Heterologous Expression of α1-Integrin cDNA Generates Variable Ligand Specificities and Alterations in Cell Shape

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Integrins can mediate a diverse variety of functions that are regulated by unknown mechanisms. Integrin α1β1 can serve as a receptor for laminin-1 and collagen in certain cell types, but is a receptor for only collagen in others. To examine the molecular basis of this difference in specificity, three cell types were transfected with cDNA for the rat α1 subunit. Following transfection with rat α1, pluripotential hematopoietic human K562 cells exhibited α1β1-dependent attachment to collagen IV, but not laminin-1, unless activating antibody TS2/16 was added. The attachment to collagen IV stimulated the elaboration of a spread morphology resembling a differentiated megakaryocyte with extensive processes which were absent in response to all other substrates.

When MRC-5 cells, a human fibroblastic cell, or RD cells, a human rhabdomyosarcoma line, were transfected with the identical α1-integrin construct, rat α1β1-dependent attachment to both collagen IV and laminin-1 was seen. Therefore differences in ligand specificity can be generated by translation of an identical integrin α1β1 mRNA in different cell types. Despite differences in ligand binding, α1 cDNA-transfected K562 and RD cells express an α1 subunit that appears to be antigenically and electrophoretically similar. Small differences in glycosylation were apparent, and correlated with changes in ligand specificity. Together these results show for the first time that identical cDNAs, absent activating antibodies or other manipulations, can change ligand selectivity and better establish the importance of cellular context in determining integrin function. Moreover they show that select integrins can shift the differentiated state of pluripotential cells.

Keywords: Extracellular Matrix, Laminin, Collagen, Cell Adhesion, Megakaryocyte Differentiation

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ABBREVIATIONS

CMF = calcium and magnesium free
endo F/N = endoglycosidase F/N-glycosidase F
endo H = endoglycosidase H
pdl = poly-D-lysine
ECM = extracellular matrix
FACS = fluorescence-activated cell sorting
mAb = monoclonal antibody

INTRODUCTION

Integrins are a large set of heterodimeric cell surface receptors for extracellular matrix (ECM) proteins and some cellular ligands (for review, see Hynes, 1992). Many receptors can bind more than one ligand, some as many as five or six. At least one integrin receptor appears to be expressed on every cell in the body, and the cellular response to ECM binding appears to be extremely diverse (reviewed in Albelda and Buck, 1990). Neurons plated on laminin or collagen substrates elaborate long neurites, while lymphocytes respond to the same substrates by attachment and migration. The diversity of the ECM-induced cellular responses suggests that integrins do not function in isolation, but rather have their activity modified by cell type-specific cofactors that culminate in unique cellular responses.

Underscoring the importance of cellular context in determining integrin function is the observation that individual integrin heterodimers can show variations in ligand specificities when expressed by different cell types. Studies have shown that human platelets use α2β1 as a collagen receptor, while other cells, including endothelial and melanoma cells, use a electrophoretically and immunologically indistinguishable protein as both a laminin-1 and collagen receptor (Elices and Hemler, 1989; Kirchhofer et al., 1987). It is apparent that cells can modulate their integrin function, but it is unclear whether these changes are due to differences undetectable by gel electrophoresis, by the action of coexpressed effector molecules, or other unknown pre- and post-translational modifications. Appearance of these differentially ligated ligand states with identical cDNAs transfected into multiple cells, without associated effector molecules or activating antibody, would suggest that the cellular context alone can determine integrin function.

However experiments with transfected integrin receptors to date have failed to fully resolve this issue. When rhabdomyosarcoma cells are transfected with α2 cDNA, the cells are able to bind both laminin-1 and collagen (Chan et al., 1991). However, manipulation of α2-transfected K562 cells is needed to generate three ligand binding states, termed O, C and CL, corresponding to the lack of ligand binding, the ability to bind collagen alone, or the binding of collagen and laminin-1 together. The CL-form of the receptor requires the integrin activating antibody TS2/16, while selection on Matrigel generates attachment solely to collagen IV (Chan and Hemler, 1993). Similarly, α6- and α4-transfected K562 cells require, activation to elicit laminin and fibronectin binding, respectively (Delwel et al., 1993; Masumoto and Hemler, 1995). All of the integrins may modulate their ligand binding through the recruitment of associated cofactors or alterations in conformational state by activating antibodies. Interestingly, no integrin expressed in K562 cells can bind ECM ligands without some associated treatment.

Antibody function-blocking assays and affinity columns have established α1β1 as a receptor for laminin-1 and collagens I and IV in several neuronal cell types (Ignatius and Reichardt, 1988; Turner et al., 1989), in smooth muscle cells (Belkin et al., 1990), on foreskin microvascular endothelial cells (Defilippi et al., 1991) in rat hepatocytes (Forsberg et al., 1990; Gullberg et al., 1990) and when expressed in NIH 3T3 cells (Kern et al., 1994). Using inhibitory antibodies, α1β1 appears to function as just a collagen I and IV receptor in sympathetic neurons (Lein et al., 1991), while in MG-63 cells, induced to express α1 by treatment with IL-1β or tumor necrosis factor, α1β1 may function solely as a receptor for laminin-1 (Santala and Heino, 1991). No direct evidence that α1β1 functions as a laminin-1 receptor on sympathetic neurons was obtained, since the presence of additional laminin-1-binding integrins on these neurons was not ruled out.
The pattern of integrin expression by the pluripotential hematopoietic K562 cells changes depending on the particular cell fate path induced by exogenous agents. Erythroid differentiation stimulated by hemin or sodium butyrate reduces expression of the sole integrin expressed by these cells, α5β1 (Jarvinen et al., 1993). Induction of the megakaryocyte lineage with phorbol ester increases expression of α2, α3, αvβ3 (Symington, 1989; Zutter et al., 1992; Jarvinen et al., 1993) along with distinct forms of phosphotyrosyl-specific protein phosphatases (Butler et al., 1990) and unknown associated cofactors. Induction of these proteins is correlated with the acquisition of a spread morphology and the elaboration of short processes indicative of proplatelet megakaryocytes. Unresolved in these studies is whether integrin expression and attachment to certain ligands alone can induce this differentiated state in the absence of cofactors.

In an effort to determine the molecular interactions that regulate α1β1 integrin ligand specificity and function, as well as its potential effect on cell morphology, we have expressed cDNA for rat α1 in three human cell lines: a pluripotent hematopoietic cell line, K562 (Lozzio and Lozzio, 1979), established from a myelogenous leukemia, and MRC-5, and an embryonal rhabdomyosarcoma line, RD. By this approach, active integrin receptor in K562 cells was attained and allowed differences in ligand specificity for the receptor to be observed between all three cell types, without the complication of activating antibodies or Matrigel-induced selection producing unknown effects. Furthermore, striking alterations in cell shape were observed when stable transfectants of K562 cells expressing α1 were cultured on collagen IV indicating a direct role for this receptor in eliciting a complex differentiated state.

METHODS

Materials, Cells and Antibodies

K562, MRC-5, and RD cells were obtained from the ATCC (Rockville, MD), propagated in RPMI-1640 or DMEM (high glucose) with 10% heat-inactivated fetal bovine serum, and maintained in a 5% CO2/95% air mix at 37°C. MRC-5 and RD cells were harvested with 5mM EDTA in Ca2+/Mg2+-free PBS (CMF-PBS: 200 mg/l KCl, 200 mg/l K2SO4, 8 g/l NaCl, 2.16 g/l Na2HPO4·7H2O, pH 7.4; PBS contains 1mM CaCl2, 0.5mM MgCl2) or with Cell Dissociation Buffer (GIBCO, BRL, Gaithersburg, MD). K562 cells do not adhere to tissue culture plastic; therefore cells were maintained in flasks pre-treated with 100μg/ml poly-D-lysine (pdl) type V (Sigma, St Louis, MO) for transfections. Drug-resistant cells remained attached and were later harvested by trituration, while dead, unattached cells were washed off every other day. Two function-blocking monoclonal antibodies (mAb) were used: 3A3, directed against the rat α1β1 receptor, and BIIG2, which blocks the human α5β1 receptor. mAb 3A3 was generously provided by Drs. D. Turner (SUNY, New York) and S. Carbonetto (McGill University, Quebec) and used as purified IgG from ascites. (The species specificity of mAb 3A3 for rat and not human α1 was established by staining of cultures of human cells (RD), known to express some human α1, that had been transfected with the rat α1 cDNA.) Clusters of 3A3-positive cells were seen along side 3A3-negative cells (data not shown). Anti-human α5 mAb, BIIG2, was courtesy of Dr. C. Damsky (UCSF, CA), and used as conditioned media, while anti-human α2 mAb, P1E6, was purchased from GIBCO BRL (Gaithersburg, MD). Activating mAb TS2/16 to the human β1 subunit was a generous gift of Dr. Martin Hemler (Dana Farber Cancer Inst., MA) and was from ascites. Laminin-1 was prepared from Engelbreth-Holm-Swarm (EHS) sarcoma tumors according to published methods (Timpl et al., 1982) or purchased, along with human fibronectin and collagens I and IV, from Collaborative Research, Inc. (Lexington, MA). Protease inhibitors used were aprotinin, benzylchloride, leupeptin, antipain, pepstatin A and phenylmethylsulfonyl fluoride, all from Sigma Chemical Co. (St. Louis, MO). Chemicals for SDS-PAGE, sequencing and agarose gels were obtained from BioRad Laboratories (Richmond, CA).

Endoglycosidase F/N-glycosidase F (EC 3.2.1.96) at 6 units/120μl and recombinant endoglycosidase H (endo-β-N-acetylglucosaminidase H, EC 3.2.1.96) at
0.2 U/200μl were purchased from Boehringer Mannheim (Indianapolis, IN); Lipofectin and Lipofectamine, DH5α competent cells and restriction endonucleases were from Gibco BRL. Secondary antibodies were from Accurate Chemical Co. (Westbury, NY) or Sigma; all eukaryotic cell culture media was from BioWhittaker (Walkersville, MD); bacterial media from DIFCO Labs (Detroit, MI); radioactive and deoxynucleotides from U.S. Biochemical Corp. (Cleveland, OH). All other reagents not specified were purchased from Sigma.

**Vector Construction**

A full-length cDNA clone of rat α1 in lambda ZAP has been isolated and described previously (Ignatius et al., 1990). The full-length insert was excised and placed behind an actin promoter in a mammalian expression vector, pHβAPr-2-neo (Figure 1), carrying G418 and ampicillin resistance (Gunning et al., 1987) as follows. For more efficient expression the full-length sequence was truncated at a Cfr10I site at nucleotide position 385 in untranslated 5' sequence of the original α1. Digests were then blunt-ended, cut with EcoRI to excise the α1 cDNA from lambda ZAP, then purified by agarose gel electrophoresis. EcoRI linkers were added and digested with EcoRI; the insert was then ligated into lambda ZAP and selected by ampicillin resistance and color on X-gal plates. Ends were sequenced to determine orientation. Correctly oriented clones were used to transform E. coli strain BB4; plasmid was purified by CsCl gradient centrifugation, digested with EcoRI and ligated into predigested EcoRI pHβAPr-2-neo vector. This construct was used to transform E. coli strain DH5α and selected with ampicillin. Insert orientations were determined by NdeI digestion and mapping. CsCl-purified plasmid preparations were used with Lipofectin or Lipofectamine to transfect K562, MRC-5 and RD cells.

**Heterologous Expression and Cell Sorting**

Stable transformants of K562 and RD cells expressing α1 receptor were isolated by Lipofectin- or Lipofectamine-mediated transfection. Transfected cells were first screened for acquisition of G418 resistance, and then enriched by several rounds of fluorescence-activated cell sorting (FACS) using the 3A3 mAb (anti-α1) as follows. K562 and RD cells were grown in RPMI-1640 or DMEM (high glucose), respectively, with 10% heat-inactivated fetal bovine serum. When cells reached 60% confluence they were washed 3 times in serum-free media. Plasmid (10 μg/ml) and Lipofectin or Lipofectamine at 8 μl/ml in serum-free medium was added; after 5 hours at 37°C, an equal volume of whole media was added. After 3 days, media was changed and drug selection with 0.5–0.7 mg/ml (active fraction) G418 (Geneticin) was begun. Prior to sorting, live cells attached to pld-coated coverslips were screened for the presence of α1-positive cells by immunofluorescence with the 3A3 mAb. This initial screen on live cells established the optimum conditions for live staining and cell surface expression (Figure 2). Positive clones in this screen were then prepared for sorting.

![Diagram](Cell%20Commun%20Adhes%20Downloaded%20from%20informahealthcare.com%20by%20University%20of%20California%20San%20Francisco%20on%2001/07/11)

**Figure 1** Vector construct for rat α1 transfaction. A 3.6 kB insert for rat α1 was placed behind the human β-actin promoter in a vector containing resistance to Neomycin. G418 was added to transfected cells to select for α1-expressing cells, and cell sorting with mAb 3A3 was used to isolate the most abundant expressors.
FIGURE 2  A–C: Indirect immunofluorescence of α1-transfected K562 cells plated on poly-D-lysine, labeled with mAb 3A3 to rat α1 and FITC goat anti-mouse IgG. A) 3A3 immunostaining of unsorted α1-transfected K562 cells reveals abundant cell surface expression on groups of cells. B) Immunostaining with normal mouse serum. C) 3A3 staining of K562 cells after several rounds of sorting. Scale bar equals 20 μm. D–F) FACS profiles of rat α1-K562 transfectants following four rounds of sorting for mAb 3A3-positive cells. D) mAb 3A3-enriched cells following four rounds of sorting. Subsequent sorts have yielded a narrower peak of similar intensity (K. Zachow and MJI, unpublished results). E) Control staining of mock-transfected K562 cells with mAb 3A3 and Fab'2 fragments of goat anti-mouse IgG. F) K562 cells labeled by mAb BIIG2 to the human α5β1 receptor.
For FACS, cells were first selected in G418 for two weeks, harvested, washed, resuspended in 1 ml of 0.3% heat-inactivated normal goat serum with 20 µg/ml mAb 3A3, and incubated for 20 min. at 4°C to slow receptor internalization. After washing, cells were incubated for 20 min. at 4°C with F(ab')₂ fragments of goat anti-mouse IgG conjugated to fluorescein isothiocyanate (FITC) (Accurate Chemicals, NY) at 1:200. Use of F(ab')₂ fragments was found to be critical for sorting of K562 cells since they express high levels of Fc receptors. Prior to sorting, dead cells were labeled with propidium iodide at 1 µg/ml. In this manner, a first round enrichment of 2–5% was attained. By four rounds of FACS, 98% of K562 cells and >60% of RD cells were receptor-positive. Transient transfections were performed on MRC-5 cells, with transfectants assayed for alterations in ligand binding 3 to 5 days posttransfection. Transfections were performed as described above, except drug selection with G418 was eliminated. Transiently transfected cells varied in the number of rat α1-expressing cells, from 40 to 60% (unpublished results).

Immunocytochemistry and Receptor Characterization

For antibody staining, live cells anchored to pdl treated glass coverslips were incubated with 20 µg/ml 3A3 mAb at 4°C in 0.3% heat-inactivated normal goat serum for 15 min., washed for 5 min. with PBS, then FITC-conjugated goat anti-mouse secondary antibody was added at 1:200 in 0.3% heat-inactivated normal goat serum. After 15 min., cells were washed briefly then fixed for 10 min. at 20°C in 4% paraformaldehyde, mounted in gelvatol/PBS with N-propyl-gallate as an antioxidant to retard bleaching, and photographed.

[^S]-methionine was used to metabolically label α1β1 receptor from stable K562-α1 and RD-α1 transfectants and then characterized by immunoprecipitation with mAb 3A3 and analyzed by SDS-PAGE, all according to Ignatius and Reichardt (1988). In addition, K562-α1 immunoprecipitates were analyzed following digestion with glycosidases. Digestions with endoglycosidase F/N-glycosidase F (endo F) or endoglycosidase H (recombinant form, endo H) were performed on 3A3 mAb-precipitated antigen with and without a cocktail of protease inhibitors. Antibody-antigen complexes were first dissociated with 50 mM glycine, pH 3.0 for 15 min. at 20°C, then neutralized to pH 5.0 by adjusting buffer concentration to 110 mM Tris, using 2.5 M Tris pH 9.0. Samples were split into separate microfuge tubes (16 µl each) and 1 µl 250 mM EDTA was added with either 2 µl Endo F (6 U/120 µl) or 8 µl of endo H (0.1 U/µl). Samples were incubated at 37°C overnight, then SDS sample buffer was added and samples analyzed by SDS-PAGE and autoradiography.

Cell Attachment and Antibody Inhibition Assays

Attachment was assayed by coating the bottoms of 96-well non-tissue culture dishes (Linbro #0150153, Flow Labs, VA) with varying concentrations of mouse laminin-1, human fibronectin, or 0.5 mg/ml pdl in CMF-PBS or collagens I or IV in PBS with Ca²⁺/Mg²⁺, all overnight at 4°C. The next day, wells were washed and blocked with 1% IgG-free BSA in PBS for 2 hr at 20°C. Plates were again washed 3 times with sterile CMF-PBS, then 50 µl of media, with either 0.2% normal mouse serum in PBS, 50 µg/ml 3A3 in 0.2% BSA/PBS, 3µg/ml TS2/16 in 0.2% BSA/PBS, or 50 µl anti-α5 mAb BIIG2 supernatant added. Transfected cells were harvested, washed and pelleted by centrifugation 3 times, resuspended in medium at a concentration of 4 × 10⁵ cells/ml, and 50 µl of cell suspension was added to each well. The 96-well plate containing the cells was centrifuged at 500 × g for 3 min. to uniformly apply cells to test substrate. The plate was then incubated at 37°C in a 5% CO₂ environment. After 30 minutes, unattached cells were washed off by repeated 50 µl streams of PBS from each of 4 polar coordinates and once from directly above. Attachment was monitored in control wells of BSA-blocked plastic alone, to which cells cannot attach, until no cells remained there. Retained, attached cells were assayed in two
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Ways, first for appearance of spread cells with processes over 1 cell body diameter, and later for total cell number by staining with crystal violet and measuring absorbance values at 540 nm using an automated microtiter plate reader. For crystal violet staining, cells were first fixed with 4% paraformaldehyde in 5% sucrose/PBS for 20 min., stained with crystal violet (0.5% in 20% MeOH) for 15 min., washed 5 times with deionized H2O, then extracted with 1% SDS. Attachment values are expressed as percentage of a BSA-blocked pdl positive control, after subtracting background binding to BSA-blocked plastic alone.

RESULTS

Heterologous Expression of Rat α1 in K562 Cells

The cDNA for the rat α1 subunit was expressed in three human cell types, two adherent and one nonadherent, in order to i) establish the properties of the normal or wild type receptor, and ii) examine the effect of varying the cellular context on αβ1 ligand specificity. Others have shown successful cell surface expression and retention of ligand binding of rat-human αβ heterodimers in human cells which already express a pl integrin (for example, Bodary and McLean, 1990). Thus only α1 cDNA was transfected into these cells which express endogenous human α5.

The full length cDNA for the wild type rat α1 subunit was placed behind an actin promoter in a mammalian expression vector conferring neomycin (G418) resistance (Gunning et al., 1987) as shown in Figure 1. K562 human erythroleukemia cells, which normally express α5β1 integrin (for example, Bodary and McLean, 1990). Thus only α1 cDNA was transfected into these cells which express endogenous human β1.

The full length cDNA for the wild type rat α1 subunit was placed behind an actin promoter in a mammalian expression vector conferring neomycin (G418) resistance (Gunning et al., 1987) as shown in Figure 1. K562 human erythroleukemia cells, which under standard conditions express the fibronectin-binding integrin α5β1 but not other β1 integrins (Hemler, 1988; Symington, 1990), were transfected with the rat α1 using Lipofectin construct. Stable transfectants were selected by the addition of G418 beginning 3 days post-transfection. Surviving cells were then screened for cell surface expression by indirect immunofluorescence with the rat α1-specific mAb 3A3. Abundant cell surface labeling was seen on live, intact cells, incubated with antibody at 4°C to ensure a cell surface distribution and to minimize clustering of IgG (Figure 2A). Three observations confirmed the specificity and reliability of this staining: i) adjacent cells, presumably not expressing receptor, were unstained, ii) control antibody showed no reactivity (Figure 2B) and iii) pairs and small clumps of cells were either mAb positive or negative, indicating a clonal relationship (Figure 2A). These results established that the rat α1 receptor was successfully expressed on the surface of these cells, and that antibody staining was specific for rat α1 expressing transfectants.

FACS was used to isolate and enrich α1-expressing K562 cells. Initially, less than 5% of the newly transfected cells were rat α1-positive (Figure 2A). Following four rounds of sorting, cultures of cells expressing rat α1 on their surface were isolated (Figure 2C, 2D). Control, mock-transfected cells (Lipofectin without plasmid) were 3A3-negative (2E).

Cells expressing rat α1 continued to express normal levels of the human α5β1 fibronectin receptor (Figure 2F), indicating that expression of β1 subunits did not limit cell surface expression.

Attachment was assayed on three test substrates: laminin-1, fibronectin, and collagen IV, both before and after transfection (Figure 3A), and with and without antiserum to either rat α1 or human α5 (Figure 3B). Untransfected, nonadherent K562 cells normally attach strongly only to fibronectin, and this binding is blocked with anti-human α5. K562 cells expressing rat α1 attach to collagen IV after 15 minutes. The attachment to collagen IV is completely inhibited by anti-rat α1, while the weak attachment to laminin-1 and slight inhibition of laminin-binding with mAb 3A3 were not statistically significant. Attachment to laminin required co-incubation with 3 μg/ml TS2/16, which had no effect on untransfected cells (Figure 3C). Specific attachment to collagen IV was seen at concentrations of collagen IV as low as 0.5 μg/ml (Figure 3D), with no increase in laminin-1 binding at concentrations as high as 40 μg/ml (Figure 3E). α1-transfected K562 cells could also bind rat tail collagen I (not shown). Replacement of Ca2+/Mg2+ buffer with equivalent concentrations of...
Mn$^{2+}$ or Mg$^{2+}$ alone produced identical results, but no attachment was seen with just Ca$^{2+}$ (unpublished results). Thus, α1β1 in the K562 cell line functions as an adhesion receptor for collagens type I and IV in a Mg$^{2+}$ dependent fashion, but only to laminin-1 in the presence of TS2/16.

**Induced Morphological Changes in K562 Cells**

Integrin α1β1 is used by a variety of neuronal cell types to promote the elaboration of neuritic processes on substrates of either collagen IV or laminin-1 (reviewed in Reichardt and Tomaselli, 1991). To examine the potential contribution of α1β1 to cell shape changes, α1-transfected K562 cells were examined after longer incubation times on collagen IV-coated substrates. Untransfected K562 cells attach, flatten slightly, but maintain a circular morphology on all substrates, including collagen IV (Figure 4A), fibronectin (Figure 4B), and plastic or pdl. Transfection with rat α1 has a dramatic effect on K562 cell morphology when plated on collagen IV. α1-K562 cells flatten out extensively on collagen IV substrates (Figure 4C, 5A) and elaborate extensive microspikes, with at least 5% of the α1-transfected cells extending neurite-like processes of 20 to 40 μm within 20 minutes of plating (Figure 4D). Greater than 70% of the α1-K562 cells lose their round mor-
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K562-a1 transfectants extend processes following attachment to collagen type IV. Untransfected K562 cells, one hour after plating on either collagen IV (A) or fibronectin (B), remain rounded on all substrates. $\alpha_1$-transfected cells are shown after one hour on collagen IV (C–D). All cells were stained with crystal violet and photographed with bright field optics. Scale bar in each panel equals 20 μm.

The morphology and assume a markedly spread morphology on collagen IV, while no spreading was seen on either fibronectin (Figure 5C, Table I) or pdl (Table I).

K562 cells can be induced to differentiate along a monocytic lineage with exposure to phorbol ester (Sutherland et al., 1986). This induction induces the expression of several integrins, including one collagen IV receptor, $\alpha_2$ (Zutter et al., 1992; Jarvinen et al., 1993), which could produce the observed effects. No immunostaining for $\alpha_2$ was seen on transfected (Figure 5E) or untransfected cells (Figure 5F), establishing that upregulation was not occurring in these cells. Consistent with that, the spreading response detected in the transfected lines was not altered by incubation with human $\alpha_2$ mAb P1E6 (Table I; Figure 6C,D) at concentrations 60-fold higher than those needed to inhibit other cell types (Wayner et al., 1988). In contrast, incubation with mAb 3A3 following attachment blocked all of the spreading response in the transfected lines (Table I; Figure 6A,B).

By time-lapse video imaging, transfectants were seen to extend processes of over 100 μm on collagen IV (Figure 7C,F). The processes in panels C and D look more neuronal, while those in E and F appear similar to the proplatelet processes elaborated by megakaryocytes. The absence of staining with neu-
K562-α1 cells spread in response to collagen IV, not fibronectin, nor do they express another collagen IV receptor, α2. K562-α1 transfecants (A,C,E) or control nontransfectants (B,D,F) were plated on collagen IV-(A–B, E–F) or fibronectin-(C,D) coated glass coverslips for 2 hrs, then fixed and immunostained with mAb 3A3 mAb (A–D) or mAb P1E6 (E and F). Only cells immunoreactive with mAb 3A3 and plated on collagen IV spread and extend processes (compare A and C). In addition, the absence of immunoreactivity in (B,D,F) also shows that the nontransfected cells do not express α2 or α1, while (E) shows that the transfected cells do not express integrin α2. Cells were photographed and reproduced for identical lengths of time. Scale bar in (F) 20 µm in all panels.
TABLE I Percentage Of Control and α1-Transfected K562 Cells That Flatten and Spread In Response To Collagen IV and Fibronectin

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Substrate and Antibody treatment</th>
<th>Col IV/NMS</th>
<th>Col IV/3A3</th>
<th>Col IV/PlE6</th>
<th>FN</th>
<th>Poly-d-Lysine</th>
</tr>
</thead>
<tbody>
<tr>
<td>α1-K562</td>
<td></td>
<td>72% (0.3%)</td>
<td>4% (0.5%)</td>
<td>68% (1.2%)</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>control K562</td>
<td></td>
<td>4% (1.1%)</td>
<td>3% (0.9%)</td>
<td>3% (1.5%)</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

1Glass cover slips were covered with substrate at 10μg/ml, and cells added for four hours. Purified 3A3 was added at 50 μg/ml and anti-α2, PlE6, from whole ascites was added at 1:100. Cells were then fixed, immunostained with 3A3, and examined under epi-fluorescence for more accurate assessment of morphological changes. Cells were scored as spread if they were not round (see figure 6 for examples). Duplicate trials were done, counting 500 cells from three test wells. Errors are standard deviations.

Filament antibodies (unpublished observation) and the overall morphology of these cells suggest that these processes may be more similar to those elaborated by megakaryoblastic cells (Symington, 1990).

FIGURE 6 Spreading and process formation by K562-α1 cells is mAb 3A3-dependent and unaffected by mAb PlE6. Cells were allowed to attach for 15 minutes, then mAb added for 4 hours. Following fixation, cells were photographed as in Figure 5. Purified mAb 3A3 IgG was added at 50 μg/ml (A) or 100 μg/ml (B), while whole ascites of PlE6 was added at 1:50 (C) or 1:100 (D). mAb PlE6 has been shown to inhibit cell attachment by dilutions up to 1:6400 (Wayner et al., 1988). Scale bar in (D) is 20 μm. See Table I for quantification of these results.
FIGURE 7 Time-lapse video images of control and K562-α1 transfectants plated on collagen type IV for two to five hours. Successive images of untransfected cells (A-B) and transfected cells (C-F) after 2 hrs (left panel in all pairs) or 5 hrs (right panel). The processes in panels C and D look more neuronal, while those in E and F appear more like the proplatelet processes elaborated by megakaryocytes. In addition the processes extending from the cell in (C-D) grew during the filming, in contrast to the cell in (E-F) which was motile. Scale bar equals 20 μm.
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Heterologous Expression of Rat $\alpha_1$ in Adherent MRC-5 and RD Cells

To examine effects of cellular context on ligand binding, the identical construct and similar transfection protocol were used to transiently transfected an adherent human fibroblast cell line, MRC-5, and the human rhabdomyosarcoma line RD. Compared to pdl, 100% of untransfected MRC-5 cells can attach to fibronectin, and greater than 130% attach to collagen IV, but less than 40% can attach to laminin-1 (Figure 9A). Binding of the untransfected cells to collagen IV and laminin-1 was likely due to endogenous $\alpha_2$ (Elices and Hemler, 1989). The attachment of untransfected cells was not blocked by the anti-$\alpha_1$ mAb 3A3. Three to five days after transfection with rat $\alpha_1$, MRC-5 cells were assayed for cell surface labeling and attachment. Cell surface labeling of live MRC-5 cells with the rat $\alpha_1$-specific mAb 3A3 was high (Figure 8A), with 40% of the cells rat $\alpha_1$ receptor-positive. Receptor levels were comparable by immunofluorescence to expression in stable rat $\alpha_1$ K562 transfectants. When $\alpha_1$-transfected MRC-5 cells were assayed for attachment to a range of laminin-1 concentrations, a two-fold increase in attachment was measured on laminin-1 concentrations ranging from 2 $\mu$g/ml to 32 $\mu$g/ml (compare Figure 9A and 9B). Addition of the anti-rat $\alpha_1$ mAb 3A3 completely inhibiting this increased attachment (Figure 9B). No detectable difference in collagen IV binding could be seen in the $\alpha_1$ transfectants, with or without antibody, even with the simultaneous addition of 3A3 and anti-$\alpha_2$ mAb (unpublished results). Stable transfectants of these cells were not isolated, since they could not be FACS-enriched due to extreme clumping.

To better characterize the acquisition of laminin-1 and collagen binding in a heterologous system, stable $\alpha_1$ transfectants were isolated from another human line, RD cells, which could be sorted. Attachment of untransfected RD cells to either laminin-1 or collagen IV was similar (Figure 10A, hatched bars), and was likely due to endogenous human $\alpha_1$$\beta_1$, $\alpha_4$$\beta_1$, $\alpha_5$$\beta_1$, and $\alpha_6$$\beta_1$ (Chan et al., 1991). However, addition of anti-rat $\alpha_1$ mAb 3A3 had no inhibitory effect on this binding, allowing direct determination of the contribution of rat $\alpha_1$ to binding. Stable rat $\alpha_1$ transfect-
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FIGURE 9 Transient transfection of MRC-5 cells with rat α1 confers laminin-1 binding. (A) Attachment of control, untransfected MRC-5 cells to the designated test substrate at 10 μg/ml in the presence of normal mouse serum (open bars) or with 50 μg/ml 3A3 (filled bars). Attachment to all substrates was unaffected by either treatment. (B) Laminin dose response curve for rat α1-MRC-5 transfectants in the presence of 50 μg/ml 3A3 (filled circles) or control rat IgG (open circles). 3A3-dependent attachment to laminin-1 is increased from 40% to 80% in the transfected cells. Residual laminin-1 attachment is likely due to α2β1 present on these cells. Cells were assayed 3 days post-transfection.

Stable transfection of RD cells with rat α1 increases 3A3-dependent attachment to laminin-1 and collagen IV. (A) Attachment of untransfected cells to indicated substrates at 2 μg/ml in the presence of normal mouse serum (diagonal lines) or 50 μg/ml 3A3 (shaded bars). RD α1 transfectant attachment is shown in the presence of normal mouse serum (open bars) or 50 μg/ml 3A3 (solid bars). (B) Laminin dose response curve for rat α1-RD transfectants. Open circles show attachment of rat α1-RD transfectants with control normal mouse serum and filled circles show attachment of rat α1-RD transfectants in the presence of 50 μg/ml 3A3. 3A3-dependent attachment of the rat α1-RD transfectants is increased from 20% to 90%. Cells were assayed following several rounds of selection for α1-positive cells by FACS.

FIGURE 10 Stable transfection of RD cells with rat α1 confers laminin-1 binding. (A) Attachment of control, untransfected MRC-5 cells to the designated test substrate at 10 μg/ml in the presence of normal mouse serum (open bars) or with 50 μg/ml 3A3 (filled bars). Attachment to all substrates was unaffected by either treatment. (B) Laminin dose response curve for rat α1-MRC-5 transfectants in the presence of 50 μg/ml 3A3 (filled circles) or control rat IgG (open circles). 3A3-dependent attachment to laminin-1 is increased from 40% to 80% in the transfected cells. Residual laminin-1 attachment is likely due to α2β1 present on these cells. Cells were assayed 3 days post-transfection.

Does Cellular Context Affect Integrin Structure?

The α1β1 integrin receptor, originally isolated as laminin-1/collagen receptor, acts as a collagen IV receptor in K562 cells without activating antibodies, but as at least a laminin-1 receptor in MRC-5 cells and a collagen IV/laminin-1 receptor in RD cells. It is possible that cellular context is affecting integrin function, either by (a) association of additional molecules or (b) post-translational modifications such as differential glycosylation. This latter possibility was tested on stable isolates of K562 cells expressing rat α1. Cultures were metabolically labeled with [35S]-methionine and membranes isolated by solubilization in Triton X-100. Prior to solubilization, a cocktail of six protease inhibitors was added to one of the radiolabeled cell pellets to prevent potential proteolytic activity. α1 receptor from these detergent-solubilized preparations was immunoprecipitated with mAb 3A3, followed by goat anti-mouse IgG, coupled to agarose beads. Precipitated proteins were detected by autoradiography. As can be seen in Figure 11A, the prominent feature of this precipitated material is a doublet with Mₚs of 200kD and 180kD. Partial degradation of the protein is unlikely, since protease inhibitors did not reduce the levels of either band (lane 1 vs lane 3). Alternative transcripts, encoding two forms of the receptor, did not provide a feasible explanation since our cells expressed processed cDNA containing no intron/exon borders.

To determine whether this doublet was the result of differential glycosylation, radiolabeled anti-α1 immunoprecipitates from stable K562-α1 transfectants
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FIGURE 11 Immunoprecipitated rat a1 from stable K562-a1 and RD-a1 transfectants migrates as a doublet. The receptor was immunoprecipitated from K562 cells (A), using mAb 3A3 with six protease inhibitors added to incubations in lane 3 and none in lane 1. The a1 subunit in these cells migrates as two bands of M, 200 and 180 kD with and without protease inhibitors. A faint band of M, 120 kD is coprecipitated, and is most likely the human B1 subunit. Controls precipitations with normal mouse serum and protease inhibitors are shown in lane 4 and without inhibitors in lane 2. Lane 5 shows collapse of the a1 doublet with endo F. Lane 7 is immunoprecipitated material after digestion with endo H. Only the lower M, 160kD band appears to have been degraded. Control digests of buffer alone with endo F (lane 6) or endo H (lane 8) are shown as well. In panel (B) stable RD-a1 transfectants were immunoprecipitated with 3A3 mAb (lane 3) or normal mouse serum control (lane 2). A lower percentage acrylamide gel (6%) was used in panel B compared to 7.5% in panel A, shifting the migration pattern slightly lower.

were isolated and subjected to overnight digestion with two enzymes, endo F/N and endo H. Endoglycosidase F/N-glycosidase F specifically cleaves Asn-linked sugars with the structure Asn-(GlcNAc)2(Man)α, including N-linked high mannose and complex oligosaccharides (Tarentino et al., 1985). Endoglycosidase H is specific for high mannose oligosaccharides, but not complex oligosaccharides (Trimble et al., 1984). Figure 11A lane 5 shows the collapse of the two rat a1 bands into a single band at approximately 160kD following endo F/N treatment. Digestion with endo H, shown in lane 7, lowered the M, 180kD band to the same 160kD band generated with endo F/N, but did not reduce the mobility of the M, 200kD band. These results suggest that the carbohydrate associated with the M, 180kD band is primarily high mannose conjugates, digestible with either glycosidase. The M, 200kD protein may represent a more fully processed form of the receptor, containing complex oligosaccharide side chains, only susceptible to endo F/N, but not endo H digestion.

Similar precipitations were done with stable RD transfectants (Figure 11B). Again two bands were seen in the RD-a1 cells. Panel 11B is a 6% polyacrylamide gel, compared to 7.5% for panel 11A, thereby accounting for the shift in the migration pattern for K562s analyzed in panel A (Lane 1 or 3) versus panel B (lane 1). Comparison of the M,s of the doublets in transfected K562 (panel B, Lane 1) and RD (panel B, Lane 3) cells shows that the upper band in the RD transfectants migrates slightly higher (estimated Rf shift corresponding to an increase in M, of 5-10kD). This increase in size, likely due to an increase in glycosylation, correlates with the difference in binding specificity. More direct demonstration of this correlation awaits further study.

DISCUSSION

We have shown that variations in integrin ligand specificities can be generated from the identical message expressed in three cell types. Expression of rat a1 cDNA in a nonadherent hematopoietic line, K562, confers collagen binding and results in dramatic cell shape changes. Expression of this receptor in the adherent fibroblastic line MRC-5 increases laminin-1...
and possibly collagen binding, while in the rhabdomysosarcoma RD line it stimulates an increase in laminin-1 and collagen IV binding with no cell shape changes. In each case, attachment activity and spreading response induced in cells expressing α1β1 was blocked in all three types by treatment with species-specific anti-rat α1 mAb 3A3, while the spreading response was unaffected by anti-human α2 mAb. These results imply that cell type-specific factors can modify integrin-ECM ligand specificities, and demonstrate clearly that expression of a single integrin can effect other cellular elements to initiate cell shape change and attachment in a normally nonadherent line.

Others have shown variable ligand selectivity with transfection of integrin α2 cDNA, but variations in binding required further manipulations, such as activating antibodies and cell selection on Matrigel (Chan and Hemler, 1993), which may have upregulated native α2. Our report, independent of these other confounding procedures, is the first to show that a transfected integrin cDNA alone can account for the observed variations in ligand specificity and underscore the importance of cell context in determining eventual integrin function. However, when the activating mAb TS216 was used, laminin binding was revealed in the K562 transfectants. This result is consistent with the view that the laminin binding site is separate from the collagen binding, and its activity is regulated separately as well. Moreover, it suggests that a conformational change is required to produce this additional ligand binding site, and compels a search for modifications in the protein, or the presence of accessory molecules, that can impart this change.

**Integrin α1 Expression Directly Affects the Cell Morphology of K562 Cells**

The observed alterations in morphological shape of α1-transfected K562 cells on collagen IV substrates were consistent and dramatic. Expression of α1 in K562 cells allowed cells to attach and extend processes on collagen IV substrates, but not on other substrates such as fibronectin. In no instance were untransfected cells seen to extend processes on collagen IV. Others have shown that phorbol ester induction of megakaryocytes from K562 cells is correlated with a reduction in cell division, spreading on plastic and fibronectin, along with the upregulation of α2 and other integrins (Burger et al., 1992; Jarvinen et al., 1993). The α1-K562 cells studied here continued to divide, showed no α2 expression or α2-dependent attachment and no spreading on fibronectin, pdl or plastic. Additional α1-K562 lines have been isolated, along with both early and late sorts, and they all exhibit similar properties (unpublished observations). These results rule out the possibility that a variant line, expressing these other megakaryocytes properties, or lines expressing human α1, emerged during repeated FACS sorting for rat α1-positive cells. Instead, the total block of morphological differentiation with species-specific mAb 3A3 indicates that the morphological change is due to rat α1-mediated response to collagen IV. The general appearance of the α1-transfected cells, and in particular the elaboration of proplatelet pseudopodia-like processes, indicates that collagen binding and recruitment of downstream cofactors by α1 alone can differentiate these cells along a megakaryocyte lineage.

K562 cells have been used a model for erythropoiesis due to their capacity to shift their differentiated state in response to exogenous agents (Anderson et al., 1979). The use of phorbol ester to determine the cellular elements needed to generate the megakaryocyte-like cells is difficult to interpret. The generalized effect on cells weakens any correlation drawn between upregulation of integrin receptors and the appearance of a morphologically differentiated state. For example, megakaryocyte differentiation induced by exogeneous agents causes an increase in the expression of a collagen binding integrin, α2 (Burger et al., 1992; Jarvinen et al., 1993). Yet α2-K562 transfectants do not show any change in their differentiated state even in the presence of activating antibodies (Chan and Hemler, 1993), while α1 transfecteds do. Apparently acquisition of collagen binding through the upregulation of α2 is not sufficient. The emergence of the morphologically differentiated state...
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in response to heterologous expression of α1, independent of the global effects of PMA treatment, indicates that the specific integrin is important in determining the differentiation state of at least K562 cells. For example, expression in K562 cells of α3A alone or α6A in association with integrin activating antibodies can induce some cell spreading on kalinin (Delwel et al., 1994) although little process formation.

Normal megakaryocyte differentiation in vitro is supported by ECM proteins collagen types I and IV and laminin-1, and leads to the formation of proplatelet-processes (Tablin et al., 1990; Topp et al., 1990). These progenitor cells may be poised to differentiate and select their fate merely by acquisition of ECM binding capacity. Remarkably, the processes elaborated by α1-transfected cells in some instances were indistinguishable from neuronal processes. Laminin and collagen IV stimulate integrin-dependent neurite outgrowth from nearly every neuronal cell type tested (Reichardt and Tomaselli, 1991), and laminin alone can induce retinal epithelial cells to transdifferentiate into neurons (Reh, 1987). Interactions with the ECM are also required for neural crest differentiation (Perris and Bronner-Fraser, 1989). All of these responses can be blocked by antibodies to laminin-1/collagen-binding integrins. α1 expression, and the acquisition of collagen IV binding, may trigger the differentiation of additional cell types already poised or determined for a particular phenotypic course by recruiting cellular machinery designed for such a switch. Of particular interest is that, in all these cases, integrin expression and function stimulate the production of cellular processes that extend some distance from the cell. The recruitment and orchestration of cellular elements, in particular the cytoskeleton, appears to be directly regulated by integrin function.

What Cell Type-Specific Modifications Can Determine Integrin-Ligand Specificity?

The molecular basis underlying changes in receptor-ligand specificity is unknown (for review see Haas and Plow, 1994). Such variation may be the consequence of cell type-specific interactions such as glycosylation (Symington et al., 1989; Lampe et al., 1993; Wadsworth et al., 1993; Chammas et al., 1993; Moss et al., 1994; Veiga et al., 1995), phosphorylation (Dahl and Grabel, 1989; Schwartz, 1992), alternative splicing (Tamura et al., 1990; Hynes, 1992), association with glycolipids (Cheresh et al., 1986; 1987) or differential expression of accessory molecules (Pullman and Bodmer, 1992). Many of these modifications are associated with changes in ligand affinity, but none are known to change ligand specificity.

Native α1 protein from rat PC12 and B50 cells (Ignatius and Reichardt, 1988), neonatal rat homogenates (Tawil et al., 1990) or human cells migrates as a single band at 200kD (Hemler, 1988). It is possible that the doublet seen in K562 cells includes a form of the receptor that is unable to bind laminin-1. An identical doublet was isolated from rat hepatocytes on a laminin-I affinity column (Forsberg et al., 1990) and from a collagen I column (Gullberg et al., 1990). These authors suggest that the doublet might be a result of multiple transcripts of the receptor, or of partial degradation of the cytoplasmic portion of the receptor. Multiple transcripts are unlikely since processed cDNA, without any exon or intron boundaries or donor sites, was used to generate expressed receptor than ran as a doublet. Degradation of cytoplasmic sequence is unlikely since protease inhibitors had no effect on the observed size, and because the cytoplasmic domain is only 16 amino acids—predicted to contribute less than 2.5kD to the apparent molecular weight. Left unexplained is why this laminin-I and collagen receptor on hepatocytes is in a form electrophoretically similar to the form expressed by K562 cells that can only bind collagen. One possibility being addressed is that the lower molecular weight form is unable to bind laminin because of reduced glycosylation and that the abundance of one species over the other can determine the net ligand specificity detected.

α1 has a larger number of N-linked sugar moieties than any other known integrin alpha subunit, contributing an apparent Mr of 60kD in rat and human cells (Ignatius et al., 1990). Ligand binding could be al-
tered by changes in the sugar content of the receptor. As a precedent, phorbol ester treatment of K562 cells has been shown to reduce the sialation of α5β1, and this treatment, or desialation with tunicamycin, of control cells reduced attachment to fibronectin (Symington et al., 1989). To determine if the extent of glycosylation was altered in the rat α1 transfectants, properties of the sugar moieties were examined using two endoglycosidases. Our results establish that the observed doublet is the product of differential glycosylation of the protein, with the M, 200kD band comprised of more branched, complex oligosaccharide side chains.

Since detergent extraction of metabolically labeled cells was used in the present study, it is possible that the lower band in the doublet is an intermediate in the synthetic pathway that has yet to reach the surface. However this is unlikely since a similar intermediate has never been seen when receptor has been isolated in the same manner from a variety of other cells, (Ignatius et al., 1988; Turner et al., 1989). More significantly, others have evidence that this lower molecular weight band reflects a different glycosylation state of the α1 subunit (Lampe et al., 1993). These authors describe a 190kD pre-α1 chain that appears below the 200kD subunit when HT-29 colon adeno-carcinoma cells are treated with an inhibitor of mannosidase I or N-glycan synthesis (deoxymannojirimycin), then surface labeled and immunoprecipitated.

Removal of these N-linked sugars with metabolic inhibitors or enzymatic treatment usually correlates with a reduction in integrin-ligand affinity. For example, deoxymannojirimycin treatment of K562 cells reduces fibronectin binding by 50% and more complete removal of N-linked sugars with a mixture of endo-N-acetylgalactosaminidase F and peptide-N-asparagine amidase F reduces fibronectin binding by 95% (Zheng et al., 1994). The 5–10 kD reduction in N-linked sugars on α1 following deoxymannojirimycin treatment of HT-29 cells, creating a 190kD α subunit from the 200kD protein, parallels a 92% reduction in laminin and collagen binding (Lampe et al., 1993). In contrast, treatment of the whole HT-29 cells with deoxynojirimycin, specific for glucosidase I, while reducing cell attachment to laminin actually increases attachment to collagen IV (Lampe et al., 1993). Indeed glycosylation may affect integrin binding in more than one way. However reductions in the glycosylation state of other integrins (α2, α3, α6) on these cells by these treatments weakens any direct correlation between reduced α1 glycosylation and reduced laminin binding or increased collagen binding. Application of these inhibitors in the heterologous system described here will better establish this correlation and awaits future studies.

One potential intracellular cofactor has been described in a study that used expression cloning to screen for non-integrin proteins that could activate cell adhesion (Pullman and Bodmer, 1992). These authors identified a 142-amino acid cytoplasmic protein that enhanced β1 integrin-dependent binding of a colon cancer-derived cell line to collagen type I. Extracellular regulators of adhesion molecule function have been described as well. α4β1 binding to fibronectin is mediated by chondroitin sulfate proteoglycan, while attachment to V-CAM is unaffected by the same treatment (Iida et al., 1992). Gangliosides have also been shown to modulate integrin function (Cheresh et al., 1986; 1987), possibly by blocking one ligand binding site. Gangliosides also appear able to modulate function of other adhesion proteins, such as N-CAM or N-cadherin (Doherty et al., 1992). Finally, the lipid composition of the membrane may influence ligand specificity. The ligand specificity of the purified vitronection receptor (αvβ3) in liposomes can be modulated by variations in the ratios of several phospholipids (Conforti et al., 1990), possibly through alterations in the conformation of the receptor.

The existence of integrin activating antibodies also indicates that marked shifts in tertiary structure can be correlated with activation, shifts that could play a central role in regulating integrin avidity and specificity (cf. Neugebauer et al., 1991; Arroyo et al., 1992; Takada and Puzon, 1993). The mAb used in the present study, TS2/16, shifts the collagen only α1 receptor in K562 cells into a laminin/collagen receptor. These antibodies likely expose additional binding sites through induction of conformational changes, changes that can be monitored by the exposure of
additional mAb epitopes (cf Bazzoni et al., 1995). Accessory molecules that provide the same shift could produce similar changes in a cell type specific manner. K562 cells provide an opportunity to study the necessity of such cofactors since integrins other than α1 are unable to bind ECM ligands without activation. Since collagen binding through transfected α1 is robust in these cells without activation, this site apparently operates in an unregulated manner. It is noteworthy that α1 contains 60 kD more N-linked sugars than other integrin. This extensive glycosylation may play a role in maintaining an active conformation that allows collagen binding, with laminin binding remaining under regulation.

Many integrins have the capacity to interact with multiple ligands. As one example, integrins containing the α4 subunit have been shown to interact with at least four distinct ligands, and several of these interactions appear to reflect partially distinct but overlapping binding sites (Pulido et al., 1991; Andrew et al., 1994). Again, variations in ligand specificity may be achieved through the masking of available sites, possibly through conformational changes or complexing with associated proteins. Both α2β1 and α1β1 contain I-domains, which in α2β1 has been shown to bind collagens I, II and XI (Kamata and Takada 1994; Tuckwell et al., 1995). These studies imply that the laminin-1 recognition site may be separate from the site mediating collagen binding, although antibody blockade studies in another heterologous system suggest that the I-domain in α1β1 may bind laminin-1 as well as collagen (Kern et al., 1994). Our results suggest that cells can regulate the exposure of one or both sites by unknown accessory interactions.

The results presented here show that variations in ligand specificities and cell morphology can be produced by heterologous expression of a single recombinant integrin receptor cDNA. The heterogeneity is not the result of transcriptional alterations but may reflect differential glycosylation or cell type-specific cofactors. The system described here affords an opportunity to isolate potential cofactors and to characterize more precisely the differences in glycosylation that might produce this diversity, as well as to offer a better understanding of the role of the ECM and integrins in the differentiation of cells.

References


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