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N-terminal sequence of human leukocyte glycoprotein Mo1: conservation across species and homology to platelet IIb/IIIa

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Mo1 and gp160-gp93 are two surface membrane glycoprotein heterodimers present on granulocytes and monocytes derived from humans and guinea pigs, respectively. We purified both antigens and found that their alpha subunits had identical N-termini which were significantly homologous to the alpha subunit of the human adhesion platelet glycoprotein IIb/IIIa.

Mo1 is a surface glycoprotein heterodimer present on human granulocytes, monocytes/macrophages and null cells [1]. It consists of an alpha chain of apparent molecular mass of 155–165 kDa noncovalently linked to a beta chain of 94 kDa [2]. The beta chain is shared by two other leukocyte surface antigens, a lymphocyte-function-associated antigen, LFA-1 (alpha subunit 180 kDa) and the Leu M5 (p 150, 95) antigen (alpha subunit 130–150 kDa) [3–5]. Mo1 is identical with complement receptor type 3 (CR3) [6,7] and also promotes adhesion of granulocytes to each other [8] and to endothelial cell monolayers [9]. The biologic significance of the Mo1/LFA-1/Leu M5 glycoprotein family is underscored by the finding that genetic deficiency of all three molecules in man results in recurrent and life-threatening bacterial infections and is associated with abnormal leukocyte adhesion-dependent functions such as phagocytosis, chemotaxis, adhesion to endothelial cells, leukoaggregation, T cell-mediated cytotoxicity and natural killing [10,11].

To gain more insight into the structure-function relationship, we purified human Mo1 to homogeneity from normal granulocytes by affinity chromatography and high-performance liquid chromatography (HPLC) and determined the N-terminal amino acid sequence of its alpha subunit (Mo1). The obtained sequence was identical, except for two conservative substitutions, to that of the alpha subunit of Mac-1 antigen [12] and to gp160, the large subunit of gp160–gp93, a major guinea-pig granulocyte/macrophage glycoprotein heterodimer [13–15] of hitherto unknown function which we also purified by affinity chromatography. Significantly, the N-terminal amino acid sequence of $Mo1_{\alpha}$ was homologous to that of the alpha subunit of platelet glycoprotein IIb/IIIa [16]. The results of our study provide evidence for the evolutionary conservation of Mol_{α}. The homology of its N-terminal amino acid sequence to the alpha subunit of IIb/IIIa, a glycoprotein that subserves similar adhesion functions on platelets, suggest that additional homologies between ad-

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hesion receptors on different cell types such as granulocytes/ macrophages, platelets, lymphocytes and fibroblasts may exist.

Mol was purified from human granulocytes by affinity chromatography using anti-Mol monoclonal antibody [1] covalently linked to Sepharose 4B (Pharmacia, Sweden) [17], followed by high-performance liquid chromatography (HPLC) (see legend to Fig. 1). Gp160-gp93, a major trypsin- and plasmin-sensitive surface glycopeptide heterodimer of guinea-pig granulocytes and macrophages [13–15] was purified by lentil-lectin sepharose and monoclonal antibody-Sepharose affinity chromatography (legend to Fig. 1). When compared to each other, and to murine Mac-1 antigen [12], it is apparent that Mol_{α}, $Mac\text{-}1_{\alpha}$ and gp160 have identical N-terminal primary structures. Substitutions in a variable region at cycles 7–9 are all conservative as defined by the mutation data matrix of Dayhoff [22]. These data provide evidence for the conservation of the Mol antigen across three species, reflecting its important biologic functions.

The N-terminal sequence of human Mol_{α}, had

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Human Mol_{α}	F	N	L	D	T	E	N	A	M	T	F	Q	E	N	A								
Guinea pig gp160	F	N	L	D	T	E	N	P	I	T	F	Q	E	N	A	R	G	F	G	Q	T	V	V
Mouse $Mac\text{-}1_{\alpha}$	F	N	L	D	T	E	H	(P)	M	T	F	Z	E	N	A	R	(G)	F					

Fig. 1. N-terminal amino acid sequence of human Mol_{α} and guinea-pig gp160. Human granulocytes were purified by Ficol-Hypaque centrifugation of anticoagulated whole blood [18]. Purified cells were treated with diisopropylfluorophosphate (DFP) (5 mM end concentration) at $(0.5\text{–}1)\cdot 10^8$/ml in phosphate-buffered saline for 10 min at 0°C. The cells were washed once and then solubilized in phosphate-buffered saline (pH 7.4), containing 2 mM phenylmethylsulfonylfluoride (PMSF) and 0.5% detergent (NP40 or Triton X-100) for 10 min at 4°C. Following centrifugation at $100000 \times g$ for 30 min at 4°C, the supernatant was collected and used as a source of antigen. Mol was isolated from human granulocyte lysate by affinity chromatography followed by high-performance liquid chromatography as follows. 20 ml of anti-Mol Sepharose 4B (2–4 mg of antibody per ml) is poured into a 50 ml column and equilibrated with phosphate-buffered saline. The granulocyte lysate ($3\cdot 10^9$ cell equivalents in 30 ml) is diluted up to 90 ml in phosphate-buffered saline containing 2 mM PMSF and 0.25% NP40 and passed slowly through the column at a flow rate of about 6–8 ml/h, at 4°C. The column is then washed with 100 ml of the equilibration buffer, 50 ml of 20 mM glycine buffer (pH 9) and 50 ml of 20 mM glycine buffer (pH 10) each containing 0.1% NP40. Mol is then desorbed with 20 mM glycine (pH 11.5) containing 0.5% NP40, 2 mM PMSF and 1 mM iodoacetic acid. 3.5 ml fractions are collected and the pH immediately adjusted to 7.4 with 1 M Tris-HCl buffer, (pH 6.8). An aliquot from each fraction (50 μl) is analyzed on sodium dodecyl sulphate polyacrylamide gels [19] followed by silver staining [20]. The early fractions usually contain the beta subunit and later fractions contain the alpha subunit. Peak fractions containing Mol_{α} and Mol_{β} are pooled separately, and the protein precipitated by the addition of three volumes of acetone (−20°C) for 12 h in a 15 ml Corex tube. The precipitate is collected by centrifugation at $10000 \times g$ for 30 min at −20°C, and resuspended in 100–250 μl of buffer (1% (w/v) SDS, 20 mM *N*-ethylmorpholine acetate (pH 7.0) and 40 mM mercaptoethanol) with heating to 37°C for 5 min. Each sample is injected onto TSK 2000 (30 cm) and TSK 3000 (30 cm) columns in series, and the proteins resolved at a flow rate of 0.5 ml/min using the same buffer. Fractions containing the alpha and beta subunits of Mol are identified by absorbance at 280 nM and SDS polyacrylamide gel electrophoresis, pooled, and precipitated with acetone as above. 10–20 μg of the pure alpha or beta subunits can be generated during one purification procedure. Gp160-gp93 is purified from peritoneal guinea-pig granulocytes, as detailed elsewhere [15]. Briefly, the detergent- (0.5% NP40-) soluble granulocyte fraction is applied onto lentil lectin Sepharose as previously described [13]. The 0.1-M methyl α-mannoside-eluted fraction is applied onto anti-gp160 monoclonal antibody-Sepharose column [15] and eluted with 0.2 M sodium citrate buffer (pH 3.5) containing 0.5% NP40. After neutralization the eluate, now containing dissociated gp160 and gp93, is applied onto anti-gp93 monoclonal antibody-Sepharose [15]. Gp160, present in the drop through, is dialysed, lyophilized and precipitated in ethanol and the precipitate is resuspended in water. Preparations consisting of 22, 39 and 42 μg of human Mol_{α}, 21 and 46 μg of gp160 [21], each, in 30–60 μl of 1% (w/v) SDS were submitted to 15–21 cycles of Edman degradation, using the Applied Biosystems Model 470 A sequencer. PTA derivatives were resolved via HPLC using an IBM Cyno column and Permaphase ETH precolumn, with gradient elution (solvent A: 70 mM sodium acetate (pH 5.5)/5% (v/v) tetrahydrofuran; solvent B: acetonitrile; gradient 11–48% B over 20 min, flow rate 1 ml/min). The sequences shown here were obtained. Multiple attempts to obtain sequence for Mol_{β} and gp93 [15] were unsuccessful. The N-terminal sequence of mouse $Mac\text{-}1_{\alpha}$ was taken from Ref. 12. The single-letter notation for amino acids is used: A, Ala; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; Z, Glx and Y, Tyr.

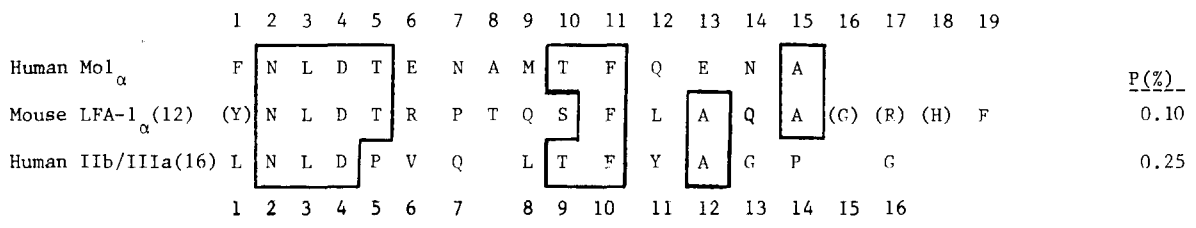

Fig. 2. Homologies between the N-termini of human Molα, mouse LFA-1α [12] and human platelet gp IIb/IIIa [16]. Residues are aligned for the N-terminus, with the introduction of a gap between residues 7 and 8 in gpIIb/IIIa. In the right column are the percentage probabilities (P%) that homology is due only to chance, as determined by the sum of the values for each amino acid pair from the log odds matrix of Dayhoff. The score for the amino acid matching the gap position is zero [22].

a significant homology with the alpha subunits of two other membrane proteins that mediate cell adhesion, mouse LFA-1 [12] (P value 0.1%) and human platelet glycoprotein IIb/IIIa [16] (P value 0.25%) (Fig. 2). LFA-1 is involved in binding of lymphocytes to each other, [23] to their targets [24], or to endothelial cells [25]. Platelet IIb/IIIa glycoprotein complex acts as a receptor for fibronectin [26–29] and has a more general adhesion role serving also as a receptor for fibrinogen [30,31] and for Von Willebrand factor [32,28,33]. Its function is inhibited by peptides related to the Arg-Gly-Asp-Ser sequence of fibronectin [28,34]. Mol is identical to CR3, a receptor for the complement C3 fragment, C3bi [6,7], and also has a more general adhesion function, promoting adhesion of granulocytes to each other or to umbilical vein endothelial cell monolayers [8,9]. Certain monoclonal antibodies directed against Mol also inhibit binding and ingestion of fibronectin-coated microspheres by granulocytes and monocytes [35]. Furthermore, the co-expression of platelet IIb/IIIa, Mol and LFA-1 in cells transfected with a genomic clone [36], as well as subunit molecular mass similarities, strongly suggest that Mol may, in fact, be functionally and structurally homologous to the Arg-Gly-Asp-Ser receptor family of adhesion molecules. Although the tetrapeptide Arg-Gly-Asp-Ser does not inhibit the CR3 function of Mol [37], its effect on Mol-dependent cell adhesion has not been evaluated. Further investigation into the structural basis for the cell-adhesion-promoting function of these cell-surface molecules should be of interest.

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