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THE IMPORTANCE OF THE Mac-1, LFA-1 GLYCOPROTEIN FAMILY IN ADHERENCE-DEPENDENT INFLAMMATORY FUNCTIONS: INSIGHTS FROM AN EXPERIMENT OF NATURE

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INTRODUCTION

Cell surface adherence reactions are of central importance in a wide spectrum of granulocyte, monocyte, and lymphocyte functions which contribute to host defense against infection. Granulocyte and monocyte translocation in vitro and mobilization in vivo are influenced by the nature of cell-substrate adherence interactions (Smith and Hollers 1980). Studies employing time lapse photography have shown that granulocytes adhere preferentially to vascular endothelium adjacent to a site of inflammation prior to their diapedesis into tissues (Atherton and Born 1972). This "directed" adherence is facilitated by byproducts of inflammation such as C5a which bind to specific receptors on granulocytes and monocytes and inititate a sequence of events that enhance cellular adherence (Tonnesen et al. 1984).

Adhesive interactions are fundamental to other granulocyte and monocyte functions. Specific recognition of opsonized microorganisms is facilitated by membrane receptors for IgG and for the third component of complement (C3), which mediate cell-microbe adhesion prior to triggering of endocytosis. Adhesion mediated by IgG (Fc) receptors is also required for antibody-dependent cytotoxicity of target cells. In the absence of opsonins, some microorganisms may adhere to granulocytes and monocyte-macrophages without undergoing ingestion or may be phagocytosed non-specifically, depending on the physical properties of the microorganism.

Many different cell surface proteins are important in these events. Increasing evidence indicates that the Mac-1, LFA-1 family of glycoproteins (Table1) are of ubiquitous importance in the aforementioned granulocyte and monocyte adhesion reactions, and additionally in lymphoid adhesive interactions such as those required for T lymphocyte or natural killer cell cytotoxicity. These molecules appear to synergize with other receptors or function independently to regulate or mediate a panoply of functional interactions. The wide variety of these functions, and their common dependence on cell adhesion, suggests that the Mac-1, LFA-1 glycoproteins are of general importance in leukocyte adhesion phenomena, and thus, may be analogous to the adhesion molecules of other tissues, such as the nervous system (N-CAM) or Liver (L-CAM).

Table 1. The Mac-1, LFA-1 Family[a]

	Mac-1 (OKM1,Mo1)	LFA-1	p150,95
Subunits ($M_r \times 10^{-3}$)	αM β (170, 95)	αL β (180, 95)	αX β (150, 95)
Cell Distribution	Monocytes Macrophages Granulocytes Large Gran. Lymph.	Lymphocytes Monocytes Granulocytes Large Gran. Lymph.	Monocytes Granulocytes
Stimulation Increases Surface Expression	+	-	+
Functions Inhibited by Monoclonal Antibodies	Complement Receptor Type Three. Granulocyte Adherence, Stimulated Adherence, Spreading, Aggregation, and Chemotaxis.	Cytolytic T Lymphocyte-Mediated Killing and T Helper Cell Responses. Natural Killing. Antibody-Dependent Cellular Cytotoxicity. Phorbol Ester-Stimulated Lymphocyte Aggregation.	?

Common Features: The β subunits appear identical. The α subunits αM and αL are 35% homologous in sequence. The α and β subunits are noncovalently associated in $\alpha_1\beta_1$ complexes. Both α and β subunits are glycosylated and exposed on the cell surface. All functions shown require divalent cations.

Footnote to Table 1

a. Reviewed in Sanchez-Madrid et al. 1983, Springer and Anderson 1985 a,b. An important physiologic property of selected proteins of the Mac-1 family as well as other critical leukocyte receptors (e.g. CR1) is the capacity of inflammatory stimuli to "up regulate" or enhance their surface expression.

Importantly, chemotactically relevant concentrations of the chemoattractants f-Met-Leu-Phe and C5a, and the secretagogues phorbol myristate acetate and calcium ionophore stimulate a five-seven-fold increase in the amount of Mac-1 and p150,95 expressed on the surface of monocytes and granulocytes (Springer et al. 1984). In contrast, the related LFA-1 glycoprotein is not increased. "Up regulation" is maximal after ≃ 8 minutes at 37° C, and is not impeded by inhibitors of protein synthesis (D. Anderson, L. Miller, and T. Springer, unpublished). Thus, Mac-1 and p150,95 are stored in a latent pool in granulocytes and monocytes, and can be rapidly mobilized to the cell surface by inflammatory stimuli. In granulocytes, the intracellular Mac-1 pool co-sediments in sucrose gradients with secondary granules (Todd et al. 1984), but further experiments are needed before it can be definitively established whether Mac-1 and p150,95 are stored in the membrane enclosing secondary granules or some other secretory vesicle. The location of the latent pool in monocytes has not yet been examined.

A major objective of this paper is to provide evidence that the Mac-1 glycoproteins regulate monocyte and granulocyte adherence, chemotaxis and other adherence-dependent functions, in vitro, and are required for leukocyte recruitment into inflammatory sites in vivo. Two types of studies are presented. The first utilizes leukocytes from patients with a recently discovered heritable deficiency of the entire Mac-1 glycoprotein family (Anderson, etal. 1985). The other type utilizes monoclonal antibodies (MAb) to these glycoproteins (Sanchez-Madrid et al. 1983) together with normal leukocytes in vitro. A number of MAbs have been obtained which are specific for the αM, αL, or αX subunits, and thus react only with Mac-1, LFA-1, or p150,95, respectively. Another type of MAb reacts with the common β subunit, and hence with all three of these glycoproteins (Table 1).

Mac-1, LFA-1 Deficiency Disease. Recently, a disease has been recognized in which the Mac-1, LFA-1, and p150,95 glycoproteins are deficient (Anderson et al. 1985 and Springer et al. 1984). Patients have recurrent, life-threatening bacterial infections, a lack of pus formation, and persistent granulocytosis. The deficiency affects all cell lineages which normally express the Mac-1, LFA-1 glycoprotein family, i.e., monocytes, granulocytes, and lym-

phocytes, and cell lines established from patients. Deficiency is inherited as an autosomal, recessive mutation. Each of the three α subunits, and the common β subunit, is deficient from the surface of all patients' cells, as shown by immunofluorescent flow cytometry and immunoprecipitation with MAb specific for each subunit. Two phenotypes have been defined, severe deficiency and moderate deficiency, with surface expression of < 0.2% and 5-30% respectively, of normal amounts of Mac-1, LFA-1, and p150,95 (Anderson et al. 1985) (Fig. 1). Patient granulocytes show normal surface expression of the Fc receptor, the complement receptor type 1 (CR1), and many other markers surveyed in an international monoclonal antibody workshop (Springer and Anderson 1985b).

FIGURE 1

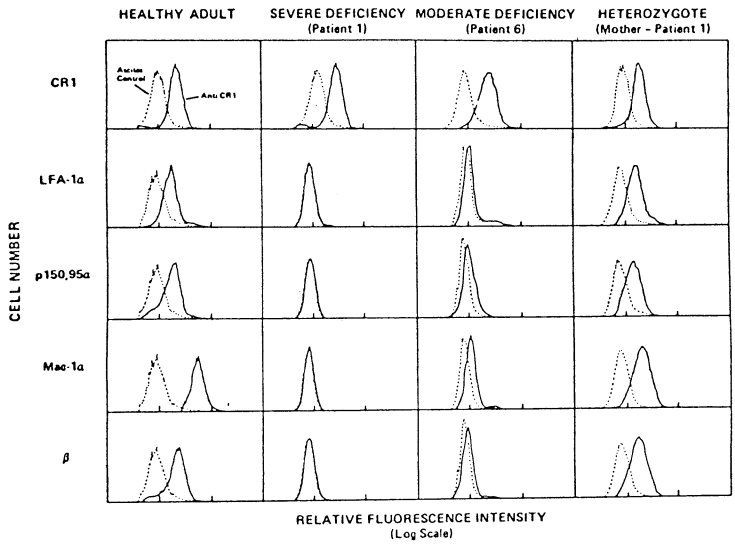
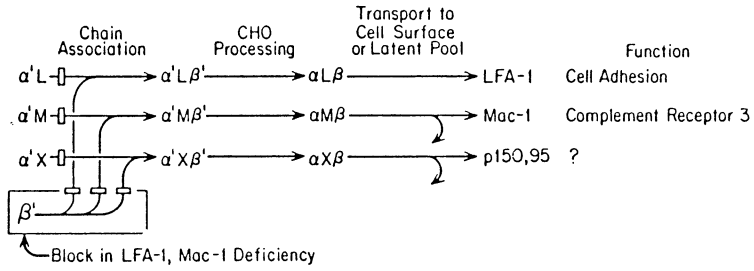


Figure 1 - Immunofluorescence flow cytometry of granulocytes of representative severe and moderate deficiency patients, a heterozygote, and a healthy adult. Unstimulated granulocytes were indirectly stained with antibodies to the CR1 or the indicated α or β subunits (solid lines) or control MAb (dashed lines).

In both phenotypes, the underlying defect is in the common β subunit (Springer et al. 1984), as summarized in Fig. 2. In normal cells, α and β subunit precursors (α L' and β ') are synthesized which become noncovalently associated, probably in the endoplasmic reticulum, and transported to the Golgi, where carbohydrate processing and a slight increase in molecular weight occurs. The mature molecules are then transported to the cell surface or to intracellular storage sites. Patient cells, however, appear to lack β subunit synthesis or to make it only in small amounts. Normal α' precursors are made, but do not undergo carbohydrate processing, suggesting biosynthesis is blocked prior to the Golgi. β association appears required for processing and transport to the surface. The α chains are not expressed on the surface (severe deficiency) or in amounts which appear stoichiometrically limited by the small quantity of β produced (moderate deficiency). In addition to surface expression, patient granulocytes and monocytes lack the intracellular pool of Mac-1 and p150,95. After stimulation with f-Met-Leu-Phe or PMA, there is little if any increase in Mac-1 and p150,95 surface expression (Springer et al 1984, Anderson et al. 1985).

FIGURE 2



Functional Consequences of Deficiency. The effects of this deficiency disease have taught us much about the importance of the Mac-1, LFA-1 glycoprotein family in leukocyte adhesion and migration (Anderson et al. 1984, 1985). The first known function of Mac-1 was as the complement receptor type 3, which mediates binding and phagocytosis of particles opsonized with the iC3b ligand (Wright et al. 1983, Anderson et al. 1984). However, the functional defects are much broader than this. The recurrent soft

tissue infections in patients appear due to an inability of granulocytes and monocytes to migrate into inflammatory sites (Anderson et al. 1984, 1985). There is an absence of pus formation and consequently, the occurance of common necrotic, ulcerative skin or mucus membrane infections. This has been confirmed by biopsies of infected tissues (gingiva, skin or Rebuck skin windows) which show profoundly impaired leukocyte mobilization. Following Rebuck skin abrasions, healthy control individuals show immigration of neutrophils at 2 and 4 hours followed by monocytes at 6 hours. Severely deficient patients showed no mobilization of neutrophils or monocytes to the site even at the 24 hour timepoint, and leukocyte mobilization in moderately deficient patients is strikingly diminished and delayed. Thus, Mac-1, LFA-1 deficiency results in a profound defect in the ability of leukocytes to diapedes i.e. to leave the circulation by migrating between endothelial cells and through the basement membrane and accumulate at inflammatory sites.

This dysfunction correlates with <u>in vitro</u> defects in adherence and adhesion-dependent functions including chemotaxis, aggregation, orientation and phagocytosis of iC3b coated particles (Table 2) (Anderson et al. 1984, 1985). In additional patient studies, defects of granulocyte antibody-dependent cellular cytotoxicity, mononuclear cytotoxicity and cytolytic T-lymphocyte responses have been consistantly demonstrated; in each assay, disturbed cytotoxic functions are related to diminished target cell binding. Observed abnormalities of lymphocyte proliferative responses to mitogens or allogeneic stimulation are presumably also related to impaired cell-cell interactions. With respect to many of these assays, the relative severity of functional abnormalities documented is directly related to the degree of molecular deficiency and severity of clinical infections or other manifestations in selected patients or kindreds.

TABLE 2

ASSESSMENTS OF ADHERENCE DEPENDENT GRANULOCYTE FUNCTIONS#

FUNCTIONAL ASSAY	SEVERE* DEFICIENCY	MODERATE** DEFICIENCY	HEALTHY ADULTS
CHEMOTAXIS			
f-Met-Leu-Phe (10^{-8}M)	43 ± 6°°	66 ± 7	105 ± 4
C5a	42 ± 5	68 ± 14	108 ± 7
ADHERENCE			
Baseline (PBS)	12 ± 2+	16 ± 9	38 ± 6
f-Met-Leu-Phe (10^{-8}M)	12 ± 3	28 ± 12	63 ± 6
PMA (5 µg/ml)	16 ± 4	31 ± 12	67 ± 9
AGGREGATION			
C5a	16 ± 11++	15 ± 12	100 ± 0
f-Met-Leu-Phe (10^{-7}M)	16 ± 4	14 ± 13	40 ± 6
PMA (10 µg/ml)	15 ± 9	22 ± 3	105 ± 7
PHAGOCYTOSIS			
Oil-Red-O-(IgG)	1.4±0.6 ¶	1.4±0.5	1.7±0.4
Oil-Red-O(iC3b)	1.9±1.2 ¶	2.4±1.2	7.0±3.1
C3-opsonized Zymosan	4.6±0.7 ¶¶	7.9±3.2	17.4±4.0

Data presented with respect to each functional assay is represented by mean ± 1 SD value for each patient category derived from individual patient mean values of 2-6 separate experiments. Summarized from Anderson et al. 1985.
* Includes assessments on severe deficiency Patients 1, 2 and 3.
**Includes assessments on moderate deficiency Patients 4, 6, 7 and 8.

°° Boyden assay values (mean ± 1 SD) for f-Met-Leu-Phe or C5a (10% Zymosan-activated serum) expressed as µm migration/40 min. incubation.
+ Percent of granulocytes adhering to serum (6%) coated glass under baseline or stimulated conditions at 21°C.
++ Granulocyte aggregation responses to C5a (10% Zymosan activated plasma), f-Met-Leu-Phe or PMA, at 37°C, measured by the increase in light transmittance and expressed as the % of the response to C5a.
¶ Dionylphthalate uptake (µg/10^6 granulocytes in 15 min).
¶¶ Slope of chemiluminescence evolution (CPM^2 x 10^{-5}).

In contrast to these characteristic abnormalities of adherence-dependent functions, adherence-independent functions of patient granulocytes or monocytes are uniformly normal. These include specific and saturable binding of the chemotactic factor, f-Met-Leu-Phe, normal expression and function of receptors for C5a, IgG Fc and CR1, cellular activiation and shape change in suspension, oxidative metabolic and secretory (primary and secondary granule markers) responses to solubule stimulants, and normal assembly or function of cytoskeletal proteins (Anderson et al. 1985).

Inhibition of Adherence-Dependent Functions by MAb. Binding of MAb to normal granulocytes largely reproduces the defects found in Mac-1, LFA-1 deficient patients. Both baseline and f-Met-Leu-Phe stimulated adherence are strikingly inhibited (Fig. 3). Spreading, chemotaxis, and aggregation are also inhibited. In most assays, the incorporation of MAbs during functional assessments are required

to demonstrate effects or to promote the most effective inhibition of cell function. These observations confirm that a continual temporal expression of new Mac-1 binding sites contributes to these functions *in vitro* and presumably *in vivo*. The general order of potency of inhibition is anti-β > anti-Mac-1α > anti-p150,95 α > anti-LFA-1α. This suggests that all members of the glycoprotein family may contribute to these reactions; the order of potency reflects their relative amounts on the granulocyte surface (Springer and Anderson 1985). These effects are quite specific. They are obtained with $F(ab')_2$ fragments (anti-β and anti-LFA-1 α) and are not given by IgG MAb bound to other surface molecules (the CR1 and HLA-A,B). Functions not deficient in patient cells are not inhibited by these MAbs, i.e. shape change in suspension, f-Met-Leu-Phe binding, superoxide generation, secondary granule secretion, and phagocytosis of oil-red-O (IgG).

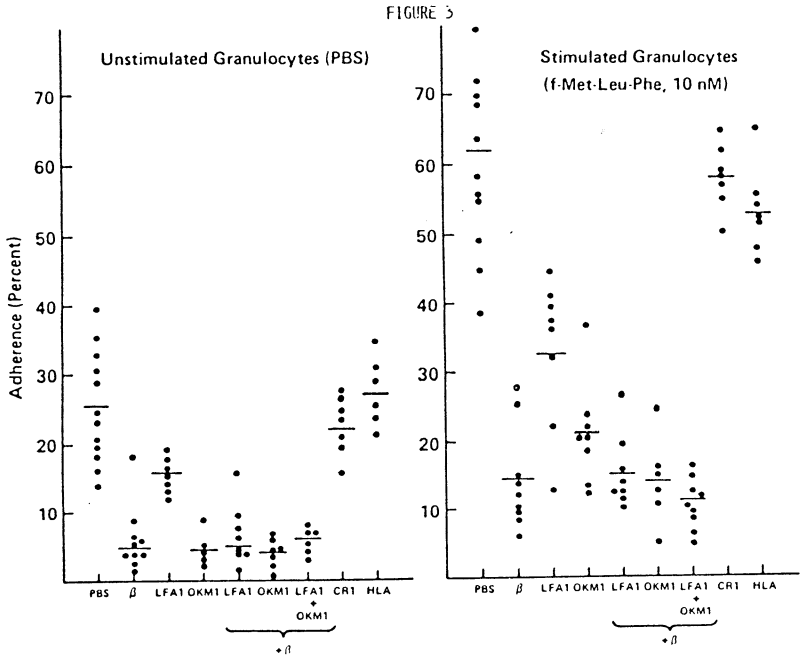


Figure 3 - Effects of MAbs to the Mac-1, LFA-1 glycoprotein family on granulocyte adherence. Granulocytes were preincubated with MAbs, washed and then incorporated into Smith Hollers adherence chambers in which they were allowed

to adhere to serum (6%) coated glass substrates under unstimulated (PBS) or stimulated (f-Met-Leu-Phe, 10 nM) conditions at 21°C. MAb preparations used for the studies shown included: the OKM1 MAb to Mac-1 α (5 µg/ml), $F(ab')_2$ fragments of the TS1/22 MAb to LFA-1α (5 µg/ml), and $F(ab')_2$ fragments of the TS1/18 MAb to the common β subunit of Mac-1 and LFA-1 (5 µg/ml). Control MAbs included saturating concentrations of a $F(ab')_2$ fragment of rabbit IgG directed against the human C3b receptor (anti-CR1) and a MAb against the HLA framework antigen (W6/32).

Conclusions and Speculations. The clinical, and histopathologic manifestations observed in Mac-1 deficient individuals as well as the specific molecular and functional deficits documented in patient cells suggest that many adherence-dependent inflammatory reactions of normal cells are mediated by the Mac-1, LFA-1 glycoprotein family, and specifically, that chemoattractant-stimulated hyperadherence and aggregation are mediated by an increased surface expression of Mac-1 and p150,95. MAb blocking experiments confirm these findings. We propose that *in vivo*, chemoattractants diffusing from sites of inflammation into the circulation induce Mac-1 and p150,95 "up regulation", leading to increased adherence of monocytes and granulocytes to endothelial substrates. We further propose that in analogy to the importance of the Mac-1, LFA-1, and p150,95 glycoproteins in chemotaxis *in vitro*, these glycoproteins mediate essential adherence functions during diapedesis and migration into the inflammatory sites. Thus, we propose that the most important clinical manifestation of Mac-1 deficiency (i.e. the inability of granulocytes to migrate into inflammatory sites and form pus) is due to a lack of "up regulation" of adhesiveness, which is normally regulated by the increased surface expression of Mac-1 and p150,95.

Further studies of the molecular mechanisms by which Mac-1 glycoproteins mediate or regulate adhesivity promise to provide important insights into the biology of inflammation. Whatever the mechanisms, the delivery of adhesive proteins to the cell surface in discrete packages, by fusion of specific granules or other secretory vesicles with the plasma membrane, allows an interesting speculation about the basis of stimulated migration. We propose that during stimulated granulocyte motility, secretory vesicles containing Mac-1 and p150,95 fuse with the plasma membrane

at the leading edge of the cell. The site of fusion is hypothesized to be directed by the same chemoattractant-sensing machinery that guides the ruffling lamellipodia toward the chemoattractant. A focal point of high Mac-1 and p150,95 concentration would thus be formed in the plasma membrane at the site of fusion, and adhesion would be initiated or strengthened at this focal point. After further cell translocation, this focal point or attachment would near the uropod, and by this time, diffusion of the adhesion proteins in the plane of the membrane would have lowered their concentration and secondarily, the strength of adhesion. This process could be superimposed on endocytic recycling of membrane, with endocytosis over the entire surace of the cell and readdition at the leading edge of the cell, thus allowing bulk membrane flow from the leading edge to the uropod, as has been hypothesized to effect cell locomotion (Bretscher 1984).

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