INHERITED DEFICIENCY OF THE Mac-1, LFA-1, p150,95 GLYCOPROTEIN FAMILY AND ITS MOLECULAR BASIS

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The lymphocyte function–associated 1 (LFA-1),[1] Mac-1, and p150,95 molecules constitute a family of structurally and functionally related, high molecular weight, human leukocyte surface glycoproteins (1). Each molecule contains an α and a β subunit noncovalently associated in an $\alpha_1\beta_1$ structure. The β subunits of M_r 95,000 in each of these three molecules are identical. The molecules are distinguished by their α subunits, which have different isoelectric points, molecular weights, and cell distributions, and are immunologically non–cross-reactive. The relative molecular weights of the Mac-1, LFA-1, and p150,95 α subunits are 165,000, 177,000, and 150,000 for αM, αL, and αX, respectively. This glycoprotein family is conserved in mouse and human (1, 2). In both species, the α and β subunits of each molecule are biosynthesized as separate α' and β' intracellular precursors (1, 3). The precursors associate into $\alpha'\beta'$ complexes, are processed to the mature $\alpha\beta$ form, and then transported to the cell surface. The murine αM and αL subunits have 33% amino acid sequence homology with one another.[2] Such homology suggests that a primordial α chain gene duplication event(s) led to the evolution of this glycoprotein family.

The LFA-1 and Mac-1 molecules contribute to multiple types of leukocyte adhesion reactions. The LFA-1 molecule participates in the formation of adhesions between effector and target cells in cytolytic T lymphocyte–mediated killing and in natural killing, as shown by monoclonal antibody (mAb) blocking experiments (4–6). LFA-1 also participates in T helper cell responses, and its distribution on B cells, granulocytes, and monocytes suggests it may function in adhesion of these cells as well (7). Mac-1 has been defined (8) by mAb as a mouse differentiation antigen present on myeloid and absent on lymphoid cells. Subse-

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[1] *Abbreviations used in this paper:* CR1, 3, complement receptor types 1 and 3; EBV, Epstein-Barr virus; FCS, fetal calf serum; FITC, fluorescein isothiocyanate; IL-2, interleukin 2; LFA-1, lymphocyte function–associated antigen 1; mAb, monoclonal antibody; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; PHA, phytohemagglutinin; SDS, sodium dodecyl sulfate.

[2] Springer, T. A., D. Teplow, and W. J. Dreyer. The LFA-1, Mac-1 family of leukocyte adhesion glycoproteins: alpha subunit sequence homology and unexpected relation to leukocyte interferon. Manuscript submitted for publication.

quently, human Mac-1 was identified by cross-reaction of an mAb with mouse Mac-1 (9), and the OKM1, Mo1, OKM9, and OKM10 mAb to other determinants on human Mac-1 were obtained (10, 11). Mac-1 appears to be identical to the complement receptor type 3 (CR3). The CR3 binds the inactivated form (iC3b) of the third component of complement and mediates adherence and phagocytosis of iC3b-coated particles by granulocytes and monocytes. Some mAb to Mac-1 inhibit the CR3 on myeloid cell surfaces (10, 12, 13). Furthermore, *Staphylococcus aureus*–mAb–Mac-1 complexes formed with noninhibitory mAb and soluble Mac-1 specifically agglutinate iC3b-opsonized erythrocytes (10).

Patients with a clinical syndrome characterized by recurrent bacterial infections, progressive periodontitis, delayed wound healing, persistent granulocytosis, and/or delayed umbilical cord separation have been described (reviewed in 14). Granulocytes and mononuclear leukocytes of these patients demonstrate severe abnormalities of adherence and functions dependent on adherence, including phagocytosis and motility (13–18). When total surface-labeled granulocyte proteins from these patients were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) (14, 17–19), a deficiency was recognized of a glycoprotein(s) variously estimated at 110,000–180,000 M_r. Subsequently (14, 20, 21), deficiency of a specific surface molecule was pinpointed using anti-Mac-1, Mo1, and OKM1 mAb to the Mac-1 α subunit. The Mac-1 β subunit was also found deficient using the TS1/18 mAb (14, 20).

In the present report, a group of unrelated patients and their kindreds was examined to determine the genetic and molecular basis of this disease. Previous knowledge (1) about the subunit organization of the Mac-1, LFA-1, p150,95 glycoprotein family has been important in guiding investigations. Since these molecules share a common β subunit, the possibility of a deficiency of LFA-1 and p150,95 as well as of Mac-1, was tested with subunit-specific mAb. The mode of inheritance of this disorder in the kindreds studied and possible variation among patients in the severity of deficiency at the cell surface were examined by quantitative imunofluorescence flow cytometry. Finally, the molecular basis of the defect was probed by immunoprecipitation of both cell surface molecules and their intracellular precursors. A novel, heritable deficiency of the entire Mac-1, LFA-1 glycoprotein family is described. The findings suggest that the primary defect is in the common β subunit. The presence of a normal α' intracellular precursor suggests that the lack of both α and β subunits on the cell surface reflects a requirement of formation of $\alpha'\beta'$ complexes for processing and transport to the surface.

Materials and Methods

Patients. Four kindreds were studied. Kindred 1 included a 6-yr-old Caucasian female patient and her parents (14). Kindred 2 included a 15-mo-old Mexican-American female patient, her 3- and 5-yr-old brothers, and her parents. Kindred 3 consisted of the parents of a 17-mo-old deceased Iranian female patient. Kindred 4 included a 17-yr-old Mexican-American male patient, his 21-yr-old sister, and mother (15). Each patient manifested recurrent soft tissue infections, progressive periodontitis, impaired wound healing, and persistent granulocytosis. Three of four patients demonstrated delayed umbilical cord separation. Severe deficits of leukocyte mobilization in vivo (Rebuck skin window) and leukocyte motility in vitro (Boyden chamber assay) were identified in each patient. Among

other family members of each kindred, no infectious susceptibility, impairment of wound healing, or other clinical diseases were recognized. Assessments of leukocyte functions among the latter individuals were generally normal.

Monoclonal Antibodies. Anti-αL mAb were either TS1/22 or an equal volume mixture of TS1/12, TS2/14, TS1/22, TS2/4, and TS2/6 culture supernatants (1). Anti-β mAb was TS1/18 (1); anti-αM mAb were OKM1, OKM10 (11) (kindly provided by Dr. G. Goldstein), or M1/70 (9). $F(ab')_2$ rabbit anti-CR1 (22) was a kind gift of Dr. D. Fearon; mouse mAb 44D to CR1 (23) was kindly given by Dr. V. Nussenzweig. mAb to HLA was W6/32 (24). Antineutrophil FcR mAb 3G8 (25) was kindly provided by Dr. J. Unkeless.

Leukocytes. Blood was drawn and transported in syringes containing citrate/phosphate/dextrose/adenine anticoagulant. Red blood cells (RBC) were sedimented with 20% (by volume) of 6% Dextran T-500 (Pharmacia Fine Chemicals, Piscataway, NJ) for 20–30 min at 20°C. All remaining steps were at 4°C to avoid "up-regulation" of surface receptors triggered by cell purification (26). Granulocytes and mononuclear cells were isolated by centrifugation for 25 min at 1,200 *g* on Ficoll-Hypaque layers of $d = 1.106$ and 1.08 (27) or on a single layer of $d = 1.08$ (14). For radiolabeling experiments, RBC contaminating the granulocytes were lysed with H_2O for 20 s. Cells were washed three times in phosphate-buffered saline (PBS) or Hanks'-Hepes + 5 mM EDTA.

B and T Cell Lines. To establish Epstein-Barr virus (EBV) transformants, 10^6 mononuclear cells/ml in RPMI 1640, 20% fetal calf serum (FCS), and 50 μg/ml gentamycin were incubated for 16 h with EBV-containing supernatant of B95-8 cells (28) (kind gift of Dr. D. Thorley-Lawson), 6.25% TS1/8 anti-LFA-2 supernatant, and 0.625% Leu-4 ascites (kindly provided by Dr. R. Evans). Cells in 0.2-ml aliquots were placed into 10 microtiter wells. Medium was replaced with RPMI 1640, 20% FCS, and 50 μg/ml gentamycin until cell growth was noted. Cells grew in most wells and were expanded in the same medium. Phytohemagglutinin (PHA) blasts were established at 10^6 cells/ml in RPMI 1640, 20% FCS containing a 1:800 dilution of PHA-P (Difco Laboratories, Inc., Detroit, MI). PHA lines were expanded with interleukin 2 (IL-2)-conditioned medium and pulsed weekly with PHA (29).

Immunofluorescence Flow Cytometry. Cells were labeled with one of the following: anti-αL mAb TS1/22, the mixture of five anti-αL mAb hybridoma supernatants, anti-β mAb TS1/18 hybridoma supernatant, anti-αM mAb (20 μg/ml of purified OKM1 or OKM10), anti-CR1 mAb (20 μg/ml of purified 44D or 5 μg/ml of $F(ab')_2$ rabbit anti-CR1), anti-HLA W6/32 mAb hybridoma supernatant, antineutrophil FcR 3G8 hybridoma supernatant, or, as control, P3X63.Ag8 γκ myeloma culture supernatant. After washing, they were stained with fluorescein isothiocyanate (FITC)-labeled affinity-purified goat anti-mouse IgG, 70 μg/ml (Zymed, Burlingame, CA), as described (30). Flow cytometry was done with an Epics V (Coulter Electronics, Inc., Hialeah, FL). Both linear and logarithmic scatter-gated fluorescence histograms were collected. Peak positions were either determined from linear histograms or determined from logarithmic histograms and converted to linear values with a log-linear calibration curve (30). The peak position of the control was subtracted to calculate specific fluorescence intensity.

Radiolabeling. Granulocytes (10^7 in 0.5 ml of Dulbecco's PBS, 0.2% glucose, 10 mM Hepes) were labeled by the addition of 0.625 U/ml glucose oxidase (from *Aspergillus niger*, type V; Sigma Chemical Co., St. Louis, MO), 0.125 U/ml of centrifuged and resuspended crystalline lactoperoxidase (Boehringer Mannheim, Federal Republic of Germany), and 5 mCi $Na^{125}I$ (New England Nuclear, Boston, MA), followed by incubation for 15 min at 20°C with occasional swirling. PHA and EBV lines ($1.5–15 \times 10^6$ cells in 5 ml) were labeled with 1 mCi [^{35}S]methionine (New England Nuclear) in methionine-free RPMI 1640, 20% dialyzed FCS, 10 mM Hepes, 50 μg/ml gentamycin, and, for PHA lines only, 2% dialyzed IL-2-containing conditioned medium, for 2 h at 37°C in duplicate 100-mm petri dishes. Cells from one dish were chased for 22 h at 37°C in complete RPMI 1640 medium, 20% FCS (with 2% IL-2 for PHA lines) after one wash with warm medium. At the end of the pulse or chase, cells were washed once in cold PBS and lysed.

Immunoprecipitation. Cells were lysed in 1% Triton X-100, 1% bovine hemoglobin, 0.14 M NaCl, 0.01 M Tris HCl, pH 8.0, with freshly added 1 mM phenylmethylsulfonyl

fluoride, for 20 min at 4°C, and centrifuged at 10,000 *g* for 10 min. Supernatants were precleared by addition of 10% (by volume) each of suspensions of 10% *S. aureus* bacteria and 50% cyanogen bromide (CNBr)-activated, glycine-quenched Sepharose. Lysates were then centrifuged at 10,000 *g* for 10 min, and supernatants ultracentrifuged at 150,000 *g* for 30 min. Additionally, for [^{35}S]methionine lysates, stock solutions of 5% sodium deoxycholate, 20% Triton X-100, and 10% SDS were added to bring the final concentration to 1% Triton X-100, 1% deoxycholate, 0.1% SDS. Lysates were incubated overnight at 4°C and subjected to a second preclearing and ultracentrifugation. For immunoprecipitation, lysates (25–50 μl) were mixed with 10 μl of a 50% suspension of mAb–Sepharose CL-4B, coupled at 2 mg/ml, or 50 μl of either hybridoma supernatants or purified mAb at 100 μg/ml. Precipitates of soluble mAb were formed with 10 μl of rabbit anti-mouse IgG serum and 50 μl of 10% *S. aureus*. Precipitates were washed and subjected to SDS-PAGE (31) and autoradiography with enhancing screens (32) or fluorography (33).

Results

mAb specific for the LFA-1 and Mac-1 αL and αM subunits, and the common β subunit, were used in immunofluorescence flow cytometry studies of patients' granulocytes (Fig. 1). Saturating concentrations of mAb and second FITC anti-mouse IgG reagents were used. Under these conditions, fluorescence intensity (after subtraction of background fluorescence) is proportional to the number of mAb-binding (antigen) surface sites per cell (30). Three unrelated patients with recurring bacterial infections were strikingly deficient in the Mac-1 α subunit (Fig. 1, *J–L*). Furthermore, the LFA-1 α subunit (Fig. 1, *F–H*) and the common β subunit (Fig. 1, *N–P*) were deficient. Thus, not only Mac-1 but also other molecules sharing its β subunit were affected. Interestingly, there were quanti-

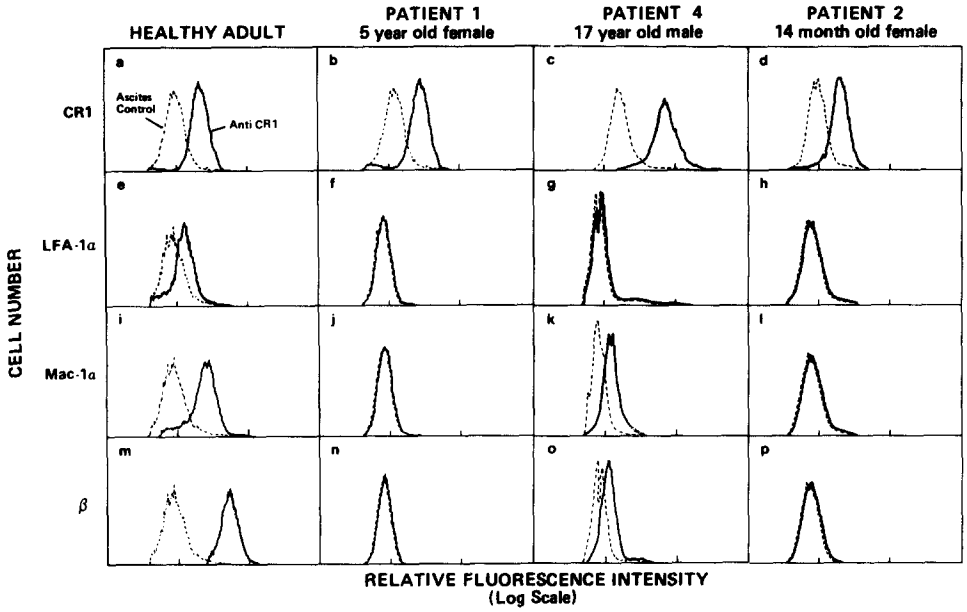

FIGURE 1. Immunofluorescence flow cytometry of granulocytes from patients with recurring bacterial infections. Granulocytes were indirectly stained with F(ab')$_2$ rabbit anti-human CR1, TS1/22 anti-LFA-1 α, OKM1 anti-Mac-1 α, or TS1/18 anti-β mAb (solid curves); or control P3X63γ/κ (dashed curves).

tative differences among the patients in the severity of deficiency. Patients 1 and 2 expressed an average of between 0.2 and 0.5% of the normal amounts of the three subunits. These patients' cells consistently showed slight staining with the specific mAb relative to irrelevant control mAb (see also Figs. 2, 4, and 5 below), indicating that the subunits are actually expressed, but at extremely low levels. In contrast to patients 1 and 2, patient 4 expressed distinctly higher but still markedly deficient amounts, 3, 9, and 7% of the αL, αM, and β subunits, respectively. The deficiency in LFA-1 and Mac-1 did not reflect the lack of only one or a few antigenic determinants, since deficiency of the αL subunit was confirmed with five mAb recognizing four spatially distinct epitopes (34), and since deficiency of αM was found with M1/70, OKM1, and OKM10 mAb, which bind to three different epitopes (11 and unpublished results). Binding assays with ^{125}I-labeled anti-αL and β F(ab')$_2$ fragments and anti-αM mAb gave the same results for patients 1, 2, and 4 (data not shown). In contrast, the complement receptor type I (CR1) (Fig. 1, *B–D*), neutrophil FcR, and HLA antigens (not shown), were present on patients' cells in normal amounts.

Next, the effect of chemotactic factors on the expression of the Mac-1, LFA-1 family on normal and patient cells was examined. *N*-formylmethionyl peptides bind to specific receptors, and stimulate granulocyte adherence, chemotaxis, granule secretion, and the mobilization of f-Met-Leu-Phe and CR1 receptors from latent intracellular pools to the cell surface (26, 35). Importantly, specific and saturable binding of f-Met-Leu-Phe by granulocytes of patients with this disorder has previously been shown to be normal (14, 15). f-Met-Leu-Phe stimulated a dramatic increase in αM and β subunit surface expression on healthy adult control granulocytes (Fig. 2, compare *b* and *c* to *e* and *f*). Similar relative increases were observed on parental granulocytes, although the absolute amounts of surface expression were lower than on control cells (Fig. 2, compare *h* and *i* to *k* and *l*; *n* and *o* to *q* and *r*). Average increases for two controls and two sets of parents were 5.5- and 3.7-fold for αM and β, respectively. The increases for LFA-1 α were consistently lower, 1.35-fold for both parental and control cells. Patient granulocytes, in contrast, did not express a latent pool of αM, β, or αL (Fig. 2, *y–dd*); patients 1 and 2 expressed $\leq$1% of normal amounts after f-Met-Leu-Phe stimulation. Similar increases in parents, and deficiency of a latent pool in patients, were observed with granulocytes stimulated with the chemoattractant C5a at 20°C relative to control cells held at 20°C (data not shown). In contrast, patient and parental granulocytes demonstrated normal increased surface expression (about sixfold) of CR1 (not shown), as previously reported for healthy adult granulocytes (26).

To detect heterozygotes and to examine the mode of inheritance, expression on cells of parents and siblings was quantitated. Granulocytes of a representative father and mother (Fig. 2, *g–r*) expressed an amount of αM and β intermediate between that on healthy control and patient cells. Levels of expression on parents, siblings, and patients relative to healthy adult controls are summarized in Fig. 3. The data are averages of two to four separate determinations on unstimulated granulocytes prepared at 4°C, each expressed as a percent of healthy adult controls. When mothers and fathers were separately averaged, mean values for maternal cells were 53–66% for the three subunits; mean values for paternal

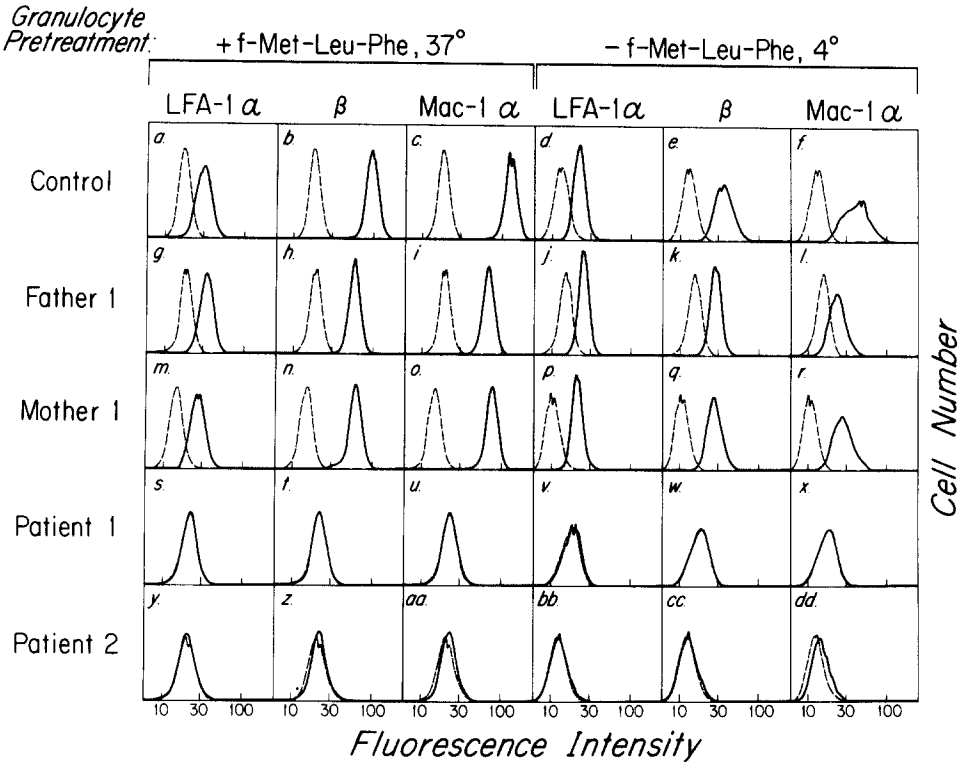

FIGURE 2. Mac-1, LFA-1 expression on patient and family member granulocytes, before or after f-Met-Leu-Phe activation. Granulocytes (10^7/ml) in Hanks'-Hepes, 5 mM EDTA were incubated with 10^{-8} M f-Met-Leu-Phe for 30 min at 37°C or were held at 4°C. They were then subjected to immunofluorescent labeling and flow cytometry. Cells were stained with a mixture of five anti-LFA-1 α mAb, TS1/18 anti-β mAb, or OKM1 anti-Mac-1 α mAb (solid curves); or P3X63 control IgG1 (dashed curves).

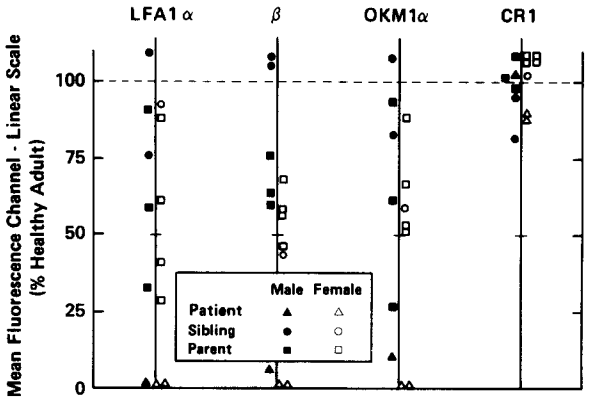

FIGURE 3. Expression of Mac-1, LFA-1, and CR1 on unstimulated granulocytes of four affected kindreds. Fluorescence intensity due to specific antibody binding was calculated as described in Materials and Methods and expressed as a percentage of healthy adult control values. Averages of two to four determinations are plotted for each individual.

TABLE I
Expression of Mac-1 α and β on f-Met-Leu-Phe-stimulated Parental Granulocytes

		Fluorescence intensity (percent of normal control)				
		Kindred 1		Kindred 2	Kindred 3	Average
		Exp. 1	Exp. 2			
Father	Mac-1					
	α	43	44	61	54	50
	β	47	74	60	61	61
Mother	Mac-1					
	α	61	56	77	ND	65
	β	74	61	81	ND	72

cells were 58–63%. Two male siblings appear to be normal homozygotes and one female sibling appears to be a heterozygote (Fig. 3). A smaller number of measurements were also made on f-Met-Leu-Phe-stimulated cells (Table I). Again, both maternal and paternal granulocytes expressed amounts of antigen intermediate between patient and normal cells, further suggesting they are heterozygous. Granulocytes were more homogeneous in fluorescence intensity after stimulation (Fig. 2, compare *left* and *right*). Overall, less variation was apparent in measurements on stimulated cells, perhaps because differences in cell activation in vivo, or those related to handling or transportation, were minimized. Assessment of surface expression under stimulated conditions may allow more accurate identification of heterozygotes. The parent with the highest level of subunit expression on unstimulated granulocytes was a father with 90% of the normal amount of Mac-1 α; the value on stimulated granulocytes was 44%.

Other leukocyte lineages were examined to determine whether Mac-1, LFA-1 deficiency was generalized or was related to cell differentiation. Normal mononuclear cells are a mixture of LFA-1$^+$ Mac-1$^-$ lymphocytes and LFA-1$^+$ Mac-1$^+$ monocytes (7) and express considerably higher levels of LFA-1 than granulocytes. Patient 1 mononuclear cells were clearly deficient in αL, β, and αM (Fig. 4, *d–f*), as were mononuclear cells of patient 2 (data not shown). Although patient 4 mononuclear cells were not examined by immunofluorescence, ^{125}I-labeled mAb binding showed 4.8 and 4.6% of normal amounts of the αL and β subunits, respectively.

PHA-activated T lymphoblasts and EBV-transformed B lymphoblastoid cell lines were established from patients as continuous sources of experimental material and to allow characterization of antigen expression on T and B lymphocyte lines. Normal PHA blasts and EBV lines expressed LFA-1 α and β (Fig. 5, *a, b, e, f*) and were Mac-1 α$^-$ (not shown). PHA blasts expressed 2.3-fold more LFA-1 than EBV lines. LFA-1 α and β were deficient in both the T and B lymphoblast lines (Fig. 5, *c, d, g–l*). For patient 4, the deficiency of αL and β appeared more severe on the EBV line than on granulocytes or mononuclear cells.

To examine the deficiency at a molecular level, granulocytes were surface-

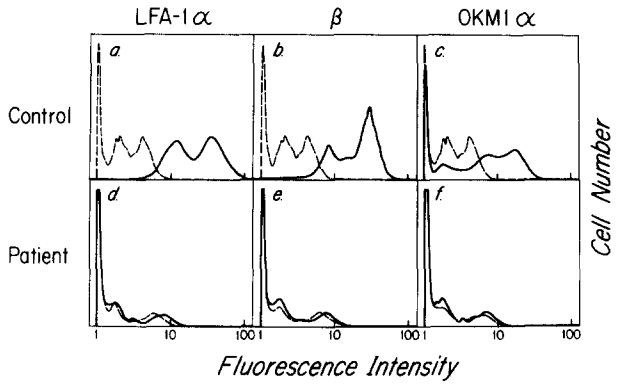

FIGURE 4. Immunofluorescence flow cytometry of patient mononuclear cells. Mononuclear cells of a normal control or patient 1 were indirectly stained with a mixture of five anti-LFA-1 α mAb, TS1/18 anti-β mAb, or OKM1 anti-Mac-1 α mAb (solid curves); or P3X63 control IgG1 (dashed curves).

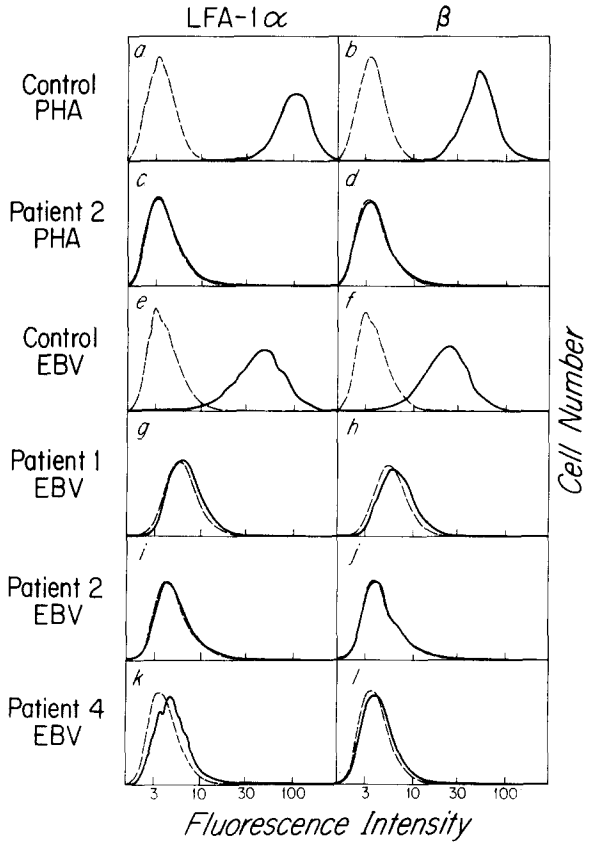

FIGURE 5. Immunofluorescence flow cytometry of patient T and B cell lines. PHA or EBV lines were indirectly stained with a mixture of five anti-LFA-1 α mAb or TS1/18 mAb (solid curves); or P3X63 control IgG1 (dashed curves).

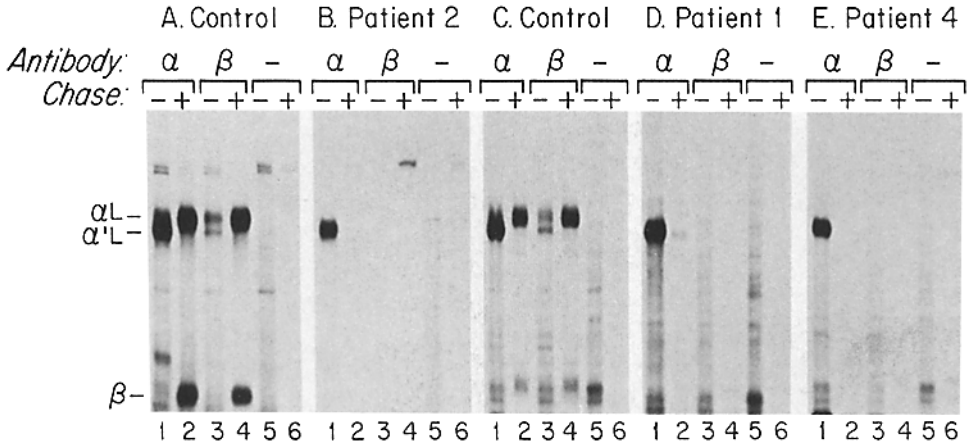

FIGURE 6. Immunoprecipitation of ^{125}I-labeled surface proteins from granulocytes. Triton X-100 lysates of each individual's ^{125}I-labeled granulocytes were immunoprecipitated with a mixture of five different anti-LFA-1 α mAb (lane *1*), anti-LFA-1 α TS1/22 mAb-Sepharose CL-4B (lane *2*), anti-β TS1/18 mAb-Sepharose (lane *3*), anti-OKM1 mAb (lane *4*), or anti-CR1 44D mAb (lane *5*). Precipitates were subjected to SDS–7% PAGE and autoradiography.

labeled with ^{125}I and subjected to immunoprecipitation and SDS-PAGE (Fig. 6). From healthy adult cells, the anti-αL mAb precipitated the LFA-1 αL subunit noncovalently associated with its β subunit (Fig. 6*A*, lanes *1* and *2*). Anti-αM mAb precipitated the αM subunit with its β subunit (Fig. 6*A*, lane *4*). The anti-β mAb precipitated the β subunit and the three types of α subunits noncovalently associated with it: αL, αM, and the αX subunit of the p150,95 molecule (Fig. 6*A*, lane *3*). In contrast, granulocytes from three patients were strikingly deficient in all these molecules (Fig. 6, *B*, *G*, *K*, lanes *1–4*). Parents and normal siblings of the patients were positive for immunoprecipitation (Fig. 6, *C–F*, *H*, *I*, *L*, *M*, lanes *1–4*). The CR1 molecule was precipitated as a positive control; it was present in similar amounts in granulocytes of healthy adults, patients, and their kindred (Fig. 6, *A–N*, lane *5*). Normal amounts of HLA antigen and neutrophil FcR were also precipitated from patients' cells (not shown).

In an attempt to detect low levels of expression on patient cells, autoradiograms were subjected to prolonged exposure. Precipitation of small amounts of LFA-1 were detected in patient 4 (Fig. 6 *O*, lanes *2* and *3*) but not in patients 1 and 2, findings consistent with the immunofluorescence cytometry results. Notably, anti-αL and anti-β mAb each precipitated both α and β subunits. This shows that when surface expression can be detected on patient cells, it is due to the presence of the normal $\alpha\beta$ complex, rather than to the presence of unassociated α or β subunits.

The biochemical basis for the lack of surface expression was studied in biosynthesis experiments. The lack of LFA-1 and p150,95 as well as Mac-1 on patients' cells suggested the primary deficiency was in the common β subunit. Although both α and β subunits were lacking on the cell surface, it was hypothesized that normal intracellular α precursors might be present, but require association with β for transport to the surface. To test this hypothesis, PHA and EBV lines were pulsed with [^{35}S]methionine to label precursors, and then either

examined immediately or chased with cold methionine to examine processing and maturation.

In healthy adult PHA blasts and EBV-transformed cells, large amounts of an α'L precursor and small amounts of mature αL were detected after a 2-h pulse by immunoprecipitation with anti-αL mAb (Fig. 7, *A* and *C*, lane *1*). Subunit association preceded processing, as shown by precipitation of α'L with anti-β (Fig. 7, *A* and *C*, lane *3*), and precipitation of all the αL produced during the pulse with anti-β (Fig. 7, *A* and *C*; compare similar amounts of αL in lanes *1* and *3*). After the 22-h chase, the mature αL and β subunits were found, and were associated with one another, as shown by precipitation with both anti-αL and anti-β mAb (Fig. 7, *A* and *C*, lanes *2* and *4*). A β' precursor was not identified by precipitation in this or four other independent labeling experiments, suggesting it had weak reactivity with the mAb and/or migrated in the same position as a background band.

In cells from all three patients, normal amounts of the intracellular α'L precursor were identified by precipitation with anti-αL mAb (Fig. 7, *B*, *D*, and *E*, lane *1*). The α'L precursor never matured, however, as demonstrated by precipitation of small amounts of α'L but not αL after the 22-h chase (Fig. 7, *B*, *D*, and *E*, lane *2*). The lack of precipitation of α'L by anti-β mAb showed it did not become β associated, nor was there any evidence of the mature β subunit (Fig. 7, *B*, *D*, and *E*, lanes *3* and *4*). Patient and control α'L were present in similar amounts after the 2-h pulse (Fig. 7, *A–E*, lane *1*), suggesting they have equal stability. The α'L precursor was largely degraded in patients' cells after the 22-h chase, showing intracellular, unassociated α'L has a shorter half-life than extracellular, associated αL, as would be expected. These findings (*a*) show that the patient lymphocytes have normal α'L precursors, (*b*) suggest that they lack normal β' precursors, and (*c*) suggest that α'L cannot be processed normally and transported to the cell surface in the absence of association with the β subunit.

Discussion

An entire family of glycoproteins that share a common β subunit, Mac-1, LFA-1, and p150,95, has been found to be deficient on leukocyte surfaces in patients with recurring bacterial infections. Surface deficiency of the α and β subunits of these molecules was independently investigated with mAb to the αL, αM, and β subunits. Since these mAb have previously been demonstrated (1) to react with ^{125}I surface-labeled subunits dissociated by brief exposure to high pH, any free subunits present on the surface of patients' cells should have been detectable. Both the αL and β subunits of LFA-1, and the αM and β subunits of Mac-1, were lacking on the cell surface. This was shown by immunofluorescence, ^{125}I-mAb binding, and immunoprecipitation of ^{125}I-labeled surface molecules. The p150,95 molecule's αX and β subunits were not detected by immunoprecipitation with anti-β mAb; thus at least its β subunit is deficient. By analogy to LFA-1 and Mac-1, one would not expect free p150,95 αX subunits to be present on the cell surface, but this remains to be directly demonstrated.

The deficiency appears to be limited to this glycoprotein family. The results could not be explained by a pleiotropic effect on high molecular weight proteins

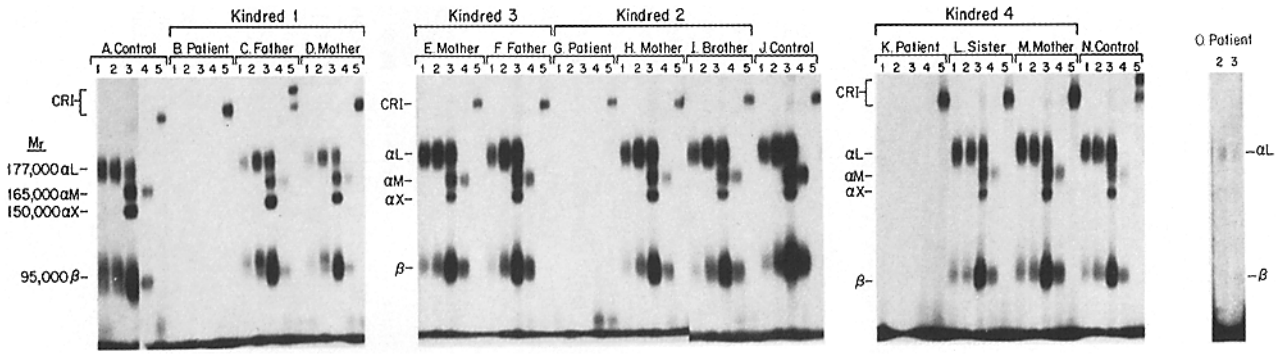

FIGURE 7. LFA-1 biosynthesis in patient or healthy adult lymphocytes. PHA-stimulated lines (*A*, *B*) or EBV-transformed lines (*C–E*) were labeled with [^{35}S]methionine for 2 h, and either harvested immediately or chased for 22 h with unlabeled methionine as indicated. Lysates with Triton X-100 and sodium deoxycholate were immunoprecipitated with purified antibodies coupled to Sepharose CL-4B: TS/22 anti-LFA-1 αL, TS1/18 anti-β, or activated, quenched Sepharose, which served as a negative control. Precipitates were subjected to SDS–6% PAGE and fluorography. Only the upper part of the gel is shown.

or on glycoproteins, since the CR1, the neutrophil Fc receptor, and HLA antigen glycoproteins were present in normal amounts and were of normal mobility in SDS-PAGE. Normal numbers of f-Met-Leu-Phe receptors, and normal granule secretion triggered by receptors for f-Met-Leu-Phe and C5a, have previously been described on these patients' cells (14, 15). Furthermore, latent CR1 (26) and FcR but not αM or β were present in patients' granulocytes and could be expressed on the surface in response to f-Met-Leu-Phe or C5a.

A number of lines of evidence show that Mac-1, LFA-1, p150,95 deficiency is a heritable autosomal recessive disease. (*a*) Mothers and fathers in these studies were found to express approximately half the normal amount of αL, αM, and β subunits, both on unstimulated and f-Met-Leu-Phe-stimulated granulocytes. (*b*) In the case of X-linked inheritance, two populations (normal and defective) of maternal granulocytes would be expected as a result of X chromosome inactivation, as is observed in the X-linked form of chronic granulomatous disease (36). However, all four mothers had a single population of granulocytes expressing intermediate levels of each glycoprotein subunit. (*c*) One recently identified family contains a cluster of an affected father, son, and daughter (not shown). (*d*) Unaffected male siblings of a female patient (kindred 2) and a female heterozygote sibling of a male patient (kindred 4) were recognized. (*e*) Equivalent numbers of male and female patients have been identified. (*f*) One patient (No. 2) was the product of a consanguinous marriage. It is interesting that of two apparently similar patients in whom expression was studied by SDS-PAGE of total granulocyte proteins and by radioimmunoassay, one was reported to have X-linked and the other somatic inheritance (17, 19, 37). Potentially, polymorphism in the level of expression could make some fathers (or mothers) appear normal. Further studies of the granulocytes of the carrier of the putative X-linked defect by immunofluorescence cytometry, for evidence of mosaicism in Mac-1, LFA-1 expression, would be of interest to provide more convincing evidence of X-linked inheritance.

The molecular pathogenesis of this deficiency was examined in biosynthesis experiments. In normal myeloid cells, the precursors for each subunit are synthesized separately and become noncovalently associated in $\alpha_1\beta_1$ complexes (1), as shown in Fig. 8. In patients' cells, the α'L subunit was synthesized in normal amounts and had normal stability, suggesting the primary defect was in

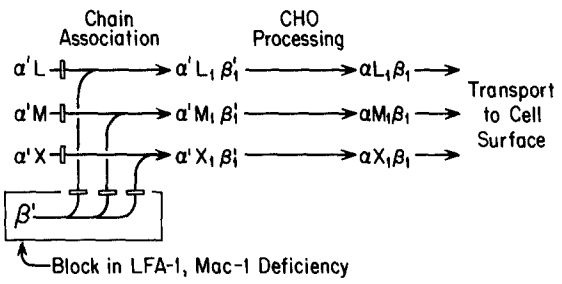

FIGURE 8. Biosynthesis of the Mac-1, LFA-1 glycoprotein family. The biosynthetic pathway in normal cells is as described in reference 1. The evidence for a primary block in β subunit synthesis, a secondary block in α'L biosynthesis due to lack of β subunit association, and hypothetically similar blocks in α'M and α'X biosynthesis, is discussed in the text.

the β subunit. Despite five independent labeling experiments and different solubilization conditions, the β′ precursor could not be identified in healthy adult or patient PHA blasts or EBV lines. β′ has been previously identified in the U937 myeloid cell line (1). The difficulty in identifying β′ in B and T lymphocyte lines may be related to the lower amount of β on the cell surface or differing glycosylation than in U937, or to lower mAb affinity for β′ than β. Nonetheless, the following lines of evidence strongly suggest that the primary deficiency is of the β subunit: (*a*) the presence of normal α′L but absence of any form of αLβ complex either at the cell surface or as an intracellular precursor detectable by precipitation of αL or α′L by anti-β mAb, and (*b*) the lack of three different surface proteins having as their only common structural entity the β subunit.

The effect of β deficiency on biosynthesis of the Mac-1, LFA-1 family is summarized in Fig. 8. The presence of α′L precursor but no αL inside patients' cells, and the absence of cell surface αL, show that association with the β subunit is required for processing and transport to the surface. Biosynthesis experiments have been done thus far in cells that express only LFA-1, but since the biosynthetic pathways of Mac-1, p150,95, and LFA-1 are similar in normal cells (1), it is proposed that α′M and α′X intracellular precursors are blocked at the same point in their biosynthetic pathway in patient myeloid cells, as shown in Fig. 8.

The lack of at least two and probably three α subunits on the cell surface as the result of β subunit deficiency represents an interesting example of the effects of gene complementation on the phenotypic expression of an inherited defect. The lack of a latent pool of Mac-1 α and β in patients' cells suggests that subunit association is also required for entry into this pool, which is presumably intracellular. Studies with endoglycosidase H digestion of Mac-1 have shown that the maturation of α′M to αM is due to the conversion of high mannose to complex-type carbohydrates (Kishimoto, Sastre, and Springer, unpublished results). This type of carbohydrate processing is known to occur in the Golgi, suggesting that lack of association with β in patients' cells blocks transport to the Golgi. Further understanding of the nature of the mutation, and of whether mRNA is quantitatively or qualitatively defective, will be dependent on DNA probes. In vitro translation assays and specific mRNA purification have been developed in the mouse system (38), and cross-hybridization with cloned mouse DNA probes should allow analysis of the defect in human cells.

Other patients appear to resemble the three described here in lacking multiple members of the Mac-1, LFA-1 glycoprotein family. Although patient 3 of this study died before testing with mAb, approximately half-normal expression of α and β subunits by her parents' granulocytes suggests she also suffered from the same disease. Four additional patients recently studied by us are also deficient in the entire Mac-1, LFA-1 family. Two patients in the eastern United States have deficiencies demonstrated with the M1/70 and Mol anti-Mac-1 mAb, the TS1/18 anti-β mAb, and anti-LFA-1 mAb (20, 21, 37). A patient in the western U. S. is deficient, as shown with an mAb of undefined subunit specificity but that appears similar to our TS1/18 anti-β mAb (39). This patient is also unreactive

with the TS1/22 anti-αL and OKM1 anti-αM mAb. Three patients in England[3] and one in France[4] are deficient in αL, αM, and β, as demonstrated by TS1/22, OKM1, and TS1/18 mAb. Although none of these patients have been characterized by biosynthetic labeling of precursors, and surface ^{125}I-labeled molecules have been characterized not at all or not to the same extent as in this report, findings in each case are suggestive of a similar defect.

Heterogeneity with respect to the severity of clinical illness and/or abnormalities of leukocyte functions observed among recognized examples of this disease may be causally related to quantitative differences observed among the patients studied here in surface expression of this critical glycoprotein family. Granulocytes of patient 4 have ~5% of normal amounts on the cell surface, and αβ complexes were detectable by immunoprecipitation. Patients 1 and 2 have a clearly more severe, but apparently not complete, deficiency. Among our patient population, patient 4 appears to have had a relatively milder clinical disease as compared with the other patients and has survived the disease longest. Differences with respect to the severity and types of functional abnormalities, including differences in CR3 activity, observed between our patient 1 and a patient reported by Arnaout and coworkers (20), may be related to a more complete Mac-1, LFA-1, p150,95 deficiency in the former patient (14). Collectively, the accrued evidence suggests that with the possible exception of the patient with the X-linked defect (17, 37), the patients reported to date represent examples of the same genetic disease. Despite some observed heterogeneity, remarkably similar clinical features, functional abnormalities, and biochemical defects have been described among all recognized individuals. However, the possibility exists that disease among different recognized kindreds results from independent mutations within or affecting the same gene. This is suggested by the quantitative variations in surface expression and the different ethnic backgrounds among the patients in this report. Patient 1 is of Anglo-Saxon descent while the parents of patient 2 emigrated from Iran. Patients 3 and 4 (and four other patients) are of Hispanic descent; families of each of these patients are living in or riginated from southern and western Texas.

Since a deficiency of the Mac-1, LFA-1 family has been clearly documented here on multiple types of leukocytes, including granulocytes, monocytes, and T and B lymphocytes, the observed functional abnormalities of both phagocytic and lymphoid cells of these patients (15) are not unexpected. Recurrent soft tissue infections secondary to both bacterial and fungal microorganisms are unquestionably related to granulocyte dysfunction in this disorder. Patient granulocytes are deficient in adhesion-dependent functions, including attachment and spreading, aggregation, antibody-dependent cytotoxicity, and CR3-mediated

[3] Ross, G. D., R. A. Thompson, M. J. Walport, T. A. Springer, J. V. Watson, R. H. R. Ward, J. Lida, S. L. Newman, R. A. Harrison, and P. J. Lachmann. Identification of a genetic deficiency of leukocyte membrane complement receptor type 3 (CR3, an iC3b receptor) and its association with increased susceptibility to bacterial infections. Manuscript submitted for publication.

[4] Fischer, A., R. Segar, A. Durandy, B. Grospierre, J. L. Virelizier, C. Griscelli, E. Fischer, M. Kazatchkine, M. C. Bohler, B. Descamps-Latscha, P. H. Trung, D. Olive, and C. Mawas. 1984. Deficiency of the adhesive protein complex LFA-1, C3bi complement receptor, p150,95, in a girl with recurrent bacterial infections. Manuscript submitted for publication.

adherence and phagocytosis (14, 15, 17, 39).[3,4] Deficiency of CR3 function as measured with iC3b-opsonized particles agrees with the previous identification of Mac-1 as the CR3 (10, 12). The wide spectrum of other adhesion abnormalities may be related to all three members of this glycoprotein family, which are normally expressed on granulocytes and monocytes. Treatment with combinations of anti-αL, αM, and β mAb reproduces these functional abnormalities in normal granulocytes (14–16, 39). Interestingly, a latent pool of Mac-1 was found in healthy adult but not in patient granulocytes, which was mobilized to the cell surface in response to the chemoattractants and secretagogues f-Met-Leu-Phe and C5a. Since β subunit expression was increased on healthy granulocytes, p150,95 may also be up-regulated, but there was only a slight increase in LFA-1, as shown with anti-αL mAb. Lack of up-regulation of these molecules may be related to the failure of patients' granulocytes to hyperadhere in response to chemoattractants (14). Since adherence is necessary for chemotaxis, adherence abnormalities could explain the failure of patients' cells to chemotact despite normal bipolarization (14), and may lead to the absence of normal pus formation at sites of infection.

Although lymphocytes express only one member of the family, LFA-1, increasing evidence supports the clinical pathologic importance of lymphoid cell dysfunction in this disorder. Patient 2 of this report died of an overwhelming viral (pico RNA virus) respiratory infection. Impaired proliferative responses of patient cells to PHA (10–25% of normal values) and diminished cytolytic T lymphocyte and natural killing activity have been documented among studies in our patients (16, 40) as well as in a patient reported elsewhere (39). Interestingly, the PHA responses and cytotoxic killing by lymphocytes of patients 1, 2, and 4 are even further decreased by anti-LFA-1 mAb, suggesting that even the small amounts of LFA-1 present on patient cells can contribute to their functional responsiveness (40).

Summary

Leukocyte surface glycoproteins that share a common β subunit have been found to be congenitally deficient in three unrelated patients with recurring bacterial infection. The glycoproteins, Mac-1, LFA-1, and p150,95, have the subunit compositions $\alpha M\beta$, $\alpha L\beta$, and $\alpha X\beta$, respectively. Using subunit-specific monoclonal antibodies, both the αM and β subunits of Mac-1, the αL and β subunits of LFA-1, and at the least the β subunit of p150,95, were found to be deficient at the cell surface by the techniques of immunofluorescence flow cytometry, radioimmunoassay, and immunoprecipitation. A latent pool of Mac-1 that can be expressed on granulocyte surfaces in response to secretory stimuli, such as f-Met-Leu-Phe, was also lacking in patients. Deficiency was found on all leukocytes tested, including granulocytes, monocytes, and T and B lymphocytes. Quantitation by immunofluorescence cytometry of subunits on granulocytes from parents of these patients and of a fourth deceased patient showed approximately half-normal surface expression, and, together with data on other siblings and a family with an affected father and children, demonstrate autosomal recessive inheritance. Deficiency appears to be quantitative rather than qualita-

tive, with two patients expressing ~0.5% and one patient ~5% of normal amounts. The latter patient had $\alpha\beta$ complexes on the cell surface detectable by immunoprecipitation. Biosynthesis experiments showed the presence of normal amounts of α'L intracellular precursor in lymphoid lines of all three patients. Together with surface deficiency of three molecules that share a common β subunit but have differing α subunits, this suggests the primary deficiency is of the β subunit. The lack of maturation of α'L to αL and the deficiency of the α subunits at the cell surface and in latent pools suggests that association with the β subunit is required for α subunit processing and transport to the cell surface or to latent pools. The molecular basis of this disease is discussed in light of adhesion-related functional abnormalities in patients' leukocytes and the blockade of similar functions in healthy cells by monoclonal antibodies.

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