Reprinted from Nature, Vol. 314, No. 6011, pp. 540-542, 11 April 1985

Sequence homology of the LFA-1 and Mac-1 leukocyte adhesion glycoproteins and unexpected relation to leukocyte interferon

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Cell-surface adherence reactions are fundamental to the biology of lymphocytes, monocytes and granulocytes. The lymphocyte function-associated 1 (LFA-1) and macrophage 1 (Mac-1) glycoproteins mediate differing types of adhesion reactions on these cells. LFA-1 participates in T-lymphocyte and natural killer-cell adhesion to target cells[1–3], whereas the Mac-1 antigen is identical to the complement receptor type 3, which mediates adhesion of monocytes and granulocytes to C3bi-sensitized particles[4]. Deficiency of these proteins, in a heritable disease, results in multiple adhesion-related leukocyte defects[5,6]. LFA-1 and Mac-1 resemble one another in overall structure, having α-subunits of relative molecular mass (M_r) 180,000 and 170,000, respectively, which are non-covalently associated with β-subunits of M_r 95,000 in $\alpha_1\beta_1$ complexes[7,8]. Peptide mapping and immunological cross-reactivity have shown that the β-subunits are highly related if not identical, but have revealed no similarities between the α-subunits[7,9,10]. Nonetheless, the shared β-subunit suggested that LFA-1 and Mac-1 might be members of a protein family containing diversified but evolutionarily related α-subunits. Therefore, we examine here the structure of the α-subunits by N-terminal amino-acid sequencing. Sequence homology shows that the α-subunits are members of a novel leukocyte adhesion protein family, and suggests that their evolution occurred by gene duplication. A search for similarities to previously sequenced proteins reveals a further unexpected homology between LFA-1 and leukocyte (α) interferons.

We purified murine LFA-1 and Mac-1 antigens by monoclonal antibody affinity chromatography from detergent lysates of EL-4 T-lymphoid and P388D$_1$ macrophage-like cell lines and separated the α-and β-subunits (see Fig. 1 legend). The N-terminal sequences of the LFA-1 and Mac-1 α-subunits are 33% homologous (Fig. 1). Four contiguous amino acids are identical at positions 2-5 and further identities occur at positions 11 and 15. Some of the differences represent relatively conservative amino-acid substitutions, namely Phe for Tyr at residue 1, Thr for Ser at 10, and Asp for Glu at 14. If a gap was introduced before the Lys at position 16 in Mac-1, it would be aligned with an Arg in LFA-1 and Phe at positions 18 and 19 would be aligned.

	1							10							19
LFA-1	(Tyr)	Asn	Leu	Asp	Thr	Arg	Pro	Thr	Gln	Ser	Phe	Leu	Ala	Gln	Ala	(Gly/Arg/His)	Phe
Mac-1	(Phe)	Asn	Leu	Asp	Thr	Glu	His	(Pro)	Met	Thr	Phe	Glx	Glu	Asn	Ala	Lys	(Gly)	Phe

Fig. 1 N-terminal sequences of LFA-1 and Mac-1 α-subunits. Homologous sequences are boxed.
Methods. LFA-1 was purified from 10- or 50-g batches of EL-4 T lymphoma cells grown *in vitro*. Cells were lysed with Triton X-100, sodium deoxycholate was added before the ultracentrifugation step and LFA-1 antigen was bound to M17/4 monoclonal antibody-Sepharose and eluted at high *p*H, as described previously[8]. The eluate in 0.5% Triton X-100, 20 mM triethylamine *p*H 11.0, was neutralized by adding 0.075 vol. of 1 M Tris-HCl *p*H 6.8. Mac-1 from P388D$_1$ cells was purified similarly on M1/70 monoclonal antibody-Sepharose, except that the neutralized eluate was subjected to a second cycle of affinity purification. SDS-polyacrylamide gel electrophoresis (PAGE) and Coomassie brilliant blue staining of purified LFA-1 and Mac-1 demonstrated that the α- and β-subunits had no contaminants, as shown previously[7,10]. Purified antigens were reduced with 20 mM diothiothreitol, alkylated with 50 mM iodoacetic acid and precipitated overnight at −20 °C with 1.5 vols 1% acetic acid in methanol. Precipitates were collected at 200,000 *g* for 0.5 h and subjected to preparative SDS-PAGE. Subunits were localized by Coomassie brilliant blue staining, electroeluted[17] and subjected to gas/liquid solid phase microsequencing[18]. Phenylthiohydantoin amino acids were determined by HPLC[19]. The sequence assignments shown were confirmed by multiple determinations. The α-subunit of LFA-1 from two different purifications was sequenced three times (200, 44 and 22 μg) and the α-subunit of Mac-1 was sequenced twice (63 and 31 μg). Residues in parentheses were found together with smaller amounts of another residue, or in low yield. No sequence was obtained from the LFA-1 and Mac-1 β-subunits, suggesting that they are blocked.

The sequence homology shows that the α-subunits constitute a novel adhesion protein family, the genes of which evolved by duplication of a primordial ancestral gene. The N-terminal sequences have diverged considerably since the primordial gene duplication event. These differences suggest that the specificity in adhesive interactions is determined by the α-subunits, in agreement with localization of a binding site of the C3bi antigen on the α-subunit of Mac-1 using α- and β-subunit-specific monoclonal antibodies[10,11]. Duplication and specialization of the α-subunits must have preceded divergence of mouse and human, as LFA-1 and Mac-1 homologues mediating the same functions occur in humans[12]. A third type of human α-subunit has been identified[12] which may represent a further member of the α-subunit protein family. The amino-acid sequences described here allow the specification of oligonucleotide probes, which should be of use in the isolation and further study of the Mac-1/LFA-1 family of α-subunit genes.

A computer search[13] for homologies with other proteins reveals an unexpected, strong homology between LFA-1 and human leukocyte interferon (Fig. 2). Multiple genes for α-interferon (IFN-α) code for a family of closely related interferon proteins[14]. Comparison of LFA-1 with 12 mouse, rat and human

		1				5				10				15			19	P(%)[b]
LFA-1	(1-19)	Tyr	Asn	Leu	Asp	Thr	Arg	Pro	Thr	Gln	Ser	Phe	Leu	Ala	Gln	Ala	Gly Arg	His Phe	-
Mouse IFN-α1	(7-25)	His	*Asn*	*Leu*	Arg	Asn	Lys	Arg	Ala	Leu	*Thr*	Leu	Leu	Val	Gln	Met	Arg	Arg Leu Ser	1.2
Mouse IFN-α2	(7-25)	Tyr	*Asn*	*Leu*	Arg	Asn	Lys	Arg	Ala	Leu	Lys	Val	Leu	Ala	Gln	Met	Arg	Arg Leu Pro	0.3
Rat IFN-α	(7-25)	His	*Asn*	*Leu*	Arg	Asn	Lys	Arg	Val	Phe	*Thr*	Leu	Leu	Ala	Gln	Met	Arg	Arg Leu Ser	2.0
Human IFN-α1	(7-25)	His	Ser	*Leu*	*Asp*	Asn	Arg	Arg	Thr	Leu	Met	Leu	Leu	Ala	Gln	Met	Ser	Arg Ile Ser	0.08
Human IFN-α2	(7-25)	His	Ser	*Leu*	Gly	Ser	Arg	Arg	Thr	Leu	Met	Leu	Leu	Ala	Gln	Met	Arg	Lys Ile Ser	0.6
Human IFN-α4	(7-25)	His	Ser	*Leu*	Gly	Asn	Arg	Arg	Ala	Leu	Ile	Leu	Leu	Ala	Gln	Met	Gly	Arg Ile Ser	0.08
Human IFN-α5	(7-25)	His	Ser	*Leu*	Ser	Asn	Arg	Arg	Thr	Leu	Met	Ile	Met	Ala	Gln	Met	Gly	Arg Ile Ser	0.16
Human IFN-α6	(7-25)	His	Ser	*Leu*	Gly	His	Arg	Arg	Thr	*Met*	Met	Leu	Leu	Ala	Gln	Met	Arg	Arg Ile Ser	0.4
Human IFN-α7	(7-25)	His	Ser	*Leu*	Arg	Asn	Arg	Arg	Ala	Leu	Ile	Leu	Leu	Ala	Gln	Met	Gly	Arg Ile Ser	0.13
Human IFN-α8	(7-25)	His	Ser	*Leu*	Gly	Asn	Arg	Arg	Ala	Leu	Ile	Leu	Leu	Ala	Gln	Met	Arg	Arg Ile Ser	0.5
Human IFN-αH	(7-25)	His	Ser	*Leu*	Asn	Asn	Arg	Arg	Thr	Leu	Met	Leu	Met	Ala	Gln	Met	Arg	Arg Ile Ser	0.5
Human IFN-αC	(7-25)	His	Ser	*Leu*	Gly	Asn	Arg	Arg	Ala	Leu	Ile	Leu	Leu	Gly	Gln	Met	Gly	Arg Ile Ser	0.1
Mouse IFN-β	(10-29)	Glu	Arg	Thr	Asn	Ile	Arg	Lys	Cys	Gln	Glu	Leu	Leu	*Glu*	Gln	Leu	Gly	Lys Ile Asn	3.2
Human IFN-β	(10-29)	Gln	Arg	Ser	Ser	Asn	Phe	Gln	Cys	Gln	Lys	Leu	Leu	Trp	Gln	Leu	Gly	Arg Leu Glu	126

Fig. 2 Sequence homology between LFA-1 and interferons. Residues homologous between interferons and LFA-1 are boxed; homologies with Mac-1 are italicized. α-, β- and γ-interferon sequences[14,20,21] were aligned as described previously[22,23]. Asp 25 of IFN-β was deleted for alignment[22]. The right-hand column describes the percentage probability that homology results from chance alone, as determined by summation of the log odds of each amino-acid pair derived from the mutation data matrix of Dayhoff[24].

Table 1 Homology between LFA-1, Mac-1 and interferons

	% Identity				
	Mac-1	Rodent IFN-α	Human IFN-α	IFN-β	IFN-γ
LFA-1	33	32	35	26	5
Mac-1	—	15	7	3	6
Rodent IFN-α	—	—	55	20	0
Human IFN-α	—	—	—	27	1
Murine and human	—	—	—	—	5

The numbers refer to the percentage homology between the sequences depicted in Figs 1 and 2. Homologies between residues 7–25 of the mouse and human interferons were calculated and averaged in the groups shown.

α-interferons reveals homology of LFA-1 with all of them (Fig. 2). Note that the LFA-1 and IFN-α sequences align without any gaps. IFN-α shows ~30% homology to fibroblast interferon (IFN-β). LFA-1 shows a lesser degree of homology with IFN-β (Fig. 2). The homologies with IFN-α are highly statistically significant (Fig. 2).

Homology with LFA-1 occurs in different positions in rodent and human IFN-αs. Despite the higher homology of LFA-1 to human IFN-α1 than to any other interferon, an additional homology found exclusively with rodent IFN-α occurs at residues 1 and 2 in LFA-1 (Fig. 2). Also, conserved substitutions occur at many positions, including 10 and 11, where no identities are found. These findings further strengthen the homology between LFA-1 and IFN-α.

The homology among LFA-1, Mac-1 and the interferons is summarized in Table 1. Mac-1 and LFA-1 are about as homologous with one another as is LFA-1 with IFN-α. The significance of the 32–35% homology between LFA-1 and IFN-αs is emphasized by the fact that it is stronger than the 20–27% homology observed between IFN-α and IFN-β in the segments compared with LFA-1, or the homology of 29% along the total lengths of IFN-α and IFN-β[15]. Furthermore, the homology between LFA-1 and the α- and β-interferons is stronger than the homology between IFN-γ and the α- and β-interferons.

Our findings suggest that the LFA-1/Mac-1 α-subunit family and the interferon family arose from a single ancestral gene and can be considered as a superfamily. This is remarkable for several reasons. First, LFA-1 and Mac-1 are surface receptors, whereas the interferons are secreted mediators. Second, there are striking differences in size. The LFA-1 and Mac-1 α-polypeptide chains (M_r 180,000 and 170,000) are roughly 10 times longer than those of the interferons ($M_r \sim 20{,}000$). Despite the difference in size, the N-terminal sequence of LFA-1 aligns only six residues away from the N-terminus of IFN-α. A common evolutionary mechanism for obtaining large proteins is tandem duplication and joining of domains. Further sequencing of LFA-1 is needed to determine whether it contains the homologue of an entire interferon domain and whether it contains duplications of these domains.

It is tempting to speculate that the LFA-1/Mac-1/interferon superfamily could have functional as well as structural interrelationships. All of these molecules function in cell interactions of the host defence system. Unlike the immunoglobulin superfamily[16], none of them is involved in specific antigen recognition and their importance in host defence could have pre-dated the evolution of specific immunity.

We thank Dr R. Doolittle for homology searches with the NEWAT data base and Mr S. Thompson for technical assistance in protein purification. This work was supported by NIH grants CA-31798, CA-31799, and GM-06965 and an American Cancer Society faculty award to T.A.S.

Received 13 November 1984; accepted 7 January 1985.

1. Davignon, D., Martz, E., Reynolds, T., Kürzinger, K. & Springer, T. A. *J. Immun.* **127**, 590–595 (1981).
2. Springer, T. A., *et al. Immun. Rev.* **68**, 111–135 (1982).
3. Pierres, M., Goridis, C. & Golstein, P. *Eur. J. Immun.* **12**, 60–69 (1982).
4. Beller, D. I., Springer, T. A. & Schreiber, R. D. *J. exp. Med.* **156**, 1000–1009 (1982).
5. Anderson, D. C. *et al. J. clin. Invest.* **74**, 536–551 (1984).
6. Springer, T. A., Thompson, W. S., Miller, L. J., Schmalstieg, F. C. & Anderson, D. C. *J. exp. Med.* **160**, 1901–1918.
7. Kürzinger, K., Ho, M. K. & Springer, T. A. *Nature* **296**, 668–670 (1982).
8. Kürzinger, F. & Springer, T. A. *J. biol. Chem.* **257**, 12412–12418 (1982).
9. Trowbridge, I. S. & Omary, M. B. *J. exp. Med.* **154**, 1517–1524 (1981).
10. Sanchez-Madrid, F., Simon, P., Thompson, S. & Springer, T. A. *J. exp. Med.* **158**, 586–602 (1983).
11. Wright, S. D. *et al. Proc. natn. Acad. Sci. U.S.A.* **80**, 5699–5703 (1983).
12. Sanchez-Madrid, F., Nagy, J., Robbins, E., Simon, P. & Springer, T. A. *J. exp. Med.* **158**, 1785–1803 (1983).
13. Doolittle, R. F. *Science* **214**, 149–159 (1981).
14. Weissmann, C. *et al. Phil. Trans. R. Soc.* **B299**, 7–28 (1982).
15. Taniguchi, T. *et al. Nature* **285**, 547–549 (1980).
16. Williams, A. F. *Nature* **308**, 12–13 (1984).
17. Hunkapiller, M. W., Lujan, E., Ostrander, F. & Hood, L. E. *Meth. Enzym.* **91**, 227–236 (1983).
18. Hewick, R. M., Hunkapiller, M. W., Hood, L. E. & Dreyer, W. J. *J. biol. Chem.* **256**, 7990–7997 (1981).
19. Hunkapiller, M. W. & Hood, L. E. *Meth. Enzym.* **91**, 486–493 (1983).
20. Higashi, Y. *J. biol. Chem.* **258**, 9522–9529 (1983).
21. Gray, P. W. & Goeddel, D. V. *Proc. natn. Acad. Sci. U.S.A.* **80**, 5842–5846 (1983).
22. Sternberg, M. J. E. & Cohen, F. E. *Int. J. biol. Macromolec.* **4**, 137–144 (1982).
23. Epstein, L. B. *Nature* **295**, 453–454 (1982).
24. Dayhoff, M. O. & Hunt, L. T. *Meth. Enzym.* **91**, 524–545 (1983).

Printed in Great Britain by Macmillan Production Ltd., Basingstoke, Hampshire