The αvβ1 Integrin Functions As a Fibronectin Receptor But Does Not Support Fibronectin Matrix Assembly and Cell Migration on Fibronectin

Zhuohua Zhang,* Alex O. Morla,* Kristiina Vuori,* Jeffrey S. Bauer,† R. L. Juliano,‡ and Erkki Ruoslahti*

* Cancer Research Center, La Jolla Cancer Research Foundation, La Jolla, California 92037; † Department of Pharmacology, University of North Carolina School of Medicine, Chapel Hill, North Carolina 27559; and ‡ Molecular Pathology Program, University of California School of Medicine, La Jolla, California 92037

Abstract. The fibronectin receptor, α5β1, has been shown to be required for fibronectin matrix assembly and plays an important role in cell migration on fibronectin. However, it is not clear whether other fibronectin binding integrins can take the place of α5β1 during matrix assembly and cell migration. To test this, we expressed the human αv subunit in the CHO cell line CHO-B2 that lacks the α5 subunit. We found that the human αv combined with CHO cell β1 to form the integrin αvβ1. Cells that expressed αvβ1 attached to and spread well on fibronectin-coated dishes, but did so less well on vitronectin-coated dishes. This, along with other data, indicated that αvβ1 functions as a fibronectin receptor in CHO-B2 cells. The αvβ1-expressing cells failed to produce a fibronectin matrix or to migrate on fibronectin, although the same cells transfected with α5 do produce a matrix and migrate on fibronectin. The affinity of the αvβ1-expressing cells for fibronectin was fourfold lower than that of the α5β1-expressing cells. In addition, αvβ1 was distributed diffusely throughout the cell surface, whereas α5β1 was localized to focal adhesions when cells were seeded onto fibronectin-coated surfaces. Thus, of the two fibronectin receptors, αvβ1 and α5β1, only α5β1 supports fibronectin matrix assembly and promotes cell migration on fibronectin in the CHO-B2 cells. Possible reasons for this difference in the activities of αvβ1 and α5β1 include the lower affinity of αvβ1 for fibronectin and the failure of this integrin to localize to focal adhesions plaques on a fibronectin substrate. These results show that two integrins with similar ligand specificities and cell attachment functions may be quite different in their ability to support fibronectin matrix assembly and cell motility on fibronectin.

The pericellular fibronectin matrix plays an important role in cell adhesion, migration, and growth control (Hynes, 1990; Ruoslahti and Giancotti, 1990). The main receptor for fibronectin in many cells is the α5β1 integrin (Pytela et al., 1985a; Ruoslahti, 1991). This integrin not only attaches cells to the fibronectin matrix, but also regulates the formation of this matrix. That α5β1 participates in matrix formation is clear from a number of observations; antibodies against α5β1 inhibit matrix formation (Akiyama et al., 1989; Fogerty and Mosher, 1990), lowering the expression of α5β1 can eliminate matrix deposition, whereas reintroduction of the integrin restores matrix deposition (Wu, C., J. S. Bauer, R. L. Juliano, and J. A. McDonald, personal communication). Moreover, the amount of matrix deposited is proportional to the amount of α5β1 on the cells; overexpression of α5β1 from transfected cDNA increases the deposition of fibronectin matrix (Giancotti and Ruoslahti, 1990). Malignant transformation often reduces the expression of the α5β1 integrin and this is accompanied by a loss of fibronectin matrix around the transformed cells (Plantefaber and Hynes, 1989). The matrix loss is thought to contribute to the migratory and invasive properties of such cells. In fact, overexpression of the α5β1 integrin in CHO cells drastically restricts the ability of these cells to migrate and makes them non-tumorigenic (Giancotti and Ruoslahti, 1990), whereas elimination of the integrin has the opposite effect (Schreiner et al., 1991).

To better understand the role of fibronectin receptors in fibronectin matrix assembly and cell motility, we tested whether a fibronectin receptor other than α5β1 could function in these processes. In addition to α5β1, other integrins have also been found to bind to fibronectin including: αvβ1, αvβ3, and αvβ6 (Ruoslahti, 1991; Busk et al., 1992). Of these integrins, αvβ1 was of particular interest because, like α5β1, it binds to the Arg-Gly-Asp (RGD) site in fibronectin (Vogel et al., 1990). We engineered an αvβ1-expressing cell line by using a CHO cell line that was selected to be essentially devoid of the α5 subunit and that has lost its ability to attach to fibronectin (Schreiner et al., 1989), to migrate on fibronectin (Bauer et al., 1992), or to form a fibronectin matrix (Wu, C., J. S. Bauer, R. L. Juliano, and J. A. McDonald, personal communication). These bio-
logical activities are restored, however, by transfecting the cells with the human α5 subunit (Bauer et al., 1992; Wu, C., J. S. Bauer, R. L. Juliano, and J. A. McDonald, personal communication). In this report we find that expressing the αβ1 integrin by transfection of the human αv into the α5-deficient cells enabled the cells to attach to fibronectin, however, in contrast to αβ5, αβ1 supported neither fibronectin matrix assembly nor cell migration on fibronectin.

**Materials and Methods**

**Materials**

α-MEM and Geneticin (G418) were purchased from GIBCO BRL (Gaithersburg, MD), methionine-deficient MEM from ICN Biomedicals (Costa Mesa, CA), PBS from Tissue Culture Biologicals (Tulare, CA), and Glutamine Pen-Strep from Irvine Scientific (Santa Ana, CA). Iodo-Gen was obtained from Pierce Chemical Co. (Rockford, IL). Acrylamide was purchased from Bethesda Research Laboratories (Gaithersburg, MD). Microtitration multi-well plates were obtained from Flow Laboratories (McLean, VA). Human plasma fibronectin was from the Finnish Red Cross and vitronectin from Telios Pharmaceuticals (La Jolla, CA). The 110-kD fibronectin fragment containing the cell binding domain was made as previously described (Pierschbacher et al., 1981). Peptides GRGDSP and GRGESP were synthesized at the Protein Chemistry Laboratory of the La Jolla Cancer Research Foundation (La Jolla, CA). All other reagents were obtained from Pierce Chemical Co. (Rockford, IL). Acrylamide was purified from Sigma (St. Louis, MO).

An α5β1-deficient CHO cell line (B2), which expresses ~2% of the wild type level of α5β1 (Schreiter et al., 1989) was used for transfection experiments. CHO-B2 cells transfected with the α5 integrin subunit (B2/α5/β27 clones) have previously been described (Bauer et al., 1992). The B2/α5/β27 clone expressed 3.4 times more α5β1 than the wild type CHO cell line. The CHO-B2 cells were grown in α-MEM with 10% FBS and 2.5 μg/ml Glutamine Pen-Strep (Costa Mesa, CA), FBS from Tissue Culture Biologicals (Tulare, CA), and Glutamine Pen-Strep from Irvine Scientific (Santa Ana, CA). Iodo-Gen was obtained from Pierce Chemical Co. (Rockford, IL). Acrylamide was purified from Sigma (St. Louis, MO).

**Transfection of αv cDNA**

A 4.2-kb human integrin αv cDNA (Suzuki et al., 1987), which includes the entire αv coding sequence, was cloned into the mammalian expression vector pcDNA1/neo (Invitrogen, La Jolla, CA; Felding-Habermann et al., 1992). The plasmid DNA was introduced into B2 cells by calcium phosphate transfection (Chen and Okayama, 1988). Cells were split 2 d after transfection and grown in medium containing 400 μg/ml G418 for 10 more days. The G418-resistant colonies were cloned and expanded, and αv-expressing clones were identified by immunoblotting of cell extracts (Giancotti and Ruoslahti, 1990) with anti-human αv mAb 157 (Freed et al., 1989). The parental vector pcDNA1/neo was also transfected into B2 cells to generate control clones.

**Integrin Analysis**

Integrin expression in the B2 cells and in the transfecteds was evaluated by surface iodination of cells and immunoprecipitation with anti-integrin antibodies (Pytel et al., 1985a; Vogel et al., 1993). Cells were lysed in RIPA buffer (1% NP-40, 0.5% DOC, 0.1% SDS, 150 mM NaCl, 50 mM Tris, pH 8.0) for 30 min on ice. After a 20-min centrifugation at 16,000 g, supernatants were used for immunoprecipitation. The antibodies used were mAb PID6 (anti-human α5; Wayner et al., 1988), mAb 147 (anti-human αv; Freed et al., 1989), an anti-α5 cytoplasmic domain polyclonal antibody (Giancotti and Ruoslahti, 1990), an anti-β3 cytoplasmic domain polyclonal antibody, and an anti-αv cytoplasmic domain polyclonal antibody (Vogel et al., 1993), mAb DH2 (anti-ganglioside GM3; Kojima and Hakomori, 1991) with anti-human αv mAb 137 (Freed et al., 1989). The parental vector pcDNA1/neo was also transfected into B2 cells to generate control clones.

**Cell Attachment Assay**

Cell attachment to coated fibronectin and vitronectin was assayed in microtiter plates as previously described (Pytel et al., 1985a; Giancotti and Ruoslahti, 1990). Quantitation of cell attachment was achieved by staining cells with 0.5% crystal violet in 20% methanol, washing, and then eluting the dye with 0.1 M sodium citrate, pH 4.2, and measuring the absorbance at 600 nm.

**Integrin Affinity Measurement**

Iodination of the cell-binding 110-kD fibronectin fragment was done as described elsewhere (Morla and Ruoslahti, 1992). The specific activity of the radiiodinated 110-kD fragment was typically 1.6 × 10⁶ μCi/mmol. The binding of the 125I-110-kD fragment to cells in suspension was performed essentially as previously described (Akiyama and Yamada, 1985). Briefly, cells were detached with 10 mM EDTA in PBS, washed three times with α-MEM and once with binding buffer (1% BSA, 25 mM Hepes, pH 7.3, in α-MEM). Cells (5–10 × 10⁶ cells/ml) were incubated with 125I-110-kD fragment (2 × 10⁶ μCi/ml), in a total volume of 150 μl of binding buffer, for 1 h at room temperature with constant shaking. Specific binding was determined by competing with unlabeled 110-kD fragment. After binding, the cells were centrifuged for 25 s at 14,000 g and washed twice with 500 μl ice-cold binding buffer. The amount of 125I-110-kD protein remaining in the cell pellet was measured.

**Cell Motility Assay**

Cell motility in the presence of fibronectin was measured using a modified Boyden chamber (NeuroProbe, Cabin John, MD) as previously described (Albini et al., 1987) with slight modifications. The undersurface of 10-μm pore polycarbonate membrane filter (Poretics, Livermore, CA) was first precoated with 10 μg/ml of fibronectin. Lower chambers were filled with α-MEM containing 0.1% BSA and 10 μg/ml of fibronectin. Cells (1 × 10⁴) were added to the upper chambers in the same media described above except without fibronectin. The cells were incubated at 37°C for 3, 6, 12, and 24 h and then the membranes were fixed in methanol and cells were stained with 0.5% toluidine blue in 3.7% formaldehyde. The cells on the upper surface of the membrane were removed, the membranes were mounted on glass slides and the number of cells that had migrated to the lower surface were counted. Four high-magnification microscopic fields were counted per well and all experiments were performed in quadruplicate. Migration results are expressed in terms of the average number of cells/high-magnification microscopic field (HMMC)³.

**Matrix Assembly Assay**

Matrix assembly assays were carried out using 125I-labeled fibronectin as described previously (McKeown-Longo and Mosher, 1985; Morla and Ruoslahti, 1992).

**Immunofluorescence**

For fibronectin matrix staining, cells were grown to confluence on coverslips, washed, and fixed in 3.7% paraformaldehyde, 10 mM sucrose in PBS (pH 7.4) for 30 min. Cells were then stained with rabbit anti-mouse fibronectin antiserum for 2 h followed by rhodamine-labeled goat anti-rabbit IgG secondary antibody (Cappel, West Chester, PA). For integrin staining, cells were fixed for 10 min, permeabilized with 0.5% NP-40 in PBS for 15 min, and then stained with either anti-vinculin, anti-αv, anti-α5, or anti-β3 cytoplasmic domain antisera followed by rhodamine-labeled secondary antibodies. After antibody treatments, coverslips were mounted onto slides with 200 mM m-propyl gallate, 50% glycerol in PBS.

**Results**

**Expression of αv in CHO Cells**

To test the role of the αβ1 integrin in fibronectin matrix as-

1. **Abbreviation used in this paper**: HMMC, high-magnification microscopic field.
assembly and cell motility on fibronectin, a full-length cDNA of human αv was introduced into an α5β1-deficient CHO cell line (B2; Schreiner et al., 1989). G418-resistant clones were picked and screened for αv expression by immunoblotting. Two clones that expressed high levels of human αv (B2/αv7 and B2/αv12) were chosen for further study. The human αv associated with the endogenous β1 to form the αvβ1 integrin in these cells (Fig. 1 A, lanes 12 and 13-15). No αvβ1 was detected in the parental B2 cell line, or in the B2/αv27 cell line which was transfected with human α5 (Fig. 1 B, lanes 3, 7, and 11), even though the B2 cell line (and the transfectants derived from this cell line) expresses an endogenous αv subunit. The endogenous αv is associated with a β subunit that is likely to be β5, since it was the size of β3 or β5, and it was not immunoprecipitated by antibodies to β3 (Fig. 1 B, lanes 4 and 8). Also, we have found that B2 cells express β5 mRNA but no detectable β3 mRNA (not shown). It seems that αv becomes associated with β1 in these cells only when the αv concentration exceeds that of β5 as is the case with the αv transfectants.

Immunoprecipitation with anti-β1 antibodies failed to reveal any β1-containing integrins in B2 cells. However, anti-β1 immunoprecipitated α5β1 from B2/α27 and αvβ1 from B2/αv7 and B2/αv12 cells (data not shown). These results confirmed that the human αv associated with the hamster β1 in the αv transfectants.

To provide an estimate of the relative amount of α5β1 and αvβ1 expressed in the αv- and αv-transfected cell lines, B2/cl, B2/αv7, B2/αv12, and B2/α27 cells were subjected to FACS analysis with mAb 147 (anti-human αv) and mAb P1D6 (anti-human α5). The results showed that the apparent level of αvβ1 expressed in B2/αv12 and B2/αv7 cells was approximately twofold higher than the level of α5β1 expressed in B2/α27 cells (results obtained with B2/αv7 and B2/α27 are shown in Fig. 2).

αvβ1 Is a Fibronectin Receptor in CHO Cells

The αvβ1 integrin has been reported to be a fibronectin receptor (Vogel et al., 1990), a vitronectin receptor (Bodary and McLean, 1990), and a receptor for type I collagen (Dedhar and Gray, 1990).

The B2/αv7 and B2/αv12 cells attached to and spread on fibronectin, while the parental B2 cells did not. Figs. 3 and 4 A show the results with the B2/αv7 cells. In contrast, the parental B2 cells attached to and spread on vitronectin better than the αv-transfected cells (Figs. 3 and 4 B). These results indicate that fibronectin rather than vitronectin is the ligand of αvβ1 in these CHO cells.

The attachment of the B2/αv7 cells to fibronectin was completely inhibited by both anti-αvβ3 and anti-α5β1 polyclonal antisera, while a preimmune serum had no effect (Fig. 5 A). The anti-αvβ3 antiserum did not inhibit the attachment of the B2/α27 (α5β1 positive) cells to fibronectin (Fig. 5 B), thereby demonstrating the specificity of the inhibition of the B2/αv7 cells. GRGDS peptide at 1 mg/ml also fully inhibited the attachment of αv-transfected cells to fibronectin, while the same concentration of GRGESP peptide had no effect (Fig. 5 A). These results indicate that the αv-transfected cells attached to fibronectin by using the αvβ1 integrin and that the RGD sequence is the binding site on fibronectin for αvβ1.

αvβ1 Cannot Replace α5β1 in Fibronectin Matrix Assembly

We next examined whether αvβ1 could replace α5β1 in fibronectin matrix assembly. Cell monolayers were incubated with 125I-labeled fibronectin for 1 h (binding of fibronectin to cell monolayers) or 24 h (matrix deposition), and the amount of radiolabeled fibronectin that was bound to the cell monolayer or incorporated into a detergent insoluble matrix was measured (McKeown-Longo and Mosher, 1985). The B2/αv27 cells were the most efficient at both binding fibronectin from the medium (Fig. 6 A), and at depositing fibronectin into the matrix (Fig 6 B). These cells bound
and deposited into the matrix approximately 10-fold more fibronectin than the parental B2 cells, and approximately fivefold more than the αv transfectants, the B2/αv7 and B2/αv12 (Fig. 6 B).

Immunofluorescence with anti-fibronectin antibodies (Fig. 7) showed fibronectin fibrils only on B2/αv27 cells. To exclude the possibility that the differences in matrix assembly would be due to differences in fibronectin expression in B2/αv7 and B2/αv27 cells, the amount of fibronectin synthesis was measured by labeling cells with [35S]methionine and immunoprecipitating medium and cell layer samples with an anti-fibronectin antiserum. Both cell lines synthesized equivalent amounts of fibronectin, however, proportionately more of the fibronectin was found associated with the cell layer in B2/αv27 cells than in B2/αv7 cells (results not shown). Thus the immunofluorescence with anti-fibronectin antibo-

---

**Figure 2.** FACS histogram of αv and α5 integrin expression levels. B2/c1 (A and B), B2/αv7 (C) and B2/αv27 (D) cells were subject to FACS analysis as described in Materials and Methods. Cells were stained with either anti-αv (mAb 147, left) or anti-α5 (mAb P1D6, right) mAbs.

**Figure 3.** Attachment and spreading of transfected cells on fibronectin and vitronectin. B2/c1, B2/αv7, or B2/αv27 cells were incubated on fibronectin (top) or vitronectin (bottom) coated plates for 40 min at 37°C. Plates were then washed, and cells were fixed, stained, and then photographed.
ies confirmed the results obtained with 125I-labeled fibronectin; B2/α27 cells deposit a fibronectin matrix but the αβ1-expressing cells do not.

αβ1-expressing CHO-B2 Cells Migrate Poorly on Fibronectin

Previous studies have shown that the rate of cell migration on fibronectin is correlated to the level of α5β1 fibronectin receptor on the cells (Bauer et al., 1992). As shown in Fig. 8, B2/α27 cells migrated significantly better than B2/α1 cells did on fibronectin. B2/α7 and B2/α12 cells also migrated better than B2/α1 cells, however their rate of migration was six- to ninefold lower than that of B2/α27 cells. Thus, while α5β1 and αβ1 both function as fibronectin receptors, α5β1 supports cell migration on fibronectin to a greater extent than does αβ1.

B2/α1, B2/α7, B2/α12, and B2/α27 did not migrate well on collagen type I, collagen type IV, or laminin. Moreover, αv expression in B2 cells did not restore vitronectin migration, as does α5 expression (Bauer et al., 1992). Since we have not found a non-fibronectin matrix protein to which the cells adhere well, we are not able to determine the migratory ability of the αv-transfected cells on other substrates.

Binding Affinities of αβ1 and α5β1

To explore possible reasons for the lack of matrix deposition and migratory ability in the αβ1 expressors, we determined the affinities of αβ1 and α5β1 for fibronectin by measuring the binding of a 125I-labeled 110-kD cell-binding fibronectin fragment to the B2/α7 and B2/α27 cells in suspension. The half-maximal binding of the 125I-110-kD fragment to B2/α7 and B2/α27 cells was reached at 20 min, with maximal binding at 60 min (data not shown), which agrees with previous results (Akiyama and Yamada, 1985). Analysis of the binding of the 125I-110-kD fragment to B2/α7 cells indicated one class of binding sites with a dissociation constant of 2.4 ± 1.4 × 10^-7 M (Fig. 9 A), and with 3.5 ± 2.4 × 10^4 binding sites per cell. The binding results obtained with B2/α27 cells were also consistent with one class of binding sites, with a dissociation constant of 5.7 ± 1.7 × 10^-8 M, and 1.8 ± 0.5 × 10^4 binding sites per cell (Fig. 9 B). The results indicate that αβ1 has a fourfold lower affinity for the 110-kD fragment than α5β1, however the B2/α7 cells had approximately twofold more available binding sites than the B2/α27 cells for the 110-kD fragment.

αβ1 and α5β1 have Different Subcellular Localizations

Immunofluorescence showed that in B2/α27 cells seeded

Figure 4. Quantitation of cell attachment. B2/α1 (■), B2/α7 (●), and B2/α27 (▲) cells were seeded onto plates coated with various concentrations of either fibronectin (A) or vitronectin (B). After allowing cells to attach for 25 min at 37°C, the extent of cell adhesion was quantitated as described in Materials and Methods. All data points are the average of duplicate samples, variations between duplicates were typically <6%.

Figure 5. Inhibition of cell attachment with peptides and anti-integrin antibodies. The B2/α7 (A) or B2/α27 cells (B) were seeded onto fibronectin coated plates in α-MEM in the absence (C) or in the presence of 1 mg/ml GRGDSP (RGD), 1 mg/ml GRGESP (RGE), 1:10 dilution of anti-αβ1 antiserum (anti-αβ1), 1:10 dilution of anti-αβ3 antiserum (anti-αβ3), or 1:10 dilution of preimmune serum (PI), and cell attachment was quantitated as described in Materials and Methods.
Figure 6. Fibronectin binding and fibronectin matrix assembly by $\alpha 5$ and $\alpha 5$ transfectants. Confluent monolayers of B2/cl, B2/v7, B2/v12, and B2/\(\alpha 27\) cells were incubated with \textsuperscript{125}I-labeled fibronectin for either 1 h (A), or 24 h (B). The total amount of \textsuperscript{125}I-labeled fibronectin bound to cells (A), or the amount of \textsuperscript{125}I-labeled fibronectin associated with a deoxycholate insoluble matrix (B) was determined as described in Materials and Methods.

Figure 7. Immunofluorescence detection of fibronectin matrix deposition by $\alpha v$ and $\alpha 5$ transfectants. B2/v7 and B2/\(\alpha 27\) cells were grown on coverslips, and then processed for indirect immunofluorescence with anti-fibronectin antiserum (top), or omitting first antibody (bottom), followed by rhodamine-labeled second antibody. Bar, 5 \(\mu m\).

Discussion

We have found that transfecting the human $\alpha v$ integrin subunit into CHO cells which are deficient in the $\alpha 5$ subunit results in the expression of a human $\alpha v$ hamster $\beta 1$ integrin, and that this integrin functions as a fibronectin receptor and not a vitronectin receptor in these cells. Moreover, although $\alpha v\beta 1$ and $\alpha 5\beta 1$ are both fibronectin receptors and bind to the RGD site, $\alpha v\beta 1$ could not substitute for $\alpha 5\beta 1$ in fibronectin matrix assembly or in cell migration on fibronectin.

In agreement with earlier results from our laboratory (Vogel et al., 1990), the present study indicates that the $\alpha v\beta 1$ integrin is a fibronectin receptor. For example, the $\alpha v\beta 1$ expressing cells (B2/v7) attached to and spread on a fibronectin substratum whereas the parental B2 cells did not. In addition, the attachment of B2/v7 cells to fibronectin was inhibited by both an anti-$\alpha v\beta 3$ antiserum and an anti-$\alpha 5\beta 1$ antiserum, which is in agreement with the conclusion that $\alpha v\beta 1$ mediated the attachment. Also, B2/v7 cell binding to fibronectin was inhibited by RGD, which has been shown to be the binding site for $\alpha v\beta 1$ (Bodary and McLean, 1990; Vogel et al., 1990). Our results do not support the previously reported designation of $\alpha v\beta 1$ as a vitronectin receptor (Bodary and McLean, 1990); the attachment of the $\alpha v\beta 1$ expressing B2/v7 and B2/v12 cells to vitronectin was actually lower than that of their parental counterpart. However, since the integrin specificities of fibronectin and vitronectin are very similar (Pytel et al., 1985b), it is possible that
Figure 9. Displacement curves and Scatchard plots for the binding of $^{125}$I-110-kD fibronectin fragment to B2/v7 and B2/α27 cells. B2/v7 cells (A) and B2/α27 cells (B) were incubated with $^{125}$I-110-kD fragment plus various concentrations of unlabeled 110-kD fragment for 1 h. The amount of labeled 110-kD fragment bound to the cells was quantitated, and the displacement curves and Scatchard plots (insets) were generated as described in Materials and Methods.

vitronectin binding by αvβ1 may occur in other cells and other experimental conditions. We conclude that αvβ1 is a fibronectin receptor when expressed in α5-deficient CHO cells.

A previous study suggested that some β1 integrin other than α5β1 may also be involved in fibronectin matrix formation (Fogerty et al., 1990). Our results show that although αvβ1 is a fibronectin receptor that binds to the RGD cell attachment site on fibronectin, αvβ1 cannot substitute for α5β1 during fibronectin matrix assembly. Another property in which the α5β1- and αvβ1-expressing cells differ is in their ability to migrate on fibronectin. Our results, and previous work (Bauer et al., 1992), indicate that the α5β1 integrin allows CHO cells to migrate on fibronectin. We found, however, that the αvβ1 integrin supported migration to a much lesser extent. Thus, the cells expressing αvβ1 are deficient both in the ability to assemble a fibronectin matrix and in their ability to migrate on fibronectin, when compared to the α5β1-expressing cells.

One possible reason for the differences in matrix assembly and migratory ability of the cells expressing α5β1 and αvβ1 is that αvβ1 has a lower affinity for fibronectin than does α5β1. It is possible that while the affinity of αvβ1 is sufficient to allow for cell attachment and spreading on fibronectin, it is too low to support either matrix assembly or cell migration on fibronectin.

Another reason for the inability of αvβ1 to support matrix assembly and cell migration, that is not mutually exclusive with the low affinity, may be that αvβ1 does not localize to focal contacts in cells that are attached to fibronectin. Previous studies have suggested that integrins interact with the cytoskeleton at focal contacts (Chen et al., 1985). The α5β1 and αvβ3 integrins have been implicated in interactions with the actin cytoskeleton, since these integrins localize to focal contacts when fibroblasts spread on either an RGD peptide, vitronectin or fibronectin (Singer et al., 1988). In our study, when cells were seeded on fibronectin, an αv-containing integrin (most likely αvβ5, results not shown) localized to focal contacts only when α5β1 was present in the cell. Little αv- or β1-containing integrin staining was found in focal contacts in the αv-transfected cells, even though these cells had focal contacts. Since interaction with cytoskeletal proteins may determine whether an integrin localizes to focal con-
topics, it is possible that αvβ1 does not interact with cytoskeletal proteins in the same manner as α5β1. The αv and α5 transfectants may be useful tools for exploring questions other than matrix assembly, such as the interactions of integrins with cytoskeletal and focal contact proteins.

Finally, it may be that the α5β1 integrin has some secondary effect on the fibronectin molecule beyond the binding of the RGD sequence, and that this effect is needed for matrix assembly and migration. A possible candidate for a mediator of such an effect is a secondary binding site discovered recently in integrins (Vogel et al., 1993). This binding site is directed toward sequences that are rich in basic amino acids and has a distinct specificity in different integrins.

In summary, our results demonstrate that αvβ1 can function as a fibronectin receptor but is not able to support either fibronectin matrix assembly or cell migration on fibronectin in CHO cells. This reveals an unexpected difference in the functions of two fibronectin binding integrins in fibronectin matrix assembly and cell migration on fibronectin. Studying this specificity should help us to understand the process of matrix assembly and cell motility in greater detail.

We thank Dr. David Cheresh, The Scripps Research Institute, for assembly of the human αv-βcatDNA 1/neo construct, John Gresens for isolation of the 110-kD fragment of fibronectin and help with cell migration assays, and Khanh Nguyen for peptide synthesis. We also thank Dr. Bingcheng Wang for critical review of the manuscript.

This work was supported by grants CA 42507, CA 28896, and Cancer Center Support Grant CA 30199 (to E. Ruoslahti) from the National Cancer Institute. A. Morla is supported by a Damon Runyon-Walter Winchell Cancer Research Fund Fellowship, DRG-1098. K. Vuori is a recipient of the European Molecular Biology Organization long-term fellowship.

Received for publication 15 January 1993 and in revised form 29 March 1993.

References

Akiyama, S. K., and K. M. Yamada. 1985. The interaction of plasma fibronectin with fibroblastic cells in suspension. J. Biol. Chem. 260:4492-4500.

Akiyama, S. K., S. S. Yamada, W. T. Chen, and K. M. Yamada. 1989. Analysis of fibronectin receptor function with monoclonal antibodies: roles in cell adhesion, migration, matrix assembly, and cytoskeletal organization. J. Cell Biol. 109:863-875.

Albini, A., Y. Iwamoto, H. K. Kleinman, G. R. Martin, S. A. Aaronson, J. M. Kozlowski, and R. N. McEwen. 1987. A rapid in vitro assay for quantitating the invasive potential of tumor cells. Cancer Res. 47:3239-3245.

Bauer, J. S., C. L. Schreiner, F. G. Giancotti, E. Ruoslahti, and R. L. Juliano. 1992. Motility of fibronectin receptor deficient cells on fibronectin and vitronectin: collaborative interactions among integrins. J. Cell Biol. 116:477-487.

Bodary, S. C., and J. W. McLean. 1990. The integrin β1 subunit associates with the vitronectin receptor αv subunit to form a novel vitronectin receptor in a human embryonic kidney cell line. J. Biol. Chem. 265:5938-5941.

Busk, M., R. Pytela, and D. Sheppard. 1992. Characterization of the integrin αvβ6 as a fibronectin-binding protein. J. Biol. Chem. 267:5790-5796.

Chen, C., and H. Okayama. 1988. Calcium phosphate-mediated gene transfer: a highly efficient system for stably transferring cells with plasmid DNA. BioTechniques. 6:632-638.

Chen, W. T., E. Hasegawa, T. Hasegawa, C. Weinstock, and K. Yamada. 1985. Development of cell surface linkage complexes in cultured fibroblasts. J. Cell Biol. 100:1103-1114.

Chernousov, M. A., F. J. Fogerty, V. E. Koteliantsky, and D. F. Mosher. 1991. Role of the I-9 and III-1 modules of fibronectin in formation of extracellular matrix. J. Biol. Chem. 266:10851-10858.

Dedhar, S., and V. Gray. 1990. Isolation of a novel integrin receptor mediating Arg-Gly-Asp-directed cell adhesion to fibronectin and type I collagen from human neuroblastoma cells. Association of a novel beta 1-related subunit with alpha v. J. Cell Biol. 110:2185-2193.

Felding-Habermann, B., B. M. Mueller, C. A. Romerdahl, and D. A. Cheresh. 1992. Involvement of integrin αv gene expression in human melanoma tumorigenicity. J. Clin. Invest. 89:2018-2022.

Fogerty, F. J., and D. F. Mosher. 1990. Mechanism for organization of fibronectin matrix. Cell Diff. Dev. 32:439-450.

Fogerty, F. J., S. K. Akiyama, K. M. Yamada, and D. F. Mosher. 1990. Inhibition of binding of fibronectin to matrix assembly sites by anti-integrin (α5β1) antibodies. J. Cell Biol. 111:699-708.

Freed, E. J., J. Gallit, P. van der Geer, E. Ruoslahti, and T. Hunter. 1989. A novel integrin β subunit is associated with the vitronectin receptor α subunit (αv) in a human osteosarcoma cell line and is a substrate for protein kinase C. EMBO (Eur. Mol. Biol. Organ.) J. 8:2955-2965.

Giancotti, F. G., and E. Ruoslahti. 1990. Elevated levels of the α5β1 fibronectin receptor suppress the transformed phenotype of Chinese hamster ovary cells. Cell. 60:849-859.

Hynes, R. O. 1990. Fibronectins. Springer-Verlag, New York. 546 pp.

Kojima, N., and S. I. Hakomori. 1991. Cell adhesion, spreading, and motility of GM3-expressing cells based on glycolipid-glycolipid interaction. J. Biol. Chem. 266:17552-17558.

McKeown-Longo, P. J., and D. F. Mosher. 1985. Interaction of 70,000-mol-wt amino-terminal fragment of fibronectin with the matrix-assembly receptor of fibroblasts. J. Cell Biol. 100:364-374.

Morla, A., and E. Ruoslahti. 1992. A fibronectin self-assembly site involved in fibronectin matrix assembly: reconstruction in a synthetic peptide. J. Cell Biol. 118:421-429.

Pierschbacher, M. D., E. G. Hayman, and E. Ruoslahti. 1981. Location of the cell-attachment site in fibronectin with monoclonal antibodies and proteolytic fragments of the molecule. Cell. 26:259-267.

Plantefaber, L. C., and R. O. Hynes. 1989. Changes in integrin receptors on oncogenically transformed cells. Cell. 56:281-290.

Pytela, R., M. D. Pierschbacher, and E. Ruoslahti. 1988a. Identification and isolation of a 140kD cell surface glycoprotein with properties expected of a fibronectin receptor. Cell. 40:191-198.

Pytela, R., M. D. Pierschbacher, and E. Ruoslahti. 1988b. A 125/115-kDa cell surface receptor specific for vitronectin interacts with the arginine-glycine-aspartic acid adhesion sequence derived from fibronectin. Proc. Natl. Acad. Sci. USA. 82:5766-5770.

Ruoslahti, E. 1991. Integrins. J. Clin. Invest. 87:1-5.

Ruoslahti, E., and F. G. Giancotti. 1990. Integrins and tumor cell dissemination. Cancer Cells. Cold Spring Harbor Laboratories, Cold Spring Harbor, NY. 1:119-126.

Schreiner, C. L., J. S. Bauer, Y. N. Danilov, S. Hussein, M. M. Szczak, and R. L. Juliano. 1989. Isolation and characterization of CHO cell variants deficient in the expression of fibronectin receptor. J. Cell Biol. 109:3157-3167.

Schreiner, C. L., M. Fisher, S. Hussein, and R. L. Juliano. 1991. Increased tumorigenicity of fibronectin receptor deficient Chinese hamster ovary cell variants. Cancer Res. 51:1758-1761.

Singer, J. I., S. Scott, D. W. Kawk, D. M. Kazazis, J. Gallit, and E. Ruoslahti. 1988. Cell surface distribution of fibronectin and vitronectin receptors depends on substrate composition and extracellular matrix accumulation. J. Cell Biol. 106:2171-2182.

Suzuki, S., W. S. A. Argoaves, H. Arai, L. R. Languino, M. D. Pierschbacher, and E. Ruoslahti. 1987. Amino acid sequence of the vitronectin receptor α subunit and comparative expression of adhesion receptor mRNAs. J. Biol. Chem. 262:14080-14085.

Vogel, B. E., G. Tomore, F. G. Giancotti, J. Gallit, and E. Ruoslahti. 1990. A novel integrin receptor with an unexpected subunit composition (αvβ1). J. Biol. Chem. 265:5934-5937.

Vogel, B. E., S. J. Lee, A. Hildebrand, W. Craig, M. Pierschbacher, F. Wong-Suah, and E. Ruoslahti. 1993. A novel integrin specificity exemplified by binding of the αvβ5 integrin to the basic domain of the HIV Tat protein and vitronectin. J. Cell Biol. 121:461-468.

Wayner, E. A., W. G. Caner, R. S. Piotrowicz, and T. J. Kunicki. 1988. The role of arginine-glycine-aspartate-directed cell adhesion to extracellular matrix: preparation of monoclonal antibodies to the fibronec-