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COMMUNICATION

Purification and α Subunit N-terminal Sequences of Human Mac-1 and p150,95 Leukocyte Adhesion Proteins[1]

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A family of three leukocyte surface proteins of broad importance in leukocyte adhesion has recently been defined in humans (1, 2). This family is comprised of the three heterodimers p150,95, Mac-1, and LFA-1, each consisting of a unique α subunit of 150,000 to 180,000 daltons noncovalently associated with a common β subunit of 95,000 daltons. p150,95 and Mac-1 are expressed on the surface of monocytes and granulocytes, and in higher amounts inside these cells. The binding of inflammatory mediators to surface receptors on these cells triggers mobilization of this intracellular pool to the cell surface (3–6). Increased surface expression of p150,95 and Mac-1 results in increased adhesiveness to endothelial cells (7–10), and localization of leukocytes in inflammatory sites in vivo, as demonstrated in patients who are genetically deficient in these leukocyte adhesion glycoproteins (5, 11).

Little is known about the biochemistry of the most recently described member of this family, p150,95 (6, 12). The relationship of p150,95 to Mac-1 has been unclear. Although they are distinct antigenically (6, 12) and in cell distribution (13), they have similar isoelectric points (1) and functions (2, 14). We report the purification of p150,95 and Mac-1 from human cells and the N-terminal amino acid sequence of their α subunits. We find that the human p150,95 and Mac-1 α subunit sequences are homologous to one another and to the previously published murine Mac-1 and LFA-1 α subunit sequences (15). The leukocyte adhesion protein α subunit sequences are also homologous to the recently sequenced human vitronectin receptor (VNR) and platelet gpIIb/IIIa α subunits[3] (16, 17). These homologies to cell surface receptors that bind to the Arg-Gly-Asp (RGD)[4] peptide sequence found in extracellular matrix proteins suggest a novel supergene family of adhesion proteins.

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[4] Abbreviation used in this paper: RGD, arginine-glycine-aspartic acid.

MATERIALS AND METHODS

p150,95 purification. SHCL3 anti-p150,95 MAb (18) (available as Leu-M5 from Becton-Dickenson, Mountain View, CA) was purified (13) and was coupled to CL-4B Sepharose (Pharmacia, Piscataway, NJ) at 3.3 mg MAb per ml of packed bed. A spleen (50 g) from a hairy cell leukemia patient (generous gift of Dr. Harvey Golumb, University of Chicago) was diced, was minced, and was lysed in 1 L of 0.01 M Tris-HCl pH 8.0, 0.15 M NaCl, 1.0% Triton X-100, 0.025% azide, 5 mM iodoacetamide, 1 mM phenylmethylsulfonyl fluoride (PMSF), and 0.22 trypsin inhibitor units (TIU)/ml aprotinin. The lysate was centrifuged at 5000 × G for 30 min, then at 16,000 × G for 2 hr, and was sequentially filtered through Whatman No. 1 filters, AP prefilters, and 45 μm millipore filters (Millipore, Bedford, MA). The hairy cell leukemia spleen lysate was loaded onto a 4 ml SHCL3 MAb-Sepharose column, was rinsed with 5 column vol of the lysis buffer, then 10 column vol of 0.1 M glycine, pH 10.0, 0.15 M NaCl, 0.1% Triton X-100, and 1 mM PMSF, and finally with 10 column vol 0.1 M Tris-HCl, pH 8.0, 0.15 M NaCl, 0.1% Triton X-100, and 1 mM PMSF. The p150,95 molecule was eluted in 0.1 M glycine, pH 3.0, 0.15 M NaCl, 0.1% Triton X-100, 1 mM PMSF, and 0.025% azide, and the pH immediately neutralized.

Mac-1 purification. LM2/1 anti-Mac-1 MAb (13) was purified (19), and was coupled to CL-4B Sepharose at 3 mg MAb per ml of packed bed. Sendai-virus induced leukocytes (25 g) were lysed in 500 ml of 0.1 M Tris-HCl, pH 8.0, 0.15 M NaCl, 1.0% Triton X-100, 0.025% azide, 5 mM iodoacetamide, 5 mM EDTA, 1 mM DFP, and 0.22 TIU/ml aprotinin, and were centrifuged 10,000 × G for 2 hr. Batch purification was performed by incubation of 4 ml LM2/1 MAb-Sepharose with the lysate for 3.5 hr at 4°C. This was poured into a column and was rinsed with 20 column vol of 0.01 M Tris-HCl, pH 8.0, 0.15 M NaCl, 0.1% Triton X-100, and 0.025% azide. Mac-1 was eluted in 10 mM MES, pH 4.0, 0.15 M NaCl, 0.1% TX-100, and 0.025% azide, and the pH immediately neutralized. Preparative SDS-PAGE and electroelution were done as described (20), except that the protein was visualized in the preparative 7% gel by immersion in 1 M KCl.

RESULTS AND DISCUSSION

Based on our previous extensive survey of the quantity of p150,95 and Mac-1 expressed on different cell types (13), we chose hairy leukemia spleen cells and neutrophils (peripheral blood leukocytes) from which to purify p150,95 and Mac-1, respectively. Procedures for the large scale purification of these antigens from TX-100 detergent lysates were developed (See *Materials and Methods*). p150,95 was purified from a hairy cell leukemia spleen lysate on an SHCL3 MAb-Sepharose column (Fig. 1, lane *1*). Mac-1 was batch purified from a pooled leukocyte lysate with LM2/1 MAb-Sepharose (Fig. 1, lane *3*). After preparative SDS-7% PAGE, the α subunits of each antigen were excised and were electroeluted (Fig. 1, lanes *2* and *4*). p150,95 αX subunit (200 pmol) and 800 pmol Mac-1 αM subunit were sequenced with a gas phase sequenator.

The sequences for the first 24 residues of the p150,95

α subunit and 28 residues of Mac-1 α subunit were obtained (Fig. 2). The p150,95 and Mac-1 α subunits are 54% identical, demonstrating a close relationship between the two α subunit genes. The differences demonstrate that the p150,95 αX subunit and the Mac-1 αM subunit are encoded by distinct genes. The greater than 50% homology of the p150,95 αX subunit to the Mac-1 αM subunit correlates with the strikingly similar isoelectric focusing patterns (1) and functions (2, 3, 5, 11, 14, 21, 22) of the two proteins. Compared with other gene families, the N-terminal homology between the p150,95 and Mac-1 α subunits is greater than that between the human α and β hemoglobin chains (43% homology), and the human immunoglobulin Cγ and Cϵ, Cμ, or Cα heavy chains (39, 31, or 31% homology, respectively) (23).

The human Mac-1 αM subunit is 75% homologous with murine Mac-1 (15, 24) (Fig. 2); conservative substitutions account for many of the mismatches. All residues identical between human p150,95 and Mac-1 α subunits are also conserved in the mouse Mac-1α subunit, indicating the presence of conserved regions. The greater similarity between mouse and human Mac-1 (75%), than between murine Mac-1 and LFA-1 (32%), or between human p150,95 and Mac-1 (54%) suggests that the three α subunits had already diverged before the mammalian radiation. Among the three α subunits, those of p150,95 and Mac-1 appear to be most closely related (Fig. 2).

While this manuscript was in preparation, N-terminal sequences of the human vitronectin receptor and platelet IIb/IIIa α subunits were reported, and homologies were suggested with our previously published N-terminal sequences of the murine LFA-1 and Mac-1 α subunits[3] (16, 17). Our comparison with the human Mac-1 and p150,95 α subunit sequences demonstrates that they are distinct from but homologous to the human vitronectin receptor and the platelet IIb/IIIa protein (Fig. 3). The vitronectin receptor and IIb/IIIa are members of a functionally defined family of cell surface receptors which bind to fibronectin and other extracellular matrix materials. These adhesion glycoproteins recognize a common sequence, RGD, present in many extracellular matrix proteins, such as fibronectin, fibrinogen, and vitronectin (25). They are also, like Mac-1, p150,95, and LFA-1, composed of two distinct subunits (26). Although it had been speculated that gpIIb/IIIa and p150,95 may be identical (27), our results clearly show that their N-terminal sequences are distinct (Fig. 3). The similarity between Mac-1 and p150,95 is much greater than between these two proteins and LFA-1, than between VNR α and gpIIb/IIIa α (36%), or between the RGD receptor α subunits and the leukocyte adhesion proteins (21 to 45%) (Fig. 3). In contrast to the leukocyte adhesion proteins, the RGD receptor α subunits are proteolytically cleaved during processing, and hence contain two disulfide-linked polypeptide chains. The leukocyte adhesion proteins and RGD receptors also have different tissue distribution (13, 18, 28), and behavior on reduced vs non-reduced SDS-PAGE (1, 28). Thus although the leukocyte adhesion proteins and extracellular matrix receptors share some similarities, they are clearly distinct and perform different functions.

The Mac-1, LFA-1, and p150,95 molecules share a common β subunit that is distinct antigenically and in sequence from the human vitronectin receptor and IIb/IIIa β subunits[3] (17, 29). We cloned the leukocyte adhesion protein β subunit gene (29) and discovered that it is 45% homologous to the β subunit of the fibronectin/laminin receptor (integrin) of chick embryonic fibroblasts (30). Together with the α subunit sequence homologies, this suggests that the leukocyte adhesion proteins and the

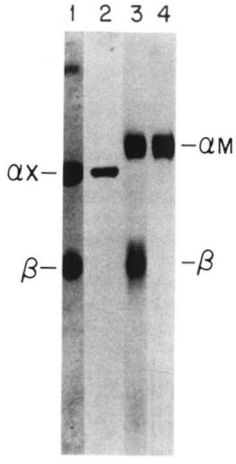

Figure 1. Purified human p150,95 and Mac-1 α subunits. Affinity-purified p150,95 (*Lane 1*), electroeluted p150,95 α subunit (*Lane 2*), affinity-purified Mac-1 (*Lane 3*), and electroeluted Mac-1 α subunit (*Lane 4*) were subjected to SDS-7% PAGE and silver staining.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Hu p150,95 α	PHE	ASN	LEU	ASP	THR	GLU	GLU	LEU	THR	ALA	PHE	ARG	VAL	ASP
Hu Mac-1α	PHE	ASN	LEU	ASP	THR	GLU	ASN	ALA	VAL	THR	PHE	GLN	GLU	ASN
Mu Mac-1α	PHE	ASN	LEU	ASP	THR	GLU	HIS	PRO	MET	THR	PHE	GLN	GLU	ASN
Mu LFA-1α	TYR	ASN	LEU	ASP	THR	ARG	PRO	THR	GLN	SER	PHE	LEU	ALA	GLN
Hu VNR α	PHE	ASN	LEU	ASP	VAL	X	SER	PRO	ALA	GLU	TYR			
Hu gpIIb	LEU	ASN	LEU	ASP	PRO	VAL	GLN	LEU	THR	PHE	TYR	ALA	GLY	PRO

Figure 3. Comparison of the α subunit sequences of the leukocyte adhesion family with two extracellular matrix receptors (16–17). Homologous residues are *boxed*.

Figure 2. α subunit N-terminal sequences. *Parentheses* denote some uncertainty with the residue assignment. Homologous residues are *boxed*. Human p150,95 and Mac-1 α subunit sequences were determined at the joint Harvard Biochemistry and Molecular Biology Department and Dana-Farber Cancer Institute Microsequencing Facility on a gas-phase sequenator (Applied Biosystems). Mouse Mac-1 and LFA-1 sequences were previously reported (15, 25).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Hup150,95 α	PHE	ASN	LEU	ASP	THR	GLU	GLU	LEU	THR	ALA	PHE	ARG	(VAL)	ASP
Hu Mac-1 α	PHE	ASN	LEU	ASP	THR	GLU	ASN	ALA	VAL	THR	PHE	GLN	GLU	ASN
Mu Mac-1α	PHE	ASN	LEU	ASP	THR	GLU	HIS	PRO	MET	THR	PHE	GLN	GLU	ASN
Mu LFA-1 α	TYR	ASN	LEU	ASP	THR	ARG	PRO	THR	GLN	SER	PHE	LEU	ALA	GLN

	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Hup150,95 α	SER	ALA	(GLY)	PHE	(GLY)	ASP	X	VAL	(VAL)	GLN				
Hu Mac-1 α	ALA	(ARG)	GLY	PHE	GLY	GLN	GLY	VAL	VAL	GLN	LEU	GLN	GLY	(SER)
Mu Mac-1α	ALA	LYS	GLY	PHE	GLY	GLN	ASP	VAL	VAL	GLN	LEU	GLY	GLY	THR
Mu LFA-1 α	ALA	(GLY)	(ARG)	(HIS)	PHE									

extracellular matrix receptors are evolutionarily related. The presence of these proteins in the human, mouse, and chicken indicates that the ancestral α and β genes duplicated and diverged quite early in evolutionary history. Even more intriguing is a possible relationship to the position-specific antigens of *Drosophila* (31), which are $\alpha\beta$ heterodimeric adhesion proteins thought to be important in guiding embryogenesis and metamorphosis (32).

As more RGD peptide binding receptors and the α subunits of the Mac-1 family of proteins are cloned and completely sequenced, the extent of homology and the relationship between the various subgroups in this supergene family will become more clearly delineated. On the basis of these homologies it is of interest to examine whether the leukocyte adhesion proteins also bind to ligands which contain the RGD sequence.

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REFERENCES

1. **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-1 (LFA-1), the C3bi complement receptor (OKM1/Mac-1), and the p150,95 molecule. *J. Exp. Med. 158:1785.*
2. **Anderson, D. C., L. J. Miller, F. C. Schmalstieg, R. Rothlein, and T. A. Springer.** 1986. Contributions of the Mac-1 glycoprotein family to adherence-dependent granulocyte functions: structure-function assessments employing subunit-specific monoclonal antibodies. *J. Immunol 137:15.*
3. **Todd, R. F., III, M. A. Arnaout, R. E. Rosin, C. A. Crowley, W. A. Peters, and B. M. Babior.** 1984. Subcellular localization of the large subunit of Mol (Molα; formerly gp110) a surface glycoprotein associated with neutrophil adhesion. *J. Clin Invest. 74:1280.*
4. **Springer, T. A., and D. C. Anderson.** 1986. The importance of the Mac-1, LFA-1 glycoprotein family in monocyte and granulocyte adherence, chemotaxis, and migration into inflammatory sites: insights from an experiment of nature. In *Biochemistry of Macrophages (Ciba Symposium 118).* Pitman, London. Pp. 102–126.
5. **Springer, T. A., W. S. Thompson, L. J. Miller, F. C. Schmalstieg, and D. C. Anderson.** 1984. Inherited deficiency of the Mac-1, LFA-1, p150,95 glycoprotein family and its molecular basis. *J Exp Med. 160:1901.*
6. **Springer, T. A., L. J. Miller, and D. A. Anderson.** 1986. p150,95, the third member of the Mac-1, LFA-1 human leukocyte adhesion glycoprotein family. *J. Immunol. 136:240.*
7. **Tonnesen, M. G., D. C. Anderson, T. A. Springer, A. Knedler, N. Avdi, and P. M. Henson.** 1986. Mac-1 glycoprotein family mediates adherence of neutrophils to endothelial cells stimulated by leukotriene B_4 and platelet activation factor. *Fed. Proc. 45:379.*
8. **Beatty, P. G., J. A. Ledbetter, P. J. Martin, T. H. Price, and J. A. Hansen.** 1983. Definition of a common leukocyte cell-surface antigen (Lp95-150) associated with diverse cell-mediated immune functions. *J. Immunol. 131:2913.*
9. **Wallis, W. J., P. G. Beatty, H. D. Ochs, and J. M. Harlan.** 1985. Human monocyte adherence to cultured vascular endothelium: monoclonal antibody-defined mechanisms. *J. Immunol. 135:2323.*
10. **Diener, A. M., P. G. Beatty, H. D. Ochs, and J. M. Harlan.** 1985. The role of neutrophil membrane glycoprotein 150 (GP-150) in neutrophil-mediated endothelial cell injury in vitro. *J. Immunol. 135:537.*
11. **Anderson, D. C., F. C. Schmalstieg, M. J. Finegold, B. J. Hughes, R. Rothlein, L. J. Miller, S. Kohl, M. F. Tosi, R. L. Jacobs, A. Goldman, W. T. Shearer, and T. A. Springer.** 1985. The severe and moderate phenotypes of heritable Mac-1, LFA-1 deficiency: their quantitaive definition and relation to leukocyte dysfunction and clinical features. *J. Infect. Dis. 152:668.*
12. **Lanier, L. L., M. A. Arnaout, R. Schwarting, N. L. Warner, and G. D. Ross.** 1985. p150/95, third member of the LFA-1/CR3 polypeptide family identified by anti-Leu M5 monoclonal antibody. *Eur. J. Immunol. 15:713.*
13. **Miller, L. J., R. Schwarting, and T. A. Springer** 1986. Regulated expression of Mac-1, LFA-1, and p150,95 during leukocyte differentiation. *J. Immunol. 137:2891.*
14. **Micklem, K. J., and R. B. Sim.** 1985. Isolation of complement fragment-iC3b-binding proteins by affinity chromatography: the identification of p150,95 as an iC3b-binding protein. *Biochem. J. 231:233.*
15. **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 inferferon. *Nature 314:540.*
16. **Suzuki, S., W. S. Argraves, R. Pytela, H. Arai, T. Krusius, M. D. Pierschbacher, and E. Ruoslahti.** 1986. cDNA and amino sequences of the cell adhesion protein receptor recognizing vitronectin reveal a transmembrane domain and homologies with other adhesion protein receptors. *Proc. Natl. Acad. Sci. USA 83:8614.*
17. **Charo, I. F., L. A. Fitzgerald, B. Steiner, S. C. Rall Jr., L. S. Beckeart, and D. R. Phillips.** 1986. Platelet glycoproteins IIb and IIIa: evidence for a family of immunologically and structurally related glycoproteins in mammalian cells. *Proc. Natl. Acad. Sci. USA 83:8351.*
18. **Schwarting, R., H. Stein, and C. Y. Wang.** 1985. The monoclonals S-HCL 1 (anti Leu 14) and anti S-HCL 3 (anti Leu M5) allow the diagnosis of hairy cell leukemia. *Blood 65:974.*
19. **Ey, P. L., S. J. Prowse, and C. R. Jenkin.** 1978. Isolation of pure IgG1, IgG2a, and IgG2b immunoglobulins from mouse serum using protein A-Sepharose. *Immunochemistry 15:429.*
20. **Hunkapillar, M. W., E. Lujan, F. Ostrander, and L. E. Hood.** 1983. Isolation of microgram quantities of proteins from polyacrylamide gels for amino acid sequence analysis. *Methods Enzymol. 91:227.*
21. **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.*
22. **Harlan, J. M., P. D. Killen, F. M. Senecal, B. R. Schwartz, E. K. Yee, R. F. Taylor, P. G. Beatty, T. H. Price, and H. D. Ochs.** 1985. The role of neutrophil membrane glycoprotein gp-150 in neutrophil adherence to endothelium in vitro. *Blood 66:167.*
23. **Dayhoff, M. O.** 1978. *Atlas of Protein Sequence and Structure, Vol. 5, Suppl. 3.* National Biomedical Research Foundation, Silver Spring, MD.
24. **Sastre, L., J. M. Roman, D. B. Teplow, W. J. Dreyer, C. E. Gee, R. S. Larson, T. M. Roberts, and T. A. Springer.** 1986. A partial genomic DNA clone for the α subunit of the mouse complement receptor type 3 and cellular adhesion molecule Mac-1. *Proc. Natl. Acad. Sci. USA 83:5644.*
25. **Pierschbacher, M. D., and E. Ruoslahti.** 1984. Cell attachment activity of fibronectin can be duplicated by small synthetic fragments of the molecule. *Nature 309:30.*
26. **Pytela, R., M. D. Pierschbacher, and E. Ruoslahti.** 1985. A 125/115 kDa cell surface receptor for vitronectin interacts with the arginine-glycine-aspartic acid adhesion sequence derived from fibronectin. *Proc. Natl. Acad. Sci. USA 82:5766.*
27. **Cosgrove, L. J., M. S. Sandrin, P. Rafasekariah, and I. F. C. McKenzie.** 1986. A genomic clone encoding the α chain of the OKM1, LFA-1, and platelet glycoprotein IIb-IIIa molecules. *Proc. Natl. Acad. Sci. USA 83:752.*
28. **Pytela, R., M. D. Pierschbacher, M. H. Ginsberg, E. F. Plow, and E. Rouslahti.** 1986. Platelet membrane glycoprotein IIb/IIIa: member of a family of Arg-Gly-Asp-specific adhesion receptors. *Science 231:1559.*
29. **Kishimoto, T. K., K. O'Conner, A. Lee, T. M. Roberts, and T. A. Springer.** 1987. Cloning of the β subunit of the leukocyte adhesion proteins: homology to an extracellular matrix receptor defines a novel supergene family. *Cell. In press.*
30. **Tamkun, J. W., S. W. DeSimone, D. Fonda, R. S. Patel, C. Buck, A. F. Horwitz and R. D. Hynes.** 1986. Structure of integrin, a glycoprotein involved in the transmembrane linkage between fibronectin and actin. *Cell 46:271.*
31. **Leptin, M.** 1986. The fibronectin receptor family. *Nature 321:728.*
32. **Wilcox, M. and M. Leptin.** 1985. Tissue-specific modulation of a set of related cell surface antigens in *Drosophila. Nature 316:351.*