0022-1767/86/1371-0015$02.00/0
THE JOURNAL OF IMMUNOLOGY

Vol. 137, No. 1, July 1, 1986
Printed in U.S.A.

CONTRIBUTIONS OF THE Mac-1 GLYCOPROTEIN FAMILY TO ADHERENCE-DEPENDENT GRANULOCYTE FUNCTIONS: STRUCTURE-FUNCTION ASSESSMENTS EMPLOYING SUBUNIT-SPECIFIC MONOCLONAL ANTIBODIES[1]

DONALD C. ANDERSON,[2]* LINDA J. MILLER,† FRANK C. SCHMALSTIEG,† ROBERT ROTHLEIN,† AND TIMOTHY A. SPRINGER[3]†

*From the *Department of Pediatrics, Cell Biology, Baylor College of Medicine, Houston, TX 77030; the †Laboratory of Membrane Immunochemistry, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02115; and the ‡Department of Pediatrics, University of Texas Medical Branch, Galveston, TX 77550*

MAb directed at the α-subunits of Mac-1 (αM), LFA-1 (αL), p150,95 (αX), or their common β-subunit were used to characterize the contributions of the Mac-1 glycoprotein family to granulocyte adherence reactions. Inhibitory effects of these MAb in incubation experiments with normal granulocytes indicated distinct adhesive contributions of each subunit. Significantly greater adherence, and inhibition of adherence by anti αM, αX, and β MAb, was observed under chemotactic conditions designed to "up-regulate" the surface expression of the αM β and αX β complexes. Adherence to protein-coated glass and binding of albumin-coated latex beads were significantly inhibited by anti-β > anti-αM (OKM-10, M1/70, LM2/1.6 and OKM-1) > anti-αX > anti-αL MAb, but no effects of anti-HLA, AB, or anti-CR-1 MAb were evident. A similar rank order of inhibition was observed in granulocyte aggregation assays in response to C5a, PMA, or f-Met-Leu-Phe. Significant inhibition of directed migration by anti-β or anti-αM (OKM-1 or OKM-10) MAb was observed in subagarose but not Boyden chemotaxis assays; inhibition was dependent on a continuous cell exposure to anti-Mac-1α or β during the assay, suggesting that a continuum of new Mac-1 expression is required for directed translocation. Phagocytosis of Oil-Red-O paraffin or zymosan selectively opsonized with C3-derived ligands was significantly inhibited by anti-αM MAb (OKM-10 > LM2/1.6 > M1/70 > OKM-1) or by combinations of anti-αM + anti-CR-1 MAb, but only minimal inhibitory effects of anti-β MAb and no effects of anti-αL or anti-αX MAb were seen. Similarly, complement-dependent phagocytosis-associated lactoferrin release, ingestion, and intracellular killing of *Staphylococcus aureus* 502A, and binding of iC3b-opsonized SRBC, were significantly inhibited by anti-αM (OKM-10, M1/70) or combinations of anti-αM + anti-CR-1 MAb, but not by anti-β, αL, or αX MAb. Notably, none of the anti-Mac-1 MAb demonstrated inhibitory effects in assays of adherence-independent functions including shape change, specific f-Met-Leu-^{3}H-Phe binding, O_2^- generation, chemiluminescence evolution, or lactoferrin release in response to PMA. These studies indicate that MAb directed at individual subunits or combinations of subunits of the Mac-1 glycoprotein family can be employed in blocking experiments to elicit functional abnormalities of granulocytes similar to those recognized in patients with a genetic deficiency of Mac-1, LFA-1, and p150,95. Thus, our findings provide additional evidence for an important physiologic role of this leukocyte glycoprotein family in the inflammatory response.

Recent reports have described a newly recognized genetic disorder which is clinically characterized by recurrent soft tissue infections, severely impaired pus formation and wound healing, and severe in vitro abnormalities of a large spectrum of adherence-dependent functions of granulocytes, monocytes, T lymphocytes, and/or NK cells (1–11). We have demonstrated previously in eight affected patients a profound deficiency of a family of three structurally and functionally related leukocyte glycoproteins, Mac-1, LFA-1, and p150,95 (1, 2, 5). These three molecules are referred to here as the Mac-1 glycoprotein family. The recognition of the clinical consequences resulting from this molecular deficiency has highlighted the biologic importance of this glycoprotein complex in mediating normal inflammatory functions.

Each of the Mac-1, LFA-1, and p150,95 molecules consists of noncovalently associated α- and β-subunits with $\alpha_1\beta_1$ stoichiometry (12). They share an identical β-subunit (M_r = 95 Kd) and are distinguished immunologically by distinct α-subunits whose relative m.w. are 165 Kd, 177 Kd, and 150 Kd for Mac-1α (αM), LFA-1α (αL), and p150,95α (αX), respectively (12). Studies employing monoclonal antibodies (MAb)[4] recognizing epitopes of Mac-1 or LFA-1 have suggested that these molecules

Received for publication December 6, 1985.
Accepted for publication March 10, 1986.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] This work was supported in part by National Institutes of Health grants AI 19031, AI 16810, AI 15877, and CA 31978, by Council for Tobacco Research Grant 1307, and by the US Department of Agriculture-Agricultural Research Service/Children's Nutrition and Research Center, Baylor College of Medicine, and Texas Children's Hospital.
[2] Dr. Anderson is the recipient of a Research Career Development Award from the National Institute of Allergy and Infectious Diseases (KO4-AI-AM 00105). Address reprint requests to: Dr. Anderson, Texas Children's Hospital, Leukocyte Biology Section, Houston, TX 77030.
[3] Dr. Springer is the recipient of an American Cancer Society Faculty Award.

[4] Abbreviations used in this paper: f-Met-Leu-^{3}H-Phe, *N*-formyl-methionyl-leucyl-^{3}H-phenylalanine; DPBS, Dulbecco's phosphate-buffered saline; ACLB, albumin-coated latex beads; BI, bactericidal indices; ZAP, zymosan-activated plasma; CTL, cytolytic T lymphocyte; CL, chemiluminescence; CFU, colony-forming units; ORO, oil-red-O; NHS, normal human serum; MAb, monoclonal antibodies.

contribute to multiple types of leukocyte adhesion reactions (12–18). The LFA-1 molecule was originally shown to participate in the formation of adhesion between effector and target cells in cytolytic T lymphocyte (CTL) mediated killing and in natural killing, as demonstrated in MAb blocking experiments (3, 12–16). Mac-1 was initially identified by MAb as a mouse differentiation antigen present on myeloid cells but absent on lymphoid cells (19). Subsequently, human Mac-1 was identified by cross-reaction of an MAb directed against mouse Mac-1 (20), and several other MAb, including OKM-1 (18), MO-1 (21), OKM-9 (12, 18), OKM-10 (12, 18), and 60.1 (16), each directed at other determinants on human Mac-1. The αM moiety recognized by these MAb appears to be identical to the complement receptor type 3 (CR-3) which binds a fragment (iC3b) of the third component of complement and mediates adherence and phagocytosis of iC3b-coated particles by granulocytes and monocytes (17, 18, 22).

Stimuli including phorbol esters and the chemoattractants f-Met-Leu-Phe and C5a induce a five- to 10-fold increase in granulocyte surface expression of Mac-1 and p150,95. LFA-1 is not increased (2, 5, 23). The increase in Mac-1 and p150,95 is maximal within 10 to 30 min, is not inhibited by cycloheximide, and appears to be due to mobilization to the cell surface of an intracellular pool of Mac-1 and p150,95, possibly from secondary granules (24, 25). Cells from patients with Mac-1 glycoprotein deficiency lack both cell surface and intracellular pools of these glycoproteins and fail to increase adhesiveness in response to the above stimuli. It has been suggested, therefore, that "up-regulation" of Mac-1 and p150,95 is of great physiologic importance in regulating granulocyte adhesiveness (25).

Studies reported here have been designated to explore the individual or collective contributions of Mac-1, LFA-1, or p150,95 to a spectrum of adherence-dependent granulocyte functions. Our findings indicate that individual MAb or combinations of MAb directed at this glycoprotein family can be employed in blocking experiments to largely reproduce the functional deficits recognized in patients with the Mac-1, LFA-1 deficiency syndrome. Our studies indicate distinct contributions of each subunit of the Mac-1, LFA-1 glycoprotein family to granulocyte adherence functions. They demonstrate significantly greater adhesive contributions of αM, αX, and β after cell exposure to chemotactic factors which "up-regulate" the surface expression of these molecules. Evidence is also presented indicating that specific iC3b recognition (assessed in rosetting and phagocytic assays) represents a functional capability of the αM molecule but not other subunits of the glycoprotein family. These studies provide the first information demonstrating adhesive properties of the p150,95α molecule, and provide new information about the mechanisms by which Mac-1, LFA-1 glycoproteins mediate granulocyte chemotaxis.

MATERIALS AND METHODS

Isolation of granulocytes. Granulocytes obtained from Mac-1, LFA-1-deficient or healthy adult individuals were purified from heparinized, dextran-sedimented venous blood samples over Ficoll-Hypaque gradients and were suspended in Dulbecco's phosphate-buffered saline (DPBS; GIBCO, Grand Island, NY), pH 7.4, containing 0.2% dextrose as described (26). For use in chemiluminescence (CL), aggregometry, or *N*-formyl-methionyl-leucyl-^{3}H-phenylalanine (f-Met-Leu-^{3}H-Phe) binding studies, erythrocytes were eliminated by hypotonic lysis (26). To prevent "up-regulation" of Mac-1 glycoprotein surface expression as a result of mechanical membrane perturbation during cell purification, anticoagulated blood samples were processed immediately at 4°C; all purification procedures were performed at 0°C to 4°C with ice-cold buffers as described by Fearon (27).

Monoclonal antibodies (MAb). MAb were prepared as described (28), directed against each subunit of the Mac-1 glycoprotein family. MAb preparations employed in these studies included hybridoma cell culture supernatants, ascites fluids, and purified IgG MAb or their Fab or F(ab')$_2$ fragments. Anti-αM MAb included: M1/70 (19), LM2/1.6.1,[5] OKM-10 (29), and OKM-1, which were obtained from Ortho Pharmaceutical Corp. (Raritan, NJ). Anti-αL MAb included the TS1/22 (12) and a mixture of anti-αL MAb (TS1/12, TS2/14, TS1/22, TS2/4, and TS2/6) (12). The SHCL-3 MAb to p150,95 α (αX) was also available for these investigations (23, 28). The TS1/18 MAb to the common β-subunit of Mac-1, LFA-1, and p150,95 was used for most experiments (12); the MHM23 anti-β MAb was also used for selected studies (30). Binding control MAb preparations included a F(ab')$_2$ fragment of rabbit IgG directed against the human C3b receptor (provided by Dr. P. S. Changelian) (27) and an anti-CR1 MAb ("YAZ-1") (provided by Dr. R. Jack, Harvard Medical School) (31), and an MAb to HLA A,B (W6/32) (32). Additional control MAb which recognize granulocyte surface antigens as enumerated in the Second International Workshop and Conference on Human Leukocyte Differentiation Antigens, including M18, M30, M42, M75, M82, M87, M100, and M103, were also employed for selected studies (33). The P3X63K nonimmune ascites was also used as a control preparation for selected investigations (12).

MAb purification. IgG1 from TS1/18 and TS1/22 ascites fluid, and IgG2b from S-HCL3 culture supernatant were obtained by passage over protein A-Sepharose (Pharmacia Fine Chemicals, Piscataway, NJ) columns as described for mouse immunoglobulins by Ey et al. (34). TS1/18 and TS1/22 were collected in the pH 6 eluate, and SHCL-3 in the pH 3.5 eluate. All fractions were neutralized immediately with 1 M Tris buffer, pH 8.6. LM2/1.6.11 IgG1 was obtained from a 2.2 M ammonium sulfate precipitation of ascites fluid, which was then passed over a DEAE-Sephacel (Pharmacia) column and was eluted with 0.05 M sodium phosphate, pH 6.8.

MAb fragmentation. TS1/18 and TS1/22 F(ab')$_2$ were prepared by digestion with cysteine-activated papain, from which the cysteine had been removed by passage over a Sephadex G-25 (Pharmacia) column, as described by Parham et al. (35). Purified IgG (10 mg/ml) in 0.1 M acetate, 3 mM EDTA, pH 5.5, were incubated at 37°C for 16 hr with cysteine -activated papain at final concentrations of 0.12 mg/ml, or 0.06 mg/ml, for TS1/18 or TS1/22, respectively. The reaction was stopped by the addition of iodoacetamide. Samples were dialyzed vs 5 mM Tris-HCl, pH 7.5, and were passed over either DE-52 (TS1/18) (Whatman, Clifton, NJ) or DEAE-Sephacel (TS1/22) columns. F(ab')$_2$ fragments were collected in the nonbinding flow-through fractions.

Digestion of SCHL-3 IgG2b to F(ab) fragments was performed as outlined by Parham (36). In brief, 10 mg of SHCL-3 IgG2b were digested for 24 hr at 37°C with a 1:50 weight ratio of papain in the presence of EDTA and cysteine. Papain was inactivated with iodoacetamide. The digest was passed over a protein A-Sepharose column, with the Fab fragments collected in the nonbinding flow-through fractions. The Fab fragments were dialyzed and were concentrated by Amicon ultrafiltration.

Incubation conditions. Freshly isolated granulocytes (1 to 2 × 10^7/ml DPBS) were incubated with MAb preparations before and/or during the performance of functional assays. Unless otherwise specified, concentrations of MAb in purified IgG preparations, hybridoma supernatants, or ascites fluids were saturating (or greater) as shown by immunofluorescence flow cytometry (2). For preincubation experiments, cell suspensions were incubated in the presence or absence of MAb for 20 min at 21°C before washing twice in DPBS. Varying incubation intervals or conditions used in selected experiments are described in *Results* with respect to each functional assay.

Final concentrations of purified polyclonal or MAb preparations employed were: a) OKM-1 (5 or 10 μg/ml); b) OKM-10 (5 μg/ml); c) LM2/1.6.1 (5 μg/ml); d) TS1/22 IgG or F(ab')$_2$ fragments (5 or 10 μg/ml); e) SHCL-3 IgG (5 μg/ml) or F(ab) fragments (10 μg/ml); f) TS1/18 IgG or F(ab')$_2$ fragments (5 μg/ml); g) polyclonal rabbit anti-CR1 F(ab')$_2$ (5–7.5 μg/ml) or anti-CR1 MAb IgG (5–7.5 μg/ml); and h) anti-HLA W6/32 IgG (5 μg/ml). Hybridoma supernatants were generally used at a final dilution of 1/2 to 1/5, and ascites preparations were generally saturating when used at 1/300 to 1/500 dilutions. When

[5] **Miller, L. J., and T. A. Springer.** 1986. Regulated expression of Mac-1, LFA-1, p150,95 proteins during leukocyte differentiation. *Submitted for publication.*

combinations of MAb were used, the final concentrations of each antibody included were similar to those when independently employed.

Adherence and aggregation assays. Adherence chambers were assembled as described (37) 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 an additional 400 sec. Cells remaining attached to the substrate surface were counted in 15 to 20 high power (× 400) fields. Chambers were again inverted and cells adhering to the untreated glass surface were counted. The results were expressed as the percentage of cells, adhering to the treated surface over the total number of cells counted (treated plus untreated surfaces).

A latex bead binding assay described previously also was employed to quantitate cell adherence (38). Latex beads (0.6 μm) (Sigma Chemical Co., St. Louis, MO) were suspended in a solution of 1% of human serum albumin in DPBS for 2 min at room temperature, were washed, and were resuspended in DPBS. After their preincubation in polyclonal or MAb preparations, control or patient granulocytes were then exposed to 1% albumin-coated latex beads (ACLB) (v/v) for 5 min at room temperature. ACLB binding was assessed either before or after exposure of cells to f-Met-Leu-Phe (5 min, 10 nM) or phorbol myristic acetate (PMA) (5 min, 10 μg/ml). Cells in suspension then were fixed in 1.5% glutaraldehyde and were examined with phase-contrast optics. The results were expressed as the percentage of cells binding ≥3 beads and as the number of beads bound per granulocyte.

Granulocyte aggregation was evaluated by a modification of the method described by Hammerschmidt et al. (39). However, granulocytes were not preincubated in cytochalasin b before assay. After 30 sec of stirring, f-Met-Leu-Phe (10^{-6} M), PMA (10 mg/ml), or zymosan-activated plasma (ZAP) was added to cell suspensions (0.5 ml, 10^7 granulocytes/ml). The characteristics of aggregation were visually evaluated by phase-contrast microscopy. Aggregation responses were expressed in arbitrary units (ZAP units) based on the relative responses of control granulocytes to varying solutions of ZAP, where undiluted ZAP = 100 ZAP units, a 1/2 dilution of ZAP = 50 ZAP units, etc. Integrals (or the area under the curve) of individual aggregation tracings were determined (2). For most experiments, the results were expressed as the percentage of control integral values.

Assays of leukocyte motility. Motility of normal granulocytes reacted with MAb preparations or those obtained from Mac-1, LFA-1-deficient patients was assessed by multiple techniques. Granulocyte motility into Micropore filters was assessed by a modified Boyden technique (26). The distributions of cells within filters were evaluated with an Optomax Image Analyzer (Optomax Inc., 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 to 60 min (40). A modification of a sub-agarose technique as described by Nelson et al. (41) was employed also. Migration values were expressed as mm/hr as determined by measurements of the distance between the cell well perimeter and the "leading front" arch directed toward the chemoattractant well. These determinations were made with a Leitz Ortholux microscope with a calibrated 40 × objective. In selected experiments employing this technique, photomicrographs were recorded to document the migratory properties of total cell populations studied under varying experimental conditions.

Phagocytosis assays. Phagocytosis of opsonized emulsified paraffin oil droplets was measured as described by Shurin and Stossel (42). 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 CL evolution as described (43).

Assessments of CR3 activity—^{51}Cr-SRBC binding assay. The functional relationships of the granulocyte iC3b receptor to Mac-1 protein subunits were studied by using healthy adult granulocytes incubated with anti-Mac-1 MAb in a ^{51}Cr SRBC binding assay. Granulocytes were allowed to adhere to flat-bottomed tissue culture plates pretreated with poly-L-lysine and quenched with bovine serum albumin as described (44). SRBC were labeled with ^{51}Cr and were treated with appropriate opsonins before addition to the assay. Assays were terminated by removing unbound SRBC by extensive washing and then lysing bound SRBC and counting ^{51}Cr in the lysate (44). All assays were performed in triplicate with less than 2% of the total input counts found in wells containing opsonized ^{51}Cr-SRBC, but no granulocytes after the final wash.

Degranulation. Untreated patient or control granulocytes (10^7/ml) or those preincubated with MAb preparations were then stimulated with PMA (10 μg/ml) or opsonized zymosan particles as described (2). Cellfree supernatants or cell pellets were then assayed for β-glucuronidase (2) or lactoferrin (45). Enzyme activities were expressed as the percentage of total cell contents released by stimulation.

Intracellular killing assay. Intracellular killing of staphylococcus 502A by patient or control granulocytes in the presence or absence of MAb preparations was assayed, employing a modification of a technique as described by Quie et al. (46). Reaction mixtures contained staphylococcus 502A (3×10^6 CFU/ml; in 0.5 ml DPBS), granulocytes (3×10^6/ml; in 0.5 ml DPBS), and antibody-deficient normal human serum (NHS) (0.5 ml) ± saturating concentrations of MAb. Reaction mixtures were tumbled at 37°C for up to 2 hr. Aliquots were removed at time 0, 30, 60, 90, and 120 min, were lysed in sterile water, were diluted, and were plated on blood agar. Control tubes containing no granulocytes were included in each experiment. The results were expressed as a bactericidal index (BI) calculated by the formula: BI = 100 − [(CFU at 120 min/CFU at 0 minutes) × 100].

Oxidative metabolic activity. Superoxide (O_2^-) generation and CL evolution by granulocytes exposed to PMA (10 μg/ml) were quantitated as described (1, 47).

f-Met-Leu-^{3}H-Phe binding. Granulocytes (10^8/ml), f-Met-Leu-^{3}H-Phe, and cold f-Met-Leu-Phe were incubated in 200 μl of DPBS at 0°C before or after incubation of cells in MAb preparations. Reactions were terminated by rapid dilution in cold (4°C) DPBS followed by filtration of mixtures through Whatman GFC filters. Radioactivity was quantitated in a liquid scintillation spectrometer, and specific binding was calculated as described (26).

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

Assessments of granulocyte adherence. The results of studies employing serum-coated glass substrates are shown in Figure 1*A* and *B*. When studied under baseline conditions (PBS), significant ($p < 0.01$) inhibition of adherence was observed for cells incubated in the presence of anti-β [TS1/18 F(ab')$_2$], anti-αM (OKM-10), or combinations of anti-β and anti-αL [TS1/22 F(ab')$_2$], αX (SCHL-3 IgG), and/or OKM-10. Slight, statistically insignificant ($p > 0.05$) inhibition was observed among cells incubated in the presence of anti-αL or anti-αX MAb alone. No inhibition was observed among binding control cell suspensions incubated in the presence of polyclonal anti-CR1 F(ab')$_2$ or anti-HLA (W6/32) IgG.

Under conditions of f-Met-Leu-Phe stimulation (Fig. 1*B*), control cells demonstrated expected hyperadherence responses, as did cell suspensions incubated in the presence of polyclonal anti-CR1 or anti-HLA A,B. A striking inhibition of stimulated adherence was observed among cells incubated in the presence of anti-β (83% ± 3 inhibition), anti-αM (68% ± 5 inhibition), or combinations of anti-β plus anti-αL, αM, or αX ($\bar{x}$ = 80 to 89% inhibition) ($p < 0.01$ for each condition). The most profound inhibition was observed when MAb directed at LFA-1α, Mac-1α, p150,95α and β-subunits were employed together (89% ± 4 inhibition) ($p < 0.001$). Similarly, low adherence (4% ± 3 of normal) was observed among genetically deficient cells tested simultaneously. Relatively less but statistically significant ($p < 0.01$) inhibition was evident among cell suspensions incubated in the presence of anti-αX (48% ± 6 inhibition). Marked but statistically insignificant ($p > 0.05$) diminution of adherence (in this assay) was observed when the anti-αL MAb was employed independently (32% ± 13 inhibition). Similar relationships were observed among cell suspensions stimulated with PMA (10 μg/ml) (data not shown). The rank order of inhibition by various MAb observed in these experiments was anti-β + anti-αL, αM and/or αX > anti-β > anti-αM > anti-αX. As also seen with f-Met-Leu-Phe-stimulated

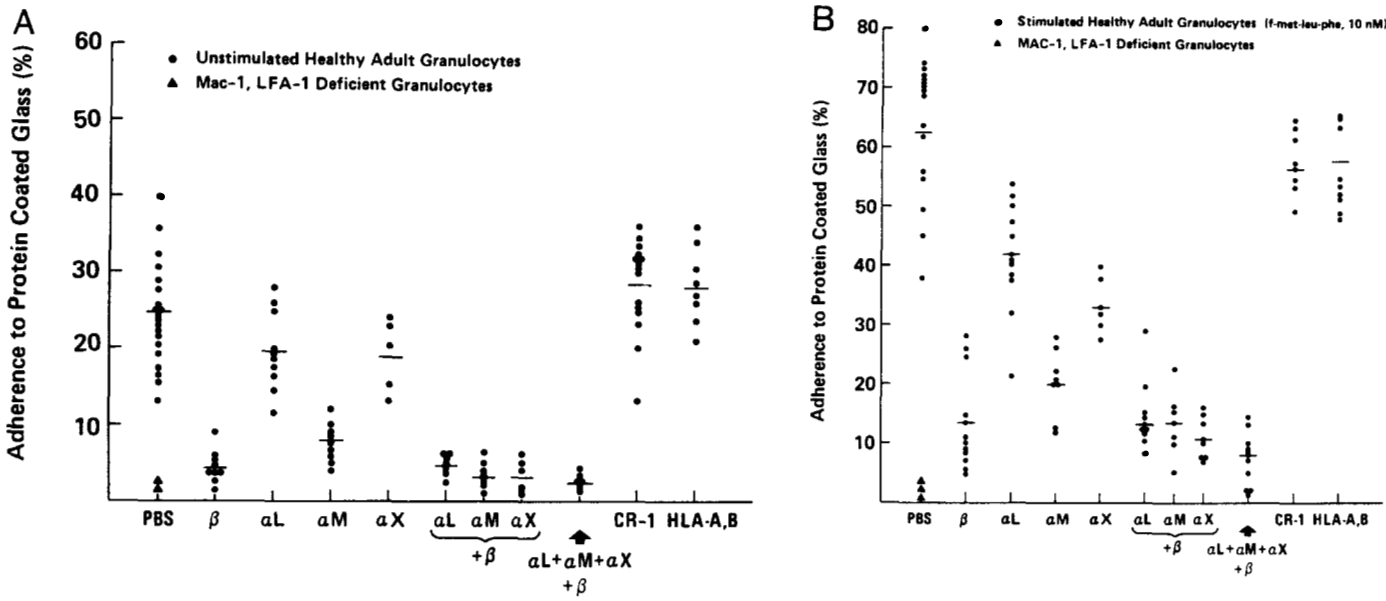

Figure 1. Assessments of granulocyte adherence to 5% serum-coated glass substrates in Smith-Hollers chambers. Unstimulated cell suspensions (*A*) or suspensions stimulated with f-Met-Leu-Phe (10 nM, 21°C) (*B*) were preincubated in and tested in the presence of saturating concentrations (5 μg/ml) of MAb directed at β (TS1/18 IgG), αM (OKM-10), αX (SHCL-3 IgG), or αL (TS1/22) subunits or combinations of these MAb. Control MAb included the anti-CR-1 "YAZ-1," and the anti-HLA A,B, W6/32 IgG. Results of individual experiments (.) and mean values (horizontal bars) are shown.

cells, minimal but statistically insignificant ($p > 0.05$) inhibition resulted from anti-αL incubation, and essentially no inhibition was observed when employing anti-CR1 or anti-HLA A,B ($p > 0.05$).

Similar findings were observed in dose-response incubation experiments employing f-Met-Leu-Phe-stimulated cell suspensions (Fig. 2). Maximal inhibition of adherence was generally observed when using MAb concentrations of 5 μg/ml; greater inhibition was seen with higher concentrations of selected MAb including TS1/22 anti-αL and OKM-1. Therefore, the TS1/22 and OKM-1 MAb were used at a final concentration of 10 μg/ml in subsequent assays. Significant ($p < 0.01$) inhibition was observed when using 5 μg/ml of the anti-αM MAb LM/2.1.6 and OKM-10 and the SHCL-3 anti-αX MAb, whereas ≥10 μg/ml of TS1/22 was required to achieve significant inhibition ($p = 0.5$). A combination of MAb directed at LFA-1α, Mac-1α, p150,95α, and the β-subunit demonstrated the most profound inhibition over the entire range of concentrations employed in incubation mixtures. These findings indicate that with the possible exception of LFA-1α, all subunits of the Mac-1 glycoprotein family contribute to the adhesive properties of granulocytes.

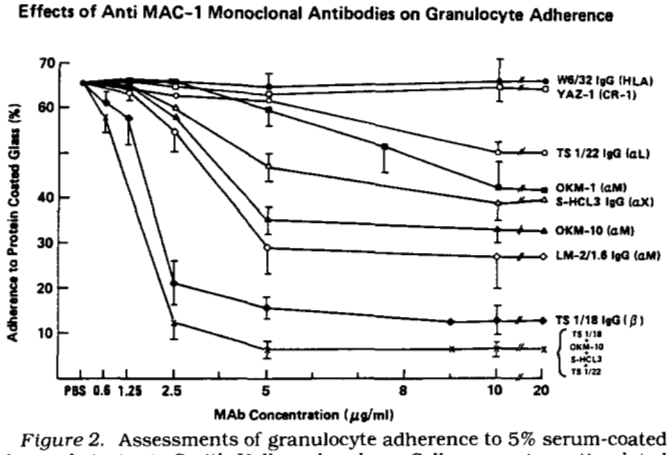

Figure 2. Assessments of granulocyte adherence to 5% serum-coated glass substrates in Smith-Hollers chambers. Cell suspensions stimulated with f-Met-Leu-Phe (10 nM, 21°C) were exposed to varying concentrations of MAb directed at individual or combinations of Mac-1 subunits or the control antigens, CR-1, or HLA A,B. Mean ± 1 SD values derived from three to seven experiments for each MAb preparation are shown.

A latex bead-binding adherence assay which allows simultaneous assessments of adhesive and morphologic characteristics of chemotactically stimulated granulocytes was also used for these studies (Table I). After stimulation with f-Met-Leu-Phe (10 nM, 5 min, 21°C) or PMA (10 μg/ml, 5 min, 21°C), granulocytes in suspension demonstrate bipolar morphology and avidly bind ACLB in a generalized distribution (Fig. 4, below); unstimulated cells are uniformly spherical and demonstrate no binding of ACLB. Anti-β MAb demonstrated significant and profound inhibitory effects on ACLB binding for both f-Met-Leu-Phe- or PMA-stimulated cell preparations (Table I). This was true for the anti-β TS1/18 IgG or its $F(ab')_2$ fragment as well as for TS1/18 supernatants containing this MAb. The incorporation of anti-αL, αM, and/or αX MAb in combination with anti-β did not further diminish ACLB binding.

Variable inhibition of ACLB binding by different anti-αM MAb was observed. However, significant ($p \leq 0.01$) inhibition was apparent for all preparations as compared to control with respect to the number of ACLB bound per cell after either f-Met-Leu-Phe or PMA stimulation. With respect to both the percentage of cells binding ACLB and the number of beads bound per cell, OKM-1 demonstrated the least inhibition, whereas OKM-10, LM2/1.6 IgG, and M1/70 IgG demonstrated greater inhibitory effects. Modest but statistically significant ($p \leq 0.01$) inhibitory effects were observed with each anti-αX SHCL-3 MAb preparation. Essentially no effects on ACLB binding by different TS1/22 anti-αL MAb preparations, the anti-HLA A,B (W6/32) MAb, the polyclonal anti-CR-1 $F(ab')_2$, or the nonbinding P3XK63 supernatant preparations were evident.

In conjunction with these assessments, the morphology

TABLE I

Effects of Mac-1 MAb on granulocyte adherence as assessed by ACLB binding

Glycoprotein Subunit(s)	Incubation Conditions	f-Met-Leu-Phe (n)	f-Met-Leu-Phe % Cells Binding Beads (≥3)	f-Met-Leu-Phe No. Beads Bound/Cell	PMA (n)	PMA % Cells Binding Beads (≥3)	PMA No. Beads Bound/Cell
	PBS	(13)	95 ± 2	21 ± 2	(2)	85	19
β	TS1/18 IgG	(3)	56 ± 4	3 ± 1	(2)	15	1
	TS1/18 F(ab')$_2$	(6)	47 ± 5	3.7 ± 1			
	TS1/18 supernatant	(3)	42 ± 4	3.5 ± 1	(2)	79	6
αM	OKM-1	(6)	89 ± 7	12 ± 4	(2)	86	10
	OKM-10	(4)	78 ± 9	7.5 ± 2	(2)	59	3
	LM2/1.6 IgG	(2)	56 ± 7	5 ± 1	(2)	55	4
	LM2/1.6 supernatant	(6)	47 ± 12	4.6 ± 2	(2)	67	6
	M1/70 supernatant	(2)	33 ± 4	3.0 ± 1	(2)	80	5
αL	TS1/22 F(ab')$_2$	(6)	100 ± 4	18 ± 1	(2)	85	19
	TS1/22 IgG	(3)	100 ± 3	19 ± 2		ND[a]	ND
	TS1/22 supernatant	(6)	94 ± 3	16 ± 6	(2)	100	20
	TS1/12, 1/14, 1/22, 2/4, 2/6 supernatant mixture	(3)	98 ± 3	16 ± 1		ND	ND
αX	SHCL-3 supernatant	(4)	85 ± 7	9.8	(2)	84	9
	SHCL-3 IgG	(2)	82 ± 4	17 ± 1	(2)	79	10
	SHCL-3 Fab	(3)	93 ± 2	14 ± 2	(2)	84	11
β, αM, αL & αX	TS1/18 + OKM-1 + S-HCL3 IgG + TS1/22 F(ab')$_2$	(3)	42 ± 8	3 ± 1	(2)	24	1
HLA	W6/32 supernatant	(4)	92 ± 4	16 ± 4	(2)	80	14
	W6/32 ascites	(2)	97	24	(2)	87	17
	W6/32 IgG	(2)	98	20	(2)	86	18
	X/63 supernatant	(2)	94	17			
CR-1	YAZ-1	(5)	94.5	18 ± 3	(2)	91	17

[a] ND, not done.

of f-Met-Leu-Phe-stimulated granulocytes incubated with each of the MAb indicated in Table I was also evaluated (36). Essentially no inhibitory effects on shape change in suspension were observed with respect to any of the MAb employed (Fig. 3). MAb binding did not promote "spontaneous" activation of unstimulated (PBS) granulocytes, nor did it impair bipolar (±uropod) shape change in response to f-Met-Leu-Phe over a range of stimulating concentrations. Thus, these findings support the specificity of anti-Mac-1 MAb effects on cell adherence properties. As shown in Figure 4*A*, anti-β (TS1/18 F(ab')$_2$) almost totally inhibited ACLB binding, but not the expected morphologic changes resulting from chemotactic stimulation (Fig. 4*B*).

Aggregation. The effects of anti-Mac-1 MAb on the characteristics of aggregation by zymosan-activated serum, f-Met-Leu-Phe (10^{-6} M), or PMA (100 μg/ml)-stimulated healthy adult granulocytes were also evaluated. Aggregation tracings of representative experiments are shown in Figure 5*A* and *B*, and the mean integrals of the area under the curve (expressed as percent of control) of all experiments are tabulated in Table II. Suspensions stimulated with ZAP demonstrated characteristic aggregation-disaggregation tracings within a 3.5-min incubation interval. Those stimulated with f-Met-Leu-Phe or PMA demonstrated a more delayed aggregatory response without disaggregation following even a prolonged interval of incubation (Fig. 5*B*).

Effects of control or anti-Mac-1 MAb on the aggregatory responses of granulocytes in response to activated plasma are shown in Figure 5*A*. Profound inhibitory effects were observed with anti-β MAb or anti-β in combination with anti-α MAb. Calculated mean aggregation integrals for cells incubated in the presence of anti-β TS1/18 F(ab')$_2$, IgG, or supernatants were approximately

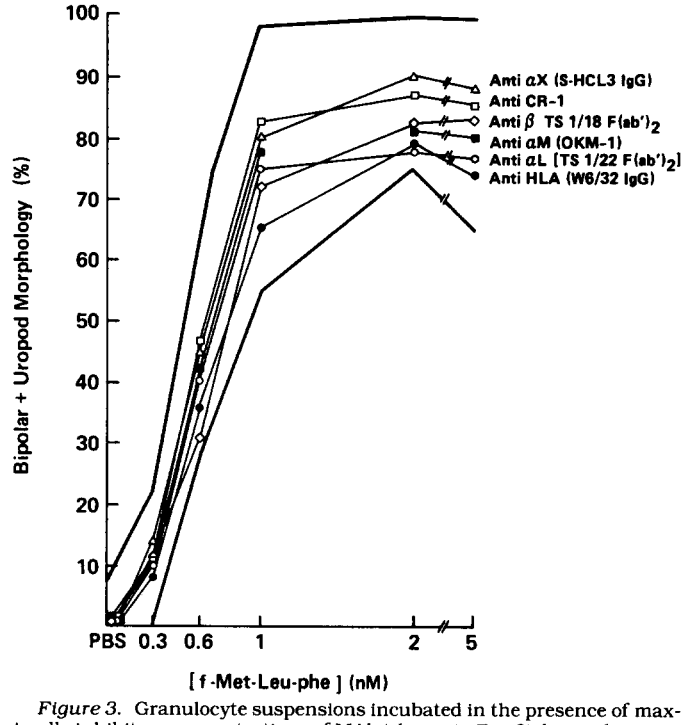

Figure 3. Granulocyte suspensions incubated in the presence of maximally inhibitory concentrations of MAb (shown in Fig. 2) directed at Mac-1 subunits or control surface proteins CR-1 or HLA A,B were stimulated with varying concentrations of f-Met-Leu-Phe for 2 min at 21°C and were then fixed in glutaraldehyde. Cells were classified morphologically and the results were expressed as the percent of cells demonstrating bipolar ± uropod morphology. A normal range of values of granulocytes obtained from 68 healthy adults is shown by the heavy solid bars.

0 to 13% of normal and were essentially comparable to those observed for Mac-1, LFA-1-deficient cell suspensions. Essentially no inhibition of cell aggregation was observed when employing anti-HLA or anti-CR-1 MAb,

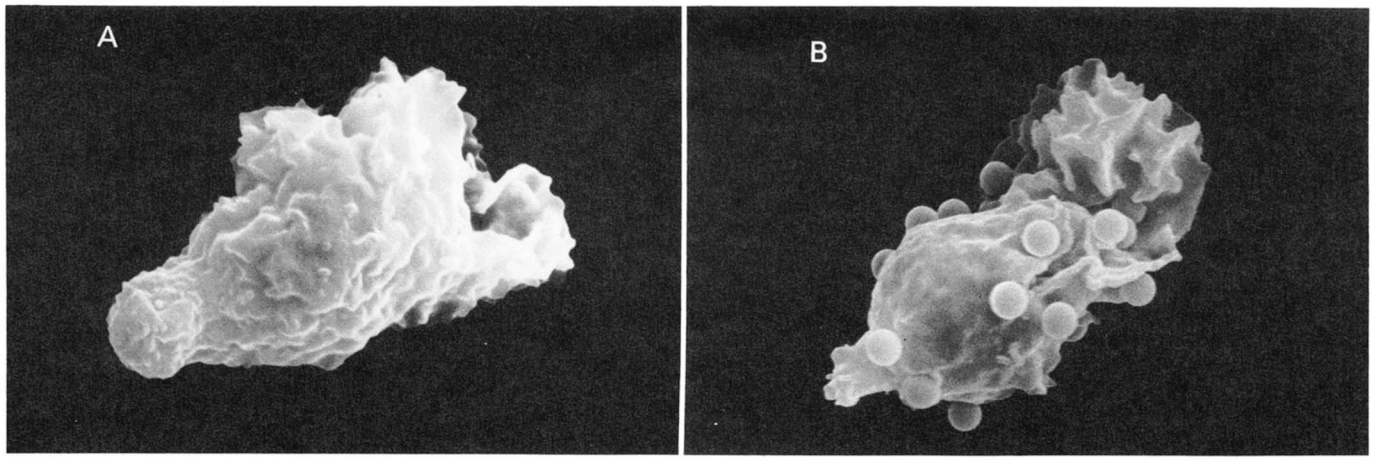

Figure 4. Granulocytes stimulated with f-Met-Leu-Phe (10 nM, 90 sec, 21°C) were exposed to ACLB in the presence or absence of MAb directed at Mac-1 subunits. These representative scanning electron micrographs demonstrate normal ACLB binding by a granulocyte incubated in the absence of anti-Mac-1 MAb or in the presence of anti-CR-1 or W6/32 MAb (*A*), and no binding of ACLB (but normal bipolar morphology) by a granulocyte preincubated with anti-Mac-1α and anti-β before exposure to ACLB under the same conditions (magnification = 6000 ×) (*B*).

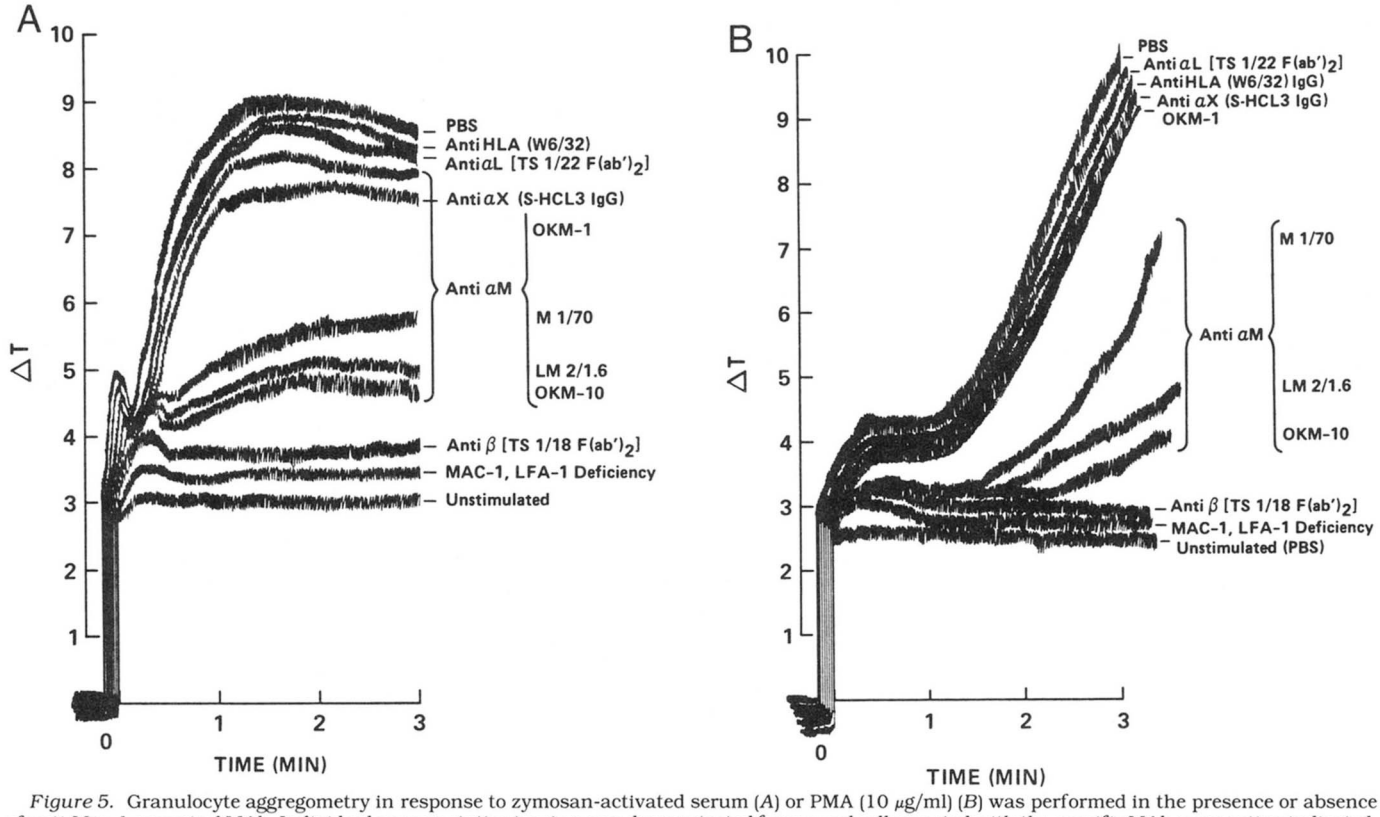

Figure 5. Granulocyte aggregometry in response to zymosan-activated serum (*A*) or PMA (10 μg/ml) (*B*) was performed in the presence or absence of anti-Mac-1 or control MAb. Individual representative tracings are demonstrated for normal cells reacted with the specific MAb preparation indicated, for Mac-1, LFA-1-deficient granulocytes tested simultaneously, and for unstimulated healthy adult cells.

TABLE II

Effects of anti-Mac-1 MAb on granulocyte aggregation

Preincubation Conditions / Stimulating Conditions	HLA A_1B_1 (W6/32)	CR-1 (YAZ-1)	β(TS1/18) IgG	β(TS1/18) $F(ab')_2$	β(TS1/18) Supernatant	αM OKM-1	αM OKM-10	αM LM2/1.6	αM M1/70	αL(TS1/22) IgG	αL(TS1/22) $F(ab')_2$	αX(S-HCL3) IgG	αX(S-HCL3) Supernatant	Mac-1, LFA-1-Deficient Granulocytes
Zymosan-activated serum	114[a]	104	0	2	3	86	26	3	69	102	89	102	96	15
PMA (10 μg/ml)	94	117	0	0	14	106	22	14	73	98	95	108	92	18
f-Met-Leu-Phe (10^{-6} M)	92	107	1	1	13	71	3	19	69	99	86	82	89	15

[a] Represents the mean integral (or area under curve) of individual aggregation tracings expressed as a percent of control (untreated) cell suspensions for each of two to six experiments.

nor were inhibitory effects observed with anti-αL or OKM-1 MAb reagents. Minimal inhibition (20%) was observed in the presence of anti-αX (SHCL-3 IgG or supernatants), and relatively greater inhibition was observed with most anti-αM reagents used, including OKM-10, LM2/1.6 IgG or supernatants, and M1/70 supernatants. With respect to all MAb demonstrating inhibitory effects, substantially less inhibition was observed when cells were preincubated in MAb and were then washed before their incorporation in the aggregometer.

The effects of binding control MAb or anti-Mac-1 MAb on aggregation of granulocytes in response to PMA stimulation are shown in Figure 5*B* and Table II. Largely similar inhibitory effects were observed for PMA-stimulated suspensions as compared to those stimulated with zymosan-activated serum. In these studies, however, essentially no effects were observed when employing the anti-Mac-1α MAb OKM-1 as well as the anti-αL TS1/22 F(ab′)$_2$, the anti-CR-1, and the anti-HLA (W6/32 IgG). Minimal effects on aggregation were observed with the anti-αX (SHCL-3 IgG), but striking inhibitory effects were observed with each of the anti-αM MAb, including M1/70, OKM-10, and LM2/1.6 (IgG, F(ab′)$_2$ or supernatant reagents). Essentially total inhibition of aggregation was observed with anti-β MAb reagents; aggregation values observed were essentially comparable to those for Mac-1, LFA-1-deficient cell suspensions or unstimulated healthy adult control suspensions incubated in the presence of DPBS. Additional binding control MAb demonstrating no inhibitory effects on granulocyte aggregation included the M18, M30, M42, M75, M82, M87, M100, and M103 MAb, as enumerated in the Second International Workshop and Conference on Human Leukocyte Differentiation Antigens (33). These MAb recognize antigens distinct from the Mac-1 family. Profound inhibition was evident when employing MHM-23, a workshop MAb which recognizes an epitope of the β-molecule (data not shown) (33).

Granulocyte motility assays. Possible effects of anti-Mac-1 MAb on random or directed granulocyte migration were evaluated in two types of chemotaxis assays which yielded strikingly different results. Random or directed migration into methylcellulose filters in a modified Boyden "leading front" assay were essentially uninfluenced by anti-Mac-1 MAb used independently or in combination (Table III). This was true for all experiments in which granulocytes were preincubated in the presence of MAb and then washed, and in most experiments when preincubated cells were incorporated into Boyden chambers in the presence of the same MAb. Thus, MAb preparations which profoundly inhibited adherence or aggregation (such as anti-β or anti-αM) failed to diminish random or directed granulocyte migration in three-dimensional matrices. Even mixtures employing MAb directed at all four molecules of the Mac-1 family failed to significantly diminish directed migration under these conditions ($p > 0.05$ compared to control). Further, directed migration values for MAb-treated cell suspensions were significantly greater than those for Mac-1, LFA-1, p150,95-deficient cell suspensions studied simultaneously ($p < 0.01$).

In contrast to findings in Boyden assays, marked inhibitory effects were observed when using a sub-agarose chemotaxis assay in which cells migrate in a two-dimen-

TABLE III
Effects of anti-Mac-1 or control MAb on granulocyte motility

Experimental Conditions	PBS	β TS 1/18	αM OKM-1	αM OKM-10	αM LM2/1.6	αM M1/70	αX SHCL-3	αL TS 1/22	β + αX, M & L	HLA W6/32	CR-1 YAZ-1
A. Boyden-Micropore Assay											
PBS (Random)	72 ± 5[a]	71 ± 4	84 ± 12	70	76 ± 11	N.D.	74	75	81 ± 6	84	67
f-Met-Leu-Phe (10 nM)	123 ± 9	114 ± 10	126 ± 8	131	121 ± 13	N.D.	125	120 ± 11	127 ± 16	124	114 ± 7
Zymosan-activated serum (10%)	110 ± 7	109 ± 9	107 ± 10	115	106 ± 7	N.D.	106	102	110 ± 6	112	113 ± 5
B. Sub-agarose Assay											
f-Met-Phe (10 μM)	2.0 ± 0.1[b]	1.0 ± 0.2[c]	1.2 ± 0.3[c]	1.6[c]	1.4 ± 0.1[c]	1.7 ± 0.3	1.8 ± 0.3	1.9 ± 0.5	1.0 ± 0.2[c]	2.0 ± 0.3	2.1 ± 0.1
Zymosan-activated serum (10%)	2.45	1.38[c]									

[a] Mean ± 1 SD μm migration/90 min incubation (three to six determinations for each MAb preparation).
[b] Mean ± 1 SD mm migration/60 min incubation (two to four determinations for each MAb preparation).
[c] $p \leq 0.05$ compared to PBS control.

sional plane between agar and plastic surfaces (41). Anti-β MAb or combinations of anti-β and anti-α MAb demonstrated significant inhibitory effects on directed migration of granulocytes to ZAP, f-Met-Phe, or f-Met-Leu-Phe (Table III). Less striking but significant inhibitory effects were also observed with the anti-αM MAb OKM-10, LM2/1.6, and OKM-1. No significant effects were seen among suspensions preincubated or tested in the presence of anti-αX or anti-αL MAb or the binding control MAb anti-CR-1 and anti-HLA,A,B.

Further studies employing the sub-agarose technique demonstrated variable inhibitory effects of anti-Mac-1α or -β, depending on the conditions employed (Fig. 6). Only modest inhibition was observed when granulocytes were preincubated and then washed before their incorporation in the assay; when preincubated cells were incorporated into the agar well in the presence of saturating concentrations of each MAb, more striking inhibitory effects were observed. Even more profound inhibition was seen when saturating concentrations of anti-β were incorporated into the agar (Fig. 6). These findings suggest that a continuum of new Mac-1 surface expression occurs during directed granulocyte migration in this experimental system.

Phagocytic ingestion of complement-opsonized particles. Results in Table IV and Figure 7 demonstrate the effects of anti-Mac-1 MAb on the characteristics of phagocytic ingestion by granulocytes. For these experiments, test particles (ORO paraffin or zymosan) were opsonized with complement, using antibody-deficient normal human serum to exclude the influence of IgG Fc receptors on phagocytic rates. When employing complement-opsonized ORO paraffin, only the anti-αM MAb OKM-10, LM2/1.6, or M1/70 or combinations of these MAb together with anti-CR-1 MAb demonstrated significant inhibitory effects on rates or extent of phagocytosis. Profoundly diminished values were observed with untreated Mac-1-deficient granulocytes as reported previously (1). The anti-CR-1 MAb "YAZ-1" demonstrated minimal but insignificant inhibitory effects in this assay. Interestingly, the anti-β MAb TS1/18 IgG (or $F(ab')_2$ or supernatant reagents) demonstrated minimal or no inhibition ($p > 0.05$) of complement-dependent phagocytosis, in contrast to its profound inhibitory effects on cell adherence, aggregation, or two-dimensional directed motility. No inhibition was observed when employing the anti-αM MAb OKM-1, anti-αL, or αX MAb as well as the anti-HLA (W6/32) binding control MAb.

Similar relationships were generally observed when using zymosan particles opsonized with C3-derived ligands in antibody-deficient serum (Table IV and Fig. 7). The anti-αM MAb OKM-10, LM2/1.6, and M1/70 demonstrated a significant ($p \leq 0.01$) inhibition of CL evolution under all conditions. The polyclonal rabbit anti-CR-1 but not the anti-CR-1 "YAZ-1" MAb demonstrated significant ($p \leq 0.05$) inhibitory influences. Even lower values were seen with Mac-1, LFA-1-deficient granulocytes tested simultaneously. With respect to each inhibitory MAb, relatively greater inhibition was observed when they were incorporated directly into reaction mixtures at the initiation of the assay in contrast to preincubation plus wash conditions (Table IV). These results suggest that Mac-1 is functionally up-regulated during particle attachment and/or phagocytosis.

These findings were further confirmed in CL titration experiments employing serial twofold dilutions of saturating concentrations of each MAb. Only OKM-10, LM2/1.6, M1/70, or polyclonal anti-CR-1 $F(ab')_2$, or combinations of OKM-10 or LM2/1.6 + anti-CR-1 demonstrated dose-dependent inhibition of CL activity. The OKM-10 and LM2/1.6 IgG MAb demonstrated significant ($p < 0.01$) inhibition when used at a concentration of 2.5 μg/ml,

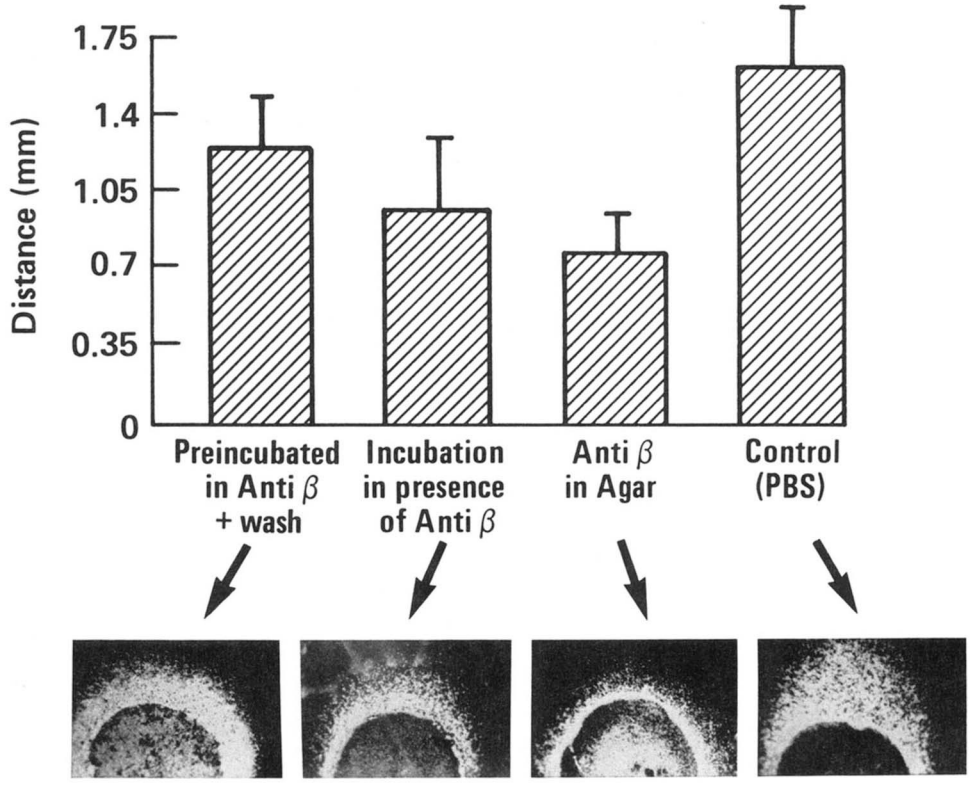

Figure 6. Effects of anti-Mac-1 MAb on granulocyte migration in a sub-agarose assay. The results shown in Figure 5 demonstrate the effects of anti-β on cells preincubated in anti-β but washed before their incorporation in cell wells; granulocytes incubated in the presence of anti-β incorporated into cell wells; and granulocytes incorporated into cell wells in the presence of anti-β in petri dishes containing additional anti-β in the agar. The results are expressed as the mean ± 1 SD values derived from three individual experiments. Photomicrographs of granulocyte populations migrating under each of the test conditions in *A* are represented in five (magnification = 100 ×).

TABLE IV

Effects of anti-Mac-1 MAb on phagocytic ingestion

Incubation Conditions—MAb	Test Particle (Opsonin)			
	ORO Paraffin (LPS-activated NHS)		Zymosan (Activated NHS)	
	5 min	15 min	preincubated in MAb and washed	MAb present in reaction mixture
PBS	5.2[a]	6.8	26.1[b]	23.6
HLA A,B (W6/32)	6.1 (111)	7.4 (108)	23.8 (91)	21.6 (91)
β (TS1/18 IgG)	4.7 (90)	6.2 (92)	24.0 (92)	16.4 (71)
αM				
(OKM-1)	4.9 (94)	7.3 (107)	27.4 (105)	13.9 (58)
(OKM-10)	2.5 (48)	5.4 (79)	12.3 (47)	9.8 (42)
(LM2/1.6 IgG)	3.7 (73)	6.0 (88)	20.2 (79)	12.9 (55)
(M1/70 IgG)	3.4 (66)	5.7 (84)	26.6 (103)	13.0 (55)
αL (TS1/22 IgG)	5.2 (100)	6.4 (94)	22.8 (87)	19.1 (82)
αX (S-HCL3 IgG)	5.1 (98)	6.8 (100)	22.9 (87)	19.3 (82)
CR-1 (YAZ-1)	4.4 (88)	6.2 (91)	20.0 (79)	18.1 (77)
αM + CR-1 (OKM-10 + YAZ-1)	2.8 (54)	2.8 (42)	9.7 (37)	8.6 (36)
Mac-1-deficient granulocytes	1.3 (25)	1.9 (28)		5.1 (21)

[a] Dionylphthalate uptake (μg/10^7 granulocytes during 5- or 15-min incubations) (percent of control). Values represent the mean of two to four individual experiments.

[b] Slope of CL evolution between t = 0 and t = 20 min (cpm × 10^{-3}) (percent of control). Values represent the mean of four to six individual experiments.

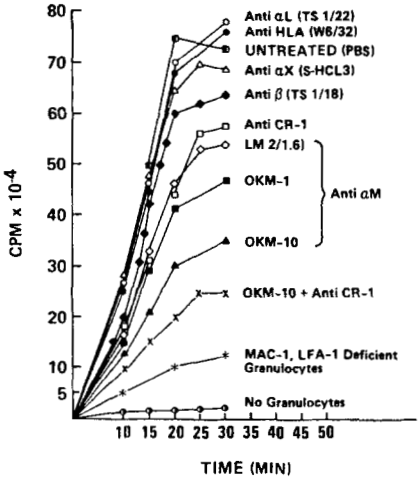

Figure 7. The effects of anti-Mac-1 MAb on the evolution of CL during granulocyte attachment-ingestion of opsonized zymosan. Anti-Mac-1 or control MAb were incorporated into phagocytic reaction mixtures (21°C) at time 0. The evolution of CL in each reaction mixture was determined over a 60-min incubation interval. Tracings shown represent the mean value for replicate samples in two individual experiments. The slope of CL evolution between t = 0 and t = 30 min was calculated by linear regression for each test mixture. Significantly diminished CL slopes were determined for cell suspensions incubated in the presence of OKM-1 ($p < 0.05$), OKM-10 ($p < 0.001$), LM2/1.6 IgG ($p < 0.001$), M1/70 ($p < 0.001$), anti-CR-1 ($p < 0.001$), and combinations of OKM-10 + anti-CR-1 ($p < 0.001$). Tracings shown represent mean values at each time period determined from two to four replicate experiments.

and concentrations of OKM-10 as low as 0.6 μg/ml were inhibitory. No MAb or MAb combination inhibited O_2^- generation or CL evolution of granulocytes stimulated with PMA ($p > 0.05$) (data not shown), indicating that inhibitory influences of anti-Mac-1 MAb in CL assays result from impairment of cell-particle interactions rather than direct effects on oxidative metabolism (1).

Similar findings were observed in phagocytosis-associated degranulation experiments. Although no inhibition of β-glucuronidase release during zymosan ingestion was observed with any MAb or MAb combination, inhibitory influences of selected MAb or MAb combinations were apparent with respect to lactoferrin release. As compared to untreated control suspensions ($\bar{x}$ = 18% release/5 min), reaction mixtures incubated in the presence of OKM-10, LM2/1.6, OKM-1, or anti-β (TS1/18) demonstrated significantly ($p < 0.05$) diminished mean values of 9%, 11%, 11%, or 15%, respectively. Anti-αX or αL MAb as well as the anti-HLA MAb W6/32 or the anti-CR-1 "YAZ-1" MAb demonstrated no inhibition of phagocytosis-associated exocytosis of lactoferrin. The extracellular release of lactoferrin from suspensions stimulated with PMA was normal in all test mixtures (data not shown).

Intracellular killing studies. Killing by granulocytes of complement-opsonized staphylococcus 502A was assayed. Relative inhibitory effects of anti-Mac-1 MAb were generally similar to those observed in ORO or degranulation assays. Most anti-αM MAb or combinations of anti-αM and anti-CR-1 MAb demonstrated significant ($p < 0.01$) inhibitory effects after incubation intervals of 30, 60, and/or 120 min. Mean (±1 SD) BI (at t = 120 min) were determined for reaction mixtures containing OKM-10, M1/70, OKM-1, and OKM-10 + anti-CR-1 were 30 ± 12, 34 ± 11, 68 ± 10, and 28 ± 11, respectively, compared to 92 ± 8 for untreated control suspensions. No inhibition was observed when employing anti-β (TS1/18 IgG) (BI = 91 ± 6), anti-αL (TS1/22 F(ab')$_2$) (BI = 88 ± 7), anti-αX (SHCL-3) (BI = 87 ± 6), or W6/32 IgG (BI = 91 ± 14) ($p \geq 0.05$ for each).

iC3b-SRBC binding study. The effects of MAb on adherence of granulocytes to iC3b-opsonized SRBC were similar to those observed in the complement-dependent phagocytosis assays. MAb directed at αM epitopes (with

the exception of OKM-1) demonstrated greater inhibitory effects than did anti-β, anti-αL, or anti-αX MAb reagents. Among anti-αM MAb used, OKM-10 demonstrated the most significant inhibition ($\bar{x}$ = 70% inhibition), whereas M1/70 ($\bar{x}$ = 50% inhibition) and LM2/1.6 ($\bar{x}$ = 40% inhibition) showed significant but less striking inhibition of SRBC binding, and OKM-1 ($\bar{x}$ = 6% inhibition) demonstrated no inhibitory effects. The anti-β MAb (TS1/18) demonstrated significant ($p < 0.01$) but overall less inhibition of binding ($\bar{x}$ = 21% inhibition) as compared to most anti-αM MAb whereas the anti-αL TS1/22 ($\bar{x}$ = 20% inhibition) and anti-αX S-HCL3 ($\bar{x}$ = 17% inhibition) MAb demonstrated even less inhibitory effects. Modest effects of anti-CR-1 ($\bar{x}$ = 26% inhibition) are consistent with the relatively small proportion of C3b (as opposed to iC3b) deposited on SRBC in this assay (2). A binding control MAb directed at HLA A,B demonstrated no effects ($\bar{x}$ = 2% inhibition).

f-Met-Leu-^{3}H-Phe binding. The possible influence of anti-Mac-1 MAb on f-Met-Leu-Phe receptor-ligand interactions was evaluated. Individual MAb directed at β, αM, αX, or αL, or combinations of MAb directed at all subunits were reacted with healthy adult granulocytes in the presence of 20 nM f-Met-Leu-^{3}H-Phe and a 100-fold excess of unlabeled f-Met-Leu-Phe at 0°C or 37°C for 12 min (26). Anti-Mac-1 MAb or control MAb demonstrated no significant influence on the number of specific binding sites for f-Met-Leu-Phe under these conditions. When tested at 0°C, suspensions of healthy adult granulocytes reacted in the presence or absence of MAb and Mac-1-deficient granulocytes demonstrated a mean of 9100, 9000, and 9700 binding sites/cell, respectively.

DISCUSSION

In these studies, subunit-specific MAb were used to determine the individual contributions of Mac-1, LFA-1, and p150,95 to adherence and adherence-dependent granulocyte functions. Previous studies showed that the Mac-1 molecule functioned as a receptor (the CR3) for the iC3b ligand, and is distinct from the CR-1 which binds to C3b (17, 18, 48). Additional studies employing MAb directed at LFA-1 have demonstrated important contributions of this molecule to CTL-mediated cytolysis (15), antigen-specific and mitogen-driven T lymphocyte proliferative responses (14, 18). NK-mediated cytolysis (14), and granulocyte antibody-dependent cellular cytotoxicity (3). Inhibition by anti-LFA-1α and -β MAb of CTL binding to target cells indicated that this molecule participates in cell-cell adhesion (15).

The incubation of normal granulocytes with MAb directed at one or more subunits of the Mac-1 glycoprotein family demonstrated reproducible, dose-dependent, and selective abnormalities of adherence, aggregation, directed migration, phagocytosis of C3 fragment-opsonized zymosan or ORO paraffin particles, and rosetting of iC3b-coated erythrocytes. Each of these abnormalities was uniformly observed here with Mac-1, LFA-1-deficient granulocytes or has been observed in patients with the heritable Mac-1, LFA-1 deficiency syndrome (2). In contrast, normal granulocytes reacted with the same MAb demonstrated no defects of adherence-independent functions including shape change in suspension, f-Met-Leu-Phe receptor binding, or oxidative metabolic activity or secretion in response to PMA. Such functions are characteristically normal among Mac-1, LFA-1-deficient granulocyte suspensions (2).

The magnitude of adherence abnormalities elicited by MAb to the Mac-1 glycoprotein family were nearly as great as those of genetically deficient granulocytes in adherence, aggregation, and phagocytosis assays. Chemotaxis was less affected by MAb than by genetic deficiency; it is possible that "up-regulation" of Mac-1 and p150,95 during chemotaxis occurs at a site on the cell surface which is closely apposed to the substrate and is inaccessible to MAb. Other explanations for the somewhat more severe impairment in the genetic deficiency than after MAb treatment of normal cells may be that binding of MAb to cell surface molecules is reversible, and may hinder but not completely block a molecule's function. However, the overall parallel functional consequences of Mac-1, LFA-1 deficiency and treatment of normal granulocytes with MAb to the Mac-1 glycoprotein family demonstrate that the functional defects observed in patient cells are a direct consequence of the deficiency of the Mac-1, p150,95, and LFA-1 glycoproteins. Furthermore, these findings provide convincing evidence of the important physiologic role of this family of adhesive glycoproteins in the inflammatory response.

Distinct contributions of the β, αM, and, to a lesser extent, αX molecules were recognized here in most adhesion-dependent granulocyte functional assays, whereas minimal or no contributions of the LFA-1 α-molecule were generally seen. The observed relative rank order of inhibition by MAb in adherence assays of anti-β > anti-αM > anti-αX > anti-αL may indicate qualitatively different adhesive properties among these molecules (12) or, alternatively, may relate to the previously reported relative quantities of each molecule expressed on granulocyte surfaces (28). The absolute amount of adherence, and the absolute inhibitory effects of anti-β, anti-αM, or anti-αX (but not anti-αL) MAb, were substantially greater among cell suspensions stimulated with f-Met-Leu-Phe as compared to unstimulated cell suspensions (Fig. 1*A* vs *B*). These findings undoubtedly reflect the fact that β, Mac-1α, and p150,95α (but not LFA-1α) are "up-regulated" five- to sevenfold on surfaces of granulocytes stimulated with chemotactic factors or secretagogues as compared to unstimulated cells (2, 5, 23, 24). The profound inhibitory influences of anti-β MAb reagents may indicate intrinsic adhesive determinants of the β-molecule, but our studies cannot exclude the possibility that anti-β blockade may secondarily impair the functional activities of α-subunits. The specificity of inhibition of adherence by anti-Mac-1 glycoprotein family MAb in these studies was shown by the absence of inhibition by binding control MAb directed at HLA A,B or the CR-1. The latter finding suggests that in contrast to αM, the CR-1 does not contribute to "nonspecific" adhesion. Moreover, the applications of anti-CR-1 in these studies provide additional evidence for functional differences betwen the CR-3 and CR-1 of human myeloid cells (48).

Our findings demonstrate that the Mac-1 molecule contributes to nonspecific leukocyte adherence reactions in addition to iC3b-dependent opsonophagocytic functions (3, 49–51). We employed multiple MAb directed at different epitopes of αM to test the hypothesis that Mac-1 may be multifunctional and to determine whether nonspecific adhesion and CR-3 receptor functional sites could be

distinguished. In both rosetting and phagocytosis CL assays employing particles or cells opsonized with C3-derived ligands, the most profound inhibitory effects were recognized with OKM-10, LM2/1.6, or M1/70; less substantial or no effects resulted from OKM-1 incubation. Similar relative effects of anti-αM MAb were generally observed with respect to nonspecific adherence and granulocyte aggregation, although some differences were evident among the spectrum of assays employed in these studies. For example, relatively greater inhibitory effects of OKM-1 MAb were seen in agarose chemotaxis assays than in other assays involving nonspecific adherence. Our findings generally demonstrated a similar relative inhibition of nonspecific adherence and CR-3 function by MAb binding to several different αM epitopes. However, because some exceptions were noted in selected assays, it is not possible to confidently exclude the possibility that distinct epitopes of the αM molecule may contribute to iC3b recognition but not "nonspecific" adherence.

Clear differences were seen, however, between the effects of anti-αM and anti-β MAb. Anti-β MAb demonstrated greater inhibition than anti-αM MAb in assays of granulocyte adherence, aggregation, or directed cell migration. In contrast, anti-αM MAb showed greater inhibition of phagocytosis, rosetting, and intracellular killing of complement-opsonized particles. In ORO phagocytosis assays, no influence of anti-β MAb was apparent even when they were incorporated directly into phagocytic reaction mixtures. Some inhibition by the anti-β MAb was apparent in opsonized zymosan-phagocytosis-CL assays, but only when it was directly incorporated into reaction mixtures, and these inhibitory effects were less than those observed for the anti-αM MAb OKM-10, LM2/1.6, M1/70, and OKM-1. The αX and αL molecules did not appear to contribute significantly to phagocytic ingestion and/or attachment of C3-opsonized test particles. Our studies suggest that all three members of the Mac-1 glycoprotein family share nonspecific adhesive properties, but that only the Mac-1 molecule and particularly its αM subunit selectively mediates specific iC3b recognition. The multifunctional capabilities of this glycoprotein family may facilitate diverse and highly integrated functions of granulocytes during inflammation in vivo (1).

Our studies provide the first evidence for an adhesive function of the p150,95α molecule. By using a subunit-specific MAb directed at this molecule, a modest but significant inhibition of granulocyte adherence was detectable under chemotactic conditions designed to "up-regulate" and to ensure maximal surface expression of this molecule (2, 5, 23, 28). Significant inhibitory influences by anti-αX MAb were also observed in iC3b-SRBC rosetting assays, but not in phagocytosis assays. Anti-αX MAb inhibited adherence substantially less than anti-β or anti-αM MAb (with the exception of OKM-1), but greater than anti-αL MAb. The findings of adhesive properties of p150,95α are consistent with its biochemical relation to LFA-1α and Mac-1α (12, 28, 52).

Studies to evaluate the contributions of Mac-1 subunits to directed cell migration were of critical importance because impaired recruitment of granulocytes into inflamed tissues represents the histopathologic hallmark of the Mac-1, LFA-1, p150,95 deficiency syndrome, and because previous investigations have documented adhesive requirements for directed cell translocation (26, 38, 39). Further, studies of Mac-1 protein expression and function provide a unique opportunity to understand the molecular mechanisms of directed migration of granulocytes which may be of general relevance to our understanding of the mechanisms regulating motility of other cell types.

Our findings demonstrated no inhibition of chemotaxis in Boyden assays employing anti-β MAb or combinations of anti-β + anti-αM MAb, regardless of the chemoattractant selected or the incubation conditions employed. In contrast, dose-dependent inhibitory influences were apparent in the sub-agarose assay. Such findings are consistent with the more profound abnormalities of directed migration of Mac-1, LFA-1-deficient granulocytes observed in the latter as opposed to the former assay (data not shown) (2). Such findings indicate a greater requirement for adhesiveness in chemotaxis on a plane surface than in a three-dimensional matrix, as previously proposed (53–55). Three-dimensional matrices may provide an appreciably greater surface for adhesive interactions, or may facilitate relatively greater "up-regulation" and/or surface redistribution of Mac-1 (54).

Findings in the sub-agarose assays are consistent with the possibility that a continual expression of new surface Mac-1 is required for cell migration. Cell suspensions exposed to MAb throughout the performance of the assay demonstrated significantly less migration than those preincubated in the same MAb and then washed before their incorporation in chemotaxis assays, or those incorporated together with MAb into cell wells at the initiation of the assay. We propose that a continual "up-regulation" of Mac-1 from intracellular pools to the surface of the cell may provide a molecular basis for the formation of focal adhesions which may subsequently dissipate or redistribute as the cell translocates (25). Studies to evaluate the spatial alterations of Mac-1 expression under conditions of chemotactic stimulation will be required to confirm this possibility, and to determine whether such alterations are similar to our previously reported findings of granulocyte "adhesion site" redistribution modulated by certain chemotactic stimuli (26, 38).

Acknowledgments. We acknowledge the technical assistance of Ms. Bonnie J. Hughes, Edward J. Burke, Beth Rudloff, and Dr. Michael Tosi. We also acknowledge the fine secretarial support provided by Marie Mason, Drenda Wayland, and Gaye Stokes, and Dr. C. Wayne Smith of the Rheumatology Department, Ciba Geigy Corp., for his preparation of the scanning electron micrographs. We appreciate the useful suggestions and continued support extended by Dr. Ralph D. Feigen.

REFERENCES

1. **Anderson, D. C., F. C. Schmalstieg, S. Kohl, M. A. Arnaout, B. J. Hughes, M. F. Tosi, G. J. Buffone, B. R. Brinkley, W. D. Dickey, J. S. Abramson, T. A. Springer, L. A. Boxer, J. M. Hollers, and C. W. Smith.** 1984. Abnormalities of polymorphonuclear leukocyte function associated with a heritable deficiency of a high molecular weight surface glycoprotein (GP138): common relationship to diminished cell adherence. *J. Clin. Invest. 74:546.*
2. **Anderson, D. C., F. C. Schmalstieg, W. Shearer, S. Kohl, and T. A. Springer.** 1985. The severe and moderate phenotypes of heritable Mac-1, LFA-1, p150,95 deficiency: their quantitative definition and relation to leukocyte dysfunction and clinical features. *J. Infect. Dis. 152(4):668.*

3. **Kohl, S., T. A. Springer, F. C. Schmalstieg, L. S. Loo, and D. C. Anderson.** 1984. Defective natural killer cytotoxicity and polymorphonuclear leukocyte antibody-dependent cellular cytotoxicity in patients with LFA-1/OKM-1 deficiency. *J. Immunol. 133:2972.*
4. **Crowley, C. A., J. T. Curnutte, R. E. Rosin, J. Andre-Schwartz, J. I. Gallin, M. Klempner, R. Snyderman, F. S. Southwick, T. P. Stossel, and B. M. Babior.** 1980. An inherited abnormality of neutrophil adhesion: its genetic transmission and its association. *N. Engl. J. Med. 302:1163.*
5. **Springer, T. A., W. S. Thompson, L. J. Miller, F. C. Schmalstieg, and D. C. Anderson.** 1984. Inherited deficiency of the Mac-1, LFA-1, p150,95 glycoprotein family and its molecular basis. *J. Exp. Med. 160:1901.*
6. **Bowen, T. J., H. D. Ochs, L. C. Altman, T. H. Price, D. E. Van Epps, D. L. Brautigan, R. E. Rosin, W. D. Perkins, B. M. Babior, S. J. Klebanoff, and R. J. Wedgwood.** 1982. Severe recurrent bacterial infections associated with defective adherence and chemotaxis in two patients with neutrophils deficient in a cell-associated glycoprotein. *J. Pediatr. 101:932.*
7. **Dana, N., R. F. Todd, J. Pitt, T. A. Springer, and M. A. Arnaout.** 1984. Deficiency of a surface membrane glycoprotein (Mol) in man. *J. Clin. Invest. 73:153.*
8. **Buescher, L. S., T. Gaither, J. Nath, and J. I. Gallin.** 1986. Abnormal adherence-related functions of neutrophils, monocytes and EB virus-transformed B cells in a patient with C3bi receptor deficiency. *Blood. In press.*
9. **Kobayashi, K., K. Fujita, F. Okino, and T. Kajii.** 1984. An abnormality of neutrophil adhesion: autosomal recessive inheritance associated with missing neutrophil glycoproteins. *Pediatrics 73:606.*
10. **Fischer, A., R. Seger, A. Durandy, B. Grospierre, J. L. Virelizier, C. Griscelli, Fisher, M. Kazatchkine, M. C. Bohler, B. Dischamps-Latscha, P. H. Trung, T. A. Springer, D. Olive, and C. Mawas.** 1986. Deficiency of the adhesion protein complex LFA-1, C3bi complement receptor, p150,95 in a girl with recurrent bacterial infections. *Blood. In press.*
11. **Ross, G. D., R. A. Thompson, M. J. Walport, T. A. Springer, J. V. Watson, R. H. R. Ward, J. Lida, S. L. Newmon, R. A. Harrison, and P. J. Lackmann.** 1986. Characterization of patients with an increased susceptibility to bacterial infections and a genetic deficiency of leukocyte membrane complement receptor type three (CR-3) and the related membrane antigen LFA-1. *Blood. In press.*
12. **Sanchez-Madrid, F., J. Nagy, E. Robbins, P. Simon, and T. A. Springer.** 1983. A human leukocyte differentiation antigen family with distinct α subunits and a common β subunit: the lymphocyte-function associated antigen (LFA-1), the C3bi complement receptor (OKM-1/Mac-1), and the p150,95 molecule. *J. Exp. Med. 158:1785.*
13. **Sanchez-Madrid, F., A. M. Krensky, C. F. Ware, E. Robbins, J. L. Strominger, S. J. Burakoff, and T. A. Springer.** 1982. Three distinct antigens associated with human T lymphocyte-mediated cytolysis: LFA-1, LFA-2, and LFA-3. *Proc. Natl. Acad. Sci. USA 79:7489.*
14. **Krensky, A. M., F. Sanchez-Madrid, E. Robbins, J. Nagy, T. A. Springer, and S. J. Burakoff.** 1983. The functional significance, distribution, and structure of LFA-1, LFA-2, and LFA-3: cell surface antigens associated with CTL-target interactions. *J. Immunol. 131:611.*
15. **Springer, T. A., D. Davignon, M. K. Ho, K. Kurzinger, E. Martz, and F. Sanchez-Madrid.** 1982. LFA-1 and Lyt-2,3 molecules associated with T lymphocyte-mediated killing; and Mac-1 and LFA-1 monologue associated with complement receptor function. *Immunol. Rev. 68:171.*
16. **Beatty, P. G., J. A. Ledbetter, P. J. Martin,** et al. 1983. Definition of a common leukocyte cell-surface antigen (Lp 95,150) associated with diverse cell-mediated immune functions. *J. Immunol. 131: 2913.*
17. **Beller, D. I., T. A. Springer, and R. D. Schreiber.** 1982. Anti-Mac-1 selectively inhibits the mouse and human type three complement receptor. *J. Exp. Med. 156:1000.*
18. **Wright, S. D., P. E. Rao, W. C. Van Voorhis, L. S. Craigmyle, K. Iida, M. A. Talle, E. F. Westberg, G. Goldstein, and S. C. Silverstein.** 1983. Identification of the C3bi receptor of human monocytes and macrophages with monoclonal antibodies. *Proc. Natl. Acad. Sci. USA 80:5699.*
19. **Springer, T., G. Galfre, D. S. Secher, and C. Milstein.** 1979. Mac-1: a macrophage differentiation antigen identified bymonoclonal antibody. *Eur. J. Immunol. 9:301.*
20. **Ault, K. A., and T. A. Springer.** 1981. Cross-reaction of a rat-anti-mouse phagocyte-specific monoclonal antibody (anti-Mac-1) with human monocytes and natural killer cells. *J. Immunol. 126:359.*
21. **Todd, R. F., III, A. Van Agthoven, S. F. Schlossman, and C. Terhorst.** 1982. Structural analysis of differentiation antigens Mo1 and Mo2 on human monocytes. *Hybridoma 1:329.*
22. **Arnaout, M. A., R. F. Todd, III, N. Dana, J. Melamed, S. F. Schlossman, and H. R. Colten.** 1983. Inhibition of phagocytosis of complement C3 or immunoglobulin G-coated particles and of C3bi binding by monoclonal antibodies to a monocyte-granulocyte membrane glycoprotein (Mo1). *J. Clin. Invest. 72:171.*
23. **Springer, T. A., L. J. Miller, and D. C. Anderson.** 1986. p150,95, the third member of the Mac-1, LFA-1 human leukocyte adhesion glycoprotein family. *J. Immunol. 136:240.*
24. **Todd, R. F., A. Arnaout, R. E. Rosin, C. A. Crowley, W. A. Peters, and B. M. Babior.** 1984. Subcellular localization of the large subunit of Mo1, a surface glycoprotein associated with neutrophil adhesion. *J. Clin. Invest. 74:1280.*
25. **Springer, T. A., and D. C. Anderson.** 1986. Functional and structural interrelationships among the Mac-1, LFA-1, and p150,95 family of leukocyte adhesion glycoproteins and their deficiency in a novel, heritable disease. In *Ciba Foundation Symposium on Human Leukocyte Function. In press.*
26. **Anderson, D. C., B. J. Hughes, and C. W. Smith.** 1981. Abnormal mobility of neonatal polymorphonuclear leukocytes. Relationship to impaired redistribution of surface adhesion sites by chemotactic factor or colchicine. *J. Clin. Invest. 68:863.*
27. **Fearon, D. T., and L. A. Collins.** 1983. Increased expression of C3b receptors on polymorphonuclear leukocytes induced by chemotactic factors and by purification procedures. *J. Immunol. 130:370.*
28. **Springer, T. A., and D. C. Anderson.** 1986. Antibodies specific for the Mac-1, LFA-1, p150-95 glycoprotein family or other granulocyte proteins. In *Leukocyte Differentiation Antigens. In press.*
29. **Todd, R. F., A. Van Agthovan, S. F. Schlussman, and C. Terhorst.** 1982. Structural analysis of differentiation antigens Mo1 and Mo2 on human monocytes. *Hybridoma 1:329.*
30. **Hildreth, J. E. K., F. M. Gotch, P. D. K. Hildreth, and A. J. McMichael.** 1983. A human lymphocyte-associated antigen involved in cell-mediated lympholysis. *Eur. J. Immunol. 13:202.*
31. **Changelian, P. S., R. M. Jack, L. A. Callin, and D. T. Fearon.** 1985. PMA induces the ligand-independent internalization of CR-1 on human neutrophils. *J. Immunol. 134:1851.*
32. **Barnstable, C. J., W. F. Bodmer, G. Brown, G. Galfre, C. Milstein, A. F. Williams, and A. Ziegler.** 1978. Production of monoclonal antibodies to group A erythrocytes, HLA and other human cell surface antigens—new tools for genetic analysis. *Cell 14:9.*
33. **Reinherz, E., J. A. Hansen, and I. Bernstein.** 1986. Human leukocyte typing. II. In *The Second International Workshop on Human Leukocyte Differentiation Antigens. In press.*
34. **Ey, P. L., S. J. Prowse, and C. R. Jenkins.** 1978. Isolation of pure IgG_1, IgG_{2a} and IgG_{2b} immunoglobulins from mouse serum using protein A-Sepharose. *Immunochemistry 15:429.*
35. **Parham, P., M. J. Androlewicz, F. M. Brodsky, N. J. Holmes, and J. P. Ways.** 1982. Monoclonal antibodies: purification, fragmentation, and application to structural and functional studies of class I MHC antigens. *J. Immunol. Methods 53:133.*
36. **Parham, P.** 1986. Preparation and purification of active fragments from mouse monoclonal antibodies. In *Handbook of Experimental Immunology. Vol. IV. 4th edition. Applications of Immunological Methods in Biomedical Sciences.* D. M. Weir, C. Blackwell, L. Herzenberg, and L. Herzenberg, eds. Blackwell, Oxford. *In press.*
37. **Smith, C. W., J. C. Hollers, R. A. Patrick, and C. Hassett.** 1979. Motility and adhesiveness in human neutrophils. Effects of chemotactic factors. *J. Clin. Invest. 63:221.*
38. **Smith, C. W., and J. C. Hollers.** 1980. Motility and adhesiveness in human neutrophils. Redistribution of chemotactic factor induced adhesion sites. *J. Clin. Invest. 65:804.*
39. **Hammerschmidt, P. E., T. K. Bowers, C. J. Kammi-Kepfe, H. S. Jacob, and P. R. Craddock.** 1980. Granulocyte aggregometry: a sensitive technique for the detection of C5a and complement activation. *Blood 55:898.*
40. **Zigmond, S. H., and J. G. Hirsch.** 1973. Leukocyte locomotion and chemotaxis. New *in vitro* method for evaluation and demonstration of a cell derived chemotactic factor. *J. Exp. Med. 137:387.*
41. **Nelson, R. D., P. G. Quie, and R. L. Simmons.** 1975. Chemotaxis under agarose: a new and simple method for measuring chemotaxis and spontaneous migration of human polymorphonuclear leukocyte and monocyte. *J. Immunol. 115:1650.*
42. **Shurin, S. B., and T. P. Stossel.** 1978. Complement (C3)-activated phagocytosis by lung macrophages. *J. Immunol. 120:1305.*
43. **Anderson, D. C., M. A. Mace, R. R. Martin, and C. W. Smith.** 1981. Recurrent infection in glycogenosis type Ib; abnormal neutrophil mobility related to impaired redistribution of adhesion sites. *J. Infect. Dis. 143:447.*
44. **Rothlein, R., and T. A. Springer.** 1982. Role of Fc receptor modulation by immobilized immune complexes in generation of nonspecific (bystander) cytotoxicity for autologous and xenogeneic targets by porcine alveolar macrophages. *J. Immunol. 129:1859.*
45. **Freeman, K. L., D. C. Anderson, and G. Buffone.** 1985. A rapid radiometric assay for lactoferrin applied to the assessment of lactoferrin in granulocytes. *Clin. Chem. 31:407.*
46. **Quie, P. G., S. G. White, B. Holmes, and R. A. Good.** 1967. *In vitro* bactericidal capacity of human polymorphonuclear leukocytes: diminished activity in chronic granulomatous diseases of childhood. *J. Clin. Invest. 46:668.*
47. **Babior, B. M., R. S. Kipnes, and J. T. Curnutte.** 1973. Biological defense mechanisms. The production by leukocytes of superoxide, a potential bacteridical agent. *J. Clin. Invest. 52:741.*
48. **O'Shea, J. S., C. S. Brown, B. E. Seligman, J. F. Metcalf, M. M. Frank, and J. I. Gallin.** 1985. Evidence for distinct intracellular pools of receptors for C3b and C3bi in human neutrophils. *J. Im-*

munol. 134(4):2580.

49. **Arnaout, M. A., R. M. Hakin, R. F. Todd, N. Dana, and H. R. Colten.** 1985. Increased expression of the adhesion promoting surface glycoprotein in the granulocytosis of hemodialysis. *N. Engl. J. Med. 312:457.*
50. **Bianco, C., and V. Nussenzweig.** 1977. Complement receptors. *Contemp. Top. Mol. Immunol. 6:145.*
51. **Perlman, H., P. Perlman, R. D. Schreiber, and H. J. Muller-Eberhard.** 1981. Interaction of target cell-bound C3bi and C3d with human lymphocyte receptors. Enhancement of antibody-mediated cellular cytotoxicity. *J. Exp. Med. 153:1592.*
52. **Lanier, L. L., M. A. Arnaout, R. Schwarting, N. L. Warner, and G. D. Ross.** 1985. p150,95, third member of the LFA-1/CR-3 polypeptide family identified by anti-Leu M5 monoclonal antibody. *Eur. J. Immunol. 15:713.*
53. **Wilkinson, P. C.** 1982. *Chemotaxis and Inflammation.* Churchill-Livingston, Edinburgh.
54. **Brown, A. F.** 1982. Neutrophil granulocytes: adhesion and locomotion on collagen substrata in collagen matrices. *J. Cell Sci. 58:455.*
55. **Schor, S. L.** 1980. Cell proliferation and migration on collagen substrata *in vitro.* J. Cell Sci. 41:159.