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The Severe and Moderate Phenotypes of Heritable Mac-1, LFA-1 Deficiency: Their Quantitative Definition and Relation to Leukocyte Dysfunction and Clinical Features

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An inherited syndrome characterized by recurrent or progressive necrotic soft-tissue infections, diminished pus formation, impaired wound healing, granulocytosis, and/or delayed umbilical cord severance was recognized in four male and four female patients. As shown with subunit-specific monoclonal antibodies in immunofluorescence flow cytometry and ^{125}I immunoprecipitation techniques, in addition to a NaB^3H_4–galactose oxidase labeling assay, granulocytes, monocytes, or lymphocytes from these individuals had a "moderate" or "severe" deficiency of Mac-1, LFA-1, or p150,95 (or a combination)—three structurally related "adhesive" surface glycoproteins. Two distinct phenotypes were defined on the basis of the quantity of antigen expressed. Three patients with severe deficiency and four patients with moderate deficiency expressed <0.3% and 2.5%–31% of normal amounts of these molecules on granulocyte surfaces, respectively. The severity of clinical infectious complications among these patients was directly related to the degree of glycoprotein deficiency. More profound abnormalities of tissue leukocyte mobilization, granulocyte-directed migration, hyperadherence, phagocytosis of iC3b-opsonized particles, and complement- or antibody-dependent cytotoxicity were found in individuals with severe, as compared with moderate, deficiency. It is proposed that in vivo abnormalities of leukocyte mobilization reflect the critical roles of Mac-1 glycoproteins in adhesive events required for endothelial margination and tissue exudation. The recognition of phenotypic variation among patients with Mac-1, LFA-1 deficiency may be important with respect to therapeutic strategies.

Recent reports [1–15] have described a newly recognized genetic disorder of adhesion-dependent leukocyte functions that is clinically characterized by recurrent soft-tissue infections and severely impaired pus formation. We have previously shown [1–3, 5,

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16] that granulocytes, monocytes, and lymphocytes of individuals with this disorder demonstrate deficiency of a family of structurally and functionally related glycoproteins, including Mac-1, LFA-1, and p150,95. Each of these molecules contains an α and a β subunit, noncovalently associated in an $\alpha_1\beta_1$ structure. These molecules share an identical β subunit and are distinguished by their α subunits, which have different isoelectric points, molecular weights, and cell distributions, and are immunologically non-cross-reactive [17]. Recent investigations of LFA-1 biosynthesis in transformed lymphocytes of patients with this disorder [5] suggest the primary genetic lesion affects the common β subunit; β appears to be required for normal α subunit maturation, intracellular transport, and cell-surface assemblage of functionally active $\alpha_1\beta_1$ molecules.

Eight patients with the Mac-1, LFA-1 glycoprotein deficiency syndrome representing white, Iranian, and Hispanic kindreds have been identified in the Houston-Galveston (Texas) referral area; they constitute approximately half of all patients reported to have this clinical entity worldwide. Thus, the recognition of this cluster of patients has allowed a unique opportunity to perform comparative studies of the clinical features, functional abnormalities, and molecular characteristics among a relatively large number of affected individuals. This report provides evidence for clinical and molecular heterogeneity in this disorder. On the basis of quantitative differences in expression of the Mac-1, LFA-1 glycoprotein family, "severe" and "moderate" deficiency phenotypes have been defined. Classification of these patients into three categories appears justified on the basis of quantitative differences of surface expresssion of these glycoproteins on granulocytes, monocytes, or lymphocytes, as shown by (*1*) fluorescence-activated cell sorting, (*2*) ^{125}I immunoprecipitation techniques employing subunit-specific monoclonal antibodies (MAbs), and (*3*) a NaB^3H_4–galactose oxidase labeling assay. Both the severity of clinical features and the degree of functional abnormalities demonstrated among affected individuals appear directly related to the magnitude of the molecular deficiencies. Documentation and confirmation of clinical heterogeneity in this disorder may have important prognostic and therapeutic implications.

Materials and Methods

Isolation of granulocytes. Granulocytes were purified from heparinized, dextran-sedimented venous blood samples over Ficoll-Hypaque gradients and suspended in Dulbecco's PBS pH 7.4 (DPBS; GIBCO, Grand Island, NY) containing 0.2% dextrose, as described [18]. For chemiluminescence (CL), aggregometry, SDS-PAGE, or *N*-formyl-methionyl-leucyl-^{3}H-phenylalanine (f-Met-Leu-^{3}H-Phe) binding studies, erythrocytes were eliminated by hypotonic lysis [18].

Preparation of reagents. Stock solutions of f-Met-Leu-Phe (Sigma, St. Louis) and f-Met-Leu-^{3}H-Phe (New England Nuclear, Boston) were prepared in DPBS. A low-molecular-weight chemotactic factor, referred to in this manuscript as complement component C5a, was prepared from activated human sera as described [19]. Neuraminidase from *Vibrio cholerae* (500 U/ml) was purchased from Schwarz/Mann (Orangeburg, NY). Galactose oxidase from *Dactylium dendroides* (Sigma) was purified by affinity chromatography over Sepharose [20]. NaB^3H_4 was obtained from New England Nuclear. Zymosan, calcium ionophore A23187, cytochalasin B, colchicine, fluorescein isothiocyanate (FITC), concanavalin A, nonidet P-40 (NP-40), phorbol myristate acetate (PMA; Sigma), rabbit antibody to human serum albumin (Calbiochem-Behring, La Jolla, Calif), lactoferrin purified from human milk (Sigma), and the IgG fraction of a goat antiserum to human lactoferrin (Atlantic Antibodies, Scarborough, Me) were obtained commercially.

SDS-PAGE. SDS-PAGE was performed with both 2.5 × 110-mm cylindrical gels and 1.4 × 120 × 140-mm slab gels [18]. For whole cell preparations, 2×10^7 cells were treated with diisopropylfluorophospate and solubilized in 0.2 ml of 1% NP-40 containing 2 m*M* phenylmethylsulfonylfluoride [21]. Whole cells, granules, or membranes were placed directly in sample buffer containing SDS.

Immunoprecipitation studies. Intact granulocytes were surface-labeled with ^{125}I by lactoperoxidase, solubilized in 0.5% NP-40 or immunoprecipitated with subunit-specific MAbs and subjected to SDS-PAGE [5].

Surface labeling of glycoproteins. Granulocytes (2×10^7) were incubated simultaneously with 0.025 ml of neuraminidase (5 U) and 0.025 ml of galactose oxidase (12.5 U) in 1 ml of DPBS for 20 min at 37 C and then washed and resuspended in 0.5 ml of DPBS [23]. One millicurie of NaB^3H_4 was added and the reaction was allowed to proceed for 30 min at 21 C. Washed cells were solubilized in 1% NP-40, and nuclei and other cellular material were removed by low-speed centrifugation. Supernatants (0.2 ml)

Table 1. Clinical features of patients with severe or moderate deficiencies of Mac-1, LFA-1.

	Severe			Moderate				
Clinical features	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8
Age, sex	6 years, F	17 months, F	18 months, F	18 years, M	15 years, M	36 years, M	7 years, M (son of patient 6)	12 years, F (daughter of patient 6)
Ethnic background	White, of English extraction	Hispanic	Iranian	Hispanic	Hispanic	Hispanic	Hispanic	Hispanic
Range of peripheral WBC count* (% neutrophils)	15–90 (64%–92%)	35–161 (68%–93%)	28–87 (53%–89%)	16–22 (75%–90%)	15 (75%)	20–30 (50%–80%)	13–57 (40%–65%)	12–45 (50%–70%)
Leukocyte mobilization into skin windows or chambers	None	None	None	Delayed and diminished	Delayed and diminished	Delayed and diminished	Delayed and diminished	Delayed and diminished
Delayed umbilical cord severance/ infection	Surgical resection of umbilicus and and patent	Surgical resection of infected umbilicus and patent urachus (*E. coli*)	Delayed separation and infection of umbilicus (*E. coli*)	–	–	–	–	–
Skin/subcutaneous infections								
Indolent/necrotic abscess or cellulitis (< 1.0 cm dia.)	+ + + Indolent lesions *Staphylococcus, Pseudomonas* spp.	+ + + Indolent lesions *Pseudomonas, Staphylococcus* spp., and *E. coli* with eschars	+ + + Indolent lesions *Staphylococcus, Pseudomonas* spp. with eschars	+ + Many lesions during infancy, fewer thereafter—most due to *Pseudomonas* spp.	+ +	+ + + Lesions beginning in infancy; *Staphylococcus, Pseudomonas* spp.	+ + + Indolent necrotic lesions, *Staphylococcus aureus* isolated most commonly	+ + Onset of recurrent lesions at 19 months
Gangrenous-bullous/ulcerative lesions with *plaque formation* (1.0–10 cm dia.)	–	+ *Pseudomonas cepacia*		+ + *Pseudomonas* spp.	–	+ + Multiple large indolent ulcers of right leg, *Staphylococcus, Pseudomonas* spp.	–	–
Cellulitis/abscess associated with wound/trauma	+ + + *Staphylococcus, Pseudomonas* spp.	+ + + *Pseudomonas, Staphylococcus* spp.	+ *Staphylococcus aureus*	–	Puncture wound (Staphylococcus spp.) × 3 surgical debridement and skin grafts	–	–	–
Otitis media	+ + +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
Ulcerative stomatitis/pharyngitis	+ + +	+ + +	+	–	+ +	+ +	+	–
Gingivitis/ periodontitis	+ + + Abnormal dentition and loss of deciduous teeth; severe demineralization of alveolar bone	Intense localized gingivitis associated with eruption of deciduous teeth	Intense localized gingivitis associated with eruption of deciduous teeth	+ + + Abnormal dentition with loss of many deciduous and secondary teeth; severe alveolar bone loss	+ + + Abnormal dentition with loss of many deciduous and secondary teeth; severe alveolar bone loss	+ + + Onset at 3 years. Generalized alveolar bone loss; surgical removal of all teeth at 13 years	+ + + Surgical removal of deciduous teeth at 4 years; abnormal permanent dentition with loss of teeth and alveolar bone	+ + + Onset at 3 years. Progressive generalized alveolar bone loss, surgical removal of deciduous and permanent dentitions at 3 and 12 years

Systemic infection	Peritonitis—*Klebsiella, Proteus, Enterococcus* spp.	Aseptic meningitis × 2	Aseptic meningitis	Frequent episodes of bronchitis and/or pneumonitis	Aseptic meningitis	–
	Perianal abscess—*Klebsiella, Proteus, Pseudomonas* spp.	Septicemia (*Pseudomonas aeruginosa*)	Necrotizing pharyngitis/tracheitis/pneumonia with encephalopathy (picornavirus)	Perianal abscess—*Klebsiella, Proteus, Pseudomonas* spp.	Frequent episodes of pneumonitis	
	Pneumonitis × 3	Pneumonitis × 4	Pneumonitis × 3			
		Necrotizing enteritis/peritonitis and septicemia (polymicrobial)	Perirectal abscess with septicemia and shock (*Pseudomonas aeruginosa*)			
		Perirectal abscess and septicemia (*Pseudomonas* spp.)				
Impaired healing and/or infection of surgical wounds	–	Delayed healing of laparotomy wound	Delayed healing and infection of laparotomy and thoracenteses wounds (*Acinetobacter* spp.) Failure to heal pleuropulmonary fistulas	–	Three skin graft procedures (1 porcine, 2 human) required to complete healing of leg ulcers	–

NOTE. +, clinical features recognized at least once; + +, clinical features recognized on several isolated occasions; + + + clinical features continuously or on numerous occasions.
* No. of cells $\times 10^3/mm^3$.

were placed in SDS sample buffer, boiled for 2 min, and subjected to SDS-PAGE. Gels were sectioned into 1.0-mm slices, and the radioactivity was determined in a scintillation counter [1].

MAbs. The OKM-1 MAb (to αM) was obtained from Ortho Pharmaceutical (Raritan, NJ). The TS1/22 MAb to LFA-1 α (αL), the SHCL-3 MAb to p150,95 α (αX) [22], and the TS1/18 MAb to the common β subunit of Mac-1, LFA-1, and p150,95 were prepared as previously described [17]. A $F(ab')_2$ fragment of rabbit IgG directed against the human C3b receptor (anti-CR1) was a generous gift of Dr. D. Fearon (Harvard Medical School, Boston) [24]. Other MAbs used in selected experiments included a mixture of MAbs to αL (TS1/12, TS2/14, TS1/22, TS2/4, and TS2/6 culture supernatants); the MAb to αM, OKM-10 [25]; a mouse MAb to CR1 (44D) [26] (kindly given by Dr. V. Nussenzweig [New York University Medical Center, New York]); and a control MAb to HLA (W6/32) [27]. $F(ab')_2$ fragments of M1/70 were prepared as described [28] for use in CR3-mediated erythrocyte lysis assays.

Immunofluorescence flow cytometry. Indirect immunofluorescence of intact granulocytes was performed by using MAbs and FITC-conjugated antibody to mouse IgG or antibody to goat IgG $F(ab')_2$. Surface-stained cells were fixed in 1% paraformaldehyde and analyzed in a flow cytometer (Coulter Epics V; Coulter Electronics, Hialeah, Fla) as previously described [1, 5]. Log values were converted to a linear scale by using a calibration curve obtained by analyzing (with both the logarithmic and linear amplifier) bead preparations conjugated with seven different concentrations of FITC. The calibration confirmed that the 256 channels of the Epics V log amplifier corresponded closely to a 1,000-fold scale. Linear specific fluorescence intensity (mean fluorescence channel of histogram) was calculated by subtraction of background fluorescence after labeling with X63 IgG1 control antibody. Values were then calculated as a percentage of the specific fluorescence intensity of healthy adult control cells. Cells from control donors demonstrated relative average intensity for the healthy population (see figure 3).

Assays of leukocyte mobilization in vivo and motility in vitro. Leukocyte tissue mobilization was assessed with a Rebuck skin window technique [29]. Granulocyte motility into Millipore filters (Bedford, Mass) was assessed by a modified Boyden technique [18]. The distributions of cells within filters were evaluated with an image analyzer (Optomax, Hollis,

NH) at various incubation intervals, and the depths at which only two cells ("leading front") were in focus in one high-power (× 40) field were measured after incubation periods of 40 min [30]. Leukotactic indices were calculated as described [1].

Shape change assay. Suspensions of granulocytes were exposed to chemotactic factors under various conditions and then fixed in cold (4 C) 1.5% glutaraldehyde. Fixed cells were examined with a ×-1000 phase-contrast objective and classified according to shape, as described by Smith [31].

f-Met-Leu-^{3}H-Phe binding. Granulocytes (10^8/ml), f-Met-Leu-^{3}H-Phe, and f-Met-Leu-Phe were incubated in 200 μl of DPBS at 0 C or 37 C. Reactions were terminated by rapid dilution in cold (4 C) DPBS followed by filtratin of mixtures through Whatman GFC filters (Whatman, Clifton, NJ). Radioactivity was quantitated in a liquid-scintillation spectrometer, and specific binding was calculated as previously described [18].

Adherence and aggregation assays. Adherence chambers were assembled as described [32] after pretreatment of one of two cover glasses for 2 min with a solution of 6% pooled human serum in DPBS. Each chamber was filled with a suspension of granulocytes (5×10^5/ml), which was allowed to settle onto the pretreated glass substrate undisturbed at 21 C for 500 sec. Chambers were then inverted for 400 sec. Cells remaining attached to the substrate surface were counted in 15–20 high-power (× 400) fields. Chambers were again inverted, and cells adhering to the untreated glass surface were counted. Results were expressed as the percentage of cells adhering to the treated surface divided by the total number of cells counted (treated plus untreated surfaces) [1]. Granulocyte aggregation was evaluated as described by Hammerschmidt et al. [33] and modified by Anderson et al. [1].

Phagocytosis assays. Phagocytosis of C3- or IgG-opsonized emulsified paraffin oil droplets was measured as described by Stossel [34]. For selective C3 opsonization, serum obtained from a patient with severe combined immunodeficiency disease was used. The rate of uptake of opsonized zymosan was calculated from the slope of chemiluminescence (CL) evolution, as described [35].

Degranulation. Untreated granulocytes (10^7/ml) or those pretreated with cytochalasin B (5 μg/ml for 5 min at 37 C) were stimulated with f-Met-Leu-Phe (20 n*M*), PMA (10 μg/ml), or opsonized zymosan [36]. Cell-free supernatants or cell pellets were then assayed for lysozyme [37], β-glucuronidase [38], or lactoferrin [39]. Enzyme activities were expressed as percentages of total cell contents released by stimulation or as the total cell contents for each enzyme.

Oxidative metabolic activity. Superoxide (O_2^-) generation of cells exposed to PMA was quantitated with a ferricytochrome C reduction assay [40]. The evolution of CL by granulocytes before or after exposure to f-Met-Leu-Phe, A23187, or PMA, was quantitated as described [35].

Binding assay of ^{51}Cr-labeled-sheep red blood cells. Flat-bottom, 96-well tissue culture plates were treated with poly-L-lysine, as previously described [41]. Granulocytes were allowed to adhere to the plate and the poly-L-lysine was quenched with bovine serum albumin. Sheep red blood cells (SRBCs) were labeled with ^{51}Cr (^{51}Cr-SRBC) as described [42] and treated with appropriate opsonins before they were added to the assay [42]. Assays were terminated by removing unbound SRBCs by extensive washing and then by lysing bound SRBCs and counting ^{51}Cr in the lysate. All assays were done in duplicate and <2% of the total input counts were found after the final wash in wells containing only opsonized ^{51}Cr-SRBCs with no granulocytes. Cytotoxicity assays were done concurrently, as described [43, 44], by using the same targets as in the ^{51}Cr-release assay.

Antibody-dependent cellular cytotoxicity (ADCC). ADCC to herpes simplex virus (HSV)–infected ^{51}Cr-labeled Chang liver target cells was assessed as previously described [3].

Statistical analysis. Data were analyzed by using Student's unpaired *t* test for summary data or Student's *t* test for paired data. Unless otherwise stated, all *P* values refer to the two-tailed test.

Results

The clinical features of the eight patients studies here are summarized in table 1, representative photographs and micrographs are shown in figure 1, and detailed case histories are presented in the appendix.

Analysis of cell surface antigens by immunofluorescence flow cytometry. MAbs specific for the αM, αL, and αX subunits of Mac-1, LFA-1, and p150,95 and their common β subunit were employed in immunofluorescence flow cytometry studies of granulocytes of patients 1, 2, 4, and 6–8. Histogram tracings of unstimulated granulocytes from representative patients, a representative heterozygote, and a healthy adult control are shown in figure 2. Granulo-

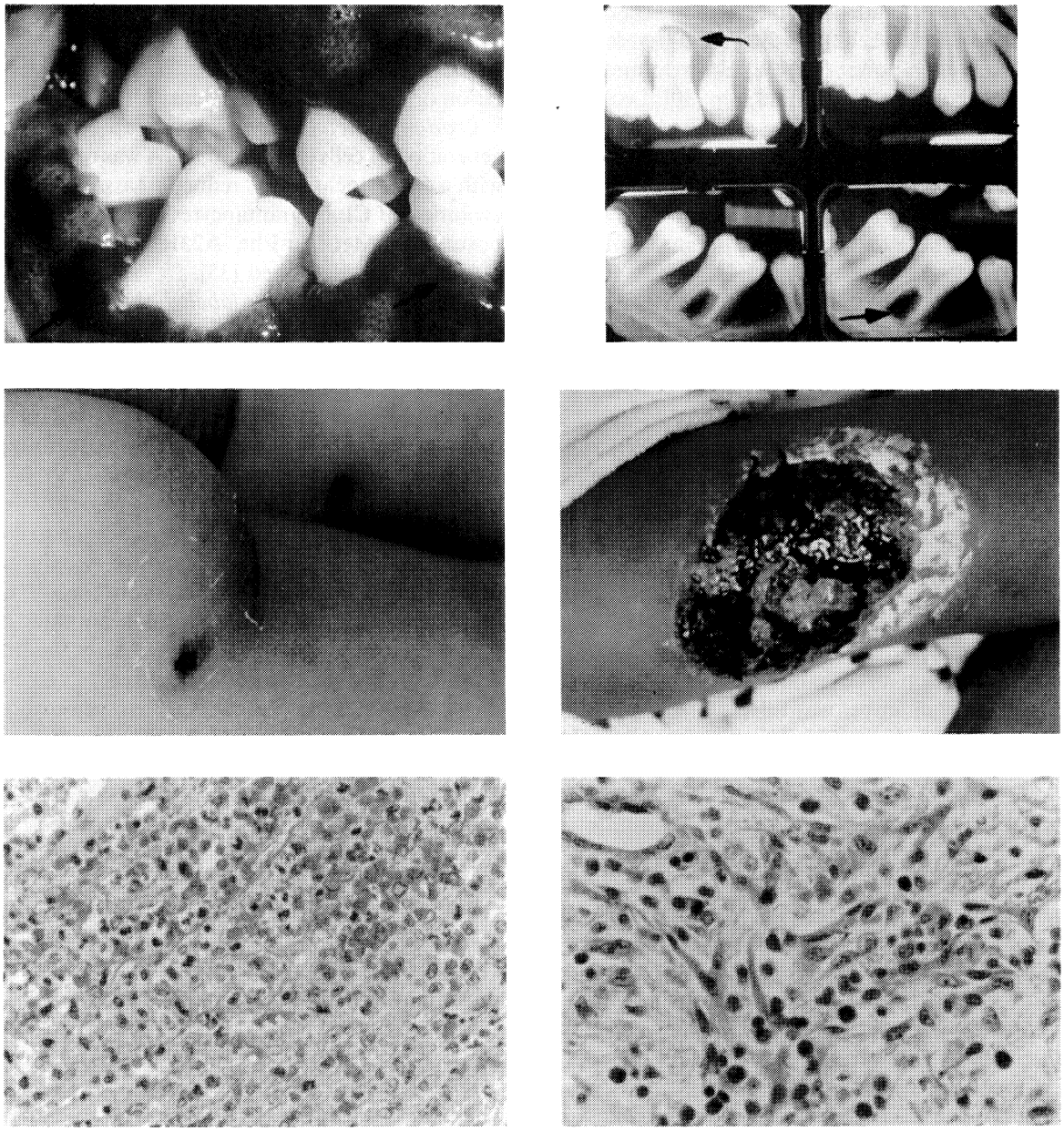

Figure 1. Clinical examples of periodontitis and cutaneous infections in patients with Mac-1, LFA-1, p150,95 deficiency: (*Top left*) Severe periodontitis involving the permanent dentition of a 12-year-old patient (patient 7). Gingivae exhibit acute inflammation, proliferation, recession, and periodontal pocket formation. All remaining teeth exhibit severe mobility. (*Top right*) Radiograph of the same area in top left demonstrates >60% alveolar bone loss around molar teeth. (*Middle left*) Early erythematous cellulitic lesions with central ulcers from which *Pseudomonas maltophilia* was cultured. (*Middle right*) Ulcerative necrotic-gangrenous lesion (5-cm diameter) on volar surface of forearm. (*Bottom left and right*) Sections of infected umbilical cord surgically resected at 18 days of life (hematoxylin-eosin, ×400). (*Bottom left*) Thrombus in an umbilical artery contains numerous neutrophils. (*Bottom right*) The inflammatory infiltrate of adjacent connective tissue is totally devoid of neutrophils but does contain eosinophils in addition to macrophages, lymphocytes, and plasma cells.

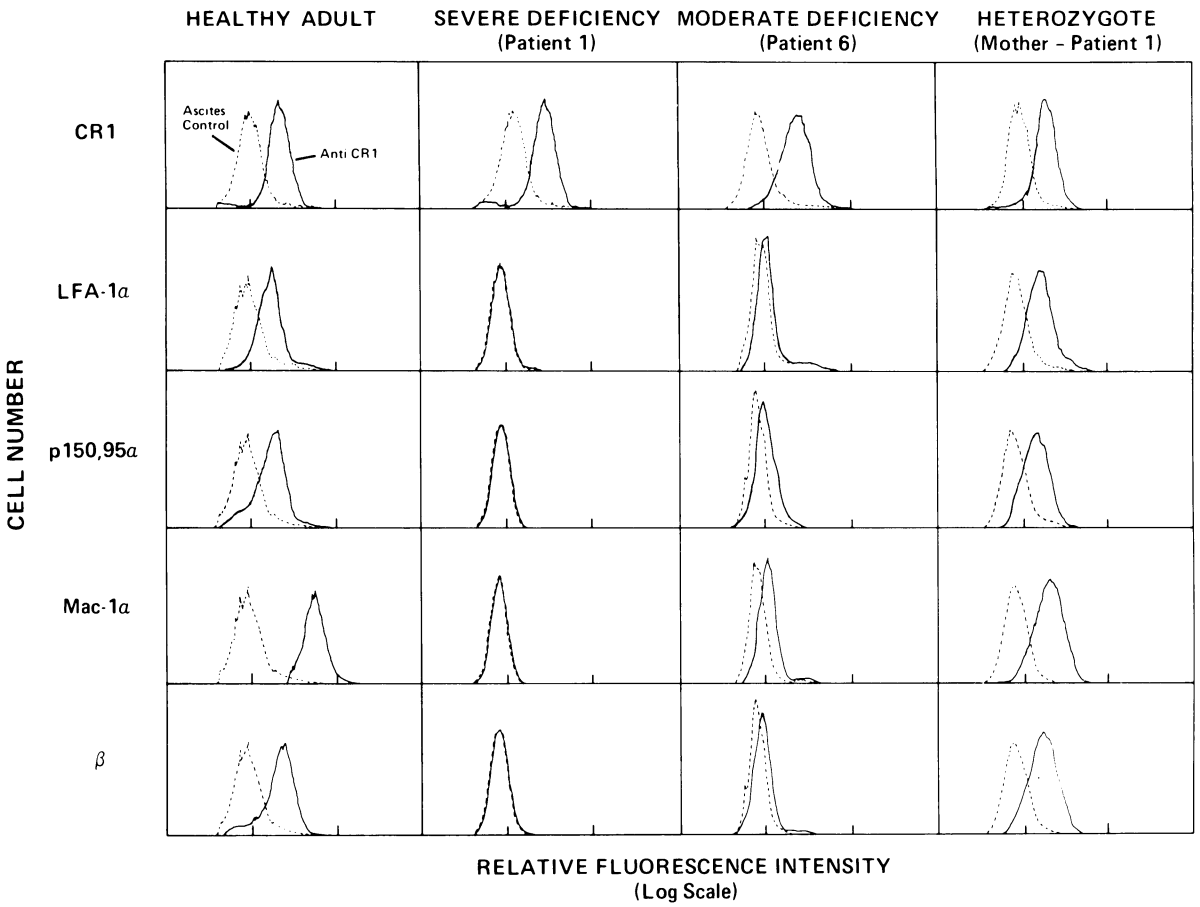

Figure 2. Immunofluorescence flow cytometry of granulocytes of representative severe deficiency (patient 1), moderate deficiency (patient 6), heterozygote (mother of patient 1), and healthy adults. Unstimulated granulocytes isolated from peripheral blood were indirectly stained with $F(ab')_2$ portion of rabbit antibody to human CR1, TS1/22 MAb to αL, OKM-1 MAb to α M, SHCL-3 MAb to α X, or TS1/18 MAb to β. Histograms (cell number vs. relative intensity of fluorescence) of cell suspensions stained with these MAbs are designated by solid lines. Histograms of suspensions reacted with P3X63K nonimmune ascites fluid are shown by dashed lines.

cytes of patients 1 and 2 demonstrated an essentially total (severe) deficiency of cell-surface expression of each protein compared with healthy adult controls. Patients 4 and 6–8 also demonstrated profoundly diminished but clearly detectable expression (moderate deficiency) of each protein subunit. Ten identifiable heterozygotes for this disorder demonstrated intermediate deficiencies of each protein, as shown in figure 2 and table 2. We determined mean baseline levels (with PBS) of 61%, 61%, 63%, and 48% of healthy adult control values for αM, αL, αX, and β subunits, respectively, although a wide range of values was noted among these individuals.

Chemotactic factors such as f-Met-Leu-Phe stimulate a dramatic and rapid increase of surface expression of the CR1 [45], of the Mac-1 α and β subunits [5, 46], and of the p150,95 α subunit [22]. For healthy adult controls employed in these studies, mean-fold increases of surface expression observed in response to f-Met-Leu-Phe (10 n*M*, at 37 C for 30 min) were 4.8, 3.7, 4.1, and 4.8 for αM, αX, β, and CR1, respectively (figure 3 and authors' unpublished data). Expression of the LFA-1 α subunit was not enhanced. Patients' granulocytes were further studied after f-Met-Leu-Phe stimulation (figure 3). Minimal or no enhancement of Mac-1 or p150,95 was observed among granulocytes of patients with either severe or moderate deficiency. However, enhancement of granulocyte CR1 among six of eight patients tested was comparable with healthy adult controls (mean-fold increase, 5.2). The increased surface expression after enhancement allowed somewhat more accurate

Table 2. Immunofluorescence flow cytometry analysis of surface Mac-1, LFA-1, and p150,95 expression on granulocytes from severely and moderately deficient patients and heterozyotes.

Status	Surface antigen				
	Mac-1 α	β	p150,95 α	LFA-1 α	CR-1
Severe deficiency					
Patient 1	0.1	0.15	0.1	0.15	94
Patient 2	0.0	0.1	0.3	0.0	100
Moderate deficiency					
Patient 4	6.0	4.4	7.0	11	92
Patient 6	4.0	2.5	3.5	31	99
Patient 7	4.0	6.0	4.0	26	87
Patient 8	3.0	4.0	2.0	24	109
Heterozygotes*	57	54.5	42.1	66.5	101
	(33–80)	(40–64)	(27–63)	(36–97)	(96–112)

NOTE. Mean values determined in 2–6 experiments expressed as percentage of healthy adult controls for each experiment. Granulocyte suspensions were preincubated with f-Met-Leu-Phe (10n*M* at 37 C for 30 min) prior to reaction with subunit-specific MAb to LFA-1 α, Mac-1 α, p150,95 α, or β, or with polyclonal rabbit and anti-CR1 $F(ab')_2$ fragments. Some determinations for patients 1, 2, and 4 were previously reported [5].

* Includes mothers of patients 1–4, 7, and 8, fathers of patients 1–3, a female sibling of patient 4, and two female siblings of patients 7 and 8. (Two unaffected healthy male siblings of patient 2 demonstrating normal surface expression of each glycoprotein are not included). Numbers in parentheses represent the range of values of individual experiments performed on each heterozygote.

measurements. Quantitation of the four subunits of f-Met-Leu-Phe–stimulated granulocytes demonstrated clear differences between the severely deficient patients (1 and 2) and moderately deficient patients (4 and 6–8; table 2). The many independent measurements on each patient's cells, both with and without f-Met-Leu-Phe stimulation, are shown for the most strongly expressed subunits, Mac-1 α and β, in figure 3. For Mac-1 α, there was no overlap between the severe and moderate groups in eight and 15 different measurements, respectively. For the β subunit, there was only one overlap, and other determinations for this patient with the same MAb or with other subunit-specific MAbs clearly indicated that she was moderately deficient. Clustering of the data points for the cells from the severe and moderate groups was evident both with and without f-Met-Leu-Phe stimulation, and the differences between these groups were highly statistically significant ($P < .001$). The correlation between measurements of each of the four different subunits on granulocytes of individual patients (table 2) confirmed their classification in the severe and moderate deficiency groups. The most striking differences between the two groups were seen with respect to LFA-1 α (table 2).

These findings were confirmed by studies on lymphocytes, which normally express the LFA-1 α and β subunits. We studied LFA-1 expression in mononuclear leukocyte suspensions [5 and data not shown], Epstein-Barr virus (EBV)–transformed and phytohemagglutinin-stimulated lymphocytes [5 and data not shown], and on cytolytic T lymphocyte (CTL) lines [47] of patients 1, 2, and 4. No expression of LFA-1 α or β subunits (<0.1%) was found for patients 1–3. Patient 4 expressed both subunits in 1.7% of normal amounts on CTL and 2% of normal amounts on an EBV line. Blast-transformed lymphocytes of patient 3 were available for immunofluorescence flow cytometry studies following her death; these cells demonstrated a total deficiency of LFA-1 α and β. For all patients studied (1, 2, and 4–8), the surface expressions of the complement receptor 1 antigen (CR1) and HLA-framework antigen (data not shown) were normal.

These immunofluorescence results were confirmed by RIA. We observed essentially no binding of radiolabeled antibody to Mac-1 α or β by unstimulated granulocytes of patient 2 (severe deficiency), but detectable low levels of binding ($\sim$10% of normal) by granulocytes of patient 4, when tested on the same date [5].

Immunoprecipitation of granulocyte membrane proteins by MAbs. To further delineate the molecular abnormalities among the study population, we surface-labeled granulocytes of each patient with ^{125}I and subjected them to immunoprecipitation and SDS-PAGE (data not shown). As shown with healthy adult cells, the MAb to Mac-1 α precipitated the αM subunit noncovalently associated with its β subunit

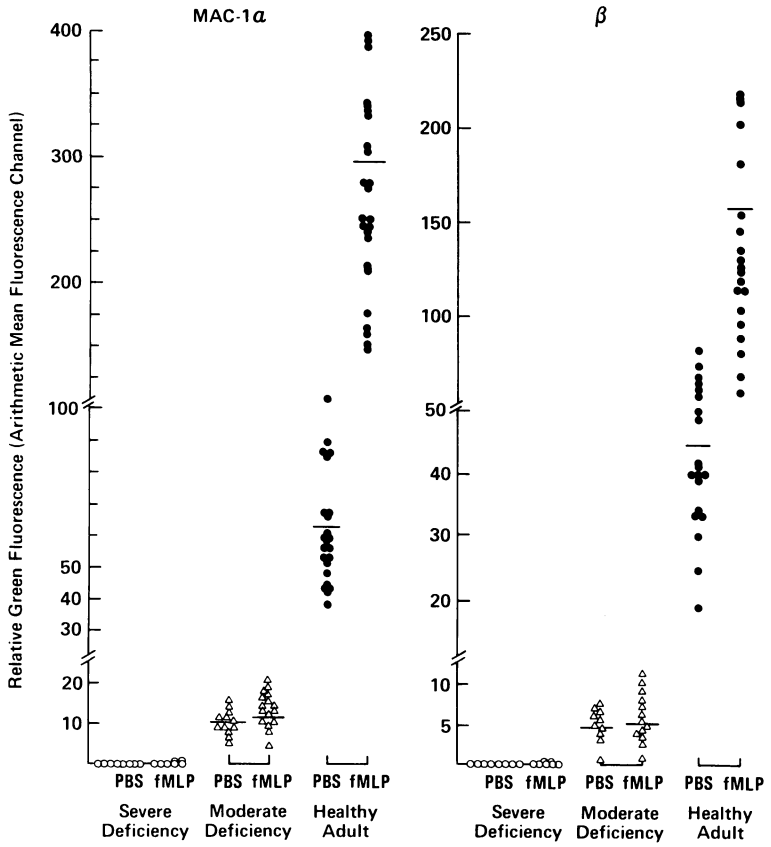

Figure 3. The surface expression of Mac-1 α and β subunit on f-Met-Leu-Phe–stimulated and unstimulated granulocytes. Unstimulated (PBS) or chemotactically stimulated (f-Met-Leu-Phe for 20 min at 37 C) suspensions of severely deficient (patients 1 and 2) or moderately deficient (patients 4 or 6–8) patients or healthy adult granulocytes were indirectly stained with OKM-1 MAb to α M or TS1/18 MAb to β, fixed in 1% paraformaldehyde, and processed in a flow cytometer. Values shown represent individual determinations performed on two to six different dates on suspensions of granulocytes from each patient and are expressed as the relative mean fluorescence of each histogram (minus nonspecific fluorescence) in arithmetic units. Mean values for each group of patients and for experiments employing seven healthy adults are designated by horizontal bars.

(figure 4 A, E, and G, lane 1); the MAb to β precipitated the β subunit and the three types of α subunits noncovalently associatd with it—αL, αM, and αX (figure 4 A, E, and G, lane 2); and the MAb to LFA-1 precipitated the αL subunit with its β subunit (figure 4 A, E, and G, lane 3). Similar results were obtained with heterozygote granulocytes (figure 4 D). In contrast, patients' granulocytes demonstrated total or marked deficiencies of all these molecules (figure 4 B, C, and F, lanes 1–3). From severely deficient patients 1 and 2, no αL, αM, αX, or β subunit precipitation was detectable, even after prolonged exposure of autoradiograms. In contrast, precipitation of small amounts of these subunits was seen in all moderately deficient patients tested (4 and 6–8), with particular clarity after prolonged exposure of autoradiograms (figure 4 F and data not shown). Precipitation of the β subunit with MAbs to α and of α subunits with the MAbs to β demonstrated that the low amounts of Mac-1, LFA-1, and p150,95 molecules are present in $\alpha_1\beta_1$ complexes on the surface of moderately deficient cells. As a positive control, the CR1 molecule was precipitable from all granulocyte preparations tested (data not shown).

SDS-PAGE and surface labeling of granulocyte glycoproteins. Deficiencies are not due to the mere absence of antigenic determinants from the Mac-1, LFA-1, p150,95 proteins. The deficiencies have been confirmed for most patients with antibodies against three determinants on the Mac-1 α subunit and four determinants on the LFA-1 α subunit [5 and data not shown]. Furthermore, direct analysis has demonstrated absence of the Mac-1 α subunit glycoprotein. Whole-cell NP-40 lysates of granulocyte suspensions of all eight patients have been assessed by SDS-PAGE, as previously described for patient 1 [1]. A major protein corresponding to the Mac-1 α subunit was noted to be severely deficient or absent in lysates from each patient, as shown by coomassie blue staining and side-by-side comparison to Mac-1 immunoprecipitates [1, 2, and data not shown]. To confirm that the deficient protein was expressed on the cell surface, we labeled surface glycoproteins with NaB^3H_4 after treatment with neuraminidase and

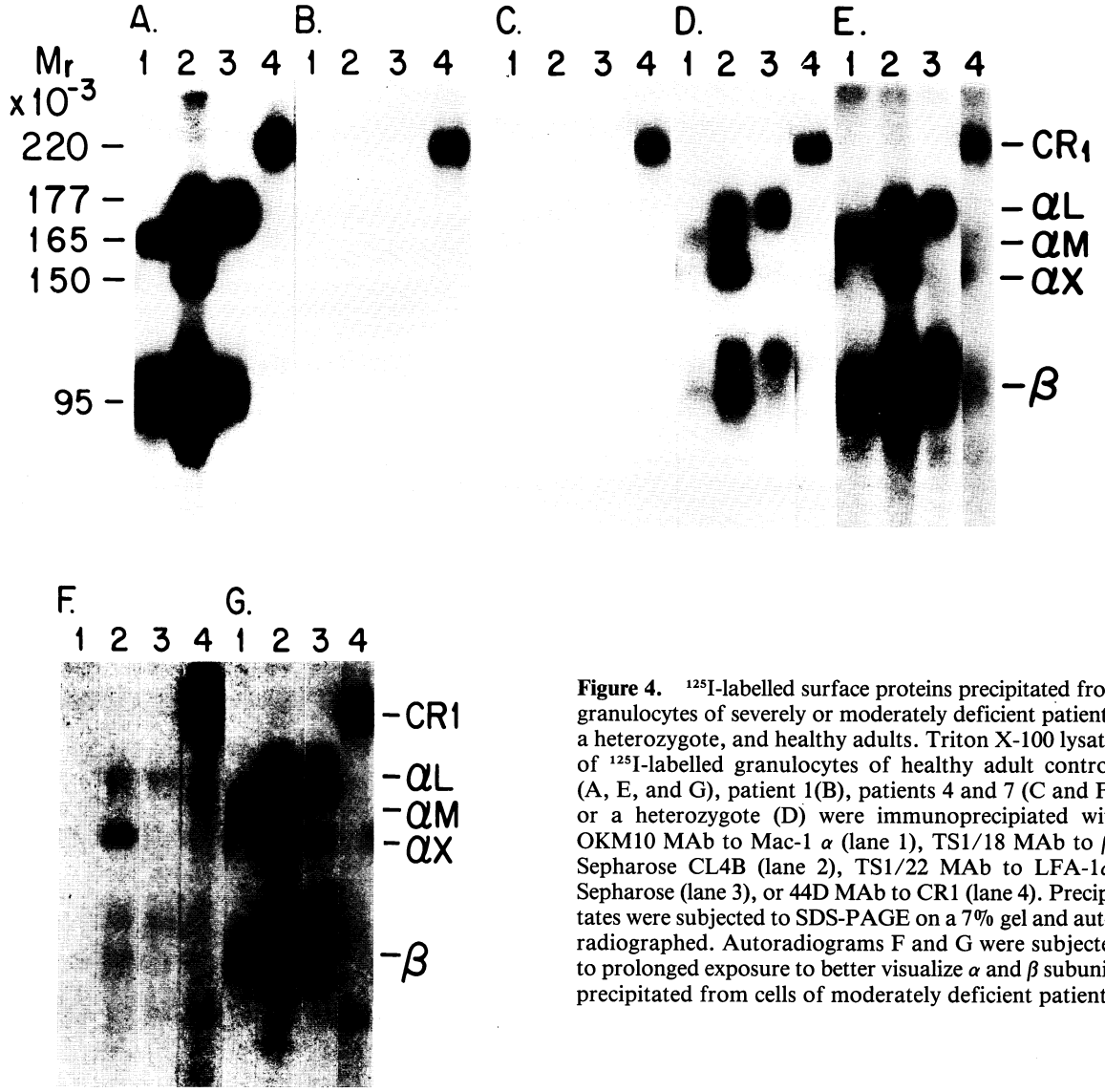

Figure 4. ^{125}I-labelled surface proteins precipitated from granulocytes of severely or moderately deficient patients, a heterozygote, and healthy adults. Triton X-100 lysates of ^{125}I-labelled granulocytes of healthy adult controls (A, E, and G), patient 1(B), patients 4 and 7 (C and F), or a heterozygote (D) were immunoprecipiated with OKM10 MAb to Mac-1 α (lane 1), TS1/18 MAb to β-Sepharose CL4B (lane 2), TS1/22 MAb to LFA-1α-Sepharose (lane 3), or 44D MAb to CR1 (lane 4). Precipitates were subjected to SDS-PAGE on a 7% gel and autoradiographed. Autoradiograms F and G were subjected to prolonged exposure to better visualize α and β subunits precipitated from cells of moderately deficient patients.

galactose oxidase. An essentially total deficiency of αM was evident among suspensions of granulocytes from patients 1–3 (severe deficiency), while diminished but clearly detectable labeling was observed in suspensions from patients 4 and 6–8 (moderate deficiency; table 3).

Leukocyte mobilization in vivo and motility in vitro. These studies and all other functional assays were performed at times when the patients were clinically well and receiving no medications. As shown with a Rebuck skin window technique, mobilization in vivo of both granulocytes and mononuclear leukocytes was profoundly diminshed in each patient as compared with controls (table 4). Patients with moderate deficiency showed some leukocyte mobilization at late time points, while patients with severe deficiency showed none; upon repeated testing and despite striking leukocytosis among the patients with severe deficiency at the time these studies were performed (mean white blood cell (WBC) count, 38,000/mm^3), essentially no granulocyte or monocyte accumulation into skin windows was detectable. Studies of two heterozygotes demonstrated mild abnormalities (data not shown).

Similar relations were observed in Boyden assays in vitro. More profound abnormalities were gener-

Table 3. Quantitation of Mac-1 α on granulocyte surfaces with tritium labeling.

Granulocyte donors	Peak area (^{3}H [cpm ± SD])
Healthy adults*	739 ± 423
Heterozygotes†	525 ± 511
Patients with	
Moderate deficiency‡	67 ± 13
Severe deficiency§	No peaks

NOTE. Tritium counts in 1-mm tube gel sections were stored as histogram data in a computer. Peaks above baseline troughs were identified by the computer. The Mac-1 α peak was located 15–22 mm into a 120-mm 7.5% gel. Counts in the peak area corresponding to Mac-1 α were calculated.

* Six normal adults, each studied once (n = 6).

† Six parents (4 mothers, 2 fathers), each studied once or twice (n = 8).

‡ Three patients with moderate deficiency were studied twice (n = 6).

§ Three patients with severe deficiency were studied twice (n = 6).

ally observed among the patients with severe deficiency compared with the patients with moderate deficiency for both random and directed migration (data not shown). Leukotactic indices determined from the entire migrating cell populations corresponded with "leading front" values (data not shown). The mean (and range) migration of cells in micropore filters in response to f-Met-Leu-Phe was 16 μm (13–18 μm), 28 μm (15–34 μm), and 49 μm, for those with severe or moderate deficiency and healthy adult groups, respectively.

Histological evidence of impaired leukocyte mobilization in tissues of patients with this syndrome is illustrated by the photomicrographs in figure 1 E and F. Sections of umbilical cord infected with *Escherichia coli* that were surgically resected from patient 2 illustrate a striking paucity of stromal neutrophilic granulocytes despite innumerable neutrophils in an adjacent vessel. Similar histopathologic features were apparant in biopsied sections of pharyngeal-glottic tissue of patient 3, gingival tissue of patient 5, and a skin window biopsy of patient 6. In contrast, infiltrates of mononuclear cells (especially plasma cells) and occasionally eosinophils were characteristically observed in these tissue sections (figure 1E and F).

Assessments of granulocyte adherence. Results of studies with serum-coated glass substrates are shown in table 4 for the two categories of patients, heterozygotes, and healthy adult controls. Mean baseline, f-Met-Leu-Phe–, and PMA-stimulated adherence values for patients in the severe or moderate deficiency groups were strikingly deficient as compared with healthy adult or heterozygote individuals. Significantly ($P \leq .01$) diminished adherence under each condition was noted for granulocytes from each patient compared with an experimental healthy adult control. Severely deficient granulocytes were less adherent than were moderately deficient cells. Differences in adherence between granulocytes from patients with severe and moderate deficiencies were statistically significant after stimulation with f-Met-Leu-Phe or PMA ($P < .01$ and $P = .05$, respectively). Mean values after f-Met-Leu-Phe or PMA stimulation for both groups of patients were not significantly greater than their mean baseline values ($P < .05$). In contrast, approximately two- to threefold increases were consistently noted in suspensions of heterozygote and healthy adult cells.

Aggregometry. Suspensions of granulocytes from patients demonstrated a profoundly diminished capacity to aggregate when exposed to chemotactic factors (table 4). As shown by immunofluorescence microscopy, a failure of patients' cells to adhere to one another but not to control granulocytes was confirmed with test mixtures of control cells and FITC-stained patients' cells. Mean group values for f-Met-Leu-Phe– or zymosan-activated serum-stimulated aggregation were significantly diminished ($P < .0001$) as compared with mean group values for heterozygotes or healthy adults. Similar relations were observed when PMA (10 μg/ml) was used as a stimulant ($P < .001$ for each suspension compared with an adult control). No significant differences were apparent between individuals with severe and moderate deficiencies when this methodology was used, and no consistent differences were observed between heterozygotes and healthy adult individuals.

Studies of phagocytic ingestion. Results in table 4 show abnormalities of phagocytic ingestion of test particles selectively opsonized with C3-derived ligands by granulocytes from patients with severe or moderate deficiencies. As previously reported in patient 1 [1], binding or ingestion of particles (Oil-Red-O paraffin or latex beads) selectively opsonized with IgG was relatively normal. However, phagocytosis of complement-zymosan particles or Oil-Red-O paraffin/lipopolysaccharide emulsions was significantly diminished ($P < .01$) among patients with severe and moderate deficiency compared with heterozygotes and healthy adult controls. Although comparable abnormalities were observed among individuals with

Table 4. Assessments of adherence-dependent granulocyte functions.

Functional assay	Patients with Severe deficiency*	Patients with Moderate deficiency†	Heterozygotes‡	Healthy adult controls
Adherence§				
Baseline (PBS, 21 C)	12 ± 2	16 ± 9	24 ± 11	38 ± 6
fMLP (10n*M*, 21 C)	12 ± 3	28 ± 12	68 ± 14	63 ± 6
PMA (5 μg/ml, 21 C)	16 ± 4	31 ± 12	60 ± 12	67 ± 9
Aggregation‖				
Zymosan activated plasma (37 C)	16 ± 11	15 ± 12	94 ± 8	100 ± 0
fMLP (100 n*M*, 37 C)	16 ± 4	14 ± 13	38 ± 9	40 ± 6
PMA (10 μg/ml, 37 C)	15 ± 9	22 ± 3	96 ± 15	105 ± 7
Phagocytosis				
Oil-Red-O-(IgG)**	0.7 ± 0.3/1.4 ± 0.6	0.7 ± 0.4/1.4 ± 0.5	0.8 ± 0.6/1.6 ± 0.3	0.8 ± 0.3/1.7 ± 0.4
Oil-Red-O-(C3)**	1.2 ± 1.0/1.9 ± 1.2	1.0 ± 0.5/2.4 ± 1.2	3.8 ± 1.6/4.5 ± 3.1	5.3 ± 1.9/7.0 ± 3.1
Opsonized zymosan (C3)††	4.6 ± 0.7	7.9 ± 3.2	18.1 ± 5.1	17.1 ± 4.0
ADCC‡‡				
100:1	4.6 ± 0.7	14.4 ± 3.4	24.4 ± 7.5	25 ± 12
60:1	4.8 ± 2.8	20.1 ± 6.7	22.8 ± 7.8	23.2 ± 9.1
30:1	4.3 ± 1.4	17.6 ± 6.1	16.9 ± 6.6	16 ± 6

NOTE. Data presented with respect to each functional assay are represented by mean ± 1 SD values (except ADCC) for each category derived from individual mean values of 2–6 separate experiments. Data included for patient 1 were previously reported [1].

* Includes assessments of patients 1–3.

† Includes assessments on patients 4, 6, 7, and 8.

‡ Includes mothers of patients 1–3, fathers of patients 1 and 3, and two affected (heterozygote) female siblings of patients 4, 7, and 8.

§ Percentage of granulocytes adhering to serum-coated (6%) glass under baseline or stimulated conditions.

‖ Granulocyte aggregation response to zymosan-activated plasma (ZAP) or fMLP expressed in ZAP units [1].

** Dionylphthalate uptake (μg/10^6 granulocytes in 5/15 min).

†† Slope of chemiluminescence evolution (cpm^2 × 10^{-5}).

‡‡ Percent ADCC expressed as ^{51}CR release at three effector-to-target cell ratios (mean ± SE). Some of the results presented for patients 1–4 were previously reported [3].

severe and moderate deficiency when the Oil-Red-O assay was used, lower values were observed among the patients with severe deficiency in the zymosan-CL assay ($P < .01$). Significantly diminished ($P < .05$) ingestion of complement-opsonized Oil-Red-O particles at both 5- and 15-min intervals was observed for granulocytes of seven heterozygotes tested compared with adult controls, but no detectable differences were observed among these two groups when the zymosan-CL assay was used. These findings are consistent with functional abnormalities of the iC3b receptor [1, 5, 9] but not the IgG Fc receptor of granulocytes from patients or heterozygotes.

Assessments of CR3 activity. ^{51}CR-SRBC (E) binding cytotoxicity assays. The complement receptor type 3 (CR3), which binds the iC3b ligand, has previously been shown to be equivalent to the Mac-1 molecule [17, 25, 48]. The functional activity of the granulocyte CR3 was tested in selected patients in adherence and lysis assays. Granulocytes from healthy adults bound 32% of E sensitized with IgM and C′ (E-IgM-C) under conditions in which C′ is deposited as iC3b [44] (table 5). In contrast, granulocytes from a severely deficient patient (2) bound only 3% of the E-IgM-C, whereas those from three moderately deficient patients (6–8) bound 12% of the E-IgM-C.

A similar pattern was seen in the ^{51}Cr-release assay designed to measure the ability of granulocytes to lyse E-IgM-C and E-IgG-C. Granulocytes from the normal and severely and moderately deficient individuals lysed 32%, 2%, and 4% of the E-IgM-C, respectively. Granulocytes of all patients evaluated also showed marked depression of ADCC activity to E-IgG when compared with normal granulocytes. When the E-IgG were further opsonized with complement, the lysis was enhanced from 14% to 24% for the severely deficient cells, from 2% to 54% when granulocytes from the moderately deficient patient were the effectors, and from 39% to 76% when

Table 5. Capacity of granulocytes deficient in Mac-1, LFA-1, p150,95 to bind to and lyse opsonized sheep red blood cells.

Status of granulocyte donor*	Adherence (^{51}Cr bound [%]†)		Lysis (^{51}Cr released [%]‡)			
	E-IgM	E-IgM-C	E-IgM	E-IgM-C	E-IgG	E-IgG-C
Severely deficient (1,1)	1	3	4	2	14	24
Moderately deficient (3,1)	3	12	0	4	2	54
Healthy adult (7,5)	3	32	2	32	39	76

NOTE. Sheep red blood cells were treated with MAb IgM antibody to SRBC alone (E-IgM) or in conjuction with serum from A/J mice (E-IgM-C) or with IgG antibody to SRBC alone (E-IgG) or in conjunction with A/J serum (E-IgG-C).

* The numbers in parentheses represent the no. of individuals for whom duplicate determinations were carried out for the binding and cytotoxicity assays, respectively.

† Granuloycte-to-erythrocyte ratio of 1:2.5; 45-min assay.

‡ Effector-to-target ratio of 1:1; 14-hr ^{51}Cr-release assay.

granulocytes from normal individuals were the effectors. Thus, granulocytes from normal individuals lysed a greater percentage of E-IgG-C than did those from moderately deficient patients, which in turn were more active than granulocytes from the severely deficient patient that were assessed in the lytic assay. The increase of lysis in the severely deficient patient when complement was added to the E-IgG was totally abrogated by the addition of $F(ab')_2$ fragments of an antiserum to CR1, a result indicating that the CR1 was responsible for the small enhancement of lysis in this situation (data not shown). In summary, the severely deficient patients tested demonstrated little or no CR3 activity, while moderately deficient patients showed intermediate levels.

ADCC. Granulocyte suspensions of severely deficient but not moderately deficient patients demonstrated profoundly diminished ADCC at effector-to-target cell ratios of 100:1 to 30:1 (table 4). Mean group values (and each individual mean value) for patients with severe deficiency were significantly diminished when compared with values from healthy adults ($P < .001$), heterozygotes ($P < .001$), or patients with moderate deficiency ($P < .05$). Mean values from patients with moderate deficiency were not significantly different from those from heterozygotes or healthy adult controls ($P > .05$). Moreover, in selected experiments, suspensions of granulocytes from patients 6–8 demonstrated essentially normal ADCC. However, assessment of natural killer cell function in all patients studied demonstrated significantly diminished activity on all test dates ($P \leq .01$, data not shown) [3]. As previously reported [1–3], abnormalities of ADCC were shown to be causally related to diminished target-cell binding, as shown in a single-cell cytotoxicity assay.

Cell activation and change of shape when in suspension. In contrast to abnormal adhesion-dependent granulocyte functions in this disorder, a variety of adherence-independent cell functions were shown to be entirely normal (table 6). Granulocytes from all patients showed a normal capacity to undergo shape change in suspension in response to C5a (data not shown) or f-Met-Leu-Phe. Comparable threshold concentrations (C5a, 1.2 μg/ml; f-Met-Leu-Phe, 0.1 n*M*) effected cell activation (membrane ruffling) of granulocytes from patients and controls [1], and at maximally effective concentrations (C5a, 4μg/ml; f-MetpLeu-Phe, 2 n*M*) granulocytes from all patients showed bipolar morphology in percentages that were comparable with that of control cells. In kinetics experiments, patients' cells also demonstrated normal bipolar shape change at all time intervals between 20 and 60 sec after exposure to 2n*M* f-Met-Leu-Phe and demonstrated normal uropod formation under all test conditions (data not shown) [1, 31].

Specific binding of f-Met-Leu-^{3}H-Phe. Under conditions of saturation at 4 C, granulocytes of patients from both groups demonstrated a normal number of specific binding sites for f-Met-Leu-^{3}H-Phe compared with values from normal adults or heterozygotes (~8,000–10,000 binding sites/granulocyte for each donor). Normal binding of 20 n*M* f-Met-Leu-^{3}H-Phe at 37 C was also observed for all patients' cells (table 6).

Oxidative metabolism. Unstimulated granulocytes from all patients tested demonstrated minimal (normal) baseline generation of O_2^- or CL activity. When stimulated with PMA or A23187 (data not shown), they demonstrated normal O_2^- production and CL activity as compared with healthy adults, heterozygotes, or age-matched controls (table 6).

Table 6. Assessments of adherence-independent granulocyte functions.

Function assessed	Severely deficient*	Moderately deficient†	Heterozyogotes‡	Healthy adults
Shape change in suspension§				
fMLP(2n*M*, 90 sec)	75 ± 8	63 ± 9	74 ± 7	77 ± 13
f-Met-Leu-^{3}H-Phe binding‖	7,800 ± 1,200	9,000 ± 1,400	7,400 ± 1,900	7,400 ± 2,400
Oxidative metabolism				
O_2^- generation**	17.6 ± 1.6	17.1 ± 3.1	19.4 ± 3.1	22.4 ± 3.4
Chemiluminescence††	65.2 ± 7.0	54.3 ± 9.3	63.6 ± 7.0	50.1 ± 8.1
Secretion‡‡				
β-Glucuronidase	3.1 ± 1.2	3.8 ± 11	4.6 ± 2.0	4.1 ± 2.1
	(11.3 ± 6.0)	(19.4 ± 9.2)	(17.1 ± 9.0)	(21.1 ± 4.1)
Lysozyme	3.5 ± 6.8	2.8 ± 0.4	3.6 ± 1.6	3.5 ± 1.0
	(22.0 ± 6.0)	(22.0 ± 1.0)	(20.1 ± 8.0)	(19.4 ± 9.2)
Lactoferrin	3.6 ± 1.2	3.9	2.9 ± 2.0	3.2 ± 1.9
	(17.5 ± 3.2)	(14.0)	(16.8 ± 4.0)	(18.2 ± 4.1)

NOTE. Date presented include assessments of patient 1 previously reported [1].

* Includes assessments of patients 1–3.

† Includes assessments of patients 4, 6, 7, and 8.

‡ Includes mothers of patients 1–3, fathers of patients 1 and 3, and two heterozygote female siblings of patients 4, 7, and 8.

§ Mean ± 1 SD percent bipolar ± uropod forms.

‖ Specific f-Met-Leu-^{3}H-Phe binding sites/granulocyte (mean ± 1 SD).

** Ferricytochrome C reduction (nmol/10 min per 10^6 granulocytes).

†† Chemiluminescence integral (area under the curve × 10^{-6}) determined between T-0 and T-60 min following exposure to PMA (5 μg/ml).

‡‡ Values represent the mean ± SD percent release of total cell content of β-glucuronidase, lysozyme, or lactoferrin following f-Met-Leu-Phe stimulation. Values in parenthesis represent granulocyte suspensions preincubated in cytochalasin B.

These findings indicate that diminished oxidative activity of patients' cells during phagocytosis (table 4) reflects abnormalities of granulocyte-particle interactions rather than intrinisic abnormalities of the respiratory apparatus.

Degranulation. Suspensions of granulocytes from each patient studied had a normal total cell content of lysozyme, β-glucuronidase, and lactoferrin, and a normal capacity to release each enzyme following stimulation with chemotactic factors or PMA. In each case, pretreatment with cytochalasin B effected significantly increased enzyme release, a finding that is consistent with the normal behavior of microfilament disassembly. In contrast, significantly diminished ($P < .01$) secretion of each enzyme by granulocytes from six of the eight patients tested was observed during phagocytic ingestion of zymosan particles [1 and data not shown]. These findings indicate diminished kinetics of ingestion by patients' cells rather than abnormalities of the secretory apparatus per se.

Relation of clinical expression to severity of molecular deficiency. Importantly, differences of clinical course and laboratory evaluation between patients with severe or moderate deficiencies were apparent (see appendix and tables 1, 3, and 4). Most notably, two of three of the patients with severe deficiency died in infancy. Patient 2 developed an invasive enterocolitis leading to shock and an ischemic encephalopathy. Patient 3 developed an acute upper-respiratory-tract infection (picornavirus) that rapidly progressd to involve the hypopharynx, glottis, trachea, and pulmonary parenchyma. Patient 1 (aged 6 years) represents the only survivor of the patients with severe deficiency; she has also had systemic life-threatening infections (peritonitis, septicemia, perirectal abscess, pneumonitis, and aseptic meningitis). Among the five patients with moderate deficiency (mean age, 19 years; range, 7–36 years), life-threatening infections have been infrequently observed despite a relatively prolonged survival. A unique feature of the patients with severe deficiency was a delayed severance or infection of the umbilical cord (or both) that required surgical resection and drainage in two of three cases. This feature was not recognized among individuals with moderate deficiency.

Impaired wound healing was observed among individuals in both groups of patients. No healing of the laparotomy wound was grossly or microscopically apparent in patient 2 at the time of her death. Delayed healing and infection of a laparotomy incision performed for reduction of an intususseption in patient 3 was also observed and, during her last

period in the hospital, a failure to heal pleural-pulmonary fistulas was an important determinant of irreversible ventilatory deterioration. Patient 6 (moderate deficiency) had impaired healing of skin ulcers on the right leg that required three skin-graft procedures, and a puncture-wound infection in patient 5 also required surgical debridement and skin grafting. Delayed healing or infection of Rebuck skin lesions or both was observed in three patients. Finally, patient 4 demonstrated delayed healing of traumatic or infected cutaneous lesions with development of striking "paper-thin" scars noted on numerous occasions over a 15-year interval.

Patients in both categories have had characteristic necrotic cutaneous/subcutaneous infections. These lesions were treated on innumerable occasions in patients 1–3; similar lesions have occurred less frequently among patients with moderate deficiency. Severe gingivitis/periodontitis was a major feature among all patients who survived infancy. Characteristic features included gingival proliferation, defective recession, mobility, pathological migration, and advanced alveolar bone loss associated with periodontal pocket formation and loss of teeth (involving the deciduous and permanent dentitions); these features were recognized in patient 1 and in all patients with moderate deficiency. Infants 2 and 3 had developed severe gingivitis associated with tooth eruption shortly before their deaths.

Discussion

The identification and characterization of these and other reported cases of Mac-1, LFA-1 deficiency provides a molecular basis for understanding normal adhesion-dependent cell functions as well as complex functional abnormalities observed in patients with this disorder [1–14]. These findings extend an increasing literature documenting the pathological importance of cell-adhesive dysfunction in leukocyte disorders [1]. The adhesion-dependent functional abnormalities recognized in our patients could be predicted on the basis of the previously recognized functional activities of Mac-1 [3, 17, 25, 48] and LFA-1 [3, 17, 49–53]. Both molecules act to strengthen adhesions between effector cells and target cells, and in both cases additional cell-surface molecules are required for optimal functional activity. Furthermore, blocking experiments employing $F(ab')_2$ fragments of αM, αL, or β subunits in vitro have demonstrated blockage of normal granulocyte adherence and adherence-dependent (but not adherence-independent) functions; more complete impairment of these functions can be achieved by using these preparations simultaneously [2]. The precise contributions of p150,95 to granulocyte/monocyte functions and the pathological significance of deficiency of this molecule remain to be determined.

Comparative evaluations of the clinical features, functional deficits, and molecular abnormalities among our patients suggest that the magnitude of cell-surface expression of Mac-1, LFA-1, and p150,95, and the severity of functional leukocyte abnormalities, as well as the severity of clinical manifestations, are directly related. As shown with immunofluorescence flow cytometry, immunoprecipitation techniques, and NaB^3H_4–galactose oxidase labeling, our patients could be categorized into two groups. The severely affected group had a virtually total deficiency of leukocyte surface Mac-1, LFA-1, and p150,95. The moderately affected group had profoundly diminished but clearly detectable expression of these proteins. Individuals with severe deficiencies consistently had higher numbers of peripheral blood granulocytes, relatively diminished tissue leukocyte mobilization, and relatively more severe in vitro abnormalities of granulocyte/monocyte motility, hyperadherence, phagocytic ingestion of opsonized zymosan, CR3 activity, and ADCC compared with the moderate deficiency group. Expression of even small quantities of Mac-1, LFA-1, or p150,95 (or any combination) on leukocyte surfaces of individuals with moderate deficiency appears to confer protection against invasive, life-threatening infections. Heterozygotes had normal functional activity (in most assays) and solid protection against clinical infections.

We previously reported [1] comparative studies of patient 1 and another male patient with Mac-1/LFA-1 deficiency described by Arnaout [8] in which analysis of Mac-1 ^{125}I $F(ab')_2$ anti-Mo-1 binding revealed that the latter patient had 7,000 ± 3,000 Mo-1 sites/cell compared with none for our patient 2 and 65,000 ± 7,000 sites/cell for normal controls. Thus, the expression of as little as 10% of normal granulocyte Mo-1 in that eight-year-old child may account for the relatively less-severe functional abnormalities reported, especially with respect to those of leukocyte motility [1, 8]. Heterogeneity with respect to the severity of clinical illness or abnormalities of leukocyte functions (or both) among other reported patients with Mac-1 deficiency may be related to quan-

titative differences of surface expression of this critical glycoprotein complex, and further comparative assessments may allow a more accurate determination of clinical prognosis and strategies for management of this disease in the future.

Recurrent infections generally occurring on body surfaces are unquestionably related to phagocyte dysfunction in this disorder. The occurrence of necrotic, cutaneous, mucous-membrane or periodontal infections and impaired wound healing observed in these patients appear to reflect a profound impairment of leukocyte mobilization (pus formation). Similar cutaneous or mucous-membrane lesions and a similar spectrum of infecting microorganisms (*Staphylococcus aureus* or gram-negative enteric organisms) are characteristic of patients with primary neutropenia syndromes [54, 55], individuals with a genetic deficiency of neutrophil-specific granules [36, 56–59], and human neonates [18, 60, 61]; these patients represent other clinical models demonstrating abnormal granulocyte adherence, chemotaxis and/or insufficient or delayed leukocyte mobilization. The absence in our patients of deep-seated granulomatous infections as characteristically occur in chronic granulomatous disease or other disorders of leukocyte oxidative metabolism [62] probably reflects a relatively normal intracellular microbicidal capacity of their peripheral blood granulocytes/monocytes and tissue macrophages. Studies of intracellular bactericidal activity (*Staphylococcus aureus* 502A) performed with suspensions of granulocytes from patients 1, 3, 4, and 6–8 in our laboratory demonstrated normal intracellular killing (data not shown). Such findings have been generally, but not always, observed among other reported patients [4, 6, 7, 10, 13].

In contrast, the impaired healing of traumatic or surgical wounds observed in our patients appears to represent a clinical feature unique to this disorder. Interestingly, unusual paper-thin cutaneous scars have been characteristic of patient 4 of this report, as well as of two patients described elsewhere. Delayed severance of the umbilical cord (requiring surgical resection) noted in several of our patients and of those reported elsewhere may represent a somewhat similar pathological process related to abnormal inflammatory contributions to "healing." Collectively, these clinical features may suggest a pathogenic role for dysfunction of monocyte-macrophages known to contribute to angiogenesis and other components of wound healing [63].

Impaired granulocyte hyperadherence and motility in this deficiency appears to be related to impaired induction of surface expression of Mac-1 and p150,95 glycoproteins by chemotactic or secretory stimuli. Essentially no or minimal enhancement of Mac-1 glycoproteins by chemotactic factors or PMA was observed on granulocytes of patients described in this study, whereas a 300%–700% increase of αM, αX, and β was consistently demonstrated when granulocytes were used from healthy adults. All other functions were normally induced by f-Met-Leu-Phe and PMA in patients' cells, including cell bipolarization, CR1 enhancement, release of granule contents, and production of O_2^-. The specific molecular and functional deficits observed in patients' cells suggests that normal granulocyte hyperadherence and aggregation is mediated by the increased surface expression of Mac-1 and p150,95. We propose that in vivo, chemoattractants diffusing from sites of inflammation into the circulation induce enhancement of granulocyte Mac-1 and p150,95, a response leading to margination and aggregation at the inflammatory site. We further propose that in analogy to the importance of Mac-1, LFA-1, and p150,95 glycoproteins in in vitro chemotaxis assays, these glycoproteins mediate essential adherence functions during granulocyte diapedesis and migration into inflammatory sites. Clearly, the profound inability of granulocytes to migrate into inflammatory sites and form pus represents an important histopathologic manifestation of Mac-1, LFA-1, p150,95 deficiency. We propose that this is due to a lack of enhancement of cell adhesiveness, which is normally mediated (in part) by the increased surface expression of Mac-1 and p150,95.

Granulocytes and monocytes normally express Mac-1, LFA-1, and p150,95; in contrast, lymphocytes normally express only LFA-1 [17]. Perhaps this is why the clinical findings point to a much more profound effect of this deficiency on granulocyte function than on lymphocyte function, despite in vitro assays that demonstrate an important role for LFA-1 in lymphocyte function. Monoclonal antibodies to LFA-1 inhibit T lymphocyte–mediated killing and proliferative responses, natural killing, and antibody-dependent killing [3, 17, 49–53]. Furthermore, after primary mixed lymphocyte culture, lymphocytes from patients 1, 2, 4, and 6–8 demonstrated diminished killing and proliferative responses [5, 64]. However, after secondary and further stimulation, responses increased to nearly normal levels. It has been speculated that this may be due to compensatory mecha-

nisms, perhaps involving an increase in the avidity of T cell–antigen receptor, and such mechanisms might also account for observations of relatively normal B and T lymphocyte function in this disorder. These include the following: (*1*) normal delayed cutaneous hypersensitivity among most of our patients and those in other reports [4, 6, 8, 10], (*2*) apparently normal and self-limiting clinical courses of varicella and other viral respiratory infectons in our patients and in those reported elsewhere, (*3*) the absence in five of our patients of untoward effects of live viral vaccine administration, and (*4*) normal synthesis of specific antibodies by most individuals with this disorder who were studied [4, 6, 8, 10]. Clinical findings in some cases, however, suggest lymphocyte dysfunction. A patient studied in France [14] failed to produce antibody to poliovirus, diphtheria, and tetanus toxoids after vaccination. This patient also developed cutaneous candidiasis. Patient 3 in this report died of an overwhelming infection with picornavirus involving orohypopharynx, glottis, trachea, and lungs. It thus appears possible that lymphocyte defects contribute to the clinical findings, particularly in severely deficient patients. Further clinical observations will be required to determine the significance of clearly documented in vitro lymphocyte abnormalities in this disorder.

Previous studies of the families of patients 1–4 showed autosomal recessive inheritance of the disease [5]. This has now been further confirmed by studies on the family of patients 6–8, the only known kindred in which the disease is present in two generations. Heterozygotes in this kindred have been identified by their expression of half-normal quantities of Mac-1 α and β on the granulocyte surface (data not shown). Both parents of patient 6 are heterozygotes. Patient 6 married a heterozygous woman, and their four tested offspring include an affected daughter (patient 7), an affected son (patient 8), and two healthy heterozygous daughters. These offspring are third cousins of patient 4. All these moderately deficient patients come from a small, isolated town and may have identical β subunit mutations.

Biosynthetic studies on patients 1, 2, and 4, and deficiency of multiple members of the glycoprotein family in all patients studied here and elsewhere suggest that the primary genetic lesion in all cases affects the common β subunit. The small amounts of β subunit obtainable from moderately deficient patients has thus far precluded determination of whether it is a normal or mutated product. We hypothesize that in moderately deficient patients only a small amount of β subunit is made and this limits the amount of α that can complex with β and be expressed as a mature $\alpha_1\beta_1$ complex on the cell surface or in intracellular latent pools. Uncomplexed, free α appears to be degraded [5]. Production of β subunit could be limited by splicing mutations, which would result in only a small percentage of correctly spliced mRNA; by regulatory mutations; or by mutations of coding sequences that affect stability. Severe deficiency is hypothesized to result from more deleterious β subunit mutations. As in other mutations for which there is no selective advantage, many independent mutations are likely to be represented in both the severely and moderately affected patients. It is thus possible that further discrimination within these groups will be possible, particularly when appropriate DNA probes become available. Severe mutations were found in three unrelated kindreds of different ethnic backgrounds among our patients. The moderately affected patients in this study may all represent a single kindred; however, further patients of distinct ethnic backgrounds reported in other studies clearly fit in the same category [1, 9], and thus it appears that this category is general.

Important quantitative differences with respect to cellular dysfunction and susceptibility to clinical infection have been defined here among our patients; these differences are directly related to variable deficiency of the Mac-1, LFA-1, and p150,95 glycoproteins on myeloid cells. Confirmation of the severe and moderate phenotypes among other recognized patients may allow the development of more appropriately tailored prophylactic or therapeutic strategies. A diminished susceptibility to infection and improved granulocyte function have been observed following bone marrow transplantation in two patients with reported Mac-1 deficiency; one is doing well clinically two years after transplantation, while demonstrating stable chimerism (40% donor cells) and normal phagocyte function [12]. When weighing the potential risks and benefits of bone marrow transplantation, classification of the deficiency as severe or moderate may be an important consideration.

Appendix—Case Reports (see table 1 and figure 1)

Patient 1

This six-year-old white girl presented at three weeks of age with delayed separation of the umbilical cord and omphalitis; the con-

dition responded promptly to antibiotics [13]. A marked leukocytosis (WBC, 70,000/mm³) with a predominance of neutrophils was noted. At five weeks of age, she presented with peritonitis secondary to infection with *Klebsiella, Proteus,* and enterococcal species. A urachal cyst was drained and the patient responded again to antibiotics. At four months she returned for surgical drainage of a perianal abscess from which were cultured species of *Klebsiella, Proteus,* and *Pseudomonas,* and enterococci. The abscess was drained and responded slowly to antibiotic therapy. During the next one to two years she developed multiple episodes of otitis media, conjunctivitis, "diaper dermatitis," and vaginitis (*Candida* spp.). At the eruption of primary dentition she developed intense inflammation of the gingivae. She subsequently developed progressive periodontal disease despite aggressive preventative dental care and prophylactic antibiotics including bactrim, penicillin, and erythromycin. By three years of age, severe periodontal disease was apparent radiographically with almost total destruction of alveolar bone. Deciduous teeth were lost and permanent teeth erupted prematurely.

The patient continued to have recurrent ulcerative stomatitis and recurrent cutaneous lesions of the face, trunk, or extremities that were nonpurulent and frequently developed a central necrotic ulcer surrounded by extensive erythema or bluish discoloration or both. *Staphylococcus aureus* and a variety of *Pseudomonas* spp. (*Pseudomonas cepacia, Pseudomonas maltophilia,* and *Pseudomonas aeruginosa*) were cultured from most of these lesions. The lesions resolved slowly in response to oral antibiotics and commonly developed into permanent eschars.

Marked granulocytosis persisted during infection-free periods. Serum concentrations of immunoglobulins, complement components, and specific antibodies to tetanus toxoid were normal for age, and delayed cutaneous hypersensitivity responses to *Candida albicans* antigen were positive at 48 and 72 hr.

The patient is an only child. Her parents are of English extraction, are not related, and have had no significant clinical disease.

Patient 2

An 18-month-old Hispanic girl presented at twelve days of age with erythema and induration of the umbilical cord from which aspirated fluid yielded *Escherichia coli.* A marked granulocytosis (WBC, 47,000/mm³) and thrombocytosis was noted at that time. Because of a poor clinical response to antibiotic therapy and continued delayed severance of the cord, a sinogram was performed; it revealed a patent urachus or omphalomesenteric remnant, which was surgically excised. A biopsy of this tissue was interpreted as fibrovascular tissue with acute inflammation and granulation; large numbers of neutrophilic granulocytes were identified within capillaries, but extravascular tissue demonstrated a striking paucity of neutrophils despite large numbers of eosinophils and bacteria. Cellulitis developed at the site of an iv canula in association with a striking granulocytosis (WBC, > 100,000/mm³) and thrombocytosis. Quantitative determinations of levels of serum immunoglobulins, C3, and C4, and the amount of complement that lyses 50% of sensitized red blood cells (CH_{50}) were normal. Examination of bone marrow revealed myeloid hyperplasia but no other abnormalities, and no morphological abnormalities of peripheral blood leukocytes were observed by light microscopy.

Readmission was required at two months of age for an upper-respiratory-tract infection and *Candida* dermatitis. Persistent and striking leukocytosis (WBC, 35,000–100,000/mm³) with a predominance of neutrophils was again noted. At five months of age, admission was required for treatment of aseptic meningitis, widespread "tinea corporis," and facial impetigenous lesions. Following the onset of diarrhea, the patient developed a severe perirectal cellulitis. The perirectal lesion desquamated and *P. aeruginosa* and *P. maltophilia* were isolated from the resultant ulcerative lesion, which slowly resolved following two weeks of antibiotic administration. Throughout this hospitalization the patient developed "nonspecific reactive dermatitis," hepatosplenomegaly, and generalized lymphadenopathy. A Rebuck skin window study demonstrated essentially no accumulation of PMNs or mononuclear leukocytes during the 24-hr interval of testing. The patient was discharged but was readmitted approximately one week later because of a new erythematous lesion on the left buttock, which ulcerated and from which *Escherichia coli* was isolated. This lesion was nonfluctuant and necrotic; it slowly granulated and healed in response to systemic antibiotics.

Over the next year, the patient developed multiple episodes of necrotic, ulcerative skin lesions usually due to *Pseudomonas cepacia,* which slowly resolved following oral antibiotic therapy. At fourteen months, she developed an infra-orbital preceptal cellulitis that responded quickly to nafcillin and chloramphenicol. At that time, a brownish discoloration of the deciduous teeth was apparent. Delayed cutaneous hypersensitivity responses to *Candida* and tetanus antigens were interpreted as normal.

At eighteen months of age the patient was admitted to the hospital because of dehydration, vomiting, and fever. Periumbilical erythema, abdominal distension, pneumatosis intestinalis, and shock rapidly evolved. Exploratory laparotomy revealed necrosis of the transverse and ascending colon and distal jejunum. Microscopy of resected bowel showed an extensive acute necrotizing vaculitis in the submucosa and serosa. The surrounding submucosa was grossly edematous and intensely inflamed. The overlying mucosa of the ileum and colon had undergone ischemic necrosis. A 0.6-cm, well-circumscribed ulcer was identified at the mesenteric portion of the colon; it showed transmural ischemic necrosis with ischemic vaculitis of the vessels. Postoperatively the patient was aggressively treated with colloid solutions, vasopressors, coricosteroids, and antibiotics for persistent hypotension and poor tissue perfusion. She remained comatose, developed progressive electroencephalographic abnormalities, and died six days after admission.

A family history and evaluations of the patient's three- and six-year-old brothers and her parents revealed no evidence of recurrent infection, other clinical disease, or consanguinity.

Patient 3

This Iranian girl presented at three weeks of age with delayed umbilical cord severance and subsequent infection with *Escherichia coli,* which responded to debridement and antibiotic therapy. The patient was well until approximately six months of age, when she presented with high fever and cellulitis of a perirectal periinguinal abscess and associated diarrhea. The patient also had many nodular erythematous facial lesions of 0.5–1-cm diameter, which subsequently ulcerated but never became fluctuant. A striking leukocytosis (WBC, 44,000/mm³ with 72% neutrophils, 3% band-forms, 18% lymphocytes, and 7% monocytes) and thrombocytosis were noted. An aspirate of the right inguinal-perirectal cellulitis yielded a *Pseudomonas* sp. This lesion slowly resolved on treatment with systemic antibiotics over a three-week interval. The patient had a persistent granulocytosis (WBC,

>33,000/mm³) throughout this period in the hospital. Laboratory studies revealed normal levels of serum immunoglobulins, C3 and C4, and normal CH_{50} activity. Delayed hypersensitivity skin tests with *Candida* antigen were interpreted as negative at 48–72 hr.

At nine months, the patient presented with a two-day history of nausea, vomiting, and abdominal distention. A radiograph showed small-bowel obstruction secondary to a iliocolic intussusception. Following surgical reduction, a wound infection developed that was successfully treated with oral antibiotic and debridement. During the next eight months she developed multiple episodes of otitis media that were successfully treated with oral antibiotics.

At eighteen months of age, she developed high fever and an ulcerative mucosal lesion on her lower lip after an exposure to family members with pharyngitis. A crusted nasal discharge, erythema and whitish exudates on the tonsils, and a whitish ulcerative lesion on the lower lip were found. Hemorrhagic and exudative lesions rapidly appeared on the pharynx, palate, oropharynx, epiglottis, and vocal cords. An emergency tracheotomy was performed to alleviate deterioration of ventilatory function. Because of concurrent deterioration of CNS function, an electroencephalograph was performed, which demonstrated a generalized encephalopathic process. Biopsies of mouth ulcers and valecula obtained at the time of the tracheotomy revealed extensive bacterial and fungal overgrowth associatd with essentially no leukocytic reaction. Electron microscopic studies of oral lesions were suggestive of herpesvirus particles; a picornavirus was cultured from these lesions.

On the third hospital day chest radiographs revealed progressive bilateral infiltrates and pneumothorax. Treatment with iv gammaglobulin and multiple antibiotics was unsuccessful, and the child subsequently died. Laboratory studies performed on the patient and her parents during these hospitalizations are described in the text.

Family history revealed that the patient was an only child and that her mother and father were first cousins who had immigrated to the United States from Iran.

Patient 4

This eighteen-year-old Hispanic man presented in the first two weeks of life with ulcerative stomatitis. During his first two years he experienced multiple episodes of otitis media, which generally responded to antibiotics. At 22 and 38 months, he developed hemorrhagic bullous lesions due to *P. aeruginosa* on the buttocks, back, and extremities. These lesions resolved after drainage and application of topical antibiotics. At three years he was referred for immunologic evaluation. At that time, we noted numerous flat eschars on the trunk and extremities; scarred, hyperemic, and retracted tympanic membranes; severe gingivitis; many mobile deciduous teeth; and hepatosplenomegaly.

Initial laboratory studies demonstrated leukocytosis with a predominance of segmented neutrophils; we saw no immature forms of neutrophils or other morphological abnormalities in blood smears by light microscopy. A bone marrow examination revealed myeloid hyperplasia. Cultures of sputum and bullous cutaneous lesions yielded *P. aeruginosa*. Serum concentrations of immunoglobulins were generally elevated (IgG, 1,800 mg/dl; IgM, 344 mg/dl; and IgA, 352 mg/dl). Isohemagglutinin titers were 1:32 for anti-A and 1:64 for anti-B. A serum agglutinin titer to the *P. aeruginosa* isolated from skin lesions was 1:128. Cutaneous hypersensitivity responses to *Candida albicans* and streptokinase-streptodornase was >12 mm of induration at 48 hr. Biopsy of the *Candida* skin test lesion at 36 hr showed perivascular infiltration of mononuclear leukocytes. Irrigations of the patient's mouth with 0.85% NaCl were examined microscopically for neutrophils. In contrast to adults with idiopathic gingivitis-periodontitis syndrome who were studied concurrently, no neutrophils were recovered. A gingival biopsy revealed stromal infiltration of mononuclear leukocytes but no neutrophils.

During the past 15 years, the patient has had multiple recurrent cutaneous infections secondary to *P. aeruginosa* but with decreasing frequency. Resultant scars are often very thin and depressed. Severe gingivitis, periodontitis, and granulocytosis (WBC, >20,000/mm³) persisted. Serum levels of immunoglobulins were normal or elevated and C3, C4, and CH_{50} levels were normal. Hepatosplenomegaly resolved and the patient has experienced no further episodes of deep-tissue infections. A family history revealed no evidence of consanguinity. The patient's mother and sister have no chronic illness.

Patient 5

This 15-year-old Hispanic boy was hospitalized during much of his first year of life for recurrent episodes of diarrheal illness. Between one and four years of age, he was treated for recurrent otitis media, severe ulcerative stomatitis, and severe persistent gingivitis. By four years of age many deciduous teeth had been lost or were mobile. A varicella infection at seven years resolved without sequelae. At 10 years he developed a vesicular skin eruption on the abdomen and extremities, which became secondarily infected. Cutaneous lesions healed with antibiotic treatment over several months, but slowly and with significant scarring. At 11 years a puncture wound on the plantar surface of the left foot became secondarily infected with *Staphylococcus aureus* and ultimately required three surgical procedures, including skin grafts, to repair the wound. Severe periodontitis and ulcerative stomatitis have persisted since that time. At 15 years the patient was referred for an immunologic evaluation. Severe periodontal disease was observed, and a gingival biopsy showed evidence of intense inflammation, with mononuclear and plasma cell infiltrates but a paucity of neutrophils. Serum immunoglobulin concentrations were increased. The patient had leukocytosis (WBC, 15,000/mm³) with a predominance of neutrophils. Nitroblue tetrazolium reduction following phagocytosis was normal. A family history revealed no evidence of consanguinity nor chronic illness among his family members.

Patient 6

This 37-year-old Hispanic man presented with a history of recurrent skin infections beginning in infancy, recurrent otitis media, and the development of severe periodontal disease by three years. Severe generalized alveolar bone loss affecting both deciduous and permanent dentitions required surgical removal of his teeth by 13 years. Over the past 22 years the patient had recurrent skin infections, characterized by impaired pus formation, a delayed response to antibiotic treatment, the development of chronic necrotic and ulcerative lesions, with subsequent scar formation, and the development of superficial hyperpigmented scars and plaques. A family history revealed that similar clinical infections have occurred in two (one male, one female) of four (one male, three female) children but no evidence of consanguinity was apparent.

At 35 years the patient presented with a chronic ulcer (10 × 6 cm in diameter) on the lateral aspect of the right leg. Cultures of this lesion yielded multiple organisms including *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Histological examination of an incisional biopsy from one of these ulcers showed a dense inflammatory infiltrate throughout the dermis and extending into subcutaneous fat; the infiltrate consisted of predominantly mononuclear cells with many plasma cells and only an occasional stromal neutrophil. No vasculitis was identified, but several small vessels were noted to have many neutrophils in their lumens. The patient's chronic leg ulcers were unsuccessfully treated with multiple antibiotic regimens, physical therapy with whirlpool baths, hyperbaric oxygen therapy, and pigskin grafting. An initial split-thickness skin graft from the thigh was unsuccessful, but successful engraftment was achieved on a second attempt.

Peripheral blood leukocyte counts were persistently >20,000–30,000/mm³, with a predominance of neutrophils and an eosinophilia (range, 700–1,500 cells/mm³). Serum IgG (1,460–2,090 mg/dl), IgA, IgM, and IgE (6,500 IU/ml on one occasion) were increased. Skin tests with mumps, *Trichophyton*, and *Candida* antigens were positive at 48 and 72 hr. Anti-B titers were 1:16, and tetanus antitoxin titers were 10 U/ml. Other laboratory studies are described in the text.

Patient 7

This 12-year-old girl, the eldest daughter of patient 6, was in relatively good health and demonstrated normal growth and development until 19 months, when she developed indolent skin lesions (0.5–2 cm in diameter) on the neck, buttocks, and thighs, and recurrent episodes of otitis media. Generalized severe periodontitis was noted at three years. Progressive loss of alveolar bone involving both the deciduous and permanent dentitions required surgical removal of the deciduous teeth by three years and selected permanent teeth by 12 years. She has continued to have recurrent necrotic, indolent skin lesions that frequently became scarred. Cultures of these lesions have generally yielded *S. aureus*. The patient has otherwise had no systemic infections and her growth and development were normal.

The peripheral WBC counts ranged from 12,000 to 45,000 cells/mm³, with a preponderance of neutrophils. Serum IgG levels (2,190–2,590 mg/dl) were consistently increased, whereas serum levels of IgA, IgM, IgE, C3 and C4, and CH_{50} activity were normal. Skin testing revealed normal delayed hypersensitivity to *Candida* and tetanus antigens at 48 and 72 hr.

Patient 8

This seven-year-old boy, the son of patient 6, developed aseptic meningitis during the neonatal period. Subsequently, the clinical course was characterized by recurrent indolent, necrotic skin infections, otitis media, and several episodes of "pneumonitis." Cultures of skin lesions yielded primarily *S. aureus*. The patient was diagnosed as having advanced generalized periodontal disease at three years; he required surgical removal of deciduous teeth at four years.

Peripheral WBC counts ranged from 13,800 to 57,000 cells/mm³, with a predominance of neutrophils. Serum IgG levels were consistently elevated (1,050–1,540 mg/dl), whereas IgA, IgM, IgE, C3 and C4, and CH_{50} were normal for age. Skin tests for delayed hypersensitivity to mumps and *Candida* antigens were interpreted as positive.

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