Synthesis and Expression of the Fibroblast Fibronectin Receptor in Human Monocytes

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Abstract

Human monocytes adhere to fibronectin, but the receptor (or receptors) mediating this interaction has not been clearly identified. To examine the nature of this receptor, human monocytes were obtained by counter-current elutriation and were found to adhere to immobilized fibronectin but not to vitronectin or von Willebrand factor. Antibodies and peptides were used to determine whether monocyte adherence to immobilized fibronectin was mediated by a receptor similar to that which mediates the adherence of fibroblasts to fibronectin. Antibodies against both the α and β subunits of the fibroblast fibronectin receptor (VLA-5) inhibited monocyte adherence to fibronectin. These antibodies immunoprecipitated two surface proteins from monocytes that migrated at 140 and 120 kD under nonreducing conditions. Surface proteins with identical apparent molecular weights were immunoprecipitated from surface-labeled MG-63 cells, a fibroblast-like cell line. A synthetic peptide containing the Arg-Gly-Asp-Ser (RGDS) sequence also inhibited monocyte adherence to fibronectin. cDNA hybridization experiments revealed the presence of mRNA for both the α and β subunits of the fibroblast fibronectin receptor in human monocytes. We conclude that circulating monocytes express a fibronectin receptor that is structurally and functionally very similar, if not identical, to the fibronectin receptor in adherent fibroblasts, and that this receptor mediates monocyte adherence to immobilized fibronectin.

Introduction

Monocyte emigration from the bloodstream is an important early step in the pathogenesis of a variety of inflammatory disorders and may also be involved in the initiation of atherosclerosis (1). To leave the vasculature, monocytes must adhere to and migrate through the endothelium and subendothelium. While crossing the subendothelium, monocytes encounter a number of adhesive proteins, such as fibronectin, laminin, collagen, elastin, vitronectin, and von Willebrand factor. Monocytes have been shown to bind specifically to laminin, elastin, and fibronectin in vitro (2, 3). While the receptors mediating cellular adherence to laminin and elastin have not been well characterized, a receptor that mediates attachment of adherent cells, such as fibroblasts, to immobilized fibronectin has been extensively studied. The fibroblast fibronectin receptor is a heterodimer complex consisting of an α and a β subunit that interacts with a restricted portion of the fibronectin molecule containing the Arg-Gly-Asp-Ser (RGDS)[1] tetrapeptide (4). This receptor is a member of a superfamily of heterodimeric adhesive protein receptors known as integrins.

The integrin superfamily consists of three families that are distinguishable from each other by their unique β subunits (5). Each member of a family possesses a unique α subunit and a β subunit, which is shared by the other members of that family. In addition to the fibroblast fibronectin receptor, the fibronectin receptor family of proteins also contains a group of closely related receptors defined immunologically as the very late activation antigens, or VLA proteins (6). The VLA proteins were initially described as antigens that appeared very late on T lymphocytes after in vitro stimulation, but it is now appreciated that these proteins are found on many cell types (6). There are at least six unique VLA α subunits (α^{1-6}) (7) that form heterodimer complexes with a common β subunit (VLA β) that is identical to the β subunit of the fibronectin receptor (FNR_β) (8). Immunological and amino-terminal sequence analyses have confirmed that the VLA α^5 subunit is identical to the α subunit of the fibroblast fibronectin receptor (FNR_α) (9). Therefore, the VLA-5 heterodimer complex is identical to the fibroblast fibronectin receptor heterodimer complex.

There is some controversy about the relationship of the fibronectin receptor on adherent cells to the receptor that mediates adherence of the circulating, normally nonadherent monocyte to immobilized fibronectin. A number of putative monocyte fibronectin receptors have been reported, some of which resemble the fibroblast fibronectin receptor and some of which are quite different (10–13). In the present report we demonstrate that circulating monocytes express surface proteins that are immunologically and biochemically very similar to the fibroblast fibronectin receptor, and that this receptor mediates monocyte adherence to immobilized fibronectin.

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Received for publication 15 September 1988 and in revised form 13 March 1989.

J. Clin. Invest.

0021-9738/89/07/0366/05 $2.00
Volume 84, July 1989, 366–370

1. *Abbreviations used in this paper:* GP, glycoprotein; GRGDSP, a hexapeptide with the sequence Gly-Arg-Gly-Asp-Ser-Pro; RGDS, a tetrapeptide with the sequence Arg-Gly-Asp-Ser; RGES, Arg-Gly-Glu-Ser; VLA, very late activation antigen.

Methods

Adhesive proteins. Vitronectin was purchased from Calbiochem-Behring Corp. (La Jolla, CA). Fibronectin was isolated from fresh human plasma by affinity chromatography on gelatin-agarose as described (14). von Willebrand factor was purified from cryoprecipitate by the method of Switzer and McKee (15). Each adhesive protein was homogeneous on SDS-polyacrylamide gels (14).

Antibodies and peptides. BIIG2 is a rat monoclonal antibody that is specific for the α subunit of the fibroblast fibronectin receptor and that selectively inhibits fibroblast attachment to fibronectin. AIIB2 is a rat monoclonal antibody that is specific for the β subunit of the fibroblast fibronectin receptor (Werb, Z., P. M. Tremble, O. Behrendtsen, E. Crowley, and C. H. Damsky. Manuscript submitted.). Conditioned media from these hybridoma cell cultures were used at the indicated dilutions. A polyclonal (rabbit) antibody that reacts with the β subunit of the fibroblast fibronectin receptor, anti-VLA β, and a monoclonal (mouse) antibody, B-5G10, that recognizes VLA-4 were gifts of Dr. Martin Hemler (Dana-Farber Cancer Institute, Boston, MA) (6, 16). Monoclonal antibody 7E3, which recognizes the platelet glycoprotein (GP) IIb-IIIa complex and the vitronectin receptor (14), was a gift of Dr. Barry Coller (State University of New York, Stony Brook, NY). The Gly-Arg-Gly-Asp-Ser-Pro (GRGDSP) and Arg-Gly-Glu-Ser (RGES) peptides were purchased from Peninsula Laboratories (Belmont, CA) and were further purified by repeated extraction with ethyl acetate.

Isolation of monocytes. For adherence experiments and RNA extraction, monocytes were isolated in suspension by counter-current elutriation, as described by Fogelman et al. (17). Peripheral venous blood from normal volunteers was collected in heparin (5 U/ml; Elkins-Sinn, Inc., Cherry Hill, NJ), mixed with Plasmagel (Laboratoire Roger Bellon, Neuilly sur Seine, France), and allowed to sediment at room temperature for 30 min. The supernatant was removed and centrifuged at 400 *g* for 10 min and resuspended in elutriation buffer consisting of Ca^{2+}- and Mg^{2+}-free PBS (140 mM NaCl, 8 mM Na_2HPO_4, 2.7 mM KCl, 1.5 mM KH_2PO_4) with 1% HSA and 15 mM dextrose. Cells were then loaded into the elutriation chamber of a Beckman J6 rotor maintained at 2,000 rpm and 15°C. 40-ml fractions were collected at flow rates ranging from 6 to 18 ml/min. The cell composition of each fraction was determined by examination of morphology, size distribution on a Coulter counter (Coulter Instruments, Inc., Hialeah, FL) and esterase staining. Fractions composed predominantly of monocytes were pooled, centrifuged at 400 *g* for 10 min, and resuspended in DME (Gibco Laboratories, Grand Island, NY). Typically, 5×10^6 monocytes were obtained at a purity of 90% from 240 ml of whole blood, the contaminating cells being almost exclusively lymphocytes. This represented a recovery of 42% of the total monocytes present in the whole blood of each donor, as determined on a Technicon H-1 Hematology System (Technicon Instruments Corp., Tarrytown, NY) provided by the clinical laboratories at San Francisco General Hospital. Monocytes for surface labeling and immunoprecipitation were obtained in suspension by the method of Recalde (18). The buffy coat from 1 U of blood was washed three times with PBS containing 1 mM EDTA. The final cell pellet was resuspended in 0.1-μm triple filtered FCS and incubated at 37°C for 30 min. During the incubation, 25 μl of 9% NaCl per milliliter of cell suspension was added. After the 30-min incubation, the cells were layered over Ficoll-Paque (Pharmacia Inc., Piscataway, NJ) (to which 2.8 mg of NaCl/ml had been added) and centrifuged at 600 *g* for 15 min. Cells at the interface were harvested, washed twice, and stained with Wright solution. 90% of the cells were monocytes.

Quantitative monocyte adhesion assay. Freshly elutriated monocytes were incubated in DME (10^6 monocytes/ml) for 30 min at 37°C with 0.5 μCi of ^{111}In-oxine per 10^6 monocytes, centrifuged twice at 400 *g* for 10 min, and resuspended in DME (250,000 monocytes/ml). Sterile glass tissue-chamber slides (Lab-Tek 4802; Nunc, Inc., Naperville, IL) were pretreated with gelatin (2 mg/ml, Bio-Rad Laboratories, Richmond, CA) at room temperature for 2 h to block the nonspecific adherence of the monocytes to glass. After removal of the excess gelatin by aspiration, we added fibronectin, vitronectin, or von Willebrand factor, each of which binds to gelatin (19–21), and incubated the slides for 1 h at room temperature. After removal of the excess, unbound adhesive proteins, 1 ml of the labeled monocytes was added to each chamber of the tissue-chamber slides and incubated for 90 min at 37°C in a 5% CO_2 incubator. In some experiments, antibodies or peptides were incubated with the monocytes for 15 min at 37°C before the monocytes were added to the slides. After incubation, nonadherent cells were aspirated, the chamber was washed twice with PBS, and the washings along with the nonadherent cells were combined (vial A). Adherent cells were lysed by incubation with 1 M NH_4OH at room temperature overnight. The lysed adherent cells were pipetted into a second scintillation vial (vial B). Each chamber was then swabbed with a cotton-tipped applicator, which was also added to vial B. The lysing and swabbing procedure was repeated one time. The fraction of monocytes that adhered to the adhesive proteins was calculated by dividing the radioactivity in vial B by the total radioactivity recovered (A + B). Specific adherence was determined by subtracting background adherence to gelatin from total adherence. Each condition was examined at least in duplicate and often in triplicate.

RNA isolation and blotting. Total RNA was isolated from freshly elutriated monocytes and cultured cells by denaturation with guanidinium thiocyanate followed by ultracentrifugation through 5.7 M CsCl as described by Chirgwin et al. (22). RNA samples were electrophoresed on 1.1% glyoxal-agarose gels and transferred to nitrocellulose by blotting (23). cDNA probes for the α and β subunits of the fibronectin receptor were isolated from an endothelial cell library by specific oligonucleotides (24, Fitzgerald, L. A., and D. R. Phillips. Unpublished results.) The α subunit probe contained nucleotide positions 2602-3281 of the published fibroblast fibronectin receptor sequence (9). The β subunit probe contained nucleotides 218-1534 of that sequence (9). These cDNA probes were labeled with [^{32}P]dCTP by using random hexanucleotides as primers in the Klenow reaction (Random Prime Labeling Kit; Amersham/Searle, Arlington Heights, IL) and incubated with the RNA blots as described by Thomas (23). The RNA blots were then washed once with 4× SSC, (1× SSC = 0.15 M NaCl, 0.015 M Na citrate), 0.1% SDS at 35°C for 15 min, three times with 1× SSC, 0.1% SDS at 35°C for 15 min, and once with a high-stringency wash of 0.3× SSC, 0.1% SDS at 56°C for 5 min with vigorous agitation. Autoradiography was performed with Trimax x-ray film and intensifying screens.

Immunoprecipitation. Freshly isolated monocytes or cultured cells were surface labeled by lactoperoxidase-catalyzed iodination using 1 mCi of ^{125}I (Amersham/Searle) and 10^{-7} M lactoperoxidase (Sigma Chemical Co., St. Louis, MO) in Tyrode's buffer as described (25). Surface-labeled cells were then washed twice with Tyrode's buffer and were lysed at room temperature in Tris-buffered saline (25 mM Tris-HCl, 150 mM NaCl, 1 mM $CaCl_2$, pH 7.2) containing 1% (vol/vol) Triton X-100. Lysates were precleared with 50 μl of goat anti-rat IgG agarose beads (Sigma) for 1 h at room temperature. Antibodies were added to aliquots of 300 μl of supernatant and incubated overnight at 4°C. Immune complexes were precipitated by incubation with 50 μl of goat anti-rat agarose. For sequential immunoprecipitation, the precleared extract was incubated with BIIG2 followed by three precipitations with goat anti-rat agarose. This cycle was repeated twice. The BIIG2-depleted extract was then incubated with AIIB2 and precipitated with goat anti-rat agarose. All samples were then washed, eluted, and analyzed as described (25).

Results

Adherence of monocytes to fibronectin. The tissue-chamber slides were precoated with gelatin to block the nonspecific adherence of monocytes to glass. Baseline monocyte adherence to this gelatin-coated surface was usually < 10%. The addition of fibronectin increased monocyte adherence four- to fivefold (Fig. 1). In contrast, coating the gelatin surface with

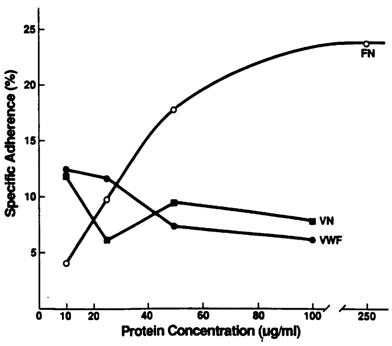

Figure 1. Monocytes adhere specifically to immobilized fibronectin. Tissue-chamber slides were precoated with gelatin (2 mg/ml), and the adhesive proteins were added in the concentrations shown. ^{111}In-labeled monocytes were incubated with the adhesive protein-coated chamber slides at 37°C for 90 min. The percent of monocyte adherence was calculated by dividing the radioactivity in adherent cells by total radioactivity (adherent + nonadherent cells). FN, fibronectin; VN, vitronectin; VWF, von Willebrand factor.

either vitronectin or von Willebrand factor did not enhance adherence of monocytes.

Antibody and peptide inhibition of monocyte adherence to fibronectin. BIIG2, a monoclonal antibody to the α subunit of the fibroblast fibronectin receptor inhibited monocyte adherence to fibronectin in a dose-dependent fashion (Fig. 2). A 1:50 dilution of this antibody inhibited virtually all of the monocyte adherence to fibronectin. Similar results were obtained with a polyclonal antibody to the β subunit of this receptor, anti-VLA β, which inhibited 88% of monocyte adherence to fibronectin at a 1:20 dilution. The monoclonal antibody B-5G10, which recognizes VLA-4 (a related receptor on monocytes that contains the same β subunit as the fibronectin receptor) had no effect on monocyte adherence to fibronectin. Monoclonal antibody 7E3, which recognizes platelet GP IIb-IIIa and the vitronectin receptor (14), also had no effect on monocyte adherence to fibronectin.

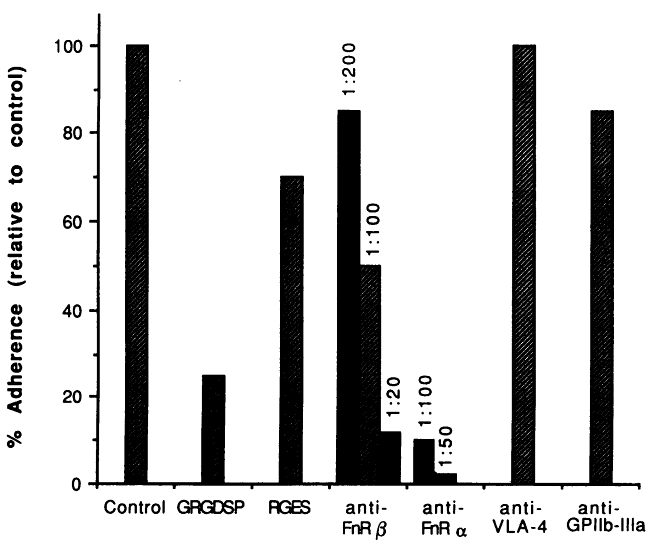

Figure 2. Inhibition of monocyte adherence to immobilized fibronectin. ^{111}In-labeled monocytes were incubated with GRGDSP (1 mg/ml), RGES (1 mg/ml), anti-FNR$_\beta$ (anti-VLA β) (1:200, 1:100, or 1:20 dilution of rabbit serum), anti-FNR$_\alpha$ (BIIG2) (1:100 or 1:50 dilution of conditioned media), anti-VLA-4 (B-5G10) (1:100 dilution), or anti-GP IIb-IIIa (7E3) (20 μg/ml) for 15 min at 37°C before being added to fibronectin-coated tissue-chamber slides. Specific adherence to fibronectin in the absence of inhibitors (control) was set at 100%.

Monocytes incubated with the synthetic peptide GRGDSP (1 mg/ml) showed a ~ 75% decline in their adherence to fibronectin (Fig. 2). A control peptide, RGES (1 mg/ml), inhibited adherence to a much smaller degree. Monocyte adherence to fibronectin thus appears to be RGDS-dependent, as is fibroblast adhesion to fibronectin (4).

Expression of the fibronectin receptor on the monocyte surface. Immunoprecipitation of ^{125}I-surface-labeled monocytes with either the β subunit monoclonal antibody (AIIB2) (Fig. 3, lane *2*) or the α subunit monoclonal antibody (BIIG2) (lane *3*) detected bands of 140 and 120 kD, which were also present on adherent MG-63 cells (lanes *13* and *14*) and which have been previously identified as the fibroblast fibronectin receptor (4). Under reducing conditions, a single 140-kD band was immunoprecipitated from ^{125}I-surface-labeled monocytes (results not shown). To examine the monocyte surface for other heterodimers sharing the same β subunit, the monocyte lysate was repeatedly incubated with the α subunit antibody (BIIG2) until all immunoreactive material was cleared (lanes *4–10*). Subsequent incubation of this lysate with the β subunit anti-

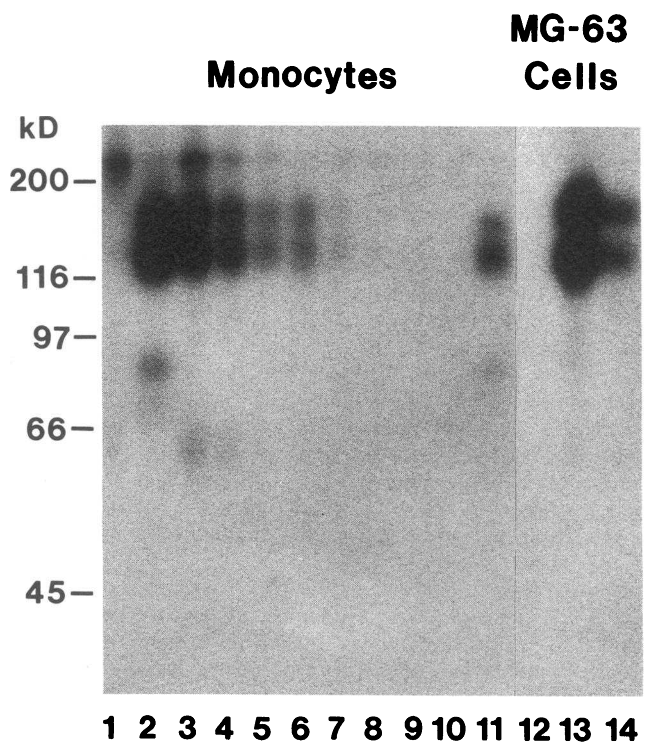

Figure 3. Expression of the fibroblast fibronectin receptor on the monocyte surface. Freshly isolated monocytes (lanes *1–11*) and MG-63 osteosarcoma cells (lanes *12–14*) were ^{125}I-surface labeled and immunoprecipitated with AIIB2, a monoclonal antibody against the β subunit of the fibroblast fibronectin receptor (lanes *2* and *13*); BIIG2, a monoclonal antibody against the α subunit of the fibroblast fibronectin receptor (lanes *3* and *14*), or control IgG (lanes *1* and *12*). The monocyte lysate immunoprecipitated with BIIG2 was repeatedly incubated with this antibody until all immunoreactive material was cleared (lanes *3–10*). A subsequent immunoprecipitation of this lysate with AIIB2 revealed the presence of an additional VLA complex, most likely VLA-4 (lane *11*). Samples were electrophoresed through 7.5% SDS-gels under nonreducing conditions and subjected to autoradiography.

body (AIIB2) detected additional bands of 135, 120, and 80 kD (lane *11*), which most likely include a complex of the VLA α^4 subunit (135 kD), the β subunit of the fibronectin (VLA) receptor family (120 kD), and a VLA α^4 proteolytic fragment (80 kD) (16).

Expression of mRNA for both the α and β subunits of the fibroblast fibronectin receptor in monocytes and endothelial cells. Hybridization of a cDNA derived from the α subunit of the fibroblast fibronectin receptor to extracts of total RNA from freshly isolated human monocytes revealed a single mRNA. The same transcript was detected in human umbilical vein endothelial cells and MG-63 osteosarcoma cells (Fig. 4, *left*). Similarly, a cDNA derived from the β subunit of the fibroblast fibronectin receptor detected a single mRNA species in monocytes that was also present in endothelial cells and osteosarcoma cells (Fig. 4, *right*).

Discussion

Monocytes bind to elements in the subendothelium of the blood vessel wall when they leave the circulation in response to a variety of physiological or pathological stimuli. Fibronectin is a major component of the blood vessel wall, and monocytes bind specifically to fibronectin in vitro. However, the nature of the receptor mediating this binding has been unclear, as has its relationship to the well-characterized fibronectin receptor in adherent cells, such as fibroblasts. Our results demonstrate that human monocytes synthesize and express a fibronectin receptor that is structurally, immunologically, and functionally very similar, if not identical, to the fibroblast fibronectin receptor.

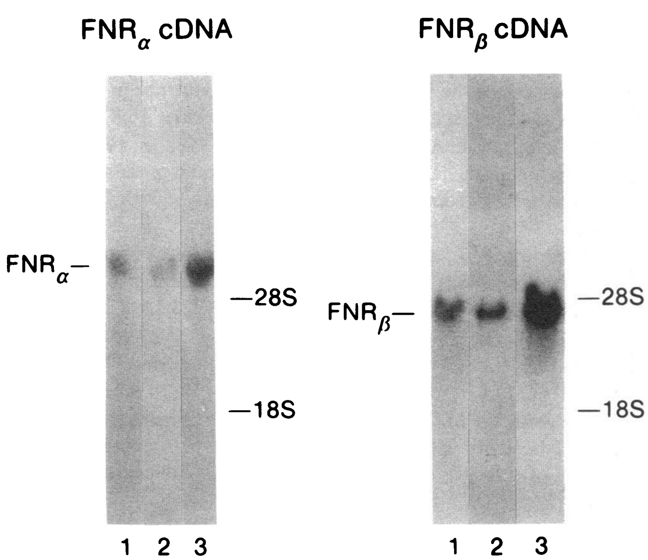

Figure 4. Human monocytes express mRNA for both the α and β subunits of the fibroblast fibronectin receptor (FNR$_\alpha$ and FNR$_\beta$). Total RNA was obtained from monocytes (lanes *1*), human umbilical vein endothelial cells (lanes *2*), and MG-63 osteosarcoma cells (lanes *3*), electrophoresed on glyoxal-agarose gels, transferred to nitrocellulose, and hybridized with ^{32}P-labeled cDNA probes for the α and β subunits of the fibronectin receptor. The positions of ribosomal RNA size markers, indicated by 18S and 28S, were determined for each gel by ethidium bromide staining.

The fibroblast fibronectin receptor was initially isolated from osteosarcoma (MG-63) cells and was found to interact specifically with the RGDS portion of the cell binding domain of fibronectin (4). This receptor is a heterodimer complex of two glycoproteins of 140 kD (α subunit) and 120 kD (β subunit), which under reducing conditions comigrate as a single 140-kD band (4). The larger, α subunit contains two disulfide-linked chains, the smaller of which contains the transmembrane domain near its carboxy terminus. The smaller, β subunit is a single polypeptide with a transmembrane segment near the carboxy terminus (9). Adherence of fibroblasts to fibronectin is inhibited by antibodies against the fibronectin receptor as well as by RGD-containing peptides (26, 27). The fibronectin receptor binds exclusively to fibronectin and not to other adhesive proteins containing an RGD sequence, such as vitronectin (14, 26, 28).

The molecular nature of putative monocyte fibronectin receptors has been a matter of controversy. Hosein and Bianco (10) described a monoclonal antibody that inhibited monocyte (but not fibroblast) adherence to fibronectin and that detected a single monocyte surface protein of M_r 110,000. This receptor was distinct from both the fibroblast fibronectin receptor and the leukocyte family of adhesive protein receptors (10). A recent abstract from this group, however, describes a heterodimer complex on monocytes that is similar in structure to the fibroblast fibronectin receptor (11). Brown and Goodwin have reported that monocytes have two fibronectin receptors, both of which recognize the RGD sequence (13). One receptor is antigenically cross-reactive with platelet GP IIIa and mediates the enhanced phagocytic response of monocytes induced by fibronectin. A second receptor, similar in size and fibronectin binding characteristics to the fibroblast fibronectin receptor, was not further characterized (13).

In this report we demonstrate that the monocyte receptor that mediates adherence to immobilized fibronectin is very similar, if not identical, to the fibroblast fibronectin receptor (VLA-5). At least four lines of evidence support this claim. First, monocytes adhere to fibronectin but not to vitronectin and von Willebrand factor, a property of the fibroblast fibronectin receptor. Second, as is true for fibroblasts, monocyte adherence to fibronectin is inhibited by an RGDS-containing peptide, but not by peptides in which the aspartic acid is replaced by glutamic acid. Third, two antibodies to the fibroblast fibronectin receptor, one to the α subunit (BIIG2) and the other to the β subunit (anti-VLA β), inhibit monocyte attachment to fibronectin. Immunoprecipitation experiments confirmed the expression of the fibroblast fibronectin receptor on the monocyte surface, as well as what appears to be VLA-4 (16). A monoclonal antibody against VLA-4 had no effect on monocyte adherence to fibronectin. Finally, using newly available cDNA probes, we have detected mRNA for both the α and β subunits of the fibroblast fibronectin receptor in monocytes. Thus, on the basis of functional, immunological, and molecular evidence, we conclude that the fibroblast fibronectin receptor (VLA-5) is present on the monocyte surface and that it mediates monocyte adherence to immobilized fibronectin in vitro.

The function of the fibronectin receptor on monocytes in vivo is unknown, but this receptor may enable monocytes to bind quickly to the extracellular matrix when the endothelium is injured or absent. It is also possible that this receptor mediates binding to the extracellular matrix during monocyte migration from the bloodstream in response to chemotactic stimuli. In this regard, the efficiency of such migration may be a function of receptor density on the monocyte surface. Studies are under way in our laboratory to determine whether pathological stimuli that are associated with monocyte infiltration of the subendothelium, such as occurs in inflammation or hyperlipidemia, increase the expression of the monocyte fibronectin receptor.

Acknowledgments

We thank Drs. Martin Hemler and Barry Coller for their generous gifts of the antibodies used in this study, and Dr. Laurence Fitzgerald for providing cDNA probes. We also thank Vicki Vasquez and Linda Parker for manuscript preparation, and Sally Gullatt Seehafer and Al Averbach for editorial assistance. A portion of this work was done during the tenure of a Clinician-Scientist Award from the American Heart Association and Squibb Corp., Princeton, NJ (Dr. Brown).

This work was supported in part by National Institutes of Health grants HL-01751 (Dr. Charo), CA42032 and HD-22210 (Dr. Damsky).

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