Alteration of Fibronectin Receptors (Integrins) in Phorbol Ester-treated Human Promonocytic Leukemia Cells

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ABSTRACT

We describe the isolation of human fibronectin receptors (integrins) from two nonadherent promonocytic cell lines and from peripheral blood monocytes. Integrins purified from U-937 and THP-1 cells exhibited identical electrophoretic migrations on sodium dodecyl sulfate gels run under reducing (~M, 150,000) and nonreducing (~M, 150,000; M, 130,000) conditions. Treatment of U-937 or THP-1 cells with phorbol esters induced these cells to express different integrins with electrophoretic mobilities (~M, 140,000; M, 115,000, nonreduced) identical to those from normal human peripheral blood monocytes. Receptors isolated from uninduced, nonadherent promonocytic leukemia cells (U-937 and THP-1) were distinct from glycoproteins IIb and IIIa and from leukocyte adhesion molecules (p150/95). However, receptors isolated here did react with an antibody known to block cell adhesion to fibronectin. The differences observed in apparent molecular masses of fibronectin receptors from uninduced and induced U-937 or THP-1 cells are removed by treatment of purified integrins with endoglycosidase F or N-glycanase. In summary, the data presented here demonstrate the purification of integrins by fibronectin affinity chromatography from human leukemia cells and normal peripheral blood monocytes. Our results suggest that these receptors differ in immature and mature monocytic cells, and are altered by glycosylation in the course of cellular maturation.

INTRODUCTION

Fibronectin is an adhesive glycoprotein important to the function of monocytes and macrophages. Macrophages ingest certain types of fibronectin-coated particles, while monocytes, when adherent to fibronectin-coated substrata, exhibit enhanced ingestion of erythrocytes opsonized with C3b or C3bi (1-6). Fibronectin also modulates macrophage migration and promotes growth factor production by these cells (7, 8). Interaction of monocytes and macrophages with fibronectin appears mediated at least in part by a domain within fibronectin which contains the tripeptide sequence RGD2 (7, 9, 10).

Receptors for this tripeptide sequence are part of a superfine family of cell surface proteins termed integrins, heterodimeric proteins comprised of one ~ (M, 140,000-200,000) and one M (95,000-120,000) subunit present in a noncovalent complex at the cell surface (11-13). Three M subunits define the three known integrin subfamilies which include the mammalian fibronectin receptors (~, the leukocyte adhesion receptors, Mac-1/Mo-1, p150/95, LFA-1 (M), and vitronectin receptors and glycoproteins IIb/IIIa (M)). These subfamilies have been elucidated by complete protein sequence data of the ~, ~, ~ proteins (14-17).

The ~ integrins, also termed VLA proteins, contain one of at least six ~ subunits in association with the M subunit (18). Of these, the ~ integrin (VLA-3 or CSAT antigen) (13) is known to associate with fibronectin, collagen, laminin, while the ~ integrin (VLA-5) has been demonstrated by fibronectin affinity chromatography to mediate binding to an RGD tripeptide sequence within fibronectin (19). This latter receptor has been identified by immunological means on cell types including fibroblasts, tumor cells, erythroid precursors, pre-B- and T-lymphocytes, and leukocytes (20-25). This fibronectin receptor may also be important to the anchorage in the bone marrow of progenitor cells of the erythroid and lymphoid lineages (21, 26, 27).

The human promonocytic cell lines, U-937 and THP-1, are useful models for monocyte/macrophage maturation. The U-937 cell line, derived from the pleural fluid of a patient with diffuse histiocytic lymphoma, and THP-1 cells, from a patient with acute monocytic leukemia, are nonadherent in culture. Under usual culture conditions, both cell lines express minimal phagocytic, peroxidase, or esterase activities, and secrete little lysozyme or interleukin-1 (28, 29). When cultured with agents known to induce maturation (e.g., phorbol esters, interferon, 1,25-dihydroxyvitamin D3), both cell lines become adherent in culture (28, 29). They also express other properties of monocytes/macrophages including augmented peroxidase and esterase activities, increased phagocytosis and specific cell surface proteins such as receptors for immunoglobulin, complement, and collagen (28-35). While increased expression of Mac-1/Mo-1 and p150/95 after induction of U-937 by phorbol esters has been reported (32, 34), no previous reports have noted alterations in the structure of integrins during induction.

We report here the isolation by affinity chromatography of fibronectin receptors from these leukemic cell lines and normal monocytes. We find that U-937 and THP-1 cells express altered receptors compared to normal peripheral blood monocytes. Treatment of these leukemic cell lines with phorbol esters results in the isolation of fibronectin receptors like those from normal monocytes.

MATERIALS AND METHODS

Materials

Human plasma and "buffy" coats were obtained from the American Red Cross, Boston, MA. Affigel-10 was purchased from Bio-Rad Labs, Richland, CA, and glycopeptidase F was from Boehringer-Mannheim, Indianapolis, IN. Na 125I(carrier-free) and endoglycosidase F were obtained from Sigma Chemical Co., St. Louis, MO. Vectorstain ABC kit for detection of rabbit IgG was purchased from Vector Labs, Burlingame, CA. Cell-binding peptide, GRGDS, was purchased from the Peptide Synthesis Facility, Children's Hospital, Boston, MA. Monoclonal antibody against the cell-binding fragment of fibronectin and rabbit antisem to platelet glycoproteins IIb and IIIa were kind gifts of J. Gardner and R. O. Hynes (36), and goat anti-hamster fibronectin receptor a generous gift of P. Brown and R. Juliano (20).

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2 The abbreviations used are: RGD, Arg-Gly-Asp; GRGDS, Gly-Arg-Gly-Asp-Ser-Pro; DMEM, Dulbecco's modified Eagle's medium; PBS, phosphate buffered saline; PMA, phorbol-12-myristate-13-acetate; PMSF, phenylmethylsulfonyl fluoride; SDS, sodium dodecyl sulfate.

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**Methods**

Cells. All cell lines, except U-937, were obtained from the American Type Culture Collection, Rockville, MD. Human U-937 cells (from P. Knudson, Beth Israel Hospital), derived from a patient with histiocytic lymphoma (28), and the osteosarcoma lines MG-63 and HOS (19, 37), were grown in DMEM with penicillin and streptomycin supplemented with fetal bovine serum (10% v/v). Human THP-1 cells (29), derived from a patient with acute monocytic leukemia, were grown in DMEM supplemented with fetal calf serum (10%) and with 50 μM 2-mercaptoethanol. U-937 or THP-1 cells were cultured in DMEM and fetal bovine serum containing PMA at 3 or 50 ng/ml for 5 or 3 days, respectively (28, 29). Human peripheral blood monocytes were purified (>90%) on sequential Ficoll and Percoll gradients and cultured in Teflon beakers (38).

Preparation of Fibronectin Fragments. The M, 120,000 fragment was purified from a chymotryptic digest of human plasma fibronectin (39, 40) and the M, 12,000 cell-binding fragment from peptic digests of the M, 120,000 fragment (36, 39).

Cell Adhesion Assays. Cell adhesion assays were carried out by the method of Johansson (41). Wells of microtiter plates were incubated (100 μl) with indicated dilutions of fibronectin, or purified M, 12 or 120,000 fragments (1 h), blocked with IgG-free bovine serum albumin (1% w/v, 30 min), and incubated with cells (1 × 10^6/ml, 37°C, indicated times). Plates were washed and attached cells quantitated.

Affinity Chromatography. Cells were iodinated (1–2 × 10^6 cells, 2–5 × 10^7 cpm, >95% viable), washed, lysed in octylglucoside, and the resulting extracts incubated with 2 ml of the M, 120,000-agarose in suspension for 60 min at room temperature (19). The column was poured, washed with column buffer (PBS containing 1 mM CaCl₂, 1 mM MgCl₂, 50 mM octyl-glucoside, and 1 mM PMSF) and in turn with 1.5 mM control synthetic peptide (Gly-Gly-Arg or Gly-His-Arg-Pro), 1.5 mM specific cell-binding peptide (GRGDSP), and 8 M urea with washing between steps. Identical results were obtained when elution was carried out with 10 mM EDTA in place of GRGDSP.

SDS-Polyacrylamide Gel Electrophoresis. Discontinuous polyacrylamide slab gel electrophoresis was performed by the method of Laemmli (42). Autoradiography was carried out at −70°C using Kodak X-Omat (XAR-2) film with a Dupont Cronex screen. Molecular weight markers were myosin (M, 200,000), β-galactosidase (M, 116,000), phosphorylase b (M, 97,000), bovine albumin (M, 67,000), egg albumin (M, 43,000).

Immunoelectroblotting. Proteins were transferred from SDS-polyacrylamide gels to nitrocellulose paper by electrophoresis at 20 V for ~18 h (43). After transfer, the nitrocellulose paper was immersed (1 h, room temperature) in 3% hemoglobin in PBS, incubated (1 h, room temperature) with rabbit anti-platelet glycoprotein IIb and IIIa sera or preimmune serum (each 1/200) in hemoglobin-PBS solution (36). The blots were washed with PBS containing 0.05% Tween-20. Bound immune complexes were then identified with anti-rabbit immunoglobulin reagents (Vectastain ABC).

Immunoprecipitation. Immunoprecipitations for p 150/95 were carried out with samples (1.6 ml) of iodinated cell extract, which were brought to 0.5% in NP-40, dialyzed against wash buffer (0.15 ml), preincubated (30 min, 37°C) with or without M, 120,000-agarose in suspension for 60 min at room temperature (19). The column was poured, washed with column buffer (PBS containing 1 mM CaCl₂, 1 mM MgCl₂, 50 mM octyl-glucoside, and 1 mM PMSF) and in turn with 1.5 mM specific cell-binding peptide (GRGDSP), and 8 M urea with washing between steps. Identical results were obtained when elution was carried out with 10 mM EDTA in place of GRGDSP.

Glycosidase Treatment. Affinity-purified integrins from U-937 cells or MG-63 cells were incubated with or without endoglycosidase F (15 U/ml, final) and 100 mM sodium phosphate (pH 6.1), 50 mM EDTA, 1% NP-40, 0.1% SDS, 1% mercaptoethanol and 2 mM PMSF overnight at 37°C (45). Sample buffer was then added, samples heated and analyzed by 7.5% SDS-gel electrophoresis under reducing conditions. Alternatively, affinity-purified integrins from THP-1 (induced with PMA), THP-1 (uninduced) or MG-63 cells were heated (90°C, 5 min, 2% w/v SDS, 1% v/v mercaptoethanol), diluted into enzyme buffer (0.2% SDS, 1% mercaptoethanol, 1% NP-40, 0.2 mM phosphate, pH 8.5 and 2 mM PMSF) and incubated (overnight, 37°C) with or without glycopeptidase F (10 U/ml final) (46). Sample buffer was then added, samples heated and analyzed on 7.5% SDS-gel electrophoresis run under reducing conditions.

**RESULTS**

Human U-937 Cells Bind to the Cell-Adhesion Fragment of Fibronectin. U-937 cells adhere to plastic wells coated with a M, 120,000 chymotryptic fragment of fibronectin in a concentration-dependent manner over a range of 1–10 μg M, 120,000 fragment per well (Fig. 1A). Experiments (not shown) carried out with the M, 12,000 cell adhesion peptide (36, 39) also demonstrated peptide-dependent adhesion of U-937 cells. Soluble fibronectin and the M, 120,000 fibronectin fragment were also examined for inhibition of cell adhesion (Fig. 1B). Native fibronectin inhibited U-937 cell adhesion to substrate-adsorbed fibronectin only at relatively high concentrations (50% inhibition at ~5 mg/ml). When soluble M, 120,000 was used as inhibitor, half-maximal binding of U-937 cells was observed at...

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**Fig. 1. Adhesion of U-937 cells to fibronectin.** A, cell adhesion to M, 120,000. U-937 cells (1 × 10^6 cells/well) were incubated (37°C, 60 min) in microtiter wells (0.15 ml), precoated with a purified M, 120,000 fragment (0–10 μg/well), followed by serum albumin (1.5 μg/well). Plates were washed and relative cell binding determined by enzyme-linked immunosorbent assay measurements at 410 nm, as described in “Materials and Methods.” Triangle at lower left, cell binding to albumin in absence of fibronectin. Results are expressed as means of triplicate samples. B, inhibition by soluble M, 120,000. Microtiter wells were precoated with fibronectin (2 μg/well), followed by serum albumin (1.5 μg/well). Samples (0.15 ml) of U-937 cells (1 × 10^6/well) were then added to each well and incubated (37°C, 60 min) containing the designated concentrations of the M, 120,000 fragment, washed, and assayed for radioactive cells. Results (from triplicate samples) are expressed as the mean percentage of cells bound to fibronectin-coated substrate in the absence of the added M, 120,000 fragment.
described in Fig. 4. Samples were analyzed by lanes 2, 5) or osteosarcoma (MG-63, lanes 3, ~ 10,000 larger than protein purified from lines at an apparent molecular mass of tors from two mononuclear phagocyte cell

Affinity-purified Proteins from Promonocytic Cells and Osteosarcoma Cells. Radiolabeled cell extracts were subjected to affinity chromatography on Mr, 120,000 agarose (19). For comparison to published results with osteosarcoma cells (MG-63, Ref. 19), we prepared proteins eluted with GRGDSP from two human osteosarcoma cells (MG-63, HOS) and from promonocytic cell lines (U-937, THP-1). A single radiolabeled peak eluted specifically with GRGDSP; no peak was eluted during prior treatment of the column with control peptides. Proteins purified from MG-63 or HOS cell extracts were observed by SDS-gel electrophoresis to migrate identically and faster (Mr, ~ 140,000, reducing conditions) than proteins purified from the

Table 1 Effect of synthetic peptides on cell attachment

Microtiter wells were precoated with fibronectin, washed, blocked, incubated with suspensions of U-937 cells containing the designated concentrations of peptide, washed again, and treated with substrate for enzyme assay (experiments I and II) or assayed for radioactive [35S]methionine cellular protein (experiment III). Results are presented as the mean of triplicate (III) or quadruplicate (I, II) samples and are expressed as the percentage of cells bound to fibronectin-coated substrate in the absence of added peptide.

<table>
<thead>
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<th>Experiment</th>
<th>Peptide*</th>
<th>Concentration (mM)</th>
<th>Cell attachment (%)</th>
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<tr>
<td>I</td>
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<td></td>
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<td>107</td>
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<tr>
<td></td>
<td>GHRP</td>
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<tr>
<td>II</td>
<td>GRGDSP</td>
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<td></td>
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* GGYR, Gly-Gly-Tyr-Arg; GHRP, Gly-His-Arg-Pro; GGA, Gly-Gly-Arg.

Affinity-purified Proteins from Uninduced U-937 Cells Differ from Induced U-937 Cells and Peripheral Blood Monocytes. We next compared the properties of affinity-purified proteins isolated from peripheral blood monocytes and from U-937 or THP-1 cells treated with phorbol ester. When cultured with phorbol esters, U-937 and THP-1 cell lines become adherent and display properties of differentiated mononuclear phagocytes (28, 29). Peripheral blood monocytes or phorbol ester-treated U-937 or THP-1 cells were iodinated and subjected to affinity chromatography. When analyzed by SDS-gel electrophoresis under nonreducing conditions, proteins from uninduced U-937 cells migrated at Mr ~ 160,000 and Mr ~ 130,000 as before (Fig. 3A, lane 1). Moreover, the proteins isolated from induced U-937 cells (lane 2) and monocytes (lane 3) comigrated with apparent molecular masses of Mr ~ 140,000 (a) and Mr ~ 115,000 (b) (Fig. 3). However, both a and b proteins isolated from these cells displayed a consistently faster migration on SDS gels than the comparable proteins isolated from uninduced U-937 cells (lane 1). Similar results were obtained with THP-1 cells [Fig. 3B, lane 1 (uninduced); lane 3 (induced)]. Affinity-purified proteins from induced cells did not comigrate with those from MG-63 cells (lane 2).

Affinity-purified Proteins from Promonocytic Leukemia Cells Are Distinct from p150/95. The proteins isolated from the uninduced U-937 and THP-1 cells and from peripheral blood monocytes had electrophoretic migrations that were similar to those observed for one member, p150/95, of a family of adhesion-related proteins (Mac-1/Mo-1, LFA-1) expressed by macrophages. We therefore carried out immune precipitations using an antibody to p150 (Leu M5) on extracts of U-937 cells (Fig. 4). Fractions containing proteins not bound to the Mr, 120,000-agarose (as in lane 1, Fig. 1) were incubated with control mouse IgG (Fig. 4, lanes 1, 5) or with Leu M5 monoclonal antibody (Fig. 4, lanes 2, 6). Affinity-purified proteins, purified from uninduced and induced U-937 cells are shown for comparison of electrophoretic mobilities (lanes 3, 4, 7, 8). Proteins immu-
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noprecipitated specifically by the Leu M5 monoclonal exhibited electrophoretic mobilities of $M$, 150,000 and 95,000 under reducing conditions (lane 6); p95 was a faint band, as reported by others (47). When analyzed under nonreducing conditions (Fig. 3), p150 (lane 2) did not comigrate with the larger $\alpha$ protein purified from either induced or uninduced U-937 cells (lanes 3, 4). Immunoprecipitated p95 (lane 6) did not comigrate with the $\beta$ protein affinity purified from either uninduced or induced U-937 cells when analyzed on SDS gels run under reducing conditions (lanes 7, 8). In our gel system run reduced/nonreduced, immunoprecipitated p95 (lanes 2, 6) migrated in a manner opposite to what one might predict based on the protein sequence (15). This electrophoretic behavior has been reported by others (48). In addition, we found that induced and uninduced U-937 proteins which eluted specifically from the $M$, 150,000-agarose with GRGDSP did not immunoprecipitate with this monoclonal antibody (not shown).

Fibronectin-binding Proteins from Uninduced U-937 and THP-1 Cells Are Distinct from Platelet Glycoprotein IIb/IIIa. Proteins immunologically related to glycoproteins IIb/IIIa have been detected on a variety of cells including leukocytes and U-937 cells (49-51). We employed rabbit antisera to compare the relative electrophoretic mobilities of human platelet glycoproteins IIb/IIIa to affinity-purified proteins from uninduced U-937 and THP-1 cells and osteosarcoma (MG-63) cells (Fig. 5). Samples of unlabeled platelet extract proteins and radiiodinated, affinity-purified proteins from the U-937 (lane 2) and THP-1 (lane 3) or fibroblastic (lane 4) cell lines were electrophoretically transferred from an SDS gel to the same nitrocellulose sheet. The platelet glycoproteins (lanes 1, 5) were detected on nitrocellulose by reaction with rabbit antisera to glycoproteins IIb and IIIa followed by incubation with horseradish peroxidase-conjugated secondary reagents (Fig. 5A). The $\alpha$ and $\beta$ fibronectin receptors isolated from both uninduced promonocytic cell lines (lanes 2, 3) migrated closer together and slower than did the platelet proteins (Fig. 5B). Our data demonstrate that the fibronectin receptors purified from U-937 and THP-1 cells are electrophoretically distinct from platelet glycoproteins IIb/IIIa.

Affinity-purified Proteins from Mononuclear Phagocytes Immunoprecipitate with Antibodies to the Fibronectin Receptor. To

![Fig. 3. Affinity-purified proteins from uninduced and induced U-937 and THP-1 cells compared to peripheral blood monocytes or osteosarcoma cells. A, U-937 cells were cultivated (5 days) in the absence (lane 1) or presence (lane 2) of phorbol ester (3 ng/ml). Peripheral blood monocytes (lane 3) were purified (>90%) by centrifugation on Ficoll and Percoll gradients. Proteins were prepared from cell extracts and subjected to SDS gel (7.5%) electrophoresis (nonreducing conditions) as in Fig. 2. Positions of molecular mass markers denoted at right (in $M$, $\times 10^3$). Note that proteins purified from induced U-937 cells and peripheral blood monocytes comigrate. B, THP-1 cells were cultivated (3 days) in the absence (lane 1) or presence (lane 3) of PMA (50 ng/ml). Affinity-purified proteins were prepared from extracts of these cells or MG-63 (lane 2) and subjected to SDS gel electrophoresis (nonreducing conditions) as in Fig. 2. Proteins purified from these cells do not comigrate.](image)

![Fig. 4. Immune precipitation of p150/95 and comparison to affinity-purified proteins from induced and uninduced U-937 cells. Samples of cell extract, precleared on $M$, 120,000 agarose, were subjected to immunoprecipitation with mouse IgG (lanes 1, 5) or Leu M5 (anti-p150 antibody, lanes 2, 6). Electrophoretic migration is compared to samples of U-937 fibronectin receptors from induced (lanes 3, 7) or uninduced (lanes 4, 8) cells. SDS-polyacrylamide (7.5%) gels run under nonreduced (A) or reduced (B) conditions. Arrowhead, position of p95.](image)
Fig. 5. Comparison of affinity-purified proteins with platelet glycoproteins IIb/IIIa by immunoblotting. A and B, same nitrocellulose paper reacted with anti-platelet glycoprotein IIb/IIa (A) or subsequently dried for autoradiography. Radioiodinated proteins were purified by affinity chromatography on M, 120,000-agarose from extracts of human U-937 (lane 2), THP-1 (lane 3), and human MG-63 cells (lane 4). Unlabeled human platelet octylglucoside extract (4 μg, lanes 1, 5) were prepared as described in “Materials and Methods.” Samples were subjected to electrophoresis on SDS-polyacrylamide (7.5%) gels followed by transfer to nitrocellulose paper. Paper was blocked, reacted with a mixture of two rabbit antisera raised separately to human platelet glycoproteins IIb and IIIa, and incubated with secondary immunoperoxidase reagents and substrate (A). Nitrocellulose paper was dried and subjected to autoradiography (B). The position of platelet glycoproteins is denoted by dots in B.

demonstrate that the proteins isolated here by functional criteria belonged to the β subfamily of integrins, we carried out immunoprecipitations with goat antibodies that inhibit cell adhesion to fibronectin (20). Cell extracts, prepared from MG-63 (lanes 1, 2) or U-937 cells (lanes 4, 5), were incubated with either goat anti-fibronectin receptor IgG-agarose (Fig. 6A, lanes 1, 4) or control IgG-agarose (lanes 2, 5). Affinity-purified protein from U-937 cells was included as a marker (lane 3). The results demonstrated that these extracts contained fibronectin receptor. Immunoprecipitations were also carried out with affinity-purified proteins from U-937 cells (Fig. 6B). Purified protein was selectively precipitated by anti-fibronectin receptor antibodies (lanes 1, 3) but not control IgG (lanes 2, 4) and were analyzed under nonreducing (lanes 1, 2) or reducing (lanes 3, 4) conditions.

Glycosylation Contributes to Differences in Apparent Fibronectin Receptor Size. We were interested in determining the structural basis for differences in the apparent molecular masses of integrins purified from these cells. Experiments were carried out with glycosidases of two types, endoglycosidase F (Fig. 7A), which cleaves within the chitobiose portion of the carbohydrate chain and glycopeptidase F (glycopeptide-N-glycosidase, Fig. 7B), which hydrolyzes at the glycosylamine junction (45, 46). When integrins from uninduced U-937 cells (Fig. 7A, lane 1) or MG-63 cells (lane 2) were incubated with endoglycosidase F, their respective electrophoretic migrations were essentially identical (lanes 3, 4). Moreover, fibronectin receptor purified from uninduced THP-1 (Fig. 7B, lane 2), induced THP-1 (lane 1) or MG-63 (lane 3) cells also migrated more closely after incubation with glycopeptidase F (lanes 4–6). The results indicate that the differences in relative migration of integrins observed in this gel system are due to glycosylation.

DISCUSSION

We have isolated fibronectin receptors from cells having properties of promonocytes (uninduced U-937 or THP-1 cells).
(28, 29), from monocytic cells (induced U-937, THP-1 cells), and peripheral blood monocytes. Our results demonstrate that human monomacrophage cells which adhere to substrata coated with fibronectin express two cell surface proteins that bind to and are specifically eluted from a cell-adhesive fragment of fibronectin. Moreover, human peripheral blood monocytes and PMA-induced U-937 or THP-1 cells express fibronectin receptors which are electrophoretically distinct from those of uninduced monomyelocytic/leukemic cells.

We found that promonocytic cells adhere to the fibronectin cell adhesion domain. U-937 cells bound to intact fibronectin and to both its \( M_r \) 120,000 and 12,000 proteolytic cleavage products (Fig. 1); this adhesion was inhibited by RGD-containing peptide (Table 1). Our results with uninduced U-937 are concordant with those obtained with a variety of cell types which adhere to the RGD-containing cell binding domain of fibronectin (52, 58).

Two cell surface proteins purified from uninduced U-937 and THP-1 cells, exhibited relative electrophoretic migrations on SDS gels of \( M_r \) \~ 150,000 under reducing conditions and \( M_r \) 160,000 (\( \alpha \)) and 130,000 (\( \beta \)) under nonreducing conditions (Fig. 2). These proteins are mononuclear phagocyte fibronectin receptors because they bound to the \( M_r \) 120,000 fibronectin fragment coupled to agarose, were specifically eluted from \( M_r \) 120,000-agarose by GRGDSP (Fig. 2), and these affinity-purified receptors as well as proteins of identical size from cell extracts were immunoprecipitated with antibodies (Fig. 6) known to block the adhesion of cells to fibronectin (20).

These fibronectin receptors isolated from uninduced THP-1 or U-937 cells exhibited identical mobility shifts on SDS gels run under reducing and nonreducing conditions (Fig. 2). For comparison, we also isolated fibronectin receptors from two human osteosarcoma cell lines, MG-63 and HOS. The fibronectin receptors purified from HOS and MG-63 cells were indistinguishable from each other on both reduced and nonreduced SDS gels but were consistently smaller by about \( M_r \) 10,000 than the corresponding proteins isolated from U-937 and THP-1 cell lines.

We extended these observations by isolating fibronectin receptors both from peripheral blood monocytes and induced U-937 and THP-1 cells (Fig. 3). Identical chromatographic elution, purity and electrophoretic migration (\( \alpha, M_r \sim 140,000; \beta, M_r \sim 115,000 \)) were observed for receptors from the induced cells and from peripheral blood monocytes. Both the \( \alpha \) and \( \beta \) subunits of fibronectin receptors from normal peripheral blood monocytes migrated faster on SDS gels than did the \( \alpha \) and \( \beta \) subunits from uninduced THP-1 and U-937 cells (\( \alpha, M_r \) 160,000; \( \beta, M_r \) 130,000). The structural basis for these differences was examined further by treating purified fibronectin receptors with endoglycosidases (Fig. 7). Using either endoglycosidase F or glycopeptidase F, we observed that receptors from uninduced and induced cells comigrated on SDS-polyacrylamide gels after enzyme treatment. Moreover, these comigrated with those isolated from MG-63 cells. These observations as well as their adhesion to and peptide-dependent elution from \( M_r \) 120,000-agarose, and their electrophoretic behavior on SDS gels run with or without reducing agent indicate that the fibronectin receptors isolated here are \( \alpha \beta_1 \) integrins (11-13, 18).

We also examined the relationship of promonocytic and monocytic receptors to glycoproteins IIb/IIIa and to several leukocyte adhesion proteins. Our data demonstrate that the proteins isolated from uninduced U-937 cells by fibronectin affinity chromatography display different electrophoretic migrations and thus are distinct from glycoproteins IIb/IIIa (Fig. 5). Because glycoprotein IIIa and the \( \beta \) subunit of the vitronectin receptor are identical (53), the fibronectin receptor from U-937 and THP-1 cells cannot be the vitronectin receptor. We also considered the possibility that the fibronectin receptors isolated here might be Mo-1/Mac-1, the \( M_r \) 170,000 and 95,000 protein complex which is the human and murine C3bi receptor or \( p_{150}/95 \) (44, 47, 54). The proteins that we have isolated from induced and uninduced U-937 cells do not comigrate with immune precipitated \( p_{150} \) or 95 when compared on reducing or nonreducing gels (Fig. 4). Further, Mac-1/Mo-1 \( \alpha M_r \sim 170,000 \) is larger than \( p_{150} \) and does not comigrate when detected by immunoblotting on a SDS gel containing radiolaabeled fibronectin receptor (not shown) (35, 44, 47). We also believe that our affinity-purified proteins are not identical to \( M_r \) 110,000 surface antigen on human monocytes (55). This \( M_r \) 110,000 antigen is expressed only at low levels on U-937 cells and exhibits identical mobility on SDS-polyacrylamide gels under reducing and nonreducing conditions. In contrast, we have isolated proteins from uninduced and induced U-937 cells and from monocytes; these receptors all show reduction-dependent shifts in electrophoretic mobility (Fig. 3).

In summary, we report that promonocytic cell lines expressed fibronectin receptors that are altered in molecular
mass during phorbol ester-induced maturation. Induced leukemic cells express fibronectin receptors of identical size to those from peripheral blood monocytes. Our data indicate that the alteration in apparent size of integrins from the induced or uninduced cells results from differential glycosylation of these fibronectin receptors. Although the consequences of this post-translational modification of integrins are uncertain, alterations in the pattern of glycosylation are known to affect the stability of other cell surface receptors (e.g., LDL receptor, 56). Because normal interactions of progenitor cells with bone marrow stromal elements are disrupted in leukemogenesis (57), it is possible that differentially processed integrins may serve an important role in this disease process.

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Alteration of Fibronectin Receptors (Integrins) in Phorbol Ester-treated Human Promonocytic Leukemia Cells

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