Requirement of the Integrin β3 Subunit for Carcinoma Cell Spreading or Migration on Vitronectin and Fibrinogen

D. I. Leavesley, G. D. Ferguson, E. A. Wayner,* and D. A. Cheresh

The Scripps Research Institute, La Jolla, California 92037; and *University of Minnesota, Department of Pathology and Laboratory Medicine, Minneapolis, Minnesota

Abstract. FG human pancreatic carcinoma cells use integrin αvβ5 as their primary vitronectin receptor since they fail to express integrin αvβ3. These cells are unable to form focal contacts, spread, or migrate on vitronectin but readily do so on collagen in a β1 integrin-dependent manner. Transfection of FG cells with a cDNA encoding the integrin β3 subunit results in the surface expression of a functional integrin αvβ3 heterodimer providing these cells with novel adhesive and biological properties. Specifically, FG cells expressing β3 acquire the capacity to attach and spread on vitronectin as well as fibrinogen with β3 localization to focal contacts. Moreover, these cells gain the capacity to migrate through a porous membrane in response to either vitronectin or fibrinogen. These results demonstrate that the β3 and β5 integrin subunits when associated with αv, promote distinct cellular responses to a vitronectin extracellular environment.

INTEGRINS are a family of cell surface molecules mediating diverse biological events involving cell-matrix as well as cell-cell interactions. The term integrin denotes a functional linkage between the extracellular matrix and the cells' interior, thus providing cellular responsiveness to the extracellular environment (Tamkun et al., 1986; Buck and Horowitz, 1987). Integrin-ligand specificity is conferred by the subunit composition of the integrin heterodimer (αβ) complex (Hynes, 1987; Ruoslahti and Pierschbacher, 1987). In some cases, a given integrin heterodimer may recognize multiple ligands or multiple integrins may recognize common ligands. The biological function underlying this intrinsic multiplicity and redundancy in ligand-integrin interaction is not well understood.

The αv integrin subunit is unique in that it associates with more than two β subunits including β1 (Bodary and McLean, 1990; Vogel et al., 1990), β3 (Cheresh and Spiro, 1987; Cheresh et al., 1989*b*), β5 (Cheresh et al., 1989*a*), β6 (Sheppard et al., 1990), and β8 (Moyle et al., 1991). While the αvβ1 and αvβ5 heterodimers appear to be restricted in their ligand binding specificity, the αvβ3 heterodimer interacts with multiple ligands in an Arg-Gly-Asp (RGD)-dependent manner (Cheresh, 1987; Cheresh and Spiro, 1987; Charo et al., 1987). We recently reported that while both αvβ3 and αvβ5 contribute to melanoma and carcinoma cell adhesion to vitronectin, only αvβ3 was found to cluster into focal contacts. In contrast, αvβ5 failed to localize to focal contacts but rather clustered in small patches distributed over much of the cell surface (Wayner et al., 1991). Focal contacts, or adhesion plaques are structures which traverse the plasma membrane linking the extracellular substrate with components of the cytoskeleton (Burridge et al., 1988; Otey and Burridge, 1990). The ability of a cell to form focal contacts correlates with its ability to assemble an organized cytoskeleton which, in turn, is prerequisite for cell spreading and migration (Dejana et al., 1987). Thus αvβ3 and αvβ5 exhibit different responses upon binding immobilized vitronectin and thereby have the potential to mediate distinct signals within the cell.

To investigate the biological role of these integrins we identified a cell line, FG human carcinoma (Cheresh et al., 1989), which fails to express αvβ3 and thus adheres to vitronectin in an αvβ5-dependent manner. These cells are unable to spread or migrate on this ligand. Upon transfection of FG cells with a cDNA encoding the human β3 integrin subunit we could express αvβ3 in a functional form resulting in the ability of these cells to spread and migrate on a vitronectin or fibrinogen matrix. Thus our results demonstrate that two homologous integrins, expressed on the same cell and recognizing the same ligand, can promote distinct signals potentiating diverse biological activities in response to a given extracellular matrix.

Materials and Methods

Cells and Cell Culture

FG is a human pancreatic carcinoma cell line that fails to express mRNA for the β3 integrin subunit (Cheresh et al., 1989). FG-A and FG-B are two stably transfected sub-lines: FG-B, is transfected with a full length cDNA encoding human β3 gene; FG-A is a subline, transfected with vector alone. M21 human melanoma cells were a gift from Dr. Donald Morton (Department of Surgery, University of California, Los Angeles, CA). All cells were grown in RPMI 1640 with 10% FBS, 50 μg/ml gentamycin, and tested free from mycoplasma during these studies.

Antibodies

Integrin specific mAbs LM609 ($\alpha v\beta 3$; Cheresh and Spiro, 1987), LM142 (αv; Cheresh and Harper, 1987), P4C10 ($\beta 1$; Carter et al., 1990); LM534 ($\beta 1$; Wayner et al., 1991), and P3G2 ($\alpha v\beta 5$; Wayner et al., 1991) were affinity purified from ascites on protein A-Sepharose (AffiGel protein A MAPS II Kit; Bio-Rad Laboratories, Richmond, CA). mAb AP3 was a generous gift from Dr. Peter Newman (Blood Center of Southwestern Wisconsin, Milwaukee, WI).

Adhesive Ligands

Vitronectin was prepared as described by Yatohgo et al. (1988). Fibrinogen was purified according to the method of Felding-Habermann et al. (1992). Collagen type I was purchased from Collaborative Research (New Bedford, MA).

cDNA Transfection of FG Cells

Full length cDNA encoding the human $\beta 3$ integrin subunit, kindly provided by Dr. Larry Fitzgerald (University of Utah, Salt Lake City, UT) was ligated into the expression vector pcDNAI*neo* (Invitrogen, San Diego, CA) and transfected into FG carcinoma cells using the lipofectin protocol (Calbiochem-Behring Corp., La Jolla, CA). In brief, sub-confluent adherent cells were incubated with 2 μg cDNA and 40 μg lipofectin for 24 h, allowed to recover for 48 h before selection in neomycin for 2 wk at a concentration of 500 μg/ml. Neomycin-resistant cells were expanded and the cell surface expression of $\alpha v\beta 3$ analyzed by flow cytometry using the $\alpha v\beta 3$ complex-specific antibody, LM609. Immunoreactive cells were enriched with four consecutive rounds of fluorescence-activated cell sorting, after which we observed stable surface expression of $\alpha v\beta 3$ in ~40% of the transfected cell line. Positive (FG-B) and negative (FG-A) transfectant populations were recovered and maintained in vitro.

Cell Surface Labeling and Immunoprecipitation

Cell surface proteins were ^{125}I-labeled using lactoperoxidase as previously described (Cheresh et al., 1989). Radiolabeled cells were lysed in *RIPA* buffer (10 mM Tris, pH 7.2, 150 mM NaCl, containing 1% Triton X-100, 1% deoxycholate, 0.1% SDS with 1% aprotinin (No. A6279; Sigma Chemical Co., St. Louis, MO) and 1 mM PMSF prior to immunoprecipitation with integrin-specific mAbs coupled to Sepharose (Pharmacia, Uppsala, Sweden) as described (Cheresh et al., 1989). Immunoprecipitates were analyzed by SDS-PAGE (Laemmli, 1970) under non-reducing conditions on 7.5% polyacrylamide gels and radiolabeled species visualized with autoradiography as previously described (Cheresh, 1987).

Adhesion Assay

Cell adhesion assays were performed as previously described with modifications (Wayner et al., 1991). In brief, sterile, untreated, bacteriological-grade polystyrene 48-well cluster plates (Costar, Cambridge, MA) were coated at 4°C overnight with adhesive ligands (200 μl at 10 μg/ml in PBS) and immediately before use blocked with 5% BSA in PBS, pH 7.4. Cells were harvested with trypsin/EDTA, washed once, and suspended in HBSS supplemented with 1% BSA, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 0.5 mM $MnCl_2$ and then added to appropriate wells. Cells were permitted to adhere for various times at 37°C in 5% CO_2 in the presence or absence of purified mAbs specific for various integrins (50 μg/ml) or the synthetic peptides (50 mM) GRGDSPK, and SPGDRGK. Non-adherent cells were removed with gentle washing and cell attachment enumerated from dye uptake. Adherent cells were fixed with 3% paraformaldehyde, stained with 0.1% crystal violet, air dried, and extracted in 10% acetic acid. Dye uptake was determined spectrophotometrically at 600 nm.

Cell spreading was evaluated before staining in the above adhesion assay and enumerated from random quadruplicate fields containing at least 100 cells.

Indirect Immunofluorescence

Cells were allowed to attach and spread on vitronectin- or fibrinogen-coated glass coverslips and stained by indirect immunofluorescence as described (Wayner et al., 1991). In brief, cells were fixed with 3% paraformaldehyde, permeabilized with 10 mM Tris, pH 7.4, 150 mM NaCl, 1 mM $CaCl_2$ containing 0.1% Triton X-100 for 1 min. Permeabilized cells were stained with mAb AP3 or antisera to human vitronectin (60 min at room temperature), washed and counter stained (60 min at room temperature) with either rhodamine-conjugated goat anti-mouse IgG, or fluorescein-conjugated goat anti-rabbit IgG (Tago, Burlingame, CA). Focal adhesion plaques were visualized by the exclusion of vitronectin-specific antibody from the cell-substrate contacts as previously described (Wayner et al., 1991). Fluorescence was detected with a Photomicroscope (Carl Zeiss, Oberkochen, Germany) fitted with epifluorescence.

Cell Migration Assay

Cell migration assays were performed using modified Boyden chambers with a 6.5-mm diam, 10-μm thickness, porous (8.0 μm) polystyrene membrane separating the two chambers (Transwell®; Costar). Soluble ligands (20 μg/ml) were placed in lower, migration chambers in 600 μl of serum-free RPMI 1640 supplemented with 1% ITS+ (Collaborative Research, New Bedford, MA), 50 μg/ml gentamycin, 2.5 μg/ml amphotericin B (Sigma Chemical Co.), and then incubated at 37°C for 30 min before the addition of cells. Sub-confluent 24-h cultures were harvested with trypsin/EDTA, washed once, and resuspended in the serum-free RPMI without adhesive ligands. Cells (50,000) were added in 100 μl to the upper chamber in the presence or absence of the synthetic peptides (50 mM) GRGDSPK, SPGDRGK, or mAbs (50 μg/ml) to various integrins. Migration is measured after 48-h incubation at 37°C by counting the number of cells recovered from the floor of the lower chamber. Non-adherent cellular debris is removed with gentle washing and cells fixed with 3% paraformaldehyde. Cell migration was quantitated from photographs taken under phase contrast of quadruplicate, random fields from duplicate samples. Random (non-specific) migration was determined using the control protein BSA (20 μg/ml). Specific cell migration toward each matrix protein was calculated by subtracting BSA-mediated migration from total cell migration. In each case random migration $\leq$7% of total matrix-dependent migration.

Results

FG human carcinoma cells fail to express mRNA encoding the integrin $\beta 3$ subunit (Cheresh et al., 1989). Thus we sought to determine the mechanism of FG cell attachment to vitronectin. FG cells were allowed to attach to vitronectin- or collagen-coated wells in the presence or absence of mAbs specific for integrins $\alpha v\beta 3$, $\alpha v\beta 5$, and $\beta 1$. As shown in Table I, FG cell attachment to vitronectin can be significantly inhibited (67%) with mAb P3G2 directed to integrin $\alpha v\beta 5$, while mAb LM609, directed to $\alpha v\beta 3$, or mAb P4C10, directed to the $\beta 1$ subunit, have little or no effect. These data indicate that $\alpha v\beta 5$ is the primary vitronectin receptor expressed by FG cells. In contrast, mAbs LM609 and P3G2 have little or no effect on FG cell adhesion to collagen, whereas mAb P4C10 abolishes (91%) cell attachment on this substrate.

M21 human melanoma cells express $\alpha v\beta 3$ as their primary vitronectin receptor (Wayner et al., 1991). Therefore, as shown in Table I, mAb LM609 significantly inhibited M21 cell adhesion to this matrix by 60%, whereas mAb P3G2, specific for $\alpha v\beta 5$, had a reduced effect (28%). The $\beta 1$-specific mAb, P4C10, had minimal effect on M21 cell attachment to vitronectin but completely inhibited attachment to collagen (97%), consistent with previous results (Strauss et al., 1989). These results collectively demonstrate that FG carcinoma cell adhesion to vitronectin is primarily mediated by $\alpha v\beta 5$ while M21 melanoma cell attachment to this ligand primarily involves $\alpha v\beta 3$.

To investigate the biological consequences of $\alpha v\beta 3$- or $\alpha v\beta 5$-dependent cell adhesion, we examined the capacity of FG cells or M21 cells to spread on vitronectin. As shown in Fig. 1, FG cells readily attach to, but do not spread on this matrix, even after 90 min. In contrast, these cells readily spread on a collagen matrix indicating that the intrinsic ability of FG cells to spread is not deficient. M21 human melanoma cells, which use primarily $\alpha v\beta 3$ to attach to vitronec-

*Table I. Effects of Anti-Integrin mABs on Cell Adhesion to Vitronectin or Collagen**

	FG cells		M21 cells	
mAb	Vitronectin	Collagen	Vitronectin	Collagen
P3G2 ($\alpha v\beta 5$)	67‡	2	28	14
LM609 ($\alpha v\beta 3$)	0	4	60	5
P4C10 ($\beta 1$)	22	91	19	97

* FG or M21 cells were allowed to attach to vitronectin- or collagen (10 μg/ml)-coated wells in the presence or absence of mAbs (50 μg/ml) for 60 min at 37°C as described in Materials and Methods. Standard deviations were routinely <10%.

‡ Represents percent inhibition of cell adhesion relative to adhesion in the absence of antibody.

tin, rapidly spread on this matrix (Fig. 1). These data suggest that the homologous integrins $\alpha v\beta 3$ and $\alpha v\beta 5$ mediate differential biological functions in response to a common ligand.

Transfection of FG Cells with β3 cDNA Results in the Expression of Integrin αvβ3

To determine whether the inability of FG cells to spread on vitronectin is a result of the specific absence of integrin $\alpha v\beta 3$, FG cells were transfected with an expression vector containing cDNA encoding the human $\beta 3$ integrin subunit and a gene for neomycin resistance. After drug selection, two stable transfectant FG sub-lines were established from populations sorted for $\alpha v\beta 3$ expression using FACS (a registered trademark of Becton Dickinson & Co., Mountain View, CA); FG-B which expresses $\beta 3$, and FG-A which fails to express $\beta 3$. As shown in Fig. 2, immunoprecipitation analysis of ^{125}I-surface-labeled FG transfectants reveals that both cell types express equivalent levels of $\alpha v\beta 5$ and $\beta 1$ integrins. mAb LM609, directed to the $\alpha v\beta 3$ complex, immunoprecipitates this receptor from the $\beta 3$ expressing FG-B sub-line and not from the FG-A (mock transfected) sub-line. mAb LM142, directed to the αv subunit, immunoprecipitates this subunit associated with multiple β subunits, including $\beta 5$ and $\beta 6$ (Busk et al., 1992). These results demonstrate that transfection of FG cells with a cDNA encoding the $\beta 3$ integrin subunit results in the stable expression of $\alpha v\beta 3$ complexes at the cell surface. Moreover these immunoprecipitation profiles indicate that de novo expression of $\beta 3$ in the FG-B population does not alter the expression of other αv-integrins, or $\beta 1$-integrins, which remain at equivalent levels in both cell populations.

Expression of β3 in FG Cells Alters Their Biological Response to Vitronectin and Fibrinogen

To establish whether FG-B cells express the $\alpha v\beta 3$ integrin in a functional form, cell adhesion assays were performed. FG-A and FG-B cells attach to vitronectin yet only FG-B cells can be inhibited with mAb LM609, directed to $\alpha v\beta 3$, (55%, not shown). This adhesion event results in FG-B cell spreading on both vitronectin (Fig. 3, *A–C*) and fibrinogen (Fig. 3 *B, left*) within 60 min. In contrast, the $\beta 3$ negative, parental FG cells (Fig. 1) or mock-transfected FG-A cells not only fail to spread on vitronectin but are unable to attach to fibrinogen (not shown). Moreover, FG-B cells which spread on both of these substrata, express $\beta 3$ in focal contacts (Fig. 3, *B* and *C*) indicating that $\alpha v\beta 3$ expression promotes the assembly of adhesion plaques and organization of the micro-

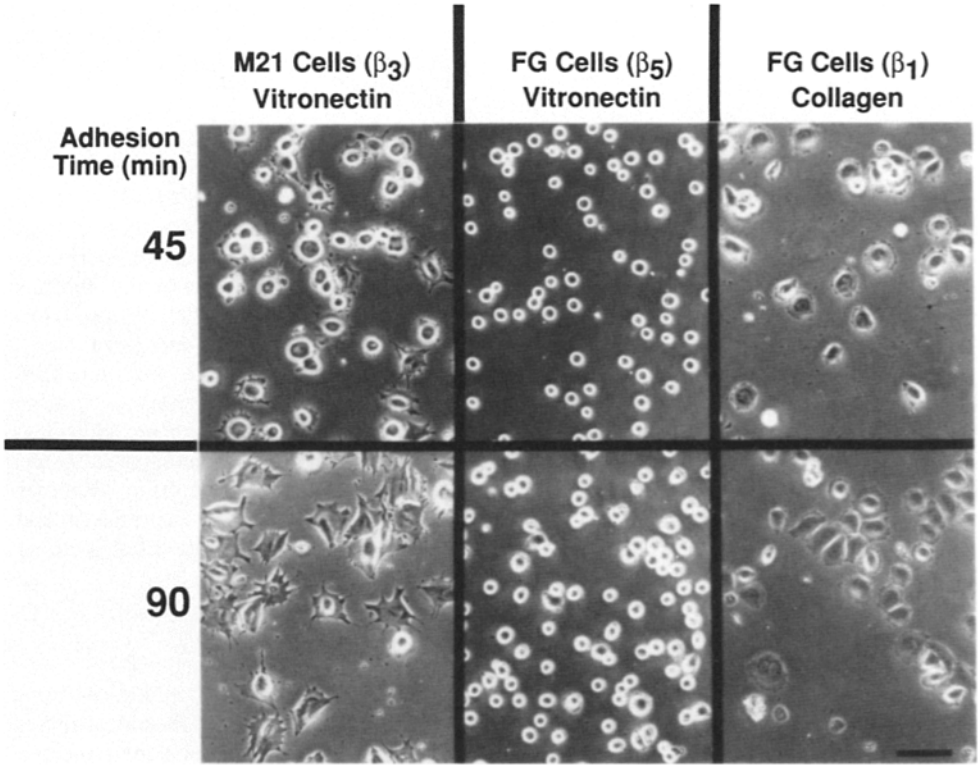

Figure 1. FG and M21 cell spreading on vitronectin and collagen. FG human carcinoma and M21 human melanoma cells were allowed to attach to vitronectin- or collagen-coated (10μg/ml) polystyrene wells at 37°C. Nonadherent cells were removed with three gentle washes as described under Materials and Methods. Attached cells were photographed at 200× under phase contrast using Kodak TMX100 (Eastman Kodak Co., Rochester, NY). Bar, 100 μm.

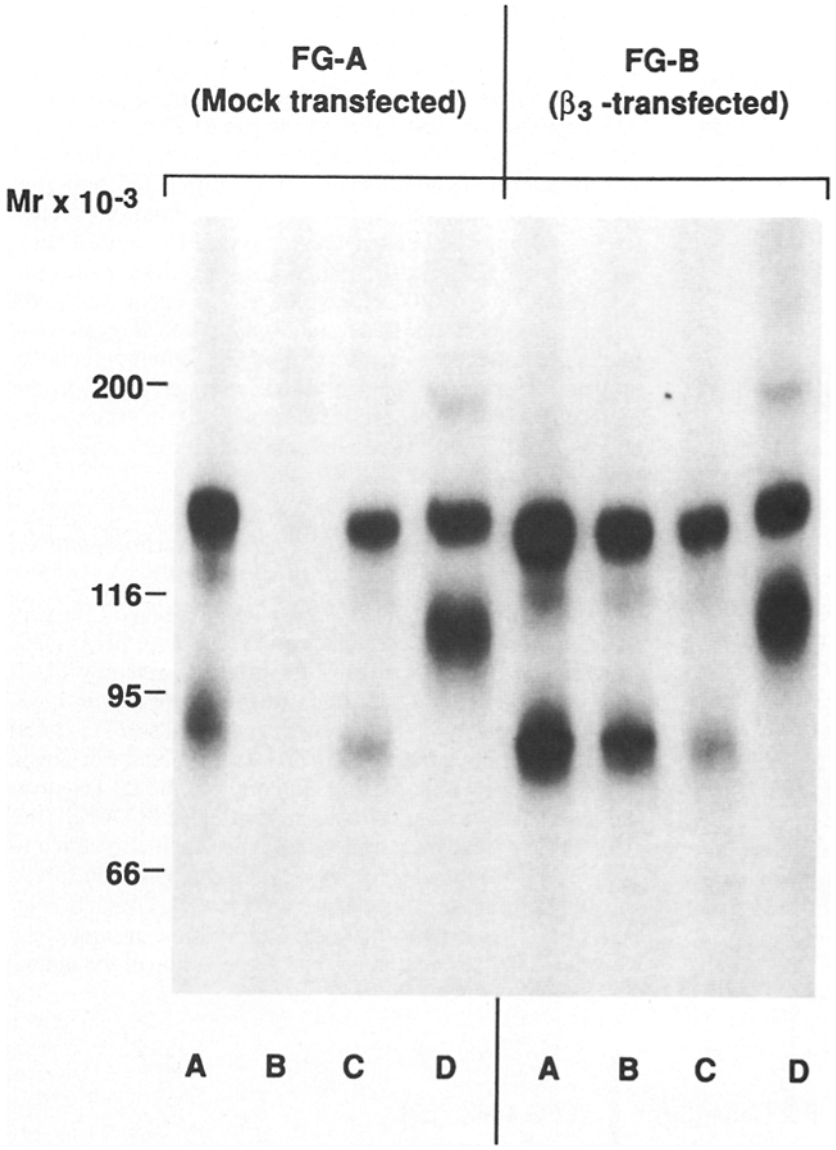

Figure 2. SDS-PAGE analysis of ^{125}I-surface labeled FG-A (mock-transfected) and FG-B (β3-transfected) cells. FG carcinoma cells were transfected with a cDNA encoding full-length human β3 and neomycin-resistance genes. After drug selection for 2 wk, cells were sorted for αvβ3 expression by FACs and stable sub-lines established. Cells were ^{125}I-surface labeled and extracted with detergent as described in Materials and Methods. Detergent extracts were immunoprecipitated with integrin-specific mAbs coupled to Sepharose beads. mAbs LM142 (αv, lane *A*), LM609 (αvβ3, lane *B*), P3G2 (αvβ5, lane *C*), and LM534 (β1, lane *D*).

filament cytoskeleton leading to cell spreading on a vitronectin or fibrinogen matrix. Approximately 40% of the FG-B cell population express β3 and only these cells were found to spread on vitronectin or fibrinogen. In addition, mAb LM609 prevents cell spreading on vitronectin or fibrinogen providing further evidence for a role for αvβ3 in this process (data not shown).

To further investigate the biological role of integrin αvβ3 we examined the capacity of this receptor to potentiate FG-B cell migration. For these experiments we used modified Boyden chambers containing an 8.0 μm porous membrane. FG cells do not demonstrate significant or specific migration through the 8.0 μm pores separating these chambers in the presence of the control ligand BSA. However, both FG-A and FG-B cells readily migrate toward a collagen source yet only FG-B cells, expressing αvβ3, are capable of migrating toward vitronectin or fibrinogen (Fig. 4). FG-B cell migration to both vitronectin and fibrinogen is completely inhibited by mAb LM609 demonstrating that αvβ3 is responsible for this event (Fig. 4). In contrast, mAb P3G2, directed to αvβ5, had no effect on FG-A or FG-B cell migration to all substrata tested (data not shown), indicating this receptor fails to potentiate migration of these cells. In addition an RGD-containing synthetic peptide which functionally inhibits αvβ3-mediated cell attachment (Cheresh et al., 1989), completely blocks αvβ3-dependent migration (data not shown). It is of interest that the migration of αvβ3-expressing FG-B cells toward collagen is not significantly inhibited by mAb P4C10, directed to β1 integrins, suggesting an additional mechanism in these cells (Fig. 4). In fact, integrin αvβ3 has been reported, in some cells, to recognize collagen (Kramer et al., 1990) suggesting that, in addition to vitronectin and fibrinogen, αvβ3, when present, may also contribute to collagen-dependent cell migration.

Discussion

This study was designed to characterize the biological properties of two structurally and functionally related integrins αvβ3 and αvβ5 which are often expressed on the same cell

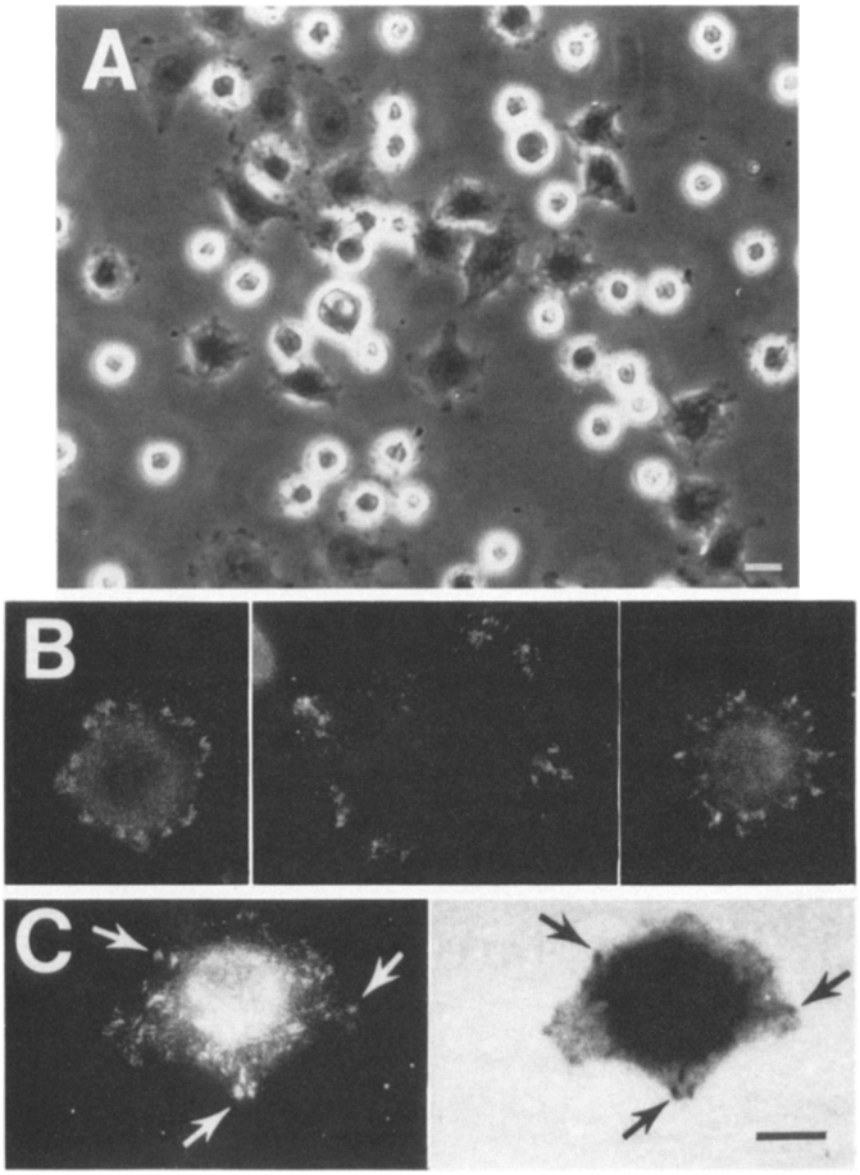

Figure 3. Expression of β3 promotes FG-B cell attachment and spreading on vitronectin and fibrinogen with β3 localization to focal contacts. β3-Transfected FG-B cells were allowed to attach and spread on vitronectin-coated coverslips for 60 min. Nonadherent cells were removed with gentle washing and attached cells evaluated for spreading with phase-contrast microscopy (*A*). For fluorescence experiments FG-B cells were allowed to attach and spread on vitronectin- or fibrinogen-coated coverslips, fixed, permeabilized, and stained with mAb AP3 directed to the β3 integrin subunit, or anti-sera to vitronectin (*B* and *C*, respectively). β3 staining in cells spread on fibrinogen (*B, left*) and vitronectin (*B, center* and *right*) exactly co-distributes with focal contacts, visualized by the exclusion of vitronectin-specific antibody from the cell-substrate contacts as described under Materials and Methods (*C*, β3, *left*; anti-vitronectin, *right*; *arrows*, co-localized staining). Representative cells were photographed with a Zeiss microscope fitted with epifluorescence. Bars: (*A*) 25 μm; (*B* and *C*) 10 μm.

(Wayner et al., 1991). These receptors bind vitronectin in an RGD-dependent manner, contain identical α subunits and structurally similar β subunits (Cheresh and Spiro, 1987; Cheresh et al., 1989; McLean et al., 1990; Ramaswamy and Hemler, 1990; Suzuki et al., 1990). However, the cytoplasmic tails of β3 and β5 are structurally distinct thus raising the possibility that αvβ3 and αvβ5 mediate differential biological signals in response to vitronectin.

In this report we provide several lines of evidence that these integrins mediate distinct biological responses to a vitronectin substrate. Firstly, FG carcinoma cells use αvβ5 as their major vitronectin receptor since they fail to express β3 mRNA or protein (Cheresh et al., 1989) and attach but fail to spread on vitronectin and are incapable of attaching to fibrinogen. This is not because of a general deficiency in FG cell spreading since these cells readily attach and spread on collagen in an integrin β1-dependent manner. Secondly, M21 cells, which express αvβ3 as their major vitronectin receptor (Wayner et al., 1991), attach and spread on both vitronectin and fibrinogen with αvβ3 expressed in focal contacts at the end of actin filament bundles (Wayner et al., 1991). Thirdly, transfection of β3 negative FG cells with a cDNA encoding β3 results in the surface expression of αvβ3 which enables these cells to spread on vitronectin and fibrinogen. Moreover, β3 localizes to focal contacts on these transfected FG cells indicating that αvβ3 is directly involved in cell spreading on these matrix proteins. Finally, FG-B cells expressing the β3 gene product not only spread, but acquire the ability to migrate in response to vitronectin and fibrinogen. That this is because of the presence of αvβ3 on these cells is demonstrated by the ability of mAb LM609 (anti-αvβ3) or an RGD-containing peptide to block this migration. These results provide a rationale for the expression of αvβ3 and αvβ5 on the same cell where αvβ5 promotes simple adhesion while αvβ3 enables cells to modify their shape and mobility on vitronectin.

Although αvβ5 is the major vitronectin-binding integrin on FG cells we cannot completely exclude the possibility that additional vitronectin-binding integrins are expressed on these cells other than αvβ3. For example, integrin αvβ1 ap-

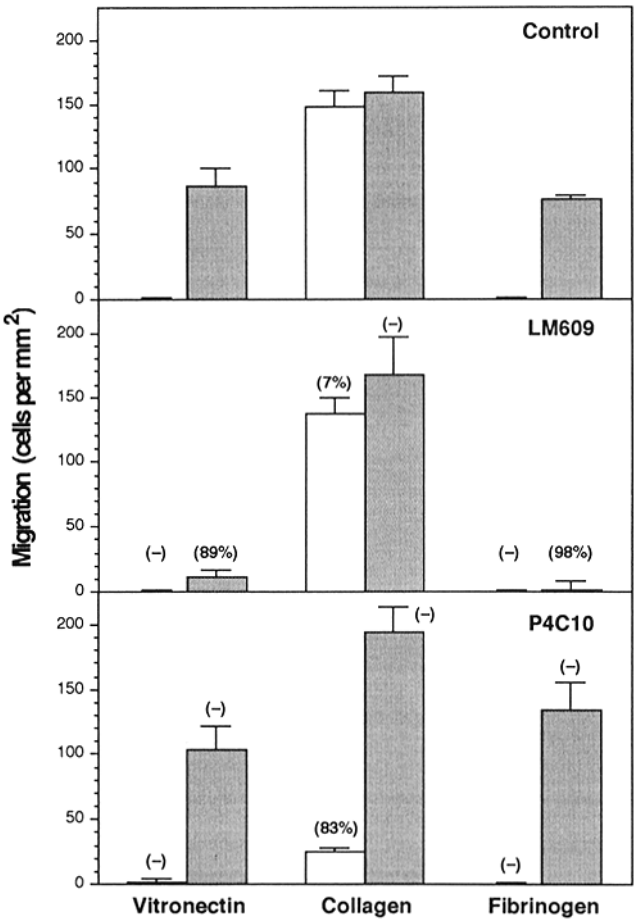

Figure 4. Antibody-specific inhibition of FG carcinoma cell migration. Vitronectin, collagen, and fibrinogen (20 μg/ml) in serum-free medium were placed into the lower chamber of a modified Boyden chamber separated by an 8.0 μm porous membrane from FG-A (□) or FG-B (■) cells placed into the upper chamber in the absence (*Control*) or presence of mAbs LM609 (anti-αvβ3) or P4C10 (anti-β1). Migratory cells which had traversed the membrane into the lower chamber were counted (mean of four random fields ± SE) after 45-h incubation at 37°C. Random, non-specific migration (when BSA is placed into the lower chamber) has been subtracted, was ⩽7% of the total migration in response to any substrate.

parently mediates vitronectin adhesion of certain cells (Bodary and McLean, 1990). It is conceivable that αvβ1 could play a minor role in FG cell and M21 cell attachment to vitronectin since mAb P4C10 directed to β1 partially inhibited the attachment of both cell types to vitronectin (~20%). However, immunoprecipitation analysis failed to detect αvβ1 on either cell type. In any event the expression of αvβ1 or another vitronectin-binding integrin on FG cells does not account for measurable cell spreading and/or migration on vitronectin.

The cytoplasmic tail of the β5 subunit is structurally distinct from those of the β1 and β3 subunits (Ramaswamy and Hemler, 1990; McLean et al., 1990; Suzuki et al., 1990) and thus may be responsible for a distinct signal transduction event. Alternatively, it is conceivable that this subunit is simply incapable of interacting with one or more cytoplasmic proteins thought to be involved in the assembly of focal contacts. For example, talin and α-actinin, two proteins found in focal contacts, have been shown to directly bind integrins (Horowitz et al., 1986). In fact, α-actinin directly binds to a peptide derived from the cytoplasmic tail of β1 (Otey et al., 1990). Based on a mutational analysis of the β1 integrin it appears that the structural basis of integrin focal contact formation depends on three domains with the cytoplasmic tail of β1 (Reszka and Horowitz, 1992). It is noteworthy that β1 and β3 are extremely well conserved in each of these regions while β5 has virtually no sequence homology in the most COOH-terminal of these domains. It is conceivable that specific residues within this particular domain fail to support the localization of αvβ5 into focal contacts. To test this hypothesis it will be necessary to examine the expression of truncated and/or chimeric integrin heterodimers in cells that normally fail to form focal contacts on a vitronectin substrate. It is also interesting to note that the cytoplasmic tail of β5 contains five serine residues not found in β1 or β3 (Ramaswamy and Hemler, 1990; McLean et al., 1990; Suzuki et al., 1990). Perhaps phosphorylation of one or more of these prevents the localization of αvβ5 to focal contacts. In fact, the βs subunit which may be related or identical to β5 becomes phosphorylated on serine in response to activators of protein kinase C (Freed et al., 1989).

The differential ability of αvβ3 and αvβ5 to promote cell spreading and migration may have profound biological implications during events associated with development, wound healing, and neoplasia where cell migration is known to take place. Thus, the genetic regulation of β3 and β5 may play a key role in determining the migratory status of a cell. Recent evidence supports this hypothesis. Non-differentiated keratinocytes, which are known to be migratory, express both αvβ3 and αvβ5 integrins while terminally differentiated keratinocytes, which do not migrate, no longer express αvβ3 (Adams and Watt, 1991). Furthermore, the β3 subunit expressed by sub-confluent embryonic lung fibroblasts, is down regulated when these cells reach confluence (Bates et al., 1991). The role of αvβ3 in melanoma cell migration may be very relevant for the metastatic phenotype of these cells. In fact, αvβ3 was found to be preferentially expressed on metastatic and vertically invasive primary lesions whereas it was not detected on normal melanocytes, nevi, or horizontal primary melanoma (Albelda et al., 1990). The promiscuous ligand-binding capacity of αvβ3 suggests that cells expressing this receptor can migrate on a wide variety of matrices and basement membranes. Our results demonstrate that fibrinogen a known ligand for αvβ3 (Cheresh, 1987) can promote the migration of β3 transfected FG cells. Therefore the expression of αvβ3 and the resulting phenotype may play an important role in a wide range of migratory events involving multiple biological phenomena.

The authors would like to thank Ms. Diane Sander for expert technical assistance.

This is manuscript number IMM 7156 from The Scripps Research Institute. D. I. Leavesley is supported by a Research Fellowship from the American Heart Association, California Affiliate, Divisions and Units. D. A. Cheresh is a recipient of the Faculty Research Award from the American Cancer Society. This work was supported by National Institutes of Health grants CA45726 and CA50286.

Received for publication 12 December 1991 and in revised form 24 January 1992.

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