A Recombinant Tail-Less Integrin β4 Subunit Disrupts Hemidesmosomes, but Does Not Suppress α6β4-mediated Cell Adhesion to Laminins

Laura Spinardi,* Steven Einheber,† Theresa Cullen,* Teresa A. Milner,§ and Filippo G. Giancotti*

*Department of Pathology and Kaplan Comprehensive Cancer Center; and †Department of Cell Biology, New York University School of Medicine, New York 10016; § Department of Neurology and Neuroscience, Cornell University Medical College, New York 10021

Abstract. To examine the function of the α6β4 integrin we have determined its ligand-binding ability and overexpressed two potentially dominant negative mutant β4 subunits, lacking either the cytoplasmic or extracellular domain, in bladder epithelial 804G cells. The results of cell adhesion and radioligand-binding assays showed that α6β4 is a receptor for several laminin isoforms, including laminin 1, 2, 4, and 5. Overexpression of the tail-less or head-less mutant β4 subunit did not suppress α6β4-mediated adhesion to laminins, as both types of transfectants adhered to these ligands in the presence of blocking anti-β4 antibodies as well as the controls. However, immunofluorescence experiments indicated that the endogenous α6β4 integrin and other hemidesmosomal markers were not concentrated in hemidesmosomes in cells overexpressing tail-less β4, while the distribution of these molecules was not altered in cells overexpressing the head-less subunit. Electron microscopic studies confirmed that cells overexpressing tail-less β4 had a drastically reduced number of hemidesmosomes, while cells expressing the head-less subunit had a normal number of these structures. Thus, expression of a tail-less, but not a head-less mutant β4 subunit leads to a dominant negative effect on hemidesmosome assembly without suppressing initial adhesion to laminins. We conclude that the α6β4 integrin binds to several laminins and plays an essential role in the assembly and/or stability of hemidesmosomes, that α6β4-mediated adhesion and hemidesmosome assembly have distinct requirements, and that it is possible to use a dominant negative approach to selectively interfere with a specific function of an integrin.

The study of laminin binding integrins is of particular relevance because many of the effects of basement membranes on the proliferation and differentiation of cells can be recapitulated in vitro by laminins (Manthorpe et al., 1983; Grant et al., 1989; Panayotou et al., 1989; von der Mark and Ocalan, 1989; Caron, 1990) or blocked by anti-laminin antibodies in cell and organ culture systems (Grover and Adamson, 1985; Klein et al., 1988; Streuli et al., 1991). Recent results indicate the existence of several laminin isoforms, expressed in a tissue-specific fashion (Engvall et al., 1990; Sanes et al., 1990; Rousselle et al., 1991; Marinkovich et al., 1992b). In addition, at least six integrins have been implicated in binding to laminins and, in several cases, their binding specificity appears to be overlapping (for review see Mercurio, 1990; Hynes, 1992). The hypothesis that individual laminin-binding integrins have distinct cytoskeletal and signaling functions may help to explain the apparent redundancy of the laminin recognition system.

There are several reasons for believing that the α6β4 integrin, which has been implicated in binding to laminin 1 (subunit composition: α6-β1-γ1) and laminin 5 (kalinin, epiligrin, nicein; α6-β1-γ1) (Lee et al., 1992; Niessen et al., 1994), is characterized by unique intracellular interactions. The cytoplasmic domain of the β4 subunit measures over 100 kD in molecular mass and bears no homology with the short cytoplasmic domains of the other known integrins (Hogervorst et al., 1990; Suzuki and Naitoh, 1990). This portion of the molecule contains two pairs of type III fibronectin-like modules connected by a sequence (Connecting Segment) which appears to be the target of multiple potential regulatory mechanisms, including alternative splicing (Tamura et al., 1990) and proteolytic processing (Giancotti et al., 1992). Furthermore, in contrast to β1 and β3 integrins which localize to focal adhesions or otherwise interact with the actin filament system (Chen et al., 1985; Damsky et al., 1985; Giancotti et al., 1986a; Dejana et al., 1988), the α6β4 integrin is found concentrated in hemidesmosomes and, thus, may interact with the keratin filament system (Carter et al., 1990; Stepp et al., 1990; Sonnenberg et al., 1991).

By expressing various deletion mutant forms of the β4...
that a critical function of the β₄ cytoplasmic domain is to mediate the association of the integrin with the hemidesmosomal cytoskeleton (Spinardi et al., 1993). As the extracellular domain of αβ₄ binds to basement membrane components, and the specialized cytoplasmic tail of β₄ subunit interacts with the hemidesmosomal cytoskeleton, this integrin may play a crucial role in the assembly of hemidesmosomes and their linkage to the keratin filament system.

Despite their functional importance, relatively little is known about the molecular composition and mechanism of assembly of hemidesmosomes. In addition to αβ₄, hemidesmosomes contain another transmembrane protein, the Bullous Pemphigoid Antibigen 2 (BPAG2). The relative roles of the α₄β₃ integrin and BPAG2 in the establishment of stable epidermal cell adhesion to the basement membrane and in the assembly of hemidesmosomes are presently unclear.

To analyze the function of the αβ₄ integrin and its role in cell adhesion and assembly of hemidesmosomes, we have examined the spectrum of ligands recognized by αβ₄ and tested two distinct strategies for creating a dominant negative mutant β₄ subunit. We report that αβ₄ binds to several laminin isoforms. Introduction of a truncated tail-less β₄ subunit in cells possessing endogenous αβ₄ integrins and hemidesmosomes led to a dominant negative effect, while comparable expression of a β₃ molecule lacking almost the entire extracellular portion did not. Cells expressing the dominant negative tail-less form of β₄ were found to have a drastically reduced number of hemidesmosomes, but did not show defective adhesion to laminins.

Materials and Methods

Cell Lines, Antibodies, and Extracellular Matrix Molecules

Rat bladder carcinoma 804G cells (Izumi et al., 1981) were cultured in DMEM with 10% bovine calf serum (BCS). RAC-11P/SD cells (Sonnenberg et al., 1993) were cultured in DMEM with 10% bovine calf serum. Rat bladder carcinoma 804G cells (Izumi et al., 1981) were cultured in DMEM-10% BCS supplemented with 400 μg/ml of G418 and cultured to densities of 1, 5, 7, or 8. Laminin 2 was purified to homogeneity from mouse heart in the laboratory of Mats Paulsson (University of North Carolina, Chapel Hill, NC). Laminin 5 was purified to homogeneity from keratinocyte cell culture medium in the laboratory of Robert Burgeson (Harvard Medical School, Boston, MA). Laminin 5 matrices were prepared as described previously (Sonnenberg et al., 1993). Electrophoretic analysis and immunoblotting with antibodies to laminin isoforms and other adhesive ligands have shown that laminin 5 is by far the predominant protein in matrices of RAC-11P/SD cells (Sonnenberg et al., 1993). Our own electrophoretic analysis indicated that the 165-kD α₅ chain, the 155-kD γ₃ chain, the 140-kD β₁ chain, and the 105-kD γ₁ chain degradation product represent more than 95% of the Coomassie blue stainable proteins present in such matrices. Most of the residual material migrates as a band of ~190 kD and possibly represents the precursor of the α₃ chain.

Constructs and Transfections

Expression constructs encoding wild-type and mutant truncated human β₄ subunits were assembled in the eukaryotic expression vector pRC-CMV (Invitrogen Corp., San Diego, CA) and were previously described (Giancotti et al., 1993). The plasmid pCMV-β₄A 854-1752 directs the expression of a β₄ molecule lacking almost the entire cytoplasmic domain. The plasmid pCMV-β₄A 470-660 encodes a β₄ subunit in which most of the extracellular sequences were replaced by a c-myc epitope tag.

Rat bladder carcinoma 804G cells stably expressing a tail-less (clones B13, B23, and B29) or a head-less (clone F28) human β₄ subunit were obtained by transfection with pCMV-β₄A 854-1752 and pCMV-β₄A 470-660, respectively (Spinardi et al., 1993). The clone expressing a full-length human β₄ subunit (clone A12) was previously described. The control cell lines (clones Z10 and Z32) were generated by transfection with pRC-CMV alone. The transfected cell lines were maintained in DMEM-10% BCS supplemented with 400 μg/ml of G418 and cultured for at least 48 h in the absence of the selective agent before all experiments.

Immunoprecipitation and Western Blotting Analysis

For immunoprecipitation of cell surface molecules, intact cells were labeled with 125I by the lactoperoxidase-H₂O₂ method, as previously described (Giancotti and Ruoslahti, 1990). After washing, the cells were extracted for 45 min on ice with lysis buffer containing 50 mM Tris (pH 7.5), 150 mM NaCl, 0.5% Triton X-100, 0.1% SDS, 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 mM sodium orthovanadate, and 1 mM EDTA. Immunoprecipitation of integrins other than αβ₄, lysis buffer contained 1 mM CaCl₂, 1 mM MgCl₂, and no EDTA. The immunoprecipitations were performed as previously described (Giancotti and Ruoslahti, 1990) and analyzed by SDS-PAGE. All
Adhesion Assay

Adhesion assays were performed essentially as previously described (Giancotti et al., 1985). After coating, all plates were blocked with PBS-0.1% BSA (Sigma). To avoid synthesis and secretion of adhesion proteins during the assay, the cells were treated with 20 μM cycloheximide (Sigma) for 1 h and 1 μM monomeric (Sigma) for 5 min before the assay. Cells were detached by incubation in 0.25% trypsin (GIBCO BRL) for 5 min. After blocking with Soybean Trypsin Inhibitor (Sigma), the cells were washed and resuspended in serum free DMEM containing 20 μM cycloheximide. For antibody inhibition experiments, the cells were preincubated with the indicated concentrations of antibodies for 15 min at 4°C before plating. Cells were added at 1-2 × 10^5 per well and incubated for the indicated times at 37°C in an atmosphere containing 5% CO2. Quantitation of the results was as previously described (Giancotti et al., 1986).

Radioligand-binding Assay

The partially recombinant wild-type and tail-less α6β4 integrins used in radioligand-binding experiments were purified from the transfected 804G clones A12 and B13, respectively. Approximately 1.5 × 10^9 cells were used for each purification. Cells were harvested by using 5 mM EDTA, washed, and solubilized with 10 ml of lysis buffer containing 50 mM Tris (pH 7.5), 150 mM NaCl, 200 mM n-Octyl-β-D-Glucopyranoside (Calbiochem, San Diego, CA), 5 mM EDTA, 0.01% Aprotinin, 4 μg/ml Pepstatin A, 10 μg/ml Leupeptin, 1 mM PMSF, and 1 mM PMSF for 45 min at 0°C. Extracts were clarified by centrifugation at 15,000 rpm and incubated with 1 ml of Sepharose-normal IgG to remove proteins which bind nonspecifically. The extracts were then applied to an affinity matrix prepared by cross-linking 5 mg of purified 3El monoclonal antibody to 1 ml of Sepharose-Protein G (Pharmacia LKB Biotechnology, Piscataway, NJ). After 4 h of incubation at 4°C, the unbound material was washed, and the column was washed with 25 bed volumes of 50 mM Tris (pH 7.5), 150 mM NaCl, 50 mM n-Octyl-β-D-Glucopyranoside. The integrins were eluted with 5 bed volumes of 50 mM Tricholamine, pH 10, 150 mM NaCl, 50 mM n-Octyl-β-D-Glucopyranoside into tubes containing neutralizing buffer (10% by volume 1 M Tris-HCl, pH 7.4). Peak fractions were analyzed by SDS-PAGE and Coomassie blue staining, and pooled. Protein concentration was estimated by comparison with known amounts of BSA. The purity was consistently higher than 95%.

Laminins 1, 2, 4, and 5 and fibronectin were radiolabeled by the iodination method and separated from free iodine by Sephadex G25 (Pharmacia) gel filtration. Protein peak fractions were analyzed by SDS-PAGE. The specific activity of all the radioligand preparations was determined by counting in a gamma counter a TCA-precipitated aliquot of the peak fractions. Specific activities were 1.3 × 10^6 CPMS/μg for laminin 1, 1.9 × 10^6 CPMS/μg for laminin 2, 3.3 × 10^6 CPMS/μg for laminin 4, 0.5 × 10^6 CPMS/μg for laminin 5, and 9.9 × 10^6 CPMS/μg for fibronectin. Purified integrins were diluted to 0.25 μg/ml with PBS containing 1 mM MnCl2. Removable microtitre wells (Microtest III, Falcon) were coated with 100 μl of receptor solution (25 mg) overnight at 4°C. After blocking with 2% BSA, the wells were incubated with the indicated amounts of radio-labeled matrix molecules diluted in PBS containing 1 mM MnCl2 for 4 h at room temperature. When indicated, synthetic peptides or EDTA were included. At the end of incubation, the wells were washed five times with PBS, 1 M NaCl and counted in a gamma counter. Non-specific binding was defined as the amount of radioligand which bound to wells coated with BSA only and was subtracted from each dose point.

To determine the dissociation constant between laminin 4 and the wild-type or truncated tail-less integrin, displacement experiments were carried out. A single concentration of radiolabeled laminin 4 (100 ng/ml) was added to each well in presence of increasing concentrations of cold ligand (0-2.5 μg/ml). The results were subjected to Scatchard analysis.

Immunofluorescence

The 804G transfectants were cultured for ~48 h on glass coverslips, and then either fixed directly with cold methanol for 2 min or treated with PBS containing 0.2% Triton X-100 for 5 min on ice before fixation with methanol. Cells were stained for 45 min with the various antibodies. The purified anti-human β3 3El and anti-c-myc Ab-1 monoclonal antibodies were used at 5 μg/ml. The anti-BPAG1 human serum and the α2 cytoplasmic domain rabbit serum were diluted 1:200. The anti-BPAG2 fusion protein IgGs were used at 25 μg/ml. The affinity-purified β4 cytoplasmic peptide antibodies were used at 5 μg/ml. After extensive washing, the cells were incubated for 45 min with 0.5-1 μg/ml of affinity-purified fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse, anti-rabbit, or anti-human IgGs (Molecular Probes Inc., Eugene, OR). The coverslips were mounted in Citifluor (Chemical Laboratory of the University of Kent, Canterbury, UK).

For double immunostaining with the 3El or Ab-1 monoclonal antibody and the anti-BPAG2 rabbit IgGs, the coverslips were incubated first with the monoclonal antibody followed by Texas red (TR)-conjugated goat anti-mouse IgGs, and then with the anti-BPAG2 IgGs followed by affinity-purified FITC-labeled goat anti-rabbit IgGs (Molecular Probes). For double immunostaining with the 3El or Ab-1 monoclonal antibody and the anti-BPAG1 human serum, the coverslips were incubated first with the monoclonal antibody followed by affinity-purified TR-conjugated goat anti-mouse IgGs (Molecular Probes), and then with the anti-BPAG1 serum followed by FITC-labeled goat anti-human IgGs. All secondary antibodies used were species-specific. Samples were examined with a Zeiss Axioshot Fluorescence Microscope.

Electron Microscopy

Cells grown on laminin 4-coated Aclar plastic coverslips were rinsed in PBS and fixed overnight at 4°C in 0.05 M Sodium Phosphate buffer, pH 7.0, containing 2% Glutaraldehyde and 0.1 M Sucrose. After washing in 0.1 M Phosphate buffer, the coverslips were incubated in 2% Osmium Tetroxide in 0.1 M Phosphate buffer for 1 h and embedded in Epon (Miller and Bank, 1989). To obtain cross-sections of the cells, pieces of the embedded coverslips were reembedded in Epon in the appropriate orientation for sectioning. Ultrathin sections (50-65 nm) were collected on copper grids and counterstained with Uranyl Acetate and Reynolds Lead Citrate. Sections were analyzed on a Philips 201 electron microscope.

Results

Overexpression of Truncated β3 Integrin Subunits

Previous results indicated that a truncated tail-less human β3 subunit (Δ cyto 874-1752) combines with endogenous α6 and reaches the cell surface, but is not incorporated in hemidesmosomes. In contrast, a truncated head-less β3 subunit (Δ exo 70-660) does not associate with endogenous α6, but is transported to the cell surface and recruited in hemidesmosomes (Spinardi et al., 1994). We reasoned that the integrin containing the tail-less recombinant β3 subunit could exert a dominant negative effect by competing with endogenous wild-type α6β4 for adhesive ligands. Conversely, the head-less recombinant β3 subunit could compete with the endogenous α6β4 receptor for binding to cytoskeletal elements or regulatory factors (Fig. 1).

To test the potential dominant negative effect of truncated β3 subunits in hemidesmosome-forming cells, we selected 804G clones with potential for high level expression of either the tail-less (Δ cyto 874-1752) or the head-less (Δ exo 70-660) human β3 subunit, as described in Materials and
Methods. The transfectants selected for study included: clones B13, B23, and B29, expressing the tail-less β4 subunit; clone F28, expressing the head-less β4 molecule; and clones Z10 and Z32, transfected with the selection marker alone.

The level of expression of recombinant tail-less β4 and endogenous wild-type β4 in the various B clones was examined by immunoprecipitation (Fig. 2 A). Control clones Z10 and Z32, clone A12 which expresses a full-length human β4 subunit and clones B13, B23, and B29 carrying the tail-less human β4 subunit were labeled at the surface with 125I and extracted. Samples containing equal amounts of TCA-precipitable counts were immunoprecipitated with saturating amounts of the 3E1 monoclonal antibody, reacting selectively with the extracellular domain of human β4 (Fig. 2 A, left). The 3E1 monoclonal antibody did not bind to any membrane protein in control cells, but immunoprecipitated high levels of recombinant full-length β4 from clone A12, similarly high levels of tail-less β4 from clone B13, and lower levels from clones B23 and B29. In accordance with previous results indicating that the α6 subunit is poorly labeled by cell surface iodination, the 110-kD α6 subunit associated with full-length and tail-less recombinant β4 could be detected only upon prolonged exposure of the gel.

To compare the expression of recombinant tail-less β4 with that of endogenous wild-type β4, the various clones were also immunoprecipitated with excess amount of a polyclonal antiserum raised against a synthetic peptide reproduc-

Figure 1. Schematic representation of wild-type and recombinant mutant αβ4 integrins. (Top) Wild-type endogenous αβ4 integrin. (Middle) Hybrid heterodimer consisting of the recombinant tail-less human β4 subunit (Δcyto) and associated endogenous α6 subunit. (Bottom) Head-less human β4 subunit (Δexo), which does not combine with endogenous α6. The heterodimer containing a tail-less β4 subunit is expected to compete with the endogenous integrin for binding to extracellular ligand. The head-less β4 poly-peptide may instead compete with endogenous wild-type αβ4 for interaction with intracellular molecules.

Figure 2. Immunoprecipitation analysis of recombinant β4 molecules expressed in 804G cells. (A) The indicated clones were labeled with 125I and extracted with lysis buffer. Aliquots containing the same amount of TCA precipitable radioactivity were immunoprecipitated with an excess amount of the 3E1 monoclonal antibody or the α6 cytoplasmic domain antiserum. Samples were separated by SDS-PAGE under reducing conditions and subjected to autoradiography. (B) The indicated clones were either directly extracted in sample buffer (TOT) or immunoprecipitated with the β4 cytoplasmic peptide antibody before electrophoresis (IP). The lanes marked TOT contain 100 μg of total proteins, while those labeled IP contain the material immunoprecipitated from 6 mg of total proteins. Both types of samples were separated by SDS-PAGE under reducing conditions and probed by immunoblotting with affinity-purified β4 cytoplasmic peptide antibodies. Bound antibodies were detected by incubation with 125I-Protein A followed by autoradiography.
ing the cytoplasmic domain of αdα. The anti-αdα antiserum was selected because αdα is the only α5 subunit isoform expressed in 804G cells (unpublished results). As shown in Fig. 2 A (right), the antiserum immunoprecipitated two types of α5 heterodimers from the B clones: those containing the 100-kD recombinant tail-less βδ molecule and those containing the 200-kD wild-type endogenous βδ subunit. Phosphorimager analysis indicated that the recombinant tail-less βδ subunit was 1.6 times more abundant than endogenous βδ at the surface of clone B13, but endogenous βδ was 3.2 and 3.4 times more abundant than the recombinant molecule in clones B23 and B29, respectively. It was concluded that tail-less βδ is overexpressed in clone B13, but not in clones B23 and B29.

The level of recombinant βδ lacking the extracellular domain expressed in clone F28 was examined by performing immunoblotting experiments with an antibody raised against a synthetic peptide designed after the COOH terminus of human βδ. This βδ cytoplasmic domain antibody was expected to react well with both the head-less βδ molecule and the endogenous rat wild-type βδ subunit, because its target sequence is conserved in rodents and humans (Kennel et al., 1993). To obtain two distinct measurements of different sensitivities, cells of the control clone Z10 and the head-less βδ expressing clone F28 were either directly extracted in sample buffer or immunoprecipitated with excess amount of the βδ cytoplasmic domain antibody before immunoblotting. The βδ cytoplasmic domain antibodies bound to the recombinant head-less βδ subunit in clone F28 and reacted with endogenous wild-type βδ in total extracts and immunoprecipitates from both control clone Z10 and clone F28. Fainter bands at ~70 kD, possibly representing proteolytic fragments of βδ, were also detected (Fig. 2 B). Phosphorimager analysis indicated that the head-less recombinant molecule is expressed at levels 5.3 times higher than endogenous βδ in clone F28. From this experiment, we concluded that the recombinant head-less subunit is overexpressed in clone F28. Although the ratios of recombinant to endogenous βδ subunits in clones F28 and B13 were determined by different methods, the results suggest that the extent of overexpression of head-less βδ in clone F28 is greater than that of tail-less βδ in clone B13.

The αβδ Integrin Is a Receptor for Laminins 1, 2, 4, and 5

To examine the effects of the truncated βδ subunits on αβδ-mediated cell adhesion, we sought to define the ligands of αβδ and determine which integrins with an overlapping ligand-binding specificity were expressed by 804G cells. The repertoire of integrins expressed by 804G cells was examined by immunoprecipitation. After labeling of the cell surface with 125I, the 804G cells were extracted and immunoprecipitated with antibodies to synthetic peptides modeled after various integrin cytoplasmic domains. As shown in Fig. 3, the results indicated that 804G cells express high levels of the αβδ and αβδ integrins and lower levels of αδβδ. A prolonged exposure of the gel (lane to the far right in Fig. 3) revealed that they also express minor levels of αδβδ. No βδ subunit could be detected in association with αδα even after prolonged exposure of the gel, indicating that 804G cells do not express the αδα integrin.

Cell adhesion assays showed that the 804G cells adhere well to fibronectin, type IV collagen, and laminin 4, but interact more weakly with laminin 1 and 2 (Fig. 4 A). In addition, time course experiments indicated that 804G cells adhere well to the laminin 5 matrix deposited by RAC-11/PD cells (Fig. 4 B). Although the matrix form of laminin 5 cannot be directly compared to the other purified soluble monomeric ligands tested, these results clearly show that the 804G cells can interact well with laminin 4 and 5, and less well with laminin 1 and 2. Since the adhesion of 804G cells to the laminin 5 matrix and to laminin 4 was not affected by antibodies reacting with rat βδ (Fig. 4 C), we concluded that adhesion to laminin 4 and 5 could involve αβδ.

To directly test the ligand-binding ability of the αβδ integrin in the absence of potentially confounding influences of other integrins or cellular regulatory factors, radioligand-binding assays were performed. A partially recombinant form of the αβδ integrin was purified from clone A12 cells by immunoaffinity chromatography on the 3E1 monoclonal antibody, as described in Materials and Methods. Microtiter wells were coated with 25-ng purified integrin, and then incubated with various concentrations of iodinated laminin 1, 2, 4, and 5 or fibronectin. The laminin 5 used in this experiment was immunopurified to homogeneity and did not contain detectable amounts of laminin 6, the laminin isoform to which laminin 5 is covalently associated in tissues. The
assay was performed in the presence of 1 mM MnCl₂, because this cation was previously reported to effectively sustain the ligand-binding function of αβ₁ (Niessen et al., 1994). The results of this experiment indicated that purified αβ₁ binds in a dose dependent way to laminin 1, 2, 4, and 5 (Fig. 4 D). Binding to laminin 4 and 5 was larger than that to laminin 1 and 2 suggesting a hierarchy of binding activities between the various αβ₁ ligands. However, affinity constants could not be directly extrapolated from these data, since no curve reached saturation. The binding of αβ₁ to laminin 1, 2, 4, and 5 was specific, since the integrin did not bind to fibronectin (Fig. 4 D) and the binding observed with laminins could be prevented by 1 mM EDTA. A synthetic peptide reproducing the major cell binding sites in fibronectin (GRGDSP) did not interfere with the binding of laminin 4 to αβ₁, suggesting that this integrin, like other laminin-binding integrins, is not RGD dependent (not shown). Taken together, these observations indicate that the αβ₁ integrin binds to several laminin isoforms, including laminin 1, 2, 4, and 5.

The Tail-Less αβ₁ Integrin Displays an Intact Affinity for Extracellular Ligand In Vitro

The hypothesis that a recombinant tail-less form of β₁ can suppress the function of endogenous αβ₁ was based on the assumption that the tail-less integrin would be able to bind effectively to extracellular ligand. To test this assumption, we purified the receptor containing a tail-less human β₁ subunit from clone B13 and the receptor containing a full-length human β₁ subunit from clone A12, and compared their binding properties by using a radioligand-binding assay. Microtiter wells were coated with 25 ng of the two receptors and incubated with 100 ng of radiiodinated laminin 4 in the presence of various concentrations of cold ligand. As shown in Fig. 5, A and B, the binding of radioactive laminin 4 to both receptors was effectively competed by excess cold ligand. In both cases complete inhibition of binding was observed with ~3.5 pmol, corresponding to a 250-fold excess of cold over radioactive laminin 4. The displacement curves generated by the tail-less and wild-type receptor were very similar, suggesting that the two receptors bind to ligand with similar kinetics. Scatchard analysis of the results indicated that the two receptors display a very similar affinity for laminin 4 (Fig. 5, C and D). Indeed, the estimated Kₘ of wild-type receptor was 8.45 × 10⁻⁴ mol/liter and that of tail-less receptor was 7.04 × 10⁻⁴ mol/liter. These results demonstrate that the truncated tail-less αβ₁ integrin retains an intact ligand-binding ability in vitro and indicate that deletion

antibodies, an extent of cell adhesion comparable to that observed on the laminin 5 matrix. The 0 point on the abscissa corresponds to the values measured in the absence of blocking antibodies. All the adhesion assays were conducted in triplicate and standard deviations, and did not exceed 22% of each mean value. (D) Microtiter wells were coated with purified, partially recombinant αβ₁ integrin (25 ng/well) and incubated with the indicated concentrations of radiolabeled ligands in presence of 1 mM MnCl₂ for 4 h at room temperature. Bound ligand was measured in a gamma counter. Each point represents the mean of duplicates from a representative experiment. Nonspecific binding did not exceed 25% of total binding and was subtracted for each dose.
of the β4 cytoplasmic domain does not result in a gross conformational change in the extracellular domain of the integrin.

**High-Level Expression of Head-Less or Tail-Less Recombinant β4 Does Not Suppress αβ4-dependent Adhesion and Spreading**

To test the effects of the two recombinant truncated β4 subunits on cell adhesion, adhesion assays were performed with the control clone Z10, the head-less β4 expressing clone F28, and the tail-less β4 expressing clones B13, B23, and B29. To selectively analyze the function of αβ4, the cells were plated on laminin 4 and 5 in the presence of antibodies capable of blocking endogenous β integrins. Fig. 6A shows that the clones B13, F28, and B29 adhered to the laminin 5 matrix deposited by the RAC-11/PD cells with kinetics and to an extent similar to that of control clone Z10. In addition, the clones B13, F28, and B29 adhered to wells

**Figure 6. Measurement of αβ4-dependent adhesion in cells expressing the head-less or tail-less recombinant β4 subunit.** The indicated clones were incubated in presence of a 1:50 dilution of the anti-beta 1 antiserum for varying times on wells coated with the laminin 5 matrix (A), in presence of a 1:50 dilution of the anti-beta 1 antiserum for 30 min on wells coated with varying amounts of laminin 4 (B), or in the absence of anti-beta 1 antiserum for 30 min on wells coated with varying amounts of fibronectin (C). The assays were conducted in triplicate and standard deviations did not exceed 18% of each mean value. The lower maximal adhesion observed in C reflects a lower input of cells in this particular experiment.
coated with increasing amounts of laminin 4 (Fig. 6 B) or fibronectin (Fig. 6 C) to an extent similar to that of control clone Z10. Thus, neither the head-less nor the tail-less β₄ subunit can suppress α₅β₄-dependent adhesion to laminin 4 or 5.

**Overexpression of a Mutant β₄ Subunit Lacking Extracellular Sequences Does Not Affect Hemidesmosome Assembly**

To determine if high level expression of the head-less β₄ integrin subunit affects the assembly and/or stability of

Figure 7. Immunofluorescent detection of recombinant full-length and head-less β₄ subunits in hemidesmosomes. Cells of the control clone Z10, the full-length β₄ expressing clone A12, and the head-less β₄ expressing clone F28 were cultured on glass coverslips for 48 h, and then were either fixed directly with cold methanol (A and B) or extracted with 0.2% Triton X-100 before fixation in methanol (C-H). Clone A12 cells were stained with the anti-human β₄ monoclonal antibody 3E1 (A) and clone Z10 with the anti-c-myc monoclonal antibody Ab-1 (B) followed by FITC-conjugated goat anti-mouse IgGs. Clone A12 cells were doubly stained with the 3E1 monoclonal antibody and the rabbit anti-BPAG2 antibody followed by Texas red-conjugated goat anti-mouse IgGs and FITC-labeled goat anti-rabbit IgGs (3E1 staining in C, and BPAG2 staining in E). Clone F28 cells were doubly stained with the monoclonal antibody Ab-1 and with the rabbit anti-BPAG2 antibody followed by Texas red-conjugated goat anti-mouse IgGs and FITC-labeled goat anti-rabbit IgGs (Ab-1 staining in D, and BPAG2 staining in F). Clone A12 and F28 cells were also stained with anti-BPAG1 human antibodies followed by FITC-labeled goat anti-human IgGs (G and H, respectively).
hemidesmosomes, we compared the subcellular localization of the head-less $\beta_4$ and that of two cytoskeletal markers of hemidesmosomes, BPAG1 and 2, in clone F28 cells with the distribution of recombinant full-length $\beta_4$ and BPAG1 and 2 in clone A12 cells. As shown in Fig. 7 A, immunofluorescent staining with the 3El monoclonal antibody indicated that the recombinant full-length $\beta_4$ subunit is concentrated at the basal surface of clone A12 cells within granular structures, possibly representing individual hemidesmosomes. These structures often merged into patches, but were excluded from circular areas thus generating a distinctive "Swiss-cheese"-like pattern. Extraction of clone A12 cells with a buffer containing 0.2% Triton X-100, before fixation and incubation with the 3El monoclonal antibody, did not affect the intensity of the staining associated with hemidesmosomes, but eliminated the immunofluorescence originating from the cytoplasm or the plasma membrane outside hemidesmosomes (Fig. 7 C), indicating that the recombinant full-length $\beta_4$ subunit within hemidesmosomes is largely resistant to extraction with nonionic detergents. Double immunofluorescent staining with the 3El monoclonal antibody and with rabbit polyclonal antibodies to BPAG2 revealed a precise colocalization of the recombinant $\beta_4$ molecule and the hemidesmosomal marker in the Triton X-100 resistant structures (Fig. 7 C and E). In addition, immunofluorescence with antibodies to BPAG1 resulted in a staining pattern similar to that generated by the 3El and BPAG2 antibodies (Fig. 7 G). These results indicate that the recombinant full-length $\beta_4$ subunit and BPAG1 and 2 colocalize in hemidesmosomes in clone A12 cells and that within these structures these mol-

Figure 8. Lack of detection of recombinant tail-less $\beta_4$ subunit and endogenous $\alpha_6$ and $\beta_4$ subunits in hemidesmosomes in clone B13. Cells of the tail-less $\beta_4$ expressing clone B13 were cultured on glass coverslips for 48 h, and then were either fixed with cold methanol (A) or extracted with 0.2% Triton X-100 before fixation in methanol (B, D, and E). Cells of the control clone Z10 were extracted with 0.2% Triton X-100 before fixation in methanol (C and F). Clone B13 cells were stained with the anti-human $\beta_4$ 3El monoclonal antibody followed by FITC-conjugated goat anti-mouse IgGs (A and D). Cells of the B13 and Z10 clones were stained with the $\alpha_6$ (B and C) or the $\beta_4$ cytoplasmic domain antibody (E and F) followed by FITC-labeled goat anti-rabbit IgGs.
ecules are largely resistant to extraction with 0.2% Triton X-100.

We next analyzed the subcellular distribution of the overexpressed recombinant head-less $\beta_4$ subunit and BPAG1 and 2 in clone F28 cells. The transfectants were extracted with Triton X-100 and stained with the monoclonal antibody Ab-1 reacting with the c-myc epitope tag included in this recombinant truncated $\beta_4$ molecule. The antibody generated negligible staining in control cells of the Z10 clone (Fig. 7B), but reacted prominently with hemidesmosomal structures in clone F28 cells (Fig. 7D). Double immunostaining experiments demonstrated a precise colocalization of the recombinant head-less $\beta_4$ with BPAG2 (Fig. 7, D and F), BPAG1 (Fig. 7 H, BPAG1 staining only is shown), and the endogenous $\alpha_6$ subunit (not shown). These results suggest that the overexpressed recombinant head-less $\beta_4$ subunit accumulates in hemidesmosomes without causing any apparent redistribution of the endogenous $\alpha_6\beta_4$ integrin and the BPAG1 and 2 antigens normally associated with hemidesmosomes.

Electron microscopic analyses were conducted to examine the structural integrity of hemidesmosomes in clone F28 cells. Cells of the control clone Z10 and the head-less $\beta_4$ expressing clone F28 were cultured for 48 h on laminin 4-coated Aclar coverslips, and then fixed. Vertical sections, cut perpendicularly to the substratum, were examined with the electron microscope. The results showed that control cells had a number of submembranous densities associated with the basal cell surface in correspondence of substratum attachment sites (see Fig. 11 A). The appearance of these structures was similar to that of the previously described hemidesmosomes in 804G cells in that they contained a relatively well defined inner plaque (Ridelle et al., 1991). A double-blind analysis indicated that the clone Z10 had an average of 6.6 hemidesmosomes per cell section. Analysis of clone F28 cells indicated that these structures were neither significantly diminished (6.2 per cell per vertical section) nor altered (Fig. 11B). Thus, Z10 and F28 cells appear to have similar numbers of normally appearing hemidesmosomes. From the immunofluorescence and electron microscopy observations, we concluded that expression of a head-less $\beta_4$ subunit, at levels 5.3-fold higher than those of endogenous wild-type $\beta_4$, does not affect hemidesmosome assembly and/or stability.

Overexpression of a Mutant $\beta_4$ Subunit Lacking the Cytoplasmic Domain Disrupts Hemidesmosomes

Immunofluorescence experiments were performed to determine if the tail-less recombinant $\beta_4$ subunit has a dominant

Figure 9. Lack of detection of BPAG1 and BPAG2 in hemidesmosomes in clone B13. Cells of the tail-less $\beta_4$ expressing clone B13 (A and B) or the control clone Z10 (C and D) were cultured on glass coverslips for 48 h, extracted with 0.2% Triton X-100, and then fixed in methanol. The cells were stained with the rabbit anti-BPAG2 antibody followed by FITC-labeled goat anti-rabbit IgGs (A and C) or with human antibodies to BPAG1 followed by FITC-labeled goat anti-human IgG (B and D).
Figure I0. Altered distribution of BPAG2 in clones expressing different levels of recombinant tail-less $\beta_4$. Control clone Z10 (A), clones B29 (B), and B23 (C) which express moderate levels of tail-less $\beta_4$, and clone B13 (D), which expresses high levels of tail-less $\beta_4$, were cultured on glass cover-slips for 48 h, extracted with 0.2% Triton X-100, and then fixed in methanol. The cells were stained with the rabbit anti-BPAG2 antibody followed by FITC-labeled goat anti-rabbit IgGs.

A negative effect on hemidesmosome assembly and/or stability. Staining of clone B13 cells with the 3El monoclonal antibody indicated that the tail-less recombinant $\beta_4$ subunit was diffusely distributed at the surface of these cells (Fig. 8 A). Extraction with 0.2% Triton X-100 before fixing and antibody incubation resulted in an almost-complete loss of staining (Fig. 8 D). Thus, in contrast to the recombinant wild-type $\beta_4$, which is largely insoluble in Triton X-100, the tail-less $\beta_4$ subunit is soluble in nonionic detergent, presumably because it cannot establish proper cytoskeletal connections.

We next wondered if expression of the tail-less mutant subunit could affect the incorporation in hemidesmosomes of wild-type endogenous $\alpha_6\beta_4$. Cells of the control clone Z10 and the tail-less $\beta_4$ expressing clone B13 were treated with Triton X-100 and stained with affinity-purified antibodies to synthetic peptides reproducing the cytoplasmic domain of either $\alpha_6$ or $\beta_4$. As shown in Fig. 8, B and E, although some punctuate staining was occasionally observed (arrows), neither antibody detected significant amounts of $\alpha_6\beta_4$ at the basal surface of clone B13 cells. In contrast, both antibodies generated a Swiss-cheese-like staining in clone Z10 cells (Fig. 8, C and F). This finding suggests that expression of tail-less $\beta_4$ prevents the incorporation of endogenous $\alpha_6\beta_4$ integrin in hemidesmosomes.

To examine the integrity of hemidesmosomes in cells over-expressing the tail-less $\beta_4$ subunit, cells of the control clone Z10 and of the tail-less $\beta_4$ expressing clone B13 were extracted with Triton X-100 and stained with antibodies to BPAG1 and 2. As shown in Fig. 9, A and B, the two hemidesmosomal proteins were largely absent from the basal surface of Triton X-100-treated cells of the B13 clone. Although some residual granular staining could be detected in a minor percentage of cells, the BPAG1 and 2 positive granules detected in clone B13 were limited to restricted areas of the basal surface (arrows in panels A and B) and rarely generated a Swiss-cheese-like pattern (open arrow in panel A). Moreover, the general distribution of BPAG2 was less altered than that of BPAG1. In contrast with the results obtained with clone B13, both the BPAG1 and the BPAG2 antibody generated a Swiss-cheese-like staining at the basal surface of control clone Z10 (Fig. 9, C and D). Control immunoprecipitation experiments from metabolically labeled cells indicated that the biosynthesis of BPAG1 and BPAG2 was not decreased in clone B13 as compared to control clones Z10 and Z32 (data not shown). These results suggest that expression of the tail-less mutant integrin subunit interferes with the assembly or stability of hemidesmosomes.

To determine if the extent of disruption of hemidesmosomal markers in cells expressing the tail-less $\beta_4$ subunit was proportional to the level of expression, we analyzed clones B29 and B23, in which the ratio of recombinant tail-less $\beta_4$ to endogenous $\beta_4$ is 5 times lower than in clone B13. Cells of the control clone Z10 and tail-less $\beta_4$ expressing clones B13, B29, and B23 were treated with Triton X-100 and stained with antibodies to BPAG2. As shown in Fig. 10, the altered distribution of BPAG2 was much less pronounced in clones B29 (panel B) and B23 (panel C) than in clone B13 (panel D), and in many instances the granular basal staining generated by the two antibodies merged at least partially into patches and occasionally into a Swiss-cheese-like pattern. Thus, the effect of tail-less $\beta_4$ on hemidesmosomes is proportional to its level of expression.
To obtain direct evidence of the effect of tail-less $\beta_4$ on hemidesmosomes, cells of the control clone Z10 and the tail-less $\beta_4$ expressing clone B13 were cultured for 48 h on laminin 4-coated Aclar coverslips and analyzed by electron microscopy. The result of these experiments indicated that clone B13 cells had a greatly diminished number of submembranous densities associated with the basal cell surface as compared with cells of the control clone Z10 (Fig. 11, C and D). A double-blind evaluation of the results indicated that clone B13 cells contain an average of 0.12 submembranous densities.

Figure 11. Electron microscopic analysis of cells expressing the recombinant head-less or tail-less $\beta_4$ subunit. The control clone Z10 (A), the head-less $\beta_4$ expressing clone F28 (B), and the tail-less $\beta_4$ expressing clone B13 (C and D) were grown for 48 h on laminin 4-coated Aclar plastic coverslips. Cross-sections of the cells were obtained and processed for electron microscopy as described in Materials and Methods. Large arrowheads in A point to hemidesmosomal structures at the basal surface of the control clone Z10. Small arrowheads in B point to similar structures at the basal surface of clone F28 cells. Bars: (A and B) 0.25 $\mu$m; (C) 1 $\mu$m; (D) 0.5 $\mu$m.
densities per vertical section. These densities were not as well organized as the hemidesmosomes of clone Z10 or 28 and rarely contacted the substratum (see Fig. 11 D for one example). Since the immunofluorescence studies indicated that the distribution of BPAG2 is less disrupted than that of other hemidesmosomal components in clone B13, it is possible that the residual submembranous densities detected in this clone during the electron microscopic analysis represent small aggregates of BPAG2. Finally, it was evident from the electron microscopic analysis that clone B13 cells did not form an extended contact with the substratum and were rounder than control cells (Fig. 11 C). Thus, although clone B13 cells can adhere well after being plated on laminin 4 and 5 for the short incubation times of the adhesion assay (see for example Fig. 6 A and B), they acquire a less adhesive morphology after a more prolonged period of culture. Taken together, the results of the immunofluorescence and electron microscopic analyses indicate that expression of a mutant tail-less β subunit disrupts the hemidesmosomes of 804G cells, and that this disruption is accompanied by the acquisition of a less adhesive morphology as compared to that of control cells.

**Discussion**

In this study we report that high-level expression of a recombinant tail-less integrin β subunit in 804G bladder epithelial cells disrupts hemidesmosomes without affecting αβ-mediated adhesion. Therefore, the tail-less β subunit is a dominant negative mutant which selectively interferes with the association of the αβ integrin with the hemidesmosomal cytoskeleton without perturbing its adhesive function. Two major conclusions can be drawn from this result. The first is that the αβ integrin plays a crucial role in promoting the assembly or maintaining the stability of hemidesmosomes: indeed αβ appears to be a necessary component of these structures, as its function cannot be replaced by the other transmembrane element of hemidesmosomes, BPAG2. The second major conclusion is that the ligand-binding function of the αβ integrin, as measured by adhesion assay, does not require stable association with the hemidesmosomal cytoskeleton. Thus, the αβ integrin appears to be regulated differently from β and β integrins which need to associate with the cytoskeleton to mediate efficient cell adhesion in vivo (Hayashi et al., 1990; Hibbs et al., 1991).

To analyze the consequences of dominant negative inhibition of αβ, we have conducted adhesion assays with the parental 804G cells and radioligand-binding studies with the purified partially recombinant αβ integrin. A major conclusion resulting from these experiments is that, in addition to laminin 5 and 1, αβ binds to laminin 2 and 4. Indeed, the affinity constant for binding to laminin 4 that we measured, 8.45 × 10⁻⁴ mol/liter, is higher than that reported for the binding of the αβ integrin to fibronectin (Hautanen et al., 1989). The observation that αβ is a receptor for laminin 2 and laminin 4 may help to understand the function of this integrin in Schwann cells. It has been proposed that αβ-mediated adhesion to the basement membrane plays a crucial role during myelination, because the expression of αβ is rapidly induced in Schwann cells at the onset of this process (Einheber et al., 1993). Since the Schwann cell base-
when expressed at levels ~5 times higher than the endogenous αβ4 integrin and did not interfere with αβ1-mediated adhesion. The lack of effect of this mutant integrin subunit is, at first glance, surprising. Mutant cadherin molecules of similar design have been shown to disrupt cell–cell adhesion in developing Xenopus embryos (Kintner, 1992). In addition, it has been recently shown that single-subunit chimeric molecules containing the β1, β1, or β3 cytoplasmic domain interfere with the ability of endogenous integrins to localize to focal adhesions, to mediate cell adhesion, migration and matrix assembly (LaFlamme et al., 1994; Lukashev et al., 1994), and to respond to intracellular regulatory signals (Chen et al., 1994). Since these chimeras localize to focal adhesions when expressed at low levels (Geiger et al., 1992; LaFlamme et al., 1992) and are capable of stimulating the activation of the focal adhesion kinase pp125 FAK (Akiyama et al., 1993; Lukashev et al., 1994), the dominant negative effect consequent to their high level expression has been attributed to the titration of intracellular factors essential for integrin activity. At least some of these factors may be common to several integrins sharing the same β subunit, as the effects observed with single-subunit chimeras is trans-dominant (LaFlamme et al., 1994; Lukashev et al., 1994). Our negative results with the head-less β4 subunit suggest that the function of the αβ4 integrin may be regulated differently than that of other integrins. If intracellular regulators of αβ1 exist, they may be different from those regulating other integrins. Alternatively, they may not be present in 804G cells in quantities low enough to allow titration.

The ability of tail-less β4 subunit to selectively interfere with hemidesmosome assembly in cultured cells suggests that it may be possible to examine the in vivo function of hemidesmosomes in transgenic mice, without disrupting initial cell adhesion to the basement membrane mediated by αβ4. It is likely that these junctions, which are formed in culture with relatively slow kinetics (our unpublished results), reinforce adhesion to the basement membrane and, indeed, our electron microscopy observations indicate that cells expressing the dominant negative β4 subunit are significantly rounder and more detached from the culture substratum than control cells. Finally, since a group of blistering skin diseases are caused by genetic or epigenetic factors acting on hemidesmosomal components (Uitto and Christiano, 1992), the introduction in transgenic mice of the dominant negative mutation described here may also provide information relevant to understanding the pathophysiology of this class of diseases.

We are indebted to Robert Burgeson and Mats Paulsson for providing purified laminin 5 and laminin 2, respectively. We are grateful to Bing-Cheng Wang, who has helped us to set up the radioligand-binding assay used in this study. We thank Paolo Bernardi, Robert Burgeson, Eva Engvall, Jo-David Fine, Erkki Ruoslahti, Arnoud Sonnenberg, Guido Tarone, and Joumi Uitto for cell lines and antibodies, and Alex Morla and James Salzer for critically reviewing the manuscript. Laura Spinardi was supported by a fellowship from the American Italian Foundation for Cancer Research. Filippo G. Giancotti is a recipient of an award from the Lucille P. Markey Charitable Trust. This work was supported by American Cancer Society grant CB-36 and National Institutes of Health grant CA-58976.

Received for publication 31 August 1994 and in revised form 23 December 1994.

References

Akiyama, S. K., S. S. Yamada, K. M. Yamada, and S. E. LaFlamme. 1994. Transmembrane signal transduction by integrin cytoplasmic domain expressed in single subunit chimeras. J. Biol. Chem. 269:15961–15964.
Bernardi, P., V. P. Patel, and H. F. Lodish. 1987. Lympoid precursor cells adhere to two different sites on fibronectin. J. Cell Biol. 105:489–498.
Caron, M. J. 1990. Induction of albumin gene transcription by extracellular matrix proteins. Mol. Cell. Biol. 10:1239–1243.
Carter, W. G., P. Kaur, S. G. Gil, P. J. Gahr, and E. A. Wayner. 1990. Distinct functions for integrins αβ1, β3, and αβ4/Bullous Pemphigoid antigen in a new stable anchoring contact (SAC) of keratinocytes: relation to hemidesmosomes. J. Cell Biol. 111:3141–3154.
Chen, Y.-P. T. E. O'Toole, T. Shipley, J. Forsyth, S. E. LaFlamme, K. M. Yamada, S. J. Shattil, and M. H. Ginsberg. 1994. Inside-out signal transduction inhibited by isolated integrin cytoplasmic domains. J. Biol. Chem. 269:18307–18310.
Chen, W. T., T. Hasegawa, C. Hasegawa, C. Weinstock, and K. M. Yamada. 1985. Development of cell surface linkage complexes in cultured fibroblasts. J. Cell Biol. 100:1103–1114.
Damsky, C. H., K. A. Knudsen, D. Bradley, C. A. Buck, and A. F. Horwitz. 1985. Distribution of the cell substratum attachment (CSAT) antigen on myogenic and fibroblastic cells in culture. J. Cell Biol. 100:1259–1259.
De Filippi, P., L. Stengo, and G. Tarone. 1992. αβ4 integrin (laminn receptor) is down-regulated by tumor necrosis factor alpha and interleukin-1 in human endothelial cells. J. Biol. Chem. 267:18303–18307.
Dejana, E., S. Colella, G. Contorfito, M. Abbodini, M. Gaboli, and P. C. Marchisio. 1988. Fibronectin and vitronectin regulate the organization of their respective Arg-Gly-Asp adhesion receptors in cultured human endothelial cells. J. Cell Biol. 107:1215–1223.
Einheber, S., T. A. Milner, F. G. Giancotti, and J. L. Salzer. 1993. Axonal regulation of Schwann cell integrin expression suggests a role for αβ1 in myelination. J. Cell Biol. 123:1223–1236.
Ehrig, K., I. Leivo, W. S. Argraves, E. Ruoslahti, and E. Engvall. 1990. Merosin, a tissue-specific basement membrane protein, is a laminin-like protein. J. Biol. Chem. 265:2326–2326.
Engvall, E., G. E. Davis, K. Dickerson, E. Ruoslahti, S. Varon, and M. Manthorpe. 1986. Mapping of domains in human laminin using monoclonal antibodies: localization of the neurite-promoting site. J. Cell Biol. 105:2457–2465.
Engvall, E., D. Earwicker, T. Haaparanta, E. Ruoslahti, and J. R. Sano. 1990. Distribution and isolation of four laminin variants; tissue restricted distribution of heterotrimers assembled from five subunits. Cell. Regulation. 1: 731–740.
Geiger, B., D. Salomon, M. Takeichi, and R. O. Hynes. 1992. A chimeric N-cadherin/β1-integrin receptor which localizes to both cell-cell and cell-matrix adhesions. J. Cell Sci. 103:943–951.
Giancotti, F. G., and E. Ruoslahti. 1990. Elevated levels of the αβ4 fibronectin receptor suppress the transformed phenotype of Chinese hamster ovary cells. Cell. 60:849–859.
Giancotti, F. G., L. Spinardi, F. Maniero, and R. Sanders. 1994. Expression of heterologous integrin genes in cultured eukaryotic cells. Methods Enzymol. 245:297–316.
Giancotti, F. G., G. Tarone, K. Knudsen, C. Damasky, and P. M. Comoglio. 1991. Cell surface glycoprotein expressed in human hemidesmosome-deficient cells. J. Clin. Invest. 87:936–943.
Hautanen, A., J. Gailit, D. M. Mann, and E. Ruoslahti. 1989. Two different iaminin domains mediate the differentiation of human endothelial cells. J. Cell Sci. 102:121–1223.
Hibbs, M. L., H. Xu, S. A. Stacke, and T. A. Springer. 1991. Regulation of adhesion to ICAM-1 by the cytoplasmic domain of LFA-1 integrin β subunit.
Hunter, D. D., V. Shah, J. P. Merlie, and J. R. Sanes. 1989. A laminin-like adhesive protein concentrated in the synaptic cleft of the neuromuscular junction. Nature (Lond.) 338:229-233.

Hynes, R. O. 1992. Integrins: versatility, modulation and signalling in cell adhesion. Cell. 69:11-25.

Izumi, K., Y. Hirao, L. Hopp, and R. Oyasu. 1981. In vitro induction of ornithine decarboxylase in urinary bladder carcinoma cells. Cancer Res. 41:405-409.

Jones, J. C. R., M. A. Kurpakus, H. M. Cooper, and V. Quarta. 1991. A function for the integrin α4β1 in the hemidesmosome. Cell Regulation. 2:427-438.

Kennel, S. J., L. J. Foote, L. Cimino, M. G. Rizzo, L.-Y. Chang, and A. Sacchi. 1993. Sequence of a cDNA encoding the β1 subunit of murine integrin. Gene (Amst.). 130:209-216.

Kintner, C. 1992. Regulation of embryonic cell adhesion by the cadherin cytoplasmic domain. Cell. 69:225-236.

Klein, G., M. Langegger, R. Timpl, and P. Ektblom. 1988. Role of laminin A chain in the development of epithelial cell polarity. Cell. 55:331-341.

Klein, S. F. G. Giancotti, M. Presta, S. M. Albelda, C. A. Buck, and D. B. Rifkind. 1993. Basic fibroblast growth factor modulates integrin expression and function for the integrin α5β1 in the hemidesmosome. J. Cell Biol. 113:907-917.

LaFlanune S. E., L. A. Thomas, S. S. Yamada, and K. M. Yamada. 1994. Integrin α4β1 complex is located in hemidesmosomes, suggesting a major role in epidermal cell-basement membrane adhesion. J. Cell Biol. 113:907-917.

LaFlanune S. E., L. A. Thomas, S. S. Yamada, and K. M. Yamada. 1994. Integrin α4β1 complex is located in hemidesmosomes, suggesting a major role in epidermal cell-basement membrane adhesion. J. Cell Biol. 113:907-917.

Lampl, S. J., L. J. Foote, L. Cimino, M. G. Rizzo, L.-Y. Chang, and A. Sacchi. 1993. Sequence of a cDNA encoding the β1 subunit of murine integrin. Gene (Amst.). 130:209-216.

Liang, C. Y., and C. E. Bacon. 1989. GABAergic neurons in rat hippocampal formation: ultrastructure and synaptic relationships with catacholaminergic terminals. J. Neurosci. 9:3410-3427.

Nies, C. M., F. Hughey, L. H. Japara, A. A. De Melker, G. O. Delwel, E. H. M. Huisman, I. Kuikman, and A. Sonnenberg. 1994. The α2β1 integrin is a receptor for both laminin and kalnin. Exp. Cell Res. 211:360-367.

Panayotou, G., P. End, M. Aumailley, R. Timpl, and J. Engel. 1989. Domains of laminin with growth factor activity. Cell. 56:93-101.

Paun, M., K. Saladin, and E. Engvall. 1991. Structure of laminin variants. J. Biol. Chem. 266:17545-17551.

Ridelle, K. S., K. J. Green, and J. C. R. Jones. 1991. Formation of hemidesmosomes in vitro by a transformed rat bladder cell line. J. Cell Biol. 112:159-168.

Rousselle, P., G. P. Lunstrum, D. R. Keene, and R. E. Burgess. 1991. Kalinin: an epithelium-specific basement membrane adhesion molecule that is a component of anchoring filaments. J. Cell Biol. 114:557-576.

Sanes, J. R., E. Engvall, R. Butkowski, and D. D. Hunter. 1990. Molecular heterogeneity of basal laminae: isoforms of laminin and collagen IV at the neuromuscular junction and elsewhere. J. Cell Biol. 111:1685-1699.

Schab-Burgier-Lever, G., C. E. Orfanos, and W. F. Lever. 1972. Electron microscopy study of Bullous Pemphigoid. Arch. Derm. 106:662-667.

Schlessinger, J., and A. Ullrich. 1992. Growth factor signalling by receptor tyrosine kinases. Neuron. 9:383-391.

Sonnenberg, A., J. Calafat, H. Janssen, H. Daams, L. M. H. van der Raaij-Helmner, R. Falcioni S. J. Keenel, J. D. Aplin, J. Baker, M. Loizidou, et al. 1991. Integrin α4β1 complex is located in hemidesmosomes, suggesting a major role in epidermal cell-basement membrane adhesion. J. Cell Biol. 113:907-917.

Sonnenberg, A., A. A. de Melker, A. M. Martinez de Velasco, H. Janssen, J. Calafat, and C. M. Niessen. 1993. Formation of hemidesmosomes in cells of a transformed murine cell line and mechanisms involved in adherence of these cells to laminin and calbindin. J. Cell Sci. 106:1083-1102.

Sprott, L., Y. L. Ren, R. Sanders, and F. G. Giancotti. 1993. The β1 subunit cytoplasmic domain mediates the interaction of α4β1 integrin with the cytoskeleton of hemidesmosomes. Mol. Biol. Cell. 4:871-884.

Stepp, M. A., S. Spurr-Michaud, A. Tisdale, J. Elwell, and J. Gipson. 1990. Alpha 6 beta 4 integrin heterodimer is a component of hemidesmosomes. Proc. Natl. Acad. Sci. USA. 87:8970-8974.

Streuli, C. H., N. Bailey, and M. J. Bissell. 1991. Control of mammalian epithelial differentiation: basement membrane induces tissue-specific gene expression in the absence of cell-cell interaction and morphological polarity. J. Cell Biol. 115:1385-1395.

Sunada, Y., S. M. Bernier, C. A. Kozak, Y. Yamada, and K. P. Campbell. 1994. Deficiency of merosin in dystrophic dy mice and genetic linkage of the murine M chain gene to dystrophic. J. Biol. Chem. 269:13729-13732.

Sunada, Y., and Y. Naitoh. 1990. Amino acid sequence of a novel integrin β1 subunit and primary expression of the mRNA in epithelial cells. EMBO (Eur. Mol. Biol. Organ.) J. 9:757-763.

Tamura, R. N., C. Rozzo, L. StarT, J. Chambers, L. Reichardt, H. M. Cooper, and V. Quarta. 1990. Epithelial integrin α6β4: complete primary structure of α and variant forms of β1. J. Cell Biol. 111:1593-1604.

Taron, G., M. A. Russo, E. Hirsch, T. Odoriso, F. Alturda, L. Silengo, and G. Siracusa. 1993. Expression of β1 integrin complexes on the surface of unfertilized mouse oocyte. Development. 117:1569-1575.

Uitto, J., and A. Christianson. 1992. Molecular genetics of the cutaneous basement membrane zone. J. Clin. Invest. 90:687-692.

Vogel, B. E., S. J. Lee, A. Hildebrand, W. Craig, M. Pierschbaeher, F. Wong-Yeh, and G. Siracusa. 1993. Expression of β1 integrin complexes on the surface of unfertilized mouse oocyte. Development. 117:1569-1575.

Uitto, J., and A. Christianson. 1992. Molecular genetics of the cutaneous basement membrane zone. J. Clin. Invest. 90:687-692.