Deficiency of a Leukocyte Surface Glycoprotein (LFA-1) in Two Patients with Mo1 Deficiency

Effects of Cell Activation on Mo1/LFA-1 Surface Expression in Normal and Deficient Leukocytes

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Abstract. Mo1, a phagocyte surface glycoprotein heterodimer, is involved in a number of phagocyte adhesion functions such as binding and ingestion of serum-opsonized particles, zymosan-induced degranulation, and superoxide generation. Deficiency of this antigen in humans has been associated with increased susceptibility to recurrent bacterial infections. The beta subunit of Mo1 is shared by another surface glycoprotein named LFA-1, which is involved in lymphocyte proliferation, cytolytic T cell, and natural killing activities. Two unrelated patients with Mo1 deficiency were found to be deficient in LFA-1 as well as in the common beta subunit. Investigation of lymphocyte functions in these two patients revealed normal mixed leukocyte culture-generated cytolytic T cell and natural killing activities and significantly reduced proliferative response to phytohemagglutinin. LFA-1-deficient cells also proliferated in response to soluble antigen and different alloantigens. These responses were partially blocked by anti-LFA-1 antibody. Whereas LFA-1 was undetectable by immunofluorescence and immunoprecipitation on the patients' resting T cells, significantly reduced (~5% of normal) but detectable amounts of the heterodimeric LFA-1 antigen were found on mitogen and alloantigen-activated T cells. On granulocytes, Mo1 surface expression was also dependent on the state of cellular activation. The amount of surface Mo1 present on resting normal granulocytes increased by 3–10-fold following exposure to stimuli that induced degranulation, suggesting the presence of a major intracellular pool for this antigen. Analysis of subcellular fractions from granulocytes showed that intracellular Mo1 is located primarily in the specific granule fraction. Activated granulocytes had little or no increase in their surface expression of LFA-1 antigen. Deficient granulocytes had significantly increased numbers of Mo1 antigen expressed on the surface following stimulation with calcium ionophore (1 μM). However, the amount expressed continued to be significantly reduced compared with normal cells. Quantitation of surface Mo1 on granulocytes exposed to calcium ionophore (1 μM) showed that both parents in one family but only the mother in the other family had significantly reduced levels of Mo1, suggesting heterogeneity in the inheritance of this disorder. Whereas LFA-1 deficiency on lymphocytes was associated with normal alloantigen-induced cytolytic T cell and natural killing activities in these two patients, functions which were in part dependent on small amounts of detectable LFA-1 antigen, the Mo1 deficiency state led to significant defects in phagocyte adhesion functions. Hence, the clinical symptoms associated with this combined defi-

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ciency state reflect a more profound phagocyte than lymphocyte disorder.

Introduction

Adherence between cells of the immune system, such as lymphocytes, monocytes, or granulocytes, and other cells or particles is an important step in the complex series of events that mediate such biologic functions as cell-mediated killing, phagocytosis, chemotaxis, degranulation, and superoxide anion production. The use of monoclonal antibodies to cell surface structures has identified two distinct surface molecules (Mo1 and LFA-1) which appear to be involved in some of these adherence reactions (1–3). Mo1, a structure closely associated or identical with the human complement C3bi receptor, consists of two noncovalently linked glycoproteins of 155 (alpha subunit) and 94 kD (beta subunit) (1, 4, 5). It is present on human monocytes, granulocytes, and null cells (5, 6). LFA-1, an antigen associated with lymphocyte functions, is also a heterodimer (alpha subunit, 177 kD; beta subunit, 94 kD) and is present on T and B cells, monocytes, and granulocytes (2, 3). Previous studies in the mouse (3, 7) and human systems (8; Borst, J., and C. Terhorst, unpublished data) demonstrated that the beta subunits of LFA-1 and Mo1 (or the related epitopes, Mac-1 and OKM1) are highly homologous or identical. Monoclonal antibodies directed to Mo1 on phagocytes or LFA-1 on lymphocytes inhibit the rate of phagocytosis of opsonized particles or lymphocyte-mediated cytolysis, respectively (1, 2), a finding that is consistent with their function as adherence molecules.

Recently, two unrelated patients with recurrent bacterial infections and Mo1 deficiency were described (9–12). The patients' phagocytes were deficient in both the alpha and beta subunits of Mo1 and had impaired phagocytosis of opsonized particles as well as defective zymosan-induced degranulation and superoxide generation. In this report, we present data showing that leukocytes from these two patients are also deficient in LFA-1. We also determined the effects of LFA-1 deficiency on lymphocyte proliferation and cytotoxic activities. The amount of LFA-1 and Mo1 expressed on the surface of lymphocytes and granulocytes, respectively, was critically dependent on the state of activation of these cells. Detectable but significantly reduced amounts of LFA-1 antigen with normal mobility on polyacrylamide gels were seen on activated but not resting T cells from these patients. Activated granulocytes from the parents of one patient but only the mother of the other patient expressed significantly reduced amounts of Mo1, suggesting heterogeneity in the mode of inheritance of this disease.

Methods

Cell separation. Human peripheral blood leukocytes (PBL) and nonadherent cells containing predominantly T lymphocytes were isolated as detailed elsewhere (5). Granulocytes were purified at 4°C on cushions of Ficol-Hypaque (specific gravity 1095) followed by hypotonic lysis of the contaminating erythrocytes (modified from reference 13). Monocytes were obtained by adherence of PBL to petri dishes (5), or by centrifugation of PBL on a Percoll gradient (14). B cell lines Laz 509, 388, Namalva, and Daudi were kindly provided by Dr. H. Lazarus, Dana Farber Cancer Institute.

Monoclonal antibodies. Mouse ascites containing monoclonal antibodies L1, L5, or L11 (all of the IgG1 noncomplement fixing subclass) directed against a lymphocyte function-associated antigen (LFA-1) (15) as well as antibodies to I-2 (Ia) (16), Mo1, Mo2, Mo5, and Mo6 (5, 6) were obtained as described. L1, L5, or L11 antibodies were not directly cytotoxic to T cells (15). Monoclonal antibodies to T3, T4, T8, and TA-1 were generously provided by Dr. S. F. Schlossman (17) (Dana Farber Cancer Institute) and Dr. T. LeBien (University of Minnesota) (18).

Other reagents. The following reagents were purchased: calcium ionophore A23187 (Eli Lilly and Co., Indianapolis, IN), phorbol myristate acetate (Consolidated Midland Chemical Co., Brewster, NY), concanavalin A (Con A)[1] (Calbiochem-Behring Corp., La Jolla, CA), phytohemagglutinin (PHA) (Wellcome Reagents Ltd., Beckenham, England), C5a desarg (Upjohn Co., Kalamazoo, MI), dimethyl sulfoxide and *f*-Met-Leu-Phe (Sigma Chemical Co., St. Louis, MO), and Percoll (Pharmacia Fine Chemicals, Piscataway, NJ).

Immunofluorescence and immunoprecipitation studies. Indirect immunofluorescence was performed by exposing test cells to saturating amounts of monoclonal antibodies followed by staining with a fluorescein-conjugated goat anti-mouse immunoglobulin reagent as previously described (5). Immunofluorescence intensity as a quantitative measure of relative antigen expression was analyzed on an Ortho cytofluorograph FC 200/4800 A (Ortho Instruments, Westwood, MA) using a logarithmic amplifier. The channel number (log scale) representing the peak fluorescence intensity of 10,000 cells exposed to either experimental or control antibodies was determined. Linear fluorescence intensity channels were computed from a logarithmic-linear calibration curve (19). Immunoprecipitation from ^{125}I-surface labeled granulocytes or PHA-generated lymphoblasts using monoclonal antibodies to LFA-1 or anti-Mo1 were performed and analyzed exactly as described elsewhere (1).

Quantitation of cell surface Mo1 antigen was also done using a direct binding assay (1). Briefly, 75 μl of granulocytes (3×10^7/ml for untreated cells, or 1×10^7/ml for calcium ionophore-treated cells) were incubated with increasing amounts of ^{125}I-Fab anti-Mo1 in the presence or absence of a 100-fold molar excess of unlabeled antibody in a total volume of 100 μl of phosphate-buffered saline (PBS), pH 7.4, containing 0.5% human serum albumin. Incubation was done for 40 min at 0°C, after which granulocytes were separated from unbound antibody on phthalate oil cushions and the specific binding calculated (1).

Lymphocyte proliferation and cytotoxicity assays. Proliferation and cytolytic T lymphocyte (CTL) assays were performed as previously described (modified from Reference 17). Briefly, PBL (2×10^5) from patient or control individuals were cultured in medium containing tetanus toxoid (TT) 1:800 final dilution (State Biological Labs, Department of Public Health, Jamaica Plain, MA), PHA (0.125–0.5 μg/ml final concentration), or Con A (6.25–62.5 μg/ml) in a total volume of 200 μl of RPMI 1640 (Microbiological Associates, Walkersville,

1. *Abbreviations used in this paper:* Con A, concanavalin A; CTL, cytolytic T lymphocyte; MLR, mixed leukocyte reaction; PHA, phytohemagglutinin; TT, tetanus toxoid.

MD) containing 10% heat-inactivated human AB serum. After 4 d (PHA or Con A) or 5 d (TT), cells were pulsed with 0.8 μCi [3H]thymidine (1.9 Ci/mM specific activity, Schwartz Mann, Spring Valley, NY) and were harvested 16 h later on a Mash 2 apparatus (Microbiologic Associates). [^{3}H]Thymidine incorporation was measured in Packard scintillation counter (Packard Instruments Co., Downers Grove, IL). To determine the effect of the IgG1 anti-LFA-1 on these proliferative responses, the monoclonal antibody or anti-Mo6, a control antibody of an identical subclass, was introduced at the start of the proliferation assay in 1:120 or 1:256 final concentration. Mixed leukocyte reaction (MLR) of patient or control cells in response to four different B cell lines Laz 509, Laz 388, Namalva, or Daudi was determined in an identical fashion except that 1.6×10^3–5×10^4 of these mitomycin C-treated cells were added per well instead of TT, PHA, or Con A, and cultures were harvested on day 6 after a 16 h [^{3}H]thymidine pulse. To measure the effect of PHA or MLR stimulation on LFA-1 surface expression, lymphoblasts were harvested on day 5 (PHA) or day 6 (MLR) and indirect immunofluorescence or immunoprecipitation from ^{125}I surface-labeled cells using anti-LFA-1 monoclonal antibodies were performed and compared with that of resting cells.

For the CTL assay, target B cell lines were selected that elicited a proliferative response in the MLR assay. To generate CTL, 2×10^5 PBL from patient or control individuals were cultured with 2.5×10^4 mitomycin C-treated Laz 388 or Laz 509 B cells in U-bottom microtiter wells (200 μl) for 6 d. To measure alloantigen-specific cytolytic activity, MLR-generated effector cells were harvested and incubated in microtiter plates at various effector to target cell ratios with the corresponding target cells that were previously labeled with sodium 51 chromate (^{51}Cr) (292 Ci/mM) (New England Nuclear, Boston, MA). The plates were centrifuged at 500 *g* for 5 min, and subsequently incubated for 4 h at 37°C. Specific ^{51}Cr-release into the supernatant as a measure of lysis was determined. To measure the effect of anti-LFA-1 on CTL activity, L1 or control monoclonal antibodies were introduced at the start of the 4 h CTL assay (1:120 final dilution). All assays were performed in triplicate.

Treatment of granulocytes with calcium ionophore, PMA, f-met-leu-phe, C5a desarg, or opsonized particles. Granulocytes (1×10^7/ml) were incubated for 10 min at 37°C in Krebs-lactated Ringer's buffer to which none or one of the following reagents were added: dimethyl sulfoxide (1 μl/ml), calcium ionophore (1 μM), *f*-Met-Leu-Phe (1 μM), PMA (0.1 μg/ml), and C5a desarg (125 ng/ml). Measurement of cobalamin-binding protein (20) in granulocytes before and after exposure to calcium ionophore showed that this treatment decreased the cellular content of cobalamin-binding protein by 65–75% but reduced beta glucoronidase activity by only 10–15% (data not shown). At the end of incubation, cells were washed twice in PBS, then treated with monoclonal antibodies to Mo1, Mo5, LFA-1, or a negative control followed by fluorescein-labeled goat anti-mouse Ig and cell surface fluorescence analysis (5).

Human granulocytes (400 μl of 2×10^7/ml) were exposed to lipopolysaccharide-coated oil red or serum-opsonized oil red O (100 μl) in a total volume of 1 ml for 10 min at 37°C. Following centrifugation at 220 $g \times 10$ min, the pelleted granulocytes were washed once and resuspended in 1 ml of PBS. Granulocytes were also incubated for 60 min at 37°C with sheep erythrocyte alone or coated with anti-sheep erythrocyte IgG antibody (Cordis Corp., Miami, FL). Noningested SE were hypotonically lysed and granulocytes were washed two times in PBS at 4°C and processed for immunofluorescence analysis. Phagocytosis was confirmed by microscopic inspection.

Preparation and iodination of granulocyte subcellular fractions. Granulocytes from a single donor were obtained by leukopheresis and purified on Ficol-Hypaque at 4°C. The alpha, beta, and gamma fractions were obtained by density centrifugation on Percoll exactly as described by Borregaard et al. (21). The composition of each fraction was determined by measuring myeloperoxidase (α-granules) (22), cobalamin-binding protein (specific or β-granules) (20), and alkaline phosphatase (plasma membrane [γ]) (22). Approximately 70% of total cobalamin-binding protein was in the specific granule fraction with 15% of total present in each of the alpha and gamma fractions, respectively. The membrane (gamma) fraction contained 73% of total alkaline phosphatase activity with 17 and 10% present in the beta and alpha granule fraction, respectively. The alpha granule fraction contained 68% of the total myeloperoxidase activity, with 19% and 13% of the total present in the beta and gamma fractions, respectively (data not shown). The protein content of each fraction was measured by the method of Lowry et al. (23) using human serum albumin (Armor Pharmaceutical Co., Kankakee, IL) as standard. 50 μg of each fraction were incubated for 10 min at 0°C in PBS containing phenylmethylsulfonyl fluoride (2 mM) and 0.5% Non-idet P40 (NP40), and then radiolabeled with ^{125}I (New England Nuclear) using the chloramine T method (24). A sample from each fraction was removed for trichloracetic acid precipitation and the rest of the samples were extensively dialyzed against ice-cold PBS. Immunoprecipitation using anti-Mo1 monoclonal antibody was performed as described (1). The specific activity of the alpha (azurophilic granule), beta (specific granule), and gamma (plasma membrane) fractions were 3.66×10^5, 2.10×10^5, and 1.60×10^5 cpm/μg, respectively.

Results

Granulocytes, monocytes, and nonadherent PBL containing both T and B lymphocytes from two patients with Mo1 deficiency had no detectable fluorescence when a monoclonal antibody to LFA-1 (L1) was used (Fig. 1) (Table I). L1 antibody immunoprecipitated from ^{125}I surface-labeled normal granulocyte lysates, two noncovalently linked peptides of 177,000 and 94,000 D (Fig. 2), similar to those immunoprecipitated from human lymphocytes and B lymphoblastoid cell lines (2, 15, 18). Using this same antibody, no detectable radiolabeled bands were immunoprecipitated from the patient's ^{125}I-labeled cells (Fig. 2) although ^{125}I-labeling of surface proteins of patient's was similar to that of control cells (data not shown).

Other antigens, such as I-2 (Ia), T3, T4, and T8, were expressed normally on the patients' circulating cells (Fig. 1, Table I, and data not shown). Other monoclonal antibodies to LFA-1 (L5, L11, and TA-1) were unreactive with the patients' granulocytes and resting T cells by immunofluorescence analysis suggesting that these two patients are probably deficient in the whole LFA-1 molecule rather than in a single epitope. In a similar manner, no detectable bands were immunoprecipitated from the patients' granulocytes using monoclonal antibodies to the alpha subunit of Mo1 and to the common beta subunit (reference 10 and data not shown).

Alloantigen, mitogen, and antigen-induced proliferation of LFA-1-deficient lymphocytes. Functionally, LFA-1 antigen appears to be involved in T lymphocyte proliferation and CTL-

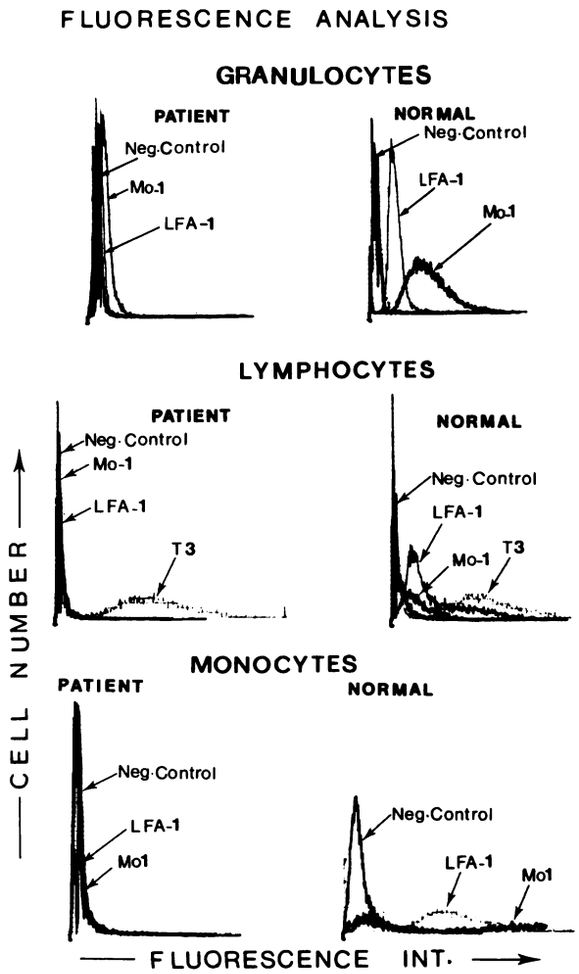


Figure 1. Immunofluorescence analysis of patient (9) and control cells using monoclonal antibodies to cell surface structures. No detectable staining on patient's granulocytes, monocytes, and PBL is noted when monoclonal antibody to LFA-1 is used. Similar analysis showed little or no staining with monoclonal antibody to Mo1 on the patient's granulocytes and monocytes, respectively.

mediated killing based on the ability of monoclonal antibodies to inhibit these functions on normal cells (2, 3, 15). The effect of LFA-1 deficiency on these functions was next examined. As shown in Table II, LFA-1-deficient lymphocytes proliferated in response to alloantigenic stimuli produced by four different B cell lines as well as to TT antigen. Mitogen-induced lymphoproliferation was normal in response to Con A (6.25–62.5 μg/ml). PHA (0.125–0.25 μg/ml) induced lymphoproliferation was significantly reduced in both LFA-1-deficient patients and was more striking at the lower concentration of the lectin (Table II and data not shown). The introduction of anti-LFA-1 (L1 or L5) inhibited the patient's proliferative responses to soluble antigen, lectins, and alloantigens by 20–60% (Table II), suggesting a partial dependence of these responses on LFA-1 antigen, despite deficient cellular expression.

*Table I. Relative Surface Expression of Mo-1 and LFA-1 on Patient 2**

Cell	Individual	Relative specific fluorescence (linear)‡		
		LFA-1	Mo-1	Mo-5
Granulocyte	Control	100	100	100
	Patient	0	23	78
		LFA-1	Mo-1	Mo-2
Monocyte	Control	100	100	100
	Patient	16	2	101
		LFA-1	T3	T4
Lymphocyte	Control	100	100	100
	Patient	1	128	99

* This patient was described by Crowley et al. (11).
‡ Numbers indicate relative specific fluorescence intensity (linear scale) computed from the peak linear fluorescence channels for cells stained with each monoclonal reagent minus the peak channels of cells stained with a nonreactive control antibody. For each antigen, the fluorescence intensity of the control individual's cells has been arbitrarily assigned 100 units.

Cytolytic T cell activity of LFA-1-deficient lymphocytes. LFA-1-deficient effector cells generated in MLR demonstrated normal CTL-mediated killing activity over a wide range of effector-to-target cell ratios (Fig. 3). Again, incubation of MLR-generated blasts with anti-LFA-1 blocked allogeneic CTL activity by 80–90% (Fig. 3).

Detection of surface LFA-1 on PHA- or MLR-generated lymphoblasts from LFA-1 deficiency patients. The fact that lymphocytes from these patients were able to generate proliferative and cytotoxic responses that were partially blocked by anti-LFA-1 antibody, despite the absence of detectable quantities of LFA-1 on the surface of resting T cells, was an enigma. Since these responses were mediated by "activated" T cells, we examined the possibility that LFA-1 antigen may be expressed on the surface of deficient cells by mitogenic or alloantigenic stimuli. We found that PHA- or MLR-generated lymphoblasts from normal individuals increased their surface expression of LFA-1 by six–eightfold compared with unstimulated cells as determined by indirect immunofluorescence analysis (Fig. 4 *A*). Whereas the patients' resting lymphocytes had no detectable levels of LFA-1, their PHA- or MLR-generated blasts had detectable amounts of both the alpha and beta subunits of LFA-1 that had normal mobility on polyacrylamide gel electrophoresis (Fig. 4 *A* and *B*). Patients' cells, however, continued to be markedly deficient (~5% of normal) in surface LFA-1 compared with controls (Fig. 4).

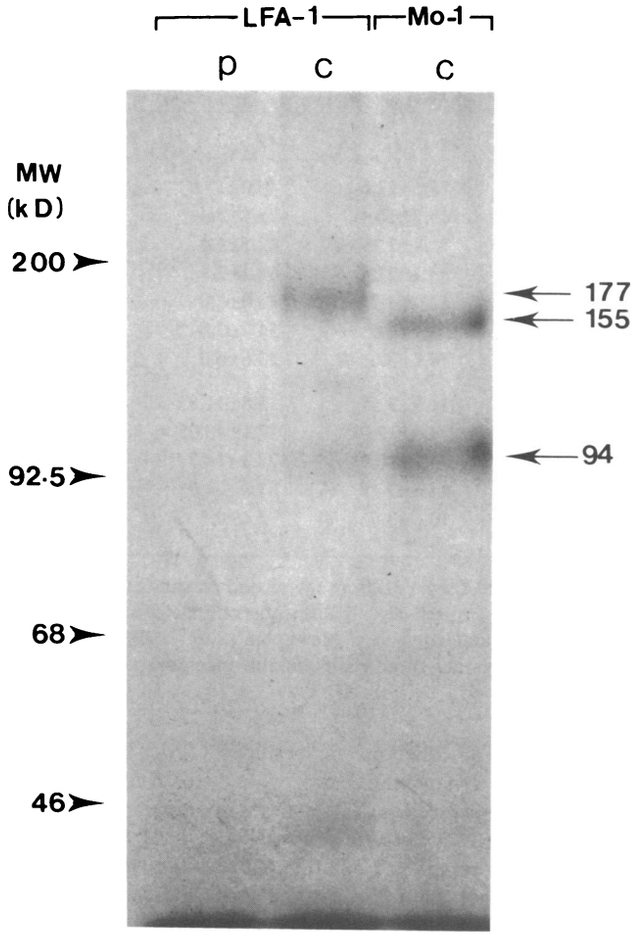


Figure 2. Radioautography of a 7% SDS-PAGE of monoclonal immunoprecipitates from ^{125}I-surface labeled granulocyte lysates. LFA-1 immuoprecipitated two radiolabeled bands of 177 and 94 kD from control (C) but not patient's (P) cells. Identical results were obtained in the other patient (not shown). The alpha (155 kD) and beta (94 kD) subunits of Mo1 immunoprecipitated from normal granulocytes are shown for comparison. Arrow heads represent molecular weight (MW) markers.

Effect of degranulation on surface expression of Mo1 in normal granulocytes. The degree of surface expression of Mo1 on normal granulocytes was also crucially dependent on the state of activation of these cells. Stimuli that induced degranulation, such as calcium ionophore, PMA, C5a, and *f*-Met-Leu-Phe, increased surface expression of Mo1 on normal granulocytes by 3–10–fold (Fig. 5). This increase in Mo1 surface expression was temperature-dependent (Fig. 5) and irreversible up to 1 h at 37°C (data not shown). Phagocytosis by granulocytes of C3 and/or IgG-opsonized particles also produced a significant increase in surface Mo1 expression (Fig. 5). Both the alpha and beta subunits of Mo1 in granulocytes were present predominantly in the specific granule fraction in addition to the plasma membrane (Fig. 6) and were expressed in a proportional manner on the plasma membrane following activation (Fig. 5, 7, and data not shown). This finding as well as the short period of incubation (10 min, 37°C) and the lack of increase in Mo1 surface expression on the myeloid cell lines KG1 and THP-1 (data not shown), which are cells that do not have specific granules, suggest that one important mechanism leading to the significant increase in surface expression of Mo1 antigen in granulocytes is probably degranulation and fusion of the specific granule fraction with the plasma membrane. None of the stimuli used led to an appreciable increase in the surface expression of LFA-1 on granulocytes, suggesting that under these conditions different mechanisms may regulate the surface expression of LFA-1 on these cells (Fig. 5).

Increased Mo1 surface expression on granulocytes from patients with Mo1 deficiency and detection of a possible carrier state. Granulocytes from the two patients with Mo1 deficiency were next examined to determine the effect of calcium ionophore on the surface expression of Mo1. As seen in Figs. 5 and 7, calcium ionophore (1 μM) produced a significant increase in Mo1 (but not LFA-1) surface expression on granulocytes from the two patients. Quantitation of expressed Mo1 sites on calcium ionophore-treated granulocytes using direct binding of ^{125}I-Fab anti-Mo1 (1) revealed that the two patients had 4.5×10^4 and 4.8×10^4 Mo1 sites/cell, respectively (Fig. 8), representing about a sevenfold increase over the levels measured on unstimulated cells (10). This number was still significantly less than that expressed on calcium ionophore-stimulated normal cells (Fig. 8). Of special interest was the finding that calcium ionophore treatment of granulocytes from the parents of the two patients led to the detection of a carrier state which was not apparent by immunofluorescence analysis of the unstimulated cells (Table III). Direct binding assays on calcium ionophore-treated cells (Fig. 8) showed that the parents of patient one (9) but only the mother of patient two (11) were possible heterozygotes for Mo1 deficiency.

Discussion

Mo1 deficiency is a phagocyte disorder that presents clinically with recurrent bacterial infections and is associated in vitro with defective granulocyte adhesion functions (1, 9–11). In this report, we found that leukocytes from two unrelated patients with Mo1 deficiency were also markedly deficient in LFA-1, another leukocyte surface glycoprotein that shares a common beta subunit with Mo1. On normal leukocytes, the surface expression of Mo1 and LFA-1 is regulated by the state of activation of the effector cell. On resting peripheral blood T cells, only a small amount of LFA-1 is expressed on the plasma membrane (Fig. 4 *A*). Upon mitogenic or alloantigenic stimulation, a marked increase in LFA-1 surface antigen is detected by immunofluorescence analysis (Fig. 4 *A*), in agreement with the observation made on human cytolytic T cell lines (2). In normal unstimulated granulocytes, a major portion

Table II. Effect of LFA-1 Monoclonal Antibody on Proliferative Response to Allogeneic MLR, TT, and Mitogens by Patient and Control PBL

		Patient's PBL*			Control PBL		
Stimulus	R:S‡	−§	+§	% Inhibition	−§	+§	% Inhibition
Namalva	1:0.5	27.3±0.5‖	10.5±1.7	62	79.1±12.6	40.0±15.0	49
Laz-388	1:0.5	54.5±3.9	42.6±3.4	22	105.7±10.4	75.4±5.4	29
Laz-388	1:0.1	16.8±4.8	18.7±1.3	−11	71.4±2.1	42.3±1.4	41
Laz-509	1:0.5	63.8±7.0	43.8±3.4	31	83.4±22.4	68.4±2.9	18
Laz-509	1:0.1	18.7±3.2	20.0±2.6	−7	62.0±3.7	43.0±7.8	31
Daudi	1:0.5	24.7±5.0	16.4±1.0	34	88.1±4.0	54.7±7.8	38
Daudi	1:0.1	5.9±2.5	5.7±1.2	3	38.2±3.0	23.6±6.0	38
PHA (0.125 μg/ml)		1.9±1.1	0.8±0.3	58	21.7±9.5¶	15.8±11.8¶	27
(0.25 μg/ml)		10.6±1.3	3.7±0.3	65	38.6±12.0¶	27.1±13.0¶	30
Con A (6.25 μg/ml)		26.8±0.6	9.1±0.7	66	24.2±10.5¶	28.2±14.3	−17
(62.5 μg/ml)		102.8±4.6	85.9±10.5	16	45.6±7.4¶	81.8±24.0¶	−79
TT (1:800 dilution)		25.1±4.7	8.4±1.5	67	16.6±3.6	2.4±0.8	83

* Measured MLR (cpm × 10^{-3}, mean±SD) to Laz 388, Laz 509, and Daudi (R:S ratio of 1:0.2) by PBL from the second patient (11) were 67.2±9.5 (control, 68.3±3.0), 57.3±4.5 (control, 88.2±4.3), and 9.3±0.3 (control, 46.7±4.3), respectively. ‡ Responder:stimulator ratio in MLR. § Proliferation obtained in the presence of anti LFA-1 (+) or a control monoclonal antibody (−). ‖ Mean cpm (×10^{-3}±SD) among triplicate determinations from a patient or a control individual. ¶ Mean cpm (×10^{-3}±SD) among mean [^{3}H]thymidine incorporation from three control individuals (both PHA and Con A responses).

of Mo1 is located intracellularly in the specific granule fraction (Fig. 6). Recently, gelatinase-containing (cobalamin-binding protein free) granules with the same density as the specific granules were described (25). Whether some of the Mo1 antigen is also present in this novel granule remains to be established. Stimuli that led to degranulation such as calcium inophore A23187, chemotactic peptides (*f*-Met-Leu-Phe or C5a), PMA, or phagocytosis produced a 3- to 10-fold increase in Mo1 surface expression. There was no comparable increase in LFA-1 surface expression although both Mo1 and LFA-1 may share the same beta subunit (8). Translocation of the Mo1 heterodimer from the specific granule fraction to the plasma membrane probably mediates the increase in Mo1 surface expression on granulocytes. The mechanism leading to an increase in surface LFA-1 on lymphocytes is unclear but may involve neosynthesis. In addition to Mo1, three other granulocyte antigens, the C3b receptor, *f*-Met-Leu-Phe receptor, and cytochrome *b* significantly increase on the surface upon stimulation (21, 26, 27). These findings suggest a common mechanism whereby granulocytes store a large proportion of functionally active molecules intracellularly and translocate them to the plasma membrane upon activation.

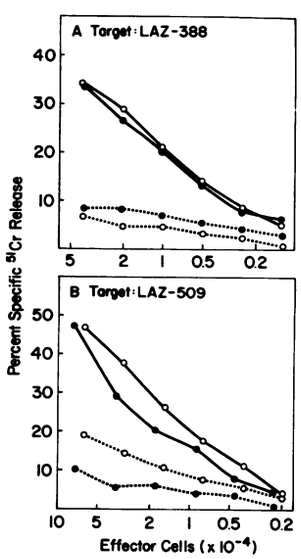
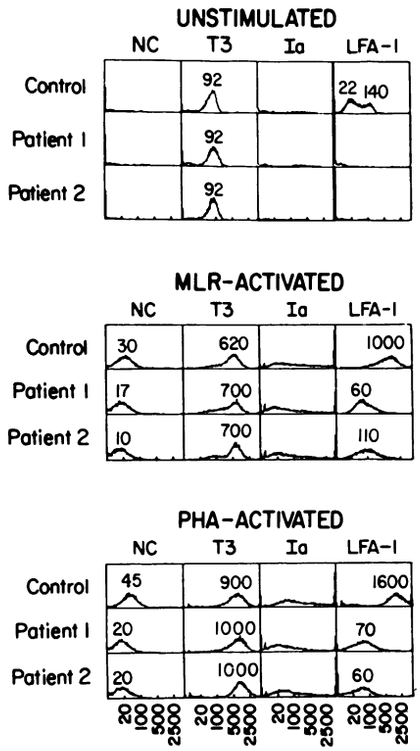
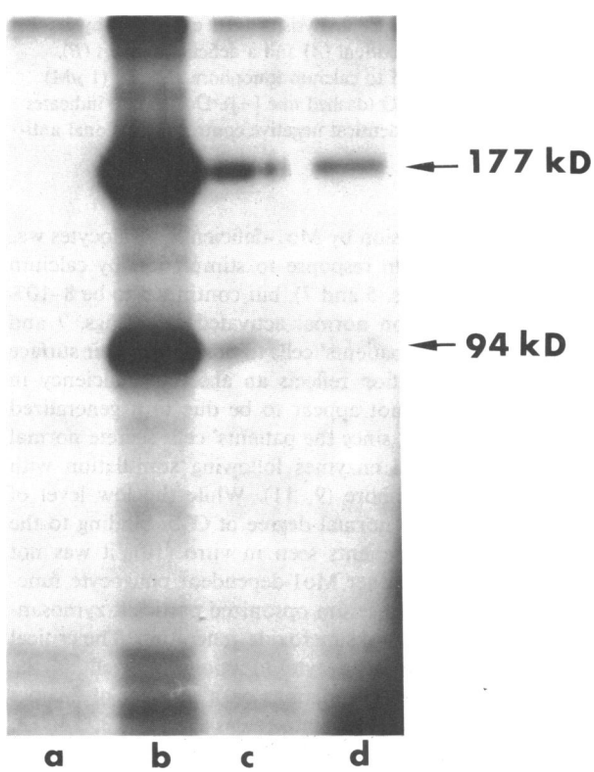


Figure 3. Effect of anti-LFA-1 monoclonal antibody on patient (9) and control CTL activity. Varying numbers of MLR-generated cytotoxic effector cells (x-axis) were cultured with 2 × 10^4 ^{51}Cr-labeled Laz-388 (*A*) or Laz-509 (*B*) target cells in medium containing anti-LFA-monoclonal antibody (- - -) or an isotype identical control antibody (——) for 4 h at 37°C. Specific ^{51}Cr-release (y-axis) as a measure of target cell lysis was determined. Closed symbols (●) and open symbols (○) indicate the mean specific ^{51}Cr-release performed by patient and control effector cells, respectively. Similar results were obtained using lymphocytes from the other patient (11) (data not shown).

LFA-1-deficient resting T cells proliferated in response to a variety of stimuli such as antigen, mitogen, and alloantigens (Table II). They also had normal T cell-mediated cytolysis of two different B cell targets and natural killing of K562 target cells (Fig. 3) (10). The partial or more complete inhibition by LFA-1 monoclonal antibodies in both patient and control cells suggested the involvement of LFA-1 antigen in these responses (Table II and Fig. 3). Whereas no detectable LFA-1 was seen on granulocytes or resting T cells (Figs. 1 and 2, Table I), PHA- or MLR-activated lymphoblasts from these patients expressed detectable amounts of LFA-1 on the cell surface (Fig. 4 *A*). Moreover, this antigen had normal mobility as determined by immunoprecipitation and sodium dodecyl sul-





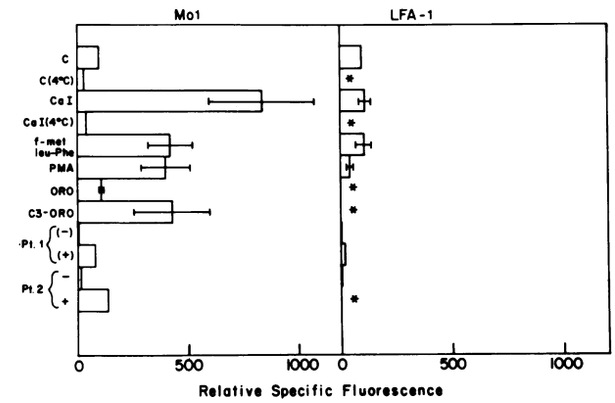


Figure 5. Surface membrane expression of Mo1 and LFA-1 after treatment of normal or deficient granulocytes with calcium ionophore, CaI, PMA, *f*-Met-Leu-Phe, or exposure to phagocytic stimuli. The fluorescence intensity of the control individual's unstimulated cells has been arbitrarily assigned 100 units. Values represent mean±SD from two to six experiments. The concentration of CaI, *f*-met-leu-phe, and C5a desarg produced half maximal surface expression of Mo1 at 5×10^{-8} M, 1×10^{-9} M, and 20 ng/ml, respectively (data not shown). Ingestion of IgG-coated sheep erythrocytes was associated with a fourfold increase in Mo1 surface expression compared with untreated or sheep erythrocyte-treated granulocytes (not shown). (−) and (+) refer to unstimulated or calcium ionophore (CaI)-treated granulocytes, respectively, from patient 1 (Reference 9) or patient 2 (Reference 11). *, not done.

fate-polyacrylamide gel electrophoresis analysis (Fig. 4 *B*). The amount of LFA-1 that was expressed on the surface of PHA- or MLR-generated lymphoblasts continued to be markedly reduced (~5% of normal) compared with normal cells (Fig. 4 *A*). The abnormally low lymphoproliferative response to PHA shown by these two patients may be a manifestation of this degree of LFA-1 deficiency (Table II). This abnormality was more apparent at lower concentrations of the mitogen (Table II), a finding which is similar to that reported recently in a

Figure 4. (*A*) Immunofluorescence analysis of LFA-1 expression relative to T3 and Ia by lymphocytes from the two patients and a control individual before and after PHA or alloantigenic (MLR) stimulation. Detectable amounts of LFA-1 were seen on stimulated (Ia positive) but not unstimulated lymphocytes from these two patients. Despite equal amounts of the T3 marker, LFA-1 expression by MLR- and PHA-activated lymphoblasts from both patients remains significantly deficient relative to control lymphoblasts. NC indicates the binding of a non-reactive control monoclonal antibody. Numbers on the X-axis and within each histogram indicate peak linear fluorescence channels. (*B*) A radioautograph of a 7.5% SDS-PAGE of LFA-1 immunoprecipitated from ^{125}I-surface labeled PHA-generated blasts from the control (lane *b*) and patients (lanes *c* and *d*). Lane (*a*) shows immunoprecipitation with a nonreactive monoclonal from ^{125}I-control cells. ^{125}I-incorporation into cell proteins from patients or control was similar (not shown).

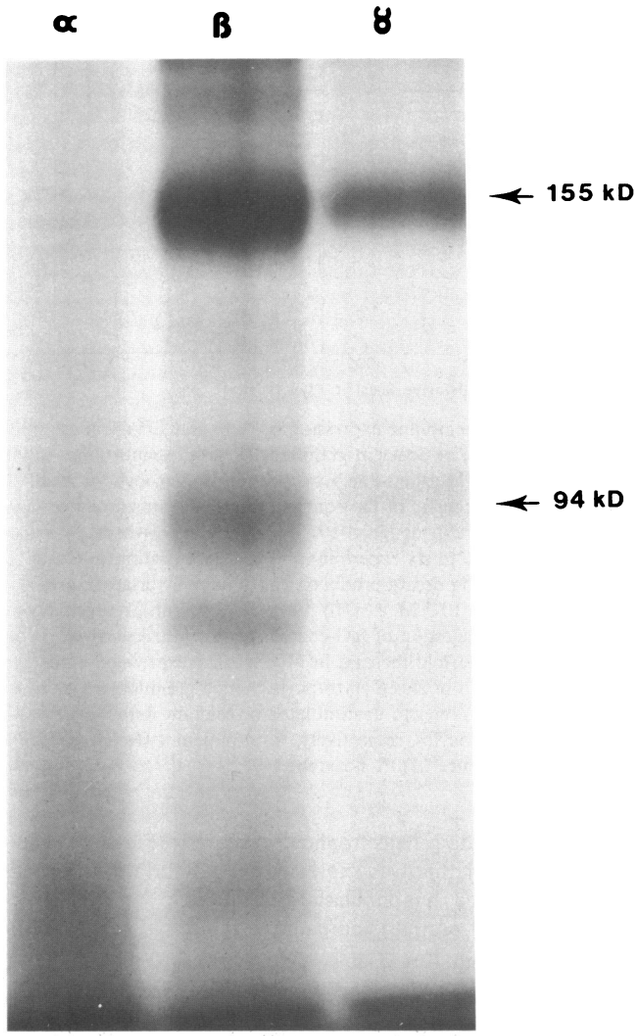


Figure 6. Radioautograph of a 9% SDS-PAGE of Mo1 immunoprecipitated from ^{125}I-labeled granulocyte subcellular fractions: alpha (primary granules), beta (specific granules), or gamma (plasma membrane). In addition to Mo1, two unidentified bands of apparent molecular weights 185 and 78 kD were seen only in the beta fraction. Mo1 was primarily detected in the plasma membrane and specific granule fractions but not in the primary granules.

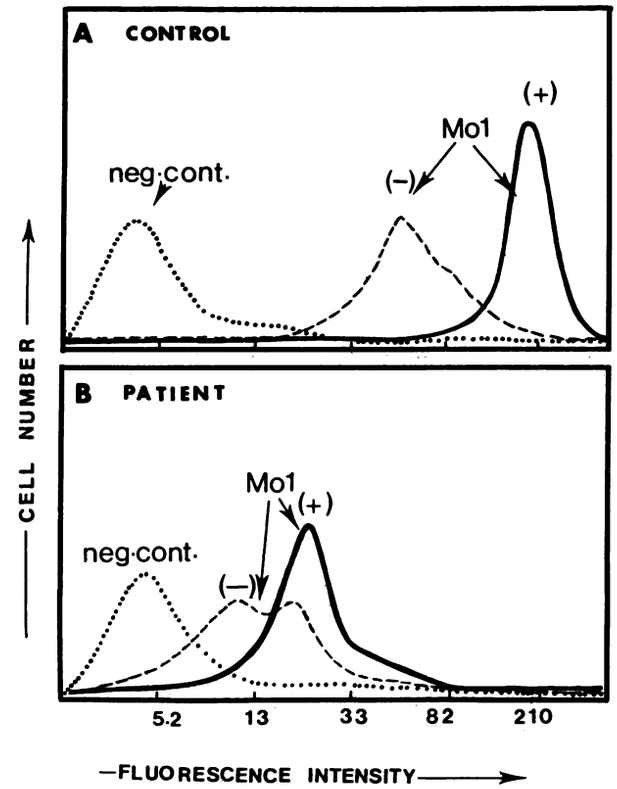


Figure 7. Immunofluorescence analysis of Mo1 expression by granulocytes from a normal individual (*A*) and a deficient patient (*B*). Granulocytes were exposed to calcium ionophore A23189 (1 μM) (solid line [+]) or to DMSO (dashed line [−]). Dotted line indicates the binding of a subclass identical negative control monoclonal antibody.

third patient with recurrent bacterial infections (28). The dependence of lymphoproliferation on the lectin concentration may also explain in part the differences seen in the ConA-induced response in our patient and that of Beatty et al. (28). While the inhibitory effect of anti-LFA-1 monoclonal antibodies suggest an involvement of LFA-1 in these T cell responses, LFA-1 deficiency may in part be compensated for by the normal expression of other functionally related molecules such as T3, T4, and T8 (29).

Surface Mo1 expression by Mo1-deficient granulocytes was significantly increased in response to stimulation by calcium ionophore A23187 (Figs. 5 and 7), but continued to be 8–10% of the levels detected on normal activated cells (Figs. 7 and 8). The inability of the patients' cells to normalize their surface Mo1 following stimulation reflects an absolute deficiency in this antigen and does not appear to be due to a generalized defect in degranulation, since the patients' cells secrete normal amounts of lyosozymal enzymes following stimulation with PMA or calcium ionophore (9, 11). While the low level of Mo1 might explain the normal degree of C3bi binding to the granulocytes of these patients seen in vitro (10), it was not sufficient to normalize other Mo1-dependent phagocyte functions such as ingestion of serum opsonized particles, zymosan-induced degranulation, and superoxide generation. The critical role of Mo1 in these phagocyte functions as well as the dependence of lymphocyte functions on a number of distinct but functionally related surface molecules such as T3, T4, T8, and LFA-1 may be one reason for the predominance of

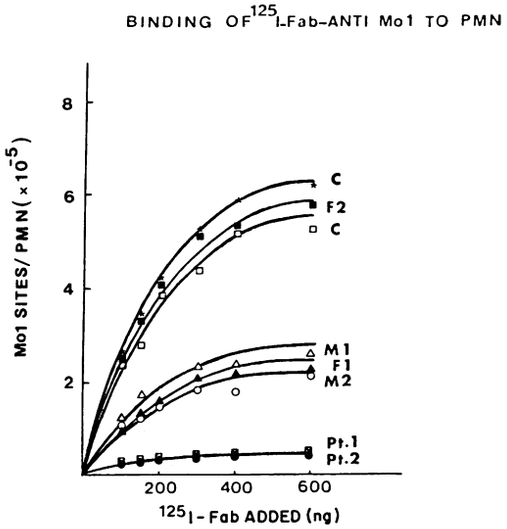


Figure 8. Binding of ^{125}I-labeled Fab anti-Mo1 monoclonal antibody to granulocytes from two normal individuals (C), a patient with Mo1 deficiency (P1) (9), his father (F1) and mother (M1), as well as the other patient described by Crowley et al. (11) (Pt2) and his parents (M2 and F2). Binding was done on granulocytes pretreated with calcium ionophore (1 μM). In all cases, half maximal saturation occurred at 1.1–1.2 μg/ml, indicating that the monoclonal antibody binds with equal affinity to Mo1 on patients, their parents and controls. Calcium ionophore-stimulated granulocytes from seven normal individuals (including the two shown in this figure) had 5.6±0.75 $\times 10^5$ (mean±SD) Mo1 binding sites/cell.

bacterial infections in these two patients with this combined immunodeficiency disorder.

Quantitation of Mo1 expressed on the surface of calcium ionophore A23187-treated cells from the parents of these two patients showed that granulocytes from the parents of one patient but only the mother of the other patient had significantly reduced numbers (~50%) of Mo1 binding sites compared with normal cells (Fig. 8). This finding may suggest (*a*) an inability of these granulocytes to fully express their normal content of Mo1 upon optimal activation, or (*b*) that these cells have ~50% of the normal total cellular content of this antigen. Previous studies (9, 11) showed that granulocytes from these patients had a normal secretory response suggesting that the differences noted in Mo1 surface expression are not a reflection of a degranulation defect. In favor of the second possibility is previous data showing that these same individuals had ~50% of the normal total granulocyte content of "gp 150" as determined by densitometric scanning of the total cell lysate (9, 11). Therefore, these studies suggest a heterogeneity in the inheritance of Mo1 deficiency in humans. They also point out the importance of standardizing the state of activation of effector cells in future studies aimed towards phenotyping relatives of these patients and understanding the molecular basis of this newly recognized disorder.

Table III. Relative Surface Membrane Expression of Mo-1 and LFA-1 on Myeloid Cells From Two Patients with Mo-1 Deficiency Relative to Controls and Family Members

			Relative specific fluorescence (linear)*		
Cell	Family	Individual	Mo-1	Mo-5	LFA-1
Granulocyte	Family 1	(Control)	100	100	100
		Patient	12	96	14
		Mother	84	115	95
		Father	73	85	86
Granulocyte	Family 2	(Control)	100	100	100
		Patient	23	78	0
		Mother	99	106	124
		Father	122	125	106

* Numbers indicate relative specific fluorescence intensity (linear scale) computed from the peak linear fluorescence channels for cells stained with each monoclonal reagent minus the peak channels of cells stained with a nonreactive control antibody. For each antigen, the fluorescence intensity of the control individual's cells has been arbitrarily assigned 100 units.

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