The Laminin α2-Chain Short Arm Mediates Cell Adhesion through Both the α1β1 and α2β1 Integrins*

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Laminin-2, a heterotrimer composed of α2, β1, and γ1 subunits, is the primary laminin isoform found in muscle and peripheral nerve and is essential for the development and stability of basement membranes in these tissues. Expression of a domain VI-truncated laminin α2-chain results in muscle degeneration and peripheral nerve dysmyelination in the dy2J dystrophic mouse. We have expressed amino-terminal domains VI through IVb of the laminin α2-chain, as well as its laminin-1 α1-chain counterpart, to identify candidate cell-interaction functions of this critical region. Using integrin-specific antibodies, recognition sites for the α1β1 and α2β1 integrins were identified in the short arms of both laminin α1- and α2-chain isoforms. Comparisons with a β-α chimeric short arm protein possessing β1-chain domain VI further localized these activities to α-chain domain VI. In addition, we found that the laminin α2-chain short arm supported neurite outgrowth independent of other laminin-2 subunits. A heparin/heparan sulfate binding activity was also localized to this region of the laminin α2 subunit. These data provide the first evidence that domain VI of the laminin α2-chain mediates interactions with cell surface receptors and suggest that these integrin and heparin binding sites, alone or in concert, may play an important role in muscle and peripheral nerve function.

Members of the laminin family of glycoproteins are thought to be important for the development and stability of basement membranes, both through architecture-forming interactions with other laminins and matrix components and through recognition of cell signaling molecules such as integrins. EHS1 laminin, or laminin-1, is currently the best understood constituent of this family (reviewed in Refs. 1–4). Laminin-1 is composed of three unique polypeptide chains, α1, β1, and γ1, that form a large ~800-kDa heterotrimer composed of three short arms and one long arm. All other known laminin isoforms adopt a similar α-β-γ trimeric composition but assemble using varying combinations of unique α, β, and γ isoforms. Laminins 2 and 4, previously referred to as merosin, both contain the α2-chain subunit (5, 6). The α2-chain subunit is the primary laminin α-chain found in skeletal muscle and peripheral nerve basement membranes, but its specific interactions are currently less well understood than those of α1-chain of laminin-1. The importance of laminins 2 and 4 in these tissues, particularly muscle, is much clearer; mutations that result in the absence of the α2-chain cause autosomal recessive congenital muscular dystrophies, both in humans and in dy mice (7–10).

Several cell-interactive functions have been assigned to the carboxyl-terminal long arm of laminins 2 and 4, including α3β1, α6β1, α1β1, and α6β4 integrin recognition (11–14). It has also been shown that the G-domain at the carboxyl terminus of the laminin α2-chain provides a linkage between the extracellular matrix and the dystrophin-associated glycoprotein complex through its interaction with α-dystroglycan (see Refs. 15 and 16; reviewed in Ref. 17). The repertoire of known laminin α2-chains functions have thus far all been associated with its long arm; however, attention has recently focused on possible function(s) of the short arm following the identification of muscular dystrophies in which the amino-terminal short arm of the α2-chain is truncated or otherwise mutated (18–21). In the dy2J mouse, muscular dystrophy develops as the result of a truncation in α2-chain domain VI within the context of an otherwise functional heterotrimeric laminin molecule (18, 19). This mutation furthermore leads to peripheral nerve defects, also seen in the allelic dy mouse where the laminin α2 subunit is completely absent (7, 8). In contrast to the long arm functions, little is currently known about the α2-chain short arm and its potential role as a ligand for cell surface receptors.

In this study, we have expressed laminin short arm α2-chain and α1-chain proteins to identify and characterize cell recognition functions specific for these amino-terminal domains. We report here that the α2-chain of laminin contains two distinct integrin binding sites within its amino-terminal domain, recognizing both the α1β1 and α2β1 integrins. These cell recognition sites are conserved in domain VI of the laminin α1-chain isoform, as reported previously in the case of α1β1 integrin recognition (22–25). Furthermore, we show that the α2-chain short arm contains heparin binding sites that may mediate interactions with cell surface proteoglycans or other charged glycosaminoglycans. These shared integrin recognition sites and heparin-binding activities may act alone or in concert with α-chain domain VI polymer-forming sites (25, 26), modulating cell activity and matrix architecture.

MATERIALS AND METHODS

Expression Constructs—pC1S, a mammalian expression vector containing the CMV promoter, was used to express all described recombinant laminin proteins (27). Mouse laminin α1-chain construct α1(VI–IVb′), encoding domains VI, V, IVb and approximately ½ of domain IIIb, was described previously (25). A human laminin α2-chain partial cDNA (nucleotides 13–6927) in Bluescript KS was generously provided by Dr. Ulla Wewer (University of Copenhagen, Denmark) and Dr. Eva Cheng, Y.-S., Champliaud, M.-F., Burgess, R. E., Marinkovich, M. P., and Yurchenco, P. D. (1997) J. Biol. Chem. 272, in press.

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1 The abbreviations used are: EHS, Engelbreth-Holm-Swarm; PMSF, phenylmethylsulfonyl fluoride; HPLC, high pressure liquid chromatography.

2 H. Colognato and P. D. Yurchenco, unpublished observations.

3 Cheng, Y.-S., Champliaud, M.-F., Burgess, R. E., Marinkovich, M. P., and Yurchenco, P. D. (1997) J. Biol. Chem. 272, in press.
Engvall (Burnham Institute, La Jolla, CA). A stop codon was inserted at base 2564 in domain IIb of the α2-chain cDNA by blunt end ligation of oligonucleotide dGCTTAATTAATAGC at an AkI site. Construct α2VI-IVb/β1(VI) was generated by cloning a 2.7-kilobase pair SpeI-Puc1 fragment corresponding to the 5′-end of the α2 cDNA into pCIS at an XhoI site after being supplemented with a linker region. A complete SpeI digest and a partial BamHI digest was used to purify a fragment containing α1(VI-IVb)/pCIS in pCIS missing 1952 bases at the 5′-end. A SpeI-SstI fragment from the 5′-end of the mouse β1-chain cDNA was inserted into this region using a linker fragment generated by polymerase chain reaction. The linker piece extended from the SstI site through the BstEII site and was used using mouse β1 cDNA as a template with the following primers: 5′-TCCGAGAGAAGTTCTATACCGTGTTATATGGTGGTTGCGAGGAACTGCAATTGTGCACG-3′ (sense) and 5′-TTGTGACAGTTGC- ATTCC-5′ (antisense).

Purification of Recombinant Proteins—Human 293 cells (ATCC CRL 1573) were transfected by the calcium phosphate precipitation method as described by Chen and Okayama (28). DNA constructs were cotransfected with pSV2pac, a puromycin resistance plasmid kindly provided by Dr. Roswitha Nischt (University of Cologne, Germany). Antibiotic-resistant clones were obtained following several days of selection using 1 μg/ml puromycin. Conditioned medium from resistant colonies was screened in Western blots for the presence of recombinant laminin protein using antibodies specific for the corresponding laminin fragment only that expressed high levels of protein. Fractions were expanded and grown for 3 days before being harvested using a combination of affinity chromatography.

Protein Analysis—Purified proteins were prepared as described previously (26). The protein concentrations were determined by the method of Lowry et al. (27), and protein content was determined by absorbance at 280 nm. The concentration of recombinant proteins α1(VI-IVb), α2VI-IVb, and β1(VIα1(VI-IVb) was determined by colormetric assay. Proteins were separated by SDS-polyacrylamide gel electrophoresis using 3.5-12% linear gradient slab gels (30) and stained with Coomassie Brilliant Blue R250 (Bio-Rad). Glycerol rotary-shadowed replicas of proteins for electron microscopy were prepared as described previously (26). Cell Adhesion—HT1080 human fibrosarcoma cells (ATCC) were maintained in Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.) containing 10% fetal bovine serum (Gemini) in a 5% CO2 humidified atmosphere at 37 °C. PC12 rat pheochromocytoma cells were also maintained in these conditions, except that Dulbecco’s modified Eagle’s medium was supplemented with 10% horse serum and 5% fetal bovine serum. Both cell types were prepared for cell adhesion studies by overnight incubation in 10 μCi/ml [methyl-3H]thymidine (specific activity 85 Ci/mmol (Amersham Corp.)). Protein-coated wells were prepared by incubating proteins in 50 mM Tris-HCl, pH 7.4, 90 mM NaCl, 1 mM CaCl2 buffer at 4 °C overnight in half-area 96-well tissue culture plates (Costar). Plates were then washed three times with PBS containing 0.5% heat-inactivated bovine serum albumin and blocked for 2 h at 37 °C in the same buffer. Labeled cells were detached with 0.1% trypsin and 0.05% EDTA, washed once in Dulbecco’s modified Eagle’s medium containing 0.5% heat-inactivated bovine serum albumin, and resuspended in the same medium at approximately 6 × 104 cells/ml. For studies using integrin-blocking antibodies, cell suspensions were pre-incubated in the presence of the appropriate antibodies for 30 min at 37 °C. Fifty μl of cell suspension was added to each protein-coated well and incubated at 37 °C for 70 min. Plates were then washed three times with PBS to remove unattached cells, followed by treatment with 2% SDS for 1 h to solubilize remaining cells. Solubilized cell suspensions were added to scintillation mixture (Ecosint A, National Diagnostics), and radioactivity was determined using a model LS6000IC scintillation counter (Beckman Instruments). One hundred percent adhesion was defined as radioactivity of the total added cell suspension. Error bars represent S.E.

Antibodies—Antibodies against human integrin subunits were used at 10 μg/ml in cell adhesion studies unless otherwise stated. Mouse monoclonal antibodies against the α1 and α2 subunits of human β1 integrins were purchased from Upstate Biotechnologies Inc. Mouse monoclonal antibody against the α3-chain of the human α5β1 integrin was purchased from Chemicon. Monoclonal rat anti-human α6 integrin subunit (GoH3) was generously provided by Dr. Arnold Sonnenberg (Netherlands Cancer Institute, Amsterdam). Monoclonal antibody 3A3, a mouse IgG specific for the α1 subunit of rat α1β1 integrin (23), was generously provided by Dr. Sal Carbonetto (McGill University, Montreal). Ascites fluid containing monoclonal 3A3 was diluted at 1:50 in cell adhesion studies.

RESULTS

Expression and Characterization of Laminin α-Chain Short Arm Proteins—Previous studies investigating the role of laminin-2 in cell adhesion and signaling have used the entire three-chain ~800-kDa molecule (11–14, 31–34). Consequently, the interaction of whole laminin-2 with many different integrins has been reported, including α3β1, α6β1, α6β4, and α7β1 (11–14). In this study, we sought to identify cell-interaction functions found exclusively in the laminin α2-chain amino-terminal region as well as to draw comparisons to those found in the analogous region of laminin-1. To analyze this region in isolation, we used a mouse laminin α1-chain short arm protein α1(VI-IVb), described and characterized previously (25), as a model to design an analogous α2-chain protein. Human recombinant α2(VI-IVb) was expressed in 293 cells transfected with a mammalian expression vector (pCIS) containing the 5′-region of the α2-chain cDNA. This protein begins at the α2-chain amino terminus and extends through domains VI, V, and IVb, ending about a third of the way into domain VIa.

Preparation of Laminins from Tissue—Mouse laminin-1 was extracted from lathyritic EHS tumor tissue and purified as described by Yurchenco and Cheng (26). A preparation containing laminin isoforms with the α2-chain subunit (a mixture of laminins 2 and 3) was purified from human placenta using a modification of the procedure developed by (29). Briefly, homogenized human placentas were digested with bacterial collagenase (Worthington), followed by extraction with EDTA-containing buffer at 0 °C. The laminin-2/4 mixture was then purified from extracted material using ion exchange, gel filtration, and heparin affinity chromatography.

Protein Analysis—The concentration of laminin-1 and laminin-2 preparations was determined by absorbance at 280 nm. The concentration of recombinant proteins α1(VI-IVb), α2VI-IVb, and β1(VIα1(VI-IVb) was determined by colormetric assay. Proteins were separated by SDS-polyacrylamide gel electrophoresis using 3.5-12% linear gradient slab gels (30) and stained with Coomassie Brilliant Blue R250 (Bio-Rad). Glycerol rotary-shadowed replicas of proteins for electron microscopy were prepared as described previously (26).
 integrin recognition functions for each molecule (Fig. 1, lanes 1 and 2, respectively). Short arm recombinant proteins were purified to homogeneity from tissue culture medium, and in the case of α1(VI-IVb) and α2(VI-IVb) were purified using heparin affinity chromatography (see “Materials and Methods”). Analysis by SDS-polyacrylamide gel electrophoresis under reducing conditions revealed similar apparent molecular masses of 120–125 kDa for proteins α1(VI-IVb) (Fig. 1, lane 3), α2(VI-IVb) (Fig. 1, lane 4) and β1(Vl)α1(V-IVb) (Fig. 1, lane 5).

Morphology and domain structure of the three short arm proteins was compared in glycerol rotary shadows (Fig. 2). Electron microscopy of platinum/carbon replicas revealed that all three molecules have a dumbbell-like appearance with a short tail visible in some species. These observations fit the predicted domain structure of the laminin short arm region, in which globular domains VI and IVb are separated by the rod-like EGF repeats of domain V. The stub-like extension seen in some molecules represents the one-third of domain IIIb present at the carboxyl terminus of these proteins.

Heparin Binds Laminin α1- and α2-Chain Short Arm Proteins through Domain VI—Studies using proteolytic fragments derived from laminin-1 have shown that both G-domain and domain VI of the laminin α1-chain contain distinct heparin binding activities (25, 37–38). HPLC heparin affinity chromatography was used to evaluate the laminin α2 subunit short arm, as well as the β1-α1 laminin short arm chimeric protein, for the ability to interact with heparin. Laminin α2-chain short arm protein α2(VI-IVb) bound heparin and was eluted from the column by 0.16 M NaCl, showing a reduced relative affinity compared with α1(VI-IVb), which required 0.19 M NaCl (Fig. 3). Chimeric protein β1(Vl)α1(V-IVb), lacking an α-chain domain VI, did not bind heparin at all. Both α-domain VI-containing proteins showed lower relative affinity than α1-chain G-domain fragment E3, which eluted at 0.27 M NaCl.

HT1080 Human Fibrosarcoma Cells Adhere to the α1- and α2-Chain Short Arms of Laminin Isoforms—Previous studies have shown that HT1080 cells interact with the carboxyl-terminal long arm of laminin-1 through the α6β1 integrin (36, 39). However, the same adhesion-blocking antibodies used to map the α6β1 integrin recognition site to this region are unable to block adhesion to full-size laminin-1, implying that other β1-class integrins participate in HT1080 adhesion to laminin-1, possibly recognizing the short arm region. HT1080 adhesion to laminin-1, a mixture of laminins 2 and 4 (both α2-chain-containing laminins), and their respective α-chain short arm proteins, α1(VI-IVb) and α2(VI-IVb), was assayed over a range of substrate concentrations (Fig. 4). The cells adhered to full-size laminin isoforms and to α1(VI-IVb) and α2(VI-IVb); however, they did not adhere to chimeric protein β1(Vl)α1(V-IVb). From these observations we concluded that laminins 1, 2, and 4 contained additional cell recognition sites(s) present in their α-chain short arm regions. Furthermore, this activity could be neutralized to the amino-terminal globule, domain VI.

Integrins αβ1, αβ3, and αβ6 Mediate Adhesion of HT1080 Cells to Laminins 1, 2, and 4—A battery of integrin-blocking antibodies was used to assess which integrins were involved in the adhesion of HT1080 cells to laminin substrates. Cells were preincubated with integrin-blocking antibodies, alone or in combination, and then assayed for adhesion to full-size laminin substrates (Fig. 5). Although α6β1 integrin blocking antibodies
could partially inhibit HT1080 adhesion to laminin 1, a combination of α2β1 and α6β1 integrin-blocking antibodies was required to completely block adhesion (Fig. 5A), indicating that both receptors participate. Additionally, a combination of α3β1 and α6β1 integrin-blocking antibodies was more effective at blocking adhesion than α6β1 integrin-blocking antibodies alone. Since adhesion could be completely inhibited without inclusion of the α3β1 integrin-blocking antibodies, we suggest that an interaction between α3β1 integrin and laminin-1 is either not required in this cell type or only occurs when additional β1-class integrins are occupied.

In contrast, HT1080 cell adhesion to laminin-2/4 was only partially inhibited in the presence of α2β1 and α6β1 integrin-blocking antibodies (Fig. 5B). Treatment of cells with either α3β1 or α6β1 integrin-blocking antibodies alone resulted in a very modest decrease in adhesion to laminin-2/4, while the combination of both antibodies further decreased adhesion. A combination of α2β1, α3β1, and α6β1 integrin-blocking antibodies was the most effective blockade against HT1080 cell adhesion to the α2-chain-containing laminins; however, adhesion was not completely inhibited as for laminin-1. Laminins 2 and 4 differ from laminin-1 in integrin specificity by utilizing the α3β1 integrin in addition to α2β1 and α6β1.

The α2β1 Integrin Mediates Adhesion to the α1- and α2-Chain Short Arms of Laminin Isoforms—We next determined which integrin(s) mediated adhesion to domain VI of the α-chain short arm proteins (see Fig. 4). HT1080 cells were preincubated with α1β1 or α2β1 integrin-blocking antibodies and then added to wells coated with either α1VI-IVb' or α2VI-IVb' (Fig. 6). Cell adhesion was completely inhibited in the presence of α2β1 integrin blocking antibodies, whereas α1β1 integrin blocking antibodies had no effect. Therefore, the α1- and α2-chains of laminin isoforms both contain recognition sites for the α2β1 integrin, located in domain VI of the aminoterminal short arms.

The α1β1 Integrin Mediates Adhesion to Domain VI of Laminin α1- and α2-Chains—PC12 rat pheochromocytoma cells were used to determine whether the α2-chain short arm of laminin isoforms 2 and 4 possessed a recognition site for the α1β1 integrin, a function previously localized to the analogous region of the laminin α1-chain (25). Laminin short arm protein α2VI-IVb' was found to support adhesion of PC12 cells similar to α1(VI-IVb') (Fig. 7), and adhesion to both substrates was completely blocked by 3A3, a monoclonal antibody specific for the rat α1 integrin subunit. Chimeric protein β1(Vn)α1(V-IVb') did not support adhesion of PC12 cells, demonstrating that the recognition site for the α1β1 integrin is located in domain VI, a finding previously suggested using domain-specific blocking antibodies against laminin-1 (25).

Neurite Outgrowth of PC12 Cells Is Supported by the α2-Chain Short Arm of Laminin Isoforms 2 and 4—The α2-chain short arm of laminin was evaluated for its ability to support neurite outgrowth of nerve growth factor-primed PC12 cells (Fig. 8). Cells were maintained overnight on dishes coated with either EHS laminin-1, human placental laminin-2/4, α1VI-IVb', α2VI-IVb', or β1-chain proteolytic fragment E4 (short
clonal antibodies specific for a domain VI of the laminin-1, the first cell recognition activities to be assigned to this functional domain. Binding activities of long arm receptors contributed to neurite outgrowth of PC12 rat pheochromocytoma cells independently of substrate. Anti- m1 sites, a microtubule binding site, were seen on all substrates, with the exception of E4.

Domains VI of the laminin-1 α2-chain integrin binding sites are mediated by the α1β1 integrin. FTG-1080 cells were plated onto wells coated with either 10 μg/ml α1(VI-IVb)' or 10 μg/ml α2(VI-IVb)'. Monoclonal antibodies specific for α1β1 and α2β1 integrins were included at 10 μg/ml as specified. 10 μg/ml rat IgG was included as a negative control. Anti-α2β1 integrin completely blocked HT1080 cell adhesion on both substrates.

In the present study, two independent integrin recognition sites, α1β1 and α2β1, have been identified in domain VI of the laminin-1 α2-chain (Fig. 9). In addition, a heparin binding activity was identified in this domain, suggesting that this domain also interacts with either cell surface or matrix-bound glycosaminoglycans. The α1β1 and α2β1 integrin binding sites are the first cell recognition activities to be assigned to this functionally rich region of skeletal muscle laminin isoforms 2 and 4. We have also shown that these recognition sites are found in domain VI of the laminin-1 α-chain. In addition, the short arm regions of laminin α1- and α2-chains mediate neurite outgrowth of PC12 rat pheochromocytoma cells independently of long arm receptor contributions.

To assign these functions to domain VI, we compared the binding activities of α1- and α2-chain short arm proteins with a chimera in which domain VI was replaced by domain VI of β1. Domain VI of the β1-chain was chosen as an ideal control for cell adhesion studies, since a proteolytic fragment containing this domain was previously unable to support adhesion of the cell types used here (25, 37). Native morphology appeared to be maintained in all three short arm molecules, appearing as similar dumbbell-shaped molecules representing globular domains VI and IVb separated by a rod-like domain V.

The ability of the α2-chain short arm to support heparin interactions was assessed using HPLC heparin affinity chromatography. We found that the short arm of the α2 subunit isoform bound heparin, although its relative affinity was less than that of its α1-chain counterpart. Both short arm proteins had a lower relative affinity than carboxyl-terminal G-domain fragment E3, which has been shown to mediate a heparin-sensitive interaction with dystroglycan, a molecule that provides a linkage between the dystrophin-glycoprotein complex and the extracellular matrix in skeletal muscle (15, 16). Arginine and lysine are two basic amino acids thought to be essential in mediating electrostatic interactions with the negatively charged sulfate and carboxylic acid groups of heparin (40). Domains VI of the laminin-1 α1- and α2-chains have net positive charges of +9 and +2, respectively. It is interesting to note that domain VI of the dystrophic dY2 mouse α2-chain contains an internal deletion that results in a net loss of basic residues, reducing the net charge to zero (19). It is possible that loss of these positively charged residues could disrupt a crucial interaction with cell surface molecules containing negatively charged groups such as heparan sulfate or polysialic acid.

We evaluated cell recognition functions of these short arm proteins in comparison with those of intact EHS laminin-1 and a mixture of human placental laminins 2 and 4 (α2-chain-containing isoforms). Previous studies have identified several integrin binding sites within the carboxyl-terminal long arms of laminins 1, 2, and 4, including α3β1, α6β1, α7β1, and α6β4 (11, 12, 14, 41–44). Studies using large proteolytic fragments of laminin-1 (E1' and E1X) have shown that the α1β1 and α2β1

![Image](https://via.placeholder.com/150)

**FIG. 6.** HT1080 cell adhesion to α1(VI-IVb)' and α2(VI-IVb)' is mediated by the α2β1 integrin. HT1080 cells were plated onto wells coated with either 10 μg/ml α1(VI-IVb)' or 10 μg/ml α2(VI-IVb)'. Monoclonal antibodies specific for α1β1 and α2β1 integrins were included at 10 μg/ml as specified. 10 μg/ml rat IgG was included as a negative control. Anti-α2β1 integrin completely blocked HT1080 cell adhesion on both substrates.

![Image](https://via.placeholder.com/150)

**FIG. 7.** PC12 cell adhesion to α1(VI-IVb)' and α2(VI-IVb)' is mediated by the α1β1 integrin. PC12 rat pheochromocytoma cells were plated onto wells coated with either 10 μg/ml α1(VI-IVb)' or 10 μg/ml α2(VI-IVb)'. Monoclonal antibody 3A3, specific for the rat α1β1 integrin, was used at 1:100; control mouse IgG was used at 10 μg/ml. Anti-α1β1 integrin mAb blocked adhesion to both substrates.

![Image](https://via.placeholder.com/150)

**FIG. 8.** Neurite outgrowth of PC12 cells is supported by both α1(VI-IVb)' and α2(VI-IVb)'. PC12 cells primed for 7 days in the presence of nerve growth factor were grown overnight at 37 °C on tissue culture dishes coated with either 0.1 μg/ml laminin-1, laminin-2/4, α1(VI-IVb)', α2(VI-IVb)', β1-chain short arm fragment E4, or bovine serum albumin. At 22 h, cells showed extensive neurite formation on laminin-1 and laminin-2/4, as well as on their representative α-chain short arm proteins, α1(VI-IVb)' and α2(VI-IVb)'. E4, a proteolytic fragment of laminin-1 consisting of domains VI and V of the β1-chain, did not support neurite outgrowth. Bovine serum albumin also did not support neurite outgrowth (not shown).

**FIG. 9.** Neurite outgrowth of PC12 cells is supported by both α1(VI-IVb)' and α2(VI-IVb)'. PC12 cells primed for 7 days in the presence of nerve growth factor were grown overnight at 37 °C on tissue culture dishes coated with either 0.1 μg/ml laminin-1, laminin-2/4, α1(VI-IVb)', α2(VI-IVb)', β1-chain short arm fragment E4, or bovine serum albumin. At 22 h, cells showed extensive neurite formation on laminin-1 and laminin-2/4, as well as on their representative α-chain short arm proteins, α1(VI-IVb)' and α2(VI-IVb)'. E4, a proteolytic fragment of laminin-1 consisting of domains VI and V of the β1-chain, did not support neurite outgrowth. Bovine serum albumin also did not support neurite outgrowth (not shown).
in integrin recognition functions reside somewhere in the short arms (22–24, 33), with the αβ1 integrin recognition site specifically localized to domain VI of the α1-chain (25). To define the repertoire of laminin isoform binding integrins in HT1080 cells, we evaluated adhesion of these cells to full-size EHS laminin-1 and placental laminin-2/4 in the presence of various integrin-blocking antibodies.

We found that a combination of antibodies against the α2β1 and α6β1 integrins completely blocked adhesion of HT1080 cells on a substrate of laminin-1. These results demonstrate that the α2β1 and α6β1 integrins are required in order for HT1080 cells to mediate adhesion to laminin-1. However, since a combination of α3β1 and α5β1 integrin-blocking antibodies was more effective than α6β1 blocking antibodies alone, we suggest that laminin-1 may weakly associate with the α3β1 integrin as long as additional β1-class integrin sites are occupied. Despite these observations, the α3β1 integrin was clearly unable to support adhesion to laminin-1 in the absence of α2β1 and α6β1 interactions. In contrast, laminin isoforms 2 and 4 were found to utilize the α2β1, α6β1, and α3β1 integrins in mediating adhesion to HT1080 cells, with a combination of antibodies against these three integrins causing the most significant decrease in adhesion. These observations may address the discrepancy apparent in the finding that although the α3β1 integrin has been described as a receptor for laminin-1 (41), K562 cells transfected with the α3β1 integrin were unable to adhere to EHS laminin-1, whereas adhesion was detected on human placental laminins (12). Also consistent with our observations are studies involving sensory neurons of the dorsal root ganglion, where the α3β1 integrin is utilized more efficiently on a laminin-2/4 substrate than on an EHS laminin substrate (45).

We next examined whether the α2β1 integrin interacted with the laminin α1- and α2-chain short arm proteins. We found both α1- and α2-chain proteins mediated adhesion to HT1080 cells, whereas our chimeric α-chain protein containing β1-chain domain VI did not. Furthermore, adhesion on both substrates was completely blocked in the presence of α2β1 integrin-specific antibodies. The α2β1 integrin was first described as a receptor for laminin in studies using human umbilical endothelial cells (46). This integrin has since been shown to participate in epithelial tissue processes such as wound repair, inflammation, regulation of keratinocyte proliferation, and establishment of polarity (reviewed in Ref. 47). Several studies examining the role of extracellular matrix proteins in these processes in vivo support an interaction between both laminin-1 and laminin-2 with the α2β1 integrin. The α2β1 integrin exhibits a highly restricted distribution during epithelial branching morphogenesis of the developing lung, appearing only at the branching tips (48), and this distribution is spatially and temporally correlated with the expression of laminin-1 and collagen IV at sites of rapid matrix deposition. Branching morphogenesis of mammary epithelial cells grown on a mixture of laminin-1 and collagen IV has also been shown to be dependent on expression of the α2β1 integrin (49). Expression of the α2β1 integrin in developing and adult peripheral nerves and Schwann cells, tissues where α2-chain-containing laminins are the predominant laminin isoforms, has been observed in several studies (50–52).

Earlier studies have shown that α1β1 integrin-mediated cell attachment occurs via the short arms of laminin-1 (22–24), specifically through domain VI of the α1-chain (25). Data shown here demonstrate that the α2-chain short arm of laminin isoforms 2 and 4 also mediated cell adhesion through the α1β1 integrin, as well as supported neurite outgrowth. PC12 rat pheochromocytoma cells adhered and spread on dishes coated with laminin short arm α2-chain protein but were inhibited in the presence of antibodies specific for the α1 integrin subunit. Nerve growth factor-primed PC12 cells extended long, branching neurites when grown on α2-chain short arm protein, appearing similar to cells grown on full-size laminin-1, -2, or -4. Analysis of the developing avian embryo has shown that the α1β1 integrin is expressed in the central nervous system, sympathetic and spinal sensory ganglia, capillary endothelium, and skeletal, smooth, and cardiac muscle; although in the adult, expression becomes restricted to muscle and endothelium (53, 54). Studies have shown that the α1β1 integrin mediates neural crest cell migration on laminin-1 (55–57), and we suggest that the α1β1 integrin may mediate cell migration or adhesion during the development of tissues where laminin-2 and -4 isoforms play a prominent role, such as muscle or peripheral nerve (5, 58). Analysis of mice engineered to lack the α1 integrin subunit do not exhibit gross muscle or nerve defects, showing only subtle disturbances in cell adhesion and migration (59); however, the α2β1 integrin has an overlapping pattern of expression and ligand binding, and its presence may prevent such defects from occurring. Targeted disruption of the gene encoding the α2 integrin subunit as well as the generation of mice lacking both α1 and α2 integrin subunits are needed to more definitively assess the in vivo role of these integrin-laminin short arm interactions.

The dystrophic mouse d2α2 expresses a mutated laminin α2-chain in which domain VI is truncated (18, 19). The mutated
α2-chain assemblies with its β and γ subunit partners into laminin-2 or -4 heterotrimers,3 is secreted by cells and appears to be localized to the extracellular matrix (18, 19). The removal of domain VI from an otherwise functional laminin molecule nonetheless leads to progressive muscle degeneration characteristic of human congenital muscular dystrophies. At least four functions can now be assigned to this critical α2-chain domain: α1β1 and α2β1 integrin recognition, heparin binding, and polymer formation (described as being analogous to the heparan sulfate proteoglycan syndecan, along with the ends of the molecule in domains VI and G, suggesting a functional basis for this spatial clustering. The transmembrane heparan sulfate proteoglycan syndecan, along with the α5β1 integrin, has been proposed to function as a co-receptor for the matrix protein fibronectin (60). The co-localization of binding sites in laminin suggests that a similar pairing of integrin binding and cell surface proteoglycan binding may be involved in laminin-mediated cell attachment and signaling. The phenotype seen in the dy2 mouse indicates that one or more of these domain VI activities may be required to maintain normal muscle stability.

Peripheral nerve function is also disrupted in the dy2 dystrophic mouse, where dysmyelination results in large bundles of naked axons (61). The spinal roots show fewer Schwann cells than normal at birth, and many of the cells that are present do not differentiate. However, these uncommitted cells are capable of differentiation when transplanted into a normal environment (62). It may be that an intact laminin α2-chain domain VI is needed either for proper Schwann cell migration during development or to provide a differentiation cue to uncommitted precursor cells. It remains unclear whether the α2-chain domain VI integrin binding sites described here are involved in this process; however, the identification of these sites represents a significant step forward in our understanding of laminin α2-chain function and its requirement in muscle and peripheral nerve development and stability.

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