.0 kb, and 0.5 kb.

Mo1 α segregates with the detergent-phase upon extraction of cells with Triton X-114, suggesting that it is an integral membrane protein (unpublished observations). The present data further suggest that, like other integrins, Mo1 α is oriented with the carboxyl terminus on the cytoplasmic side.

Two studies have reported (41, 42) isolation of clones encoding for Mo1 α . A human 20-kb genomic clone was isolated, which upon transfection into mouse fibroblasts, yielded a transfectant that expressed the human α subunits of Mo1, LFA-1, and platelet IIb on the surface of the mouse cells as determined by reactivity with mAbs specific for human but not mouse surface antigens (41). This clone was not characterized at the DNA level. The findings that Mo1 α and LFA-1 α -chain genes are on chromosome 16 (this report and ref. 43) support the concept that at least these two genes may be present on a single fragment of genomic DNA. We

FIG. 7. Protein identity of human Mo1 α sequence with that of IIb and the α chain of FNR and VNR. The first amino acid in the Mo1 α clone is numbered 1. The single letter code for amino acids is used. Numbering for each protein begins with the first amino acid of the published sequences (32, 39, 40). Identical amino acids are indicated by double dots and conservative substitutions by single dots. Asterisks identify residues identical in all four glycoproteins. The C-terminal area where conservation is observed most in all four molecules is overlined.

cannot, however, reconcile the finding that the three genes for the α chain of LFA-1, Mo1 α , and IIb are on a single 20-kb genomic fragment knowing the length of the mRNA coding for human Mo1 α , IIb, and the predicted size of the α chain of LFA-1. A second partial genomic clone encoding mouse Mo1 α (Mac-1 α) was isolated (42). Partial sequencing revealed an exon coding for 28 amino acids from the N terminus of mouse Mo1 α (24) followed by an intron, thus precluding further characterization of the clone. The size of the mouse Mo1 α mRNA, 6 kb, is larger than that of human or guinea pig Mo1 α mRNA, 5 kb (Fig. 5). Since the predicted size of Mo1 α coding region is $\approx$ 3 kb (130 kDa for the unglycosylated species, corresponding to $\approx$ 1000 amino acids), it appears likely that mouse Mo1 α mRNA may contain additional 5'- or 3'-untranslated regions.

The human genome contains a single copy of Mo1 α gene that is mapped to chromosome 16 (Fig. 6). The α chain of LFA-1 has been mapped (43) to chromosome 16 through analysis of human-mouse hybrid cells with an LFA-1 α -chain specific mAb. It is possible, therefore, that the α subunit of p150,95 is on the same chromosome and that Leu-CAM α chains, which mediate specific aspects of leukocyte adhesion and have the identical property of binding to a common β subunit, have evolved from a common ancestral gene. The significant homology of the cloned region of Mo1 α to the α subunits of other integrins also support the existence of a gene superfamily of molecules that mediate various aspects of adhesion in fibroblasts and leukocytes (19).

Despite structural similarities and common requirement for divalent cations to mediate adhesion, integrins are distinct biochemically, immunologically, and functionally. For example, calcium is required for the association of the $\alpha\beta$ complex in IIb/IIIa (44, 45). Chelating agents, however, do not dissociate Leu-CAMs. In addition, despite the homologies at the protein level in the α and β subunits of these complexes, distinct and nonoverlapping disease processes are produced by the inherited deficiency of some of these molecules. Leu-CAM deficiency is not associated with bleeding tendencies. Glanzmann thrombasthenia (46), a bleeding disorder, is not characterized by recurrent bacterial infections. With the cloning of many of these glycoproteins, it should now be possible to analyze in greater detail the organization and regulation of the corresponding genes and their specific tissue expression. Furthermore, the information derived from the sequence data should provide a more detailed understanding of the structure as it relates to the specific adhesion-promoting functions mediated by each of these molecules.

We thank Scott Pendergast, Beverly Savage, and Phil Behn for their expert technical assistance; Dr. Rachael Neve for her help in construction of the human cDNA library; Dr. Gail Bruns for providing human-hamster hybrids; and Dr. Linda Clayton for helpful discussions. This work was supported by Public Health Service Grants AI21963, CA21225, CA41456, and HD18658 and Grant 1010 from the March of Dimes. D.G.T. is a fellow of the Leukemia Society of America. M.A.A. is an established investigator of the American Heart Association.

- Todd, R. F., III, Bahn, A. K., Kabawat, S. E. & Schlossman, S. F. (1984) in *Leukocyte Typing*, eds. Bounswell, B. A., Dausset, J., Milstein, C. F. & Schlossman, S. F. (Springer, New York), pp. 424-433.
- Springer, T., Galfre, G., Secher, D. S. & Milstein, C. (1979) *Eur. J. Immunol.* **9**, 301-306.
- Remold-O'Donnell, E. & Savage, B. (1988) *Biochemistry* **27**, 37-41.
- Bernstein, I. D. & Self, S. (1985) in *Leukocyte Typing II*, eds. Reinherz, E. L., Haynes, B. S., Nadler, L. M. & Bernstein, I. D. (Springer, New York), p. 1.
- Arnaout, M. A., Todd, R. F., III, Dana, N., Melamed, J., Schlossman, S. F. & Colten, H. R. (1983) *J. Clin. Invest.* **72**, 171-179.
- Dana, N., Styrt, B., Griffin, J. D., Todd, R. F., III, Klempner, M. S. & Arnaout, M. A. (1986) *J. Immunol.* **137**, 3259-3263.
- Arnaout, M. A. & Faller, D. V. (1987) *Kidney Int.* **31**, 312a.
- Trowbridge, I. S. & Omary, M. B. (1981) *J. Exp. Med.* **154**, 1517-1524.
- Sanchez-Madrid, F., Nagy, J. A., Robins, E., Simon, P. & Springer, T. (1983) *J. Exp. Med.* **158**, 1785-1803.
- Lanier, L. L., Arnaout, M. A., Schwarting, R., Warner, N. L. & Ross, G. D. (1985) *Eur. J. Immunol.* **15**, 713-718.
- Pierce, M. W., Remold-O'Donnell, E., Todd, R. F., III, & Arnaout, M. A. (1986) *Biochim. Biophys. Acta* **874**, 368-371.
- Miller, J. L., Weibe, M. & Springer, T. A. (1987) *J. Immunol.* **138**, 2381-2383.
- Dana, N., Todd, R. F., III, Pitt, J., Springer, T. A. & Arnaout, M. A. (1984) *J. Clin. Invest.* **73**, 153-159.
- Arnaout, M. A., Spits, H., Terhorst, C., Pitt, J. & Todd, R. F., III (1984) *J. Clin. Invest.* **74**, 1291-1309.
- Anderson, D. C., Schmalsteig, F. C., Arnaout, M. A., Kohl, S., Tosi, M. F., Dana, N., Buffone, G. J., Hughes, B. J., Brinkley, B. R., Dickey, W. D., Abramson, J. S., Springer, T., Boxer, L. A., Kollers, J. M. & Smith, C. W. (1984) *J. Clin. Invest.* **74**, 536-554.
- Dana, N., Todd, R. F., III, & Arnaout, M. A. (1986) *Pathol. Immunopathol. Res.* **5**, 371-383.
- Buyon, J., Shaddick, N., Berkman, R., Hopkins, P., Dalton, J., Weissman, G., Abramson, S. & Winchester, R. (1986) *Arthritis Rheum.* **29**, 520a.
- Arnaout, M. A., Hakim, R. M., Todd, R. F., III, Dana, N. & Colten, H. R. (1985) *N. Engl. J. Med.* **312**, 457-462.
- Hynes, R. O. (1987) *Cell* **48**, 549-554.
- Law, S. K. A., Gagnon, J., Hildreth, J. E. K., Wells, C. E., Willis, A. C. & Wong, A. J. (1987) *EMBO J.* **6**, 915-919.
- Kishimoto, T. K., O'Connor, K., Lee, A., Roberts, T. M. & Springer, T. A. (1987) *Cell* **48**, 681-690.
- Tamkun, J. W., DeSimone, D. W., Fonda, D., Patel, R. S., Buck, C., Horwitz, A. F. & Hynes, R. O. (1986) *Cell* **46**, 271-282.
- Charo, I. F., Fitzgerald, L. A., Steiner, B., Rall, S. C., Jr., Bekeart, L. S. & Phillips, D. R. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 8351-8355.
- Springer, T. A., Teplow, D. B. & Dryer, W. J. (1985) *Nature (London)* **314**, 540-542.
- Remold-O'Donnell, E. (1988) *J. Immunol.* **140**, 1244-1249.
- Remold-O'Donnell, E. & Savage, B. (1988) *Biochemistry* **27**, 42-45.
- Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18**, 5294-5299.
- Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 1-545.
- Young, R. A. & Davis, R. W. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 1194-1198.
- Rijsewijk, F., Schuermann, M., Wagenaar, E., Parren, P., Weigle, D. & Nusse, R. (1987) *Cell* **50**, 649-657.
- Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463-5467.
- Poncz, M., Eisman, R., Heidenreich, R., Silver, S. M., Vilaire, G., Surrey, S., Schwartz, E. & Bennett, J. S. (1987) *J. Biol. Chem.* **262**, 8476-8482.
- Arnaout, M. A., Pierce, M. W., Dana, N. & Clayton, L. C. (1987) *Methods Enzymol.* **150**, 608-615.
- Thomas, P. S. (1983) *Methods Enzymol.* **150**, 255-266.
- Southern, E. (1975) *J. Mol. Biol.* **98**, 503-517.
- Harris, P., Lalande, M., Stroh, H., Bruns, G., Flint, A. & Latt, S. A. (1987) *Hum. Genet.* **77**, 95-103.
- Needleman, S. B. & Wunsch, C. D. (1970) *J. Mol. Biol.* **48**, 443-453.
- Dayhoff, M. O., Barker, W. C. & Hunt, L. T. (1983) *Methods Enzymol.* **91**, 524-545.
- Argaves, W. S., Suzuki, S., Arai, H., Thompson, K., Pierschbacher, M. D. & Ruoslahti, E. (1987) *J. Cell Biol.* **105**, 1183-1190.
- Suzuki, S., Argaves, W. S., Arai, H., Languino, L. R., Pierschbacher, M. D. & Ruoslahti, E. (1987) *J. Biol. Chem.* **262**, 14080-14085.
- Cosgrove, L. J., Sandrin, M. S., Rajasekariah, P. & McKenzie, I. F. C. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 752-756.
- Sastre, L., Roman, J. M., Teplow, D. B., Dreyer, W. J., Gee, C. E., Larson, R. S., Roberts, T. M. & Springer, T. A. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 5644-5648.
- Marlin, S. D., Morton, C. C., Anderson, D. C. & Springer, T. A. (1986) *J. Exp. Med.* **164**, 855-867.
- Kunicki, T. J., Pidard, D., Rosa, J. P. & Nurden, A. T. (1981) *Blood* **58**, 268-278.
- Fujimura, K. & Phillips, D. R. (1983) *Thromb. Haemostasis* **50**, 251a.
- Phillips, D. R. & Agin, P. P. (1977) *J. Clin. Invest.* **60**, 535-545.