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# Rodent Myoblast Interactions With Laminin Require Cell Surface Glycoconjugates But Not Laminin Glycosyl Groups

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**Abstract** Laminin glycosyl groups are necessary for the spreading of murine melanoma cells which become attached to this glycoprotein. Laminin has been implicated in myogenesis but the potential role of its glycosyl groups in this process has not been examined. In this study we report the effects of the carbohydrate moieties of laminin on myoblast adhesion, spreading, and differentiation. Unglycosylated laminin from tunicamycin-treated cultures of a mouse cell line, M1536 B3, was used in the experiments. Glycosylated laminin from a murine tumor and from cultures of M1563 B3 cells served as controls. Cell binding experiments with C2C12 mouse myoblasts showed that the cells preferred a laminin-coated surface, compared to the uncoated plastic surface (nontissue culture wells). Myoblasts did not distinguish between glycosylated and unglycosylated laminin substrates. Both glycosylated and unglycosylated forms of laminin promoted myoblast growth and differentiation. In contrast, cells on uncoated plastic surfaces grew very slowly and did not further differentiate. The L6 rat myoblast response to glycosylated and unglycosylated laminin was the same. These results indicate that although rodent myoblasts in culture require a laminin substratum for spreading, growth, and differentiation on a proprietary plastic surface, laminin carbohydrates are not implicated in those cellular responses. In contrast, parallel studies using the lectin, Con A, indicate that cell surface glycoconjugates of myoblasts are implicated in the response of these cells to a laminin substratum. 

**Key words:** glycosylation, laminin, myoblasts, lectins, ConA

Laminin (Ln) is a major glycoprotein of basement membranes. Several isoforms of Ln occur in different tissues and at different stages of development. The most well characterized Ln, that from a mouse Englebreth-Holm-Swarm tumor, has three polypeptide chains, A (400 kDa), B1 (220 kDa), and B2 (200 kDa) [Timpl et al., 1979].

Ln plays an important role in growth, locomotion, and differentiation of many cell types; cell/Ln interactions are important for myogenesis. Ln substrata stimulate myoblast locomotion, proliferation [Ocalan et al., 1988; Goodman et al., 1989a,b], differentiation and fusion into myotubes [Foster et al., 1987; von der Mark and Ocalan, 1989]. The ability of myoblasts to locomote over laminin surfaces diminishes as they differentiate. Reduction in the number of their specific binding sites for Ln occurs while their receptor affinity does not change [Goodman et al., 1989a]. By contrast, Song et al. [1992] showed that $\alpha 7\beta 1$, an integrin selectively bound to Ln, is a developmentally regulated membrane glycoprotein of skeletal muscle. Its mRNA expression increases upon terminal differentiation and decreases in developmentally defective myoblasts; mutants which do not express $\alpha 7$ lose their ability to differentiate.

Ln is a highly glycosylated molecule. It contains from 12–15% carbohydrate [Arumugham et al., 1986; Fujiwara et al., 1988] to as much as 25–30% carbohydrate [Knibbs et al., 1989]. Mouse Ln has sixty-eight consensus sequences for potential asparagine glycosylation. The A chain has 43 potential N-glycosylation sites [Sasaki et al., 1988], the B1 chain has 11 poten-

Abbreviations used: CEF, chick embryo fibroblasts; ConA, concanavalin A; GalTase, β-1,4 galactosyltransferase; GLn, glycosylated laminin; Ln, laminin; MHC, myosin heavy chain; SW, swainsonine; ULn, unglycosylated laminin.

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tial sites [Sasaki et al., 1987a], and the B2 chain has 14 potential sites [Sasaki et al., 1987b]. EHS Ln contains only N-linked oligosaccharides [Arumugham et al., 1986; Knibbs et al., 1989]. However, Ln chains from a mouse embryonal carcinoma cell line contain large amounts of galactosamine, presumably in O-linked oligosaccharides [Chung et al., 1979]. The role(s) of Ln glycosylation in its biological functions is just emerging. Ln carbohydrates do not protect it from proteolytic degradation and they are not important for heparin binding to Ln or for Ln molecular assembly [Wu et al., 1988]. But Ln carbohydrates probably play an important role in cell-matrix interactions. In B16-F10 melanoma cells the cell surface enzyme β-1,4 galactosyltransferase (GalTase) functions as an Ln receptor, interacting with specific N-linked oligosaccharide residues. Cell spreading is dependent upon the binding of cell surface GalTase to Ln oligosaccharides but initial cell attachment to Ln is independent of GalTase activity [Runyan et al., 1988]. Cells migrating on Ln, but not on fibronectin, have three times the level of surface GalTase of stationary cells. Antibodies against GalTase inhibit cell migration on Ln-containing matrices [Eckstein and Shur, 1989]. For avian neural crest cells, reagents that inhibit surface GalTase activity inhibit cell migration on Ln, whereas reagents that stimulate the turnover of GalTase increase the rate of cell migration [Runyan et al., 1986].

Mouse melanoma cells, B16F1, and neuron-like PC12 rat pheochromocytoma cells bind to glycosylated (GLn) and unglycosylated (ULn) laminin, but B16F1 cells fail to spread on ULn while PC12 neurite outgrowth is defective [Dean et al., 1990]. Moreover, PC12 cells transfected with GalTase cDNA have increased levels of cell surface GalTase activity and also have enhanced neurite outgrowth when plated on Ln substrates [Begovac and Huang, 1993]. These facts confirm that glycosylation of Ln is important for normal biological function of melanoma and pheochromocytoma cells on this substrate.

Some carbohydrate structures seem more important for Ln biological function than others. Ln enriched in oligomannosides supports cell spreading and neurite outgrowth more effectively than laminin enriched in hybrid oligosaccharides [Chandrasekaran et al., 1991]. Spherical murine melanoma cells, adherent to unglycosylated laminin, selectively spread when titrated with oligomannosides [Chandrasekaran et al., 1994a,b; Tanzer et al., 1993]; other types of oligosaccharides failed to initiate spreading. Thus, Ln oligomannosides as well as surface GalTase are necessary for spreading and migration of some types of cells on Ln-coated surfaces as well as for neurite outgrowth.

In this study we investigated the importance of Ln glycosylation for myoblast binding, spreading, and differentiation in vitro. ULn from tunicamycin-treated cultures of the mouse cell line, M1536 B3, and control GLn from EHS tumor or from nontreated M1536 B3 cells were used in the experiments. Despite the fact that glycosylation of the Ln substrate is important for the response of some cell types, mouse C2C12 and rat L6 myoblasts in our experiments bound, spread, grew, and differentiated equally well both on GLn and ULn coated surfaces. The composite results show that although myoblasts require a laminin substratum for spreading, growth, and differentiation on a proprietary plastic surface, laminin carbohydrates are not implicated in those cellular responses. In contrast, studies using the lectin Con A, implicate myoblast surface glycoconjugates in the response of these cells to an Ln surface.

## MATERIALS AND METHODS

### Cell Culture

C2C12 mouse muscle myoblasts and L6 rat muscle myoblasts were obtained from ATCC (Rockville, MD). They were grown in DMEM (Gibco, Grand Island, NY) supplemented with 15% fetal bovine serum (HyClone), 0.5% chick embryo extract (Gibco), and 1% antibiotic/antimycotic mixture (Gibco). Myoblasts were induced to differentiate in DMEM with 2% horse serum (HyClone) and 1% antibiotic/antimycotic mixture. Hybridoma MF 20, producing antibodies to myosin heavy chain (MHC), was obtained from the Developmental Studies Hybridoma Bank which is under contract with NICHD. It was grown in DMEM supplemented with 15% fetal bovine serum, 1% antibiotic/antimycotic mixture, 0.1 mM hypoxanthine (Sigma Chemical Co., St. Louis, MO), and 0.025 mM thymidine (Sigma).

### Substrate Preparation

GLn was isolated from EHS sarcoma by the method of Timpl et al. [1979]. ULn and GLn were isolated from M1536 mouse embryonal carcinoma cells as previously described [Dean et

al., 1990]. Nontissue culture-treated 96-well plates (Becton Dickinson, Mountain View, CA) were coated with GLn or ULn at 1 or 5 μg/well in 100 μl of phosphate-buffered saline solution, pH 7.4 (PBS), and allowed to dry overnight. Wells were rinsed with PBS and residual binding sites were blocked with 1% bovine serum albumin solution (BSA, Sigma, RIA grade, preincubated for 20 min at 80°C) for 2 h at room temperature. Where indicated, wells were then treated with 100 μg/ml of Con A lectin (Vector Laboratories, Burlingame, CA) for 2 h on a shaking platform at room temperature (100 μl/well). In some experiments, lectin was directly added to the wells during the experiment (20 μg/well). Wells were washed 3 times with PBS and used in binding experiments.

### Myoblast Adhesion and Spreading Assay

C2C12 or L6 myoblasts (subconfluent culture) were harvested by brief trypsinization. Trypsin was inactivated by washing cells in a solution of soybean trypsin inhibitor (1 μg/ml, Sigma) in PBS. Cells were washed once in PBS, resuspended in serum-free DMEM, and counted by using a hemocytometer. Ten thousand cells per well, in 100 μl of serum-free DMEM, were added to the wells which already contained 100 μl of serum-free DMEM. Where indicated, Con A lectin was added to the wells to a final concentration of 100 μg/ml. Plates were incubated for 1 h in a $CO_2$ incubator (37°C, 6% $CO_2$), washed 2 times with PBS, fixed overnight at 4°C with 3% formaldehyde in PBS, and stained with a 1% Toludine blue (Sigma) solution containing 3% formaldehyde in PBS for 2 h at room temperature. The wells were rinsed 3 times with PBS and cell images were captured using an inverted microscope (Nikon, Japan) with a video camera (Optomax, Hollis, NH) attached to a video capture board installed in a Macintosh computer. Random fields from triplicate wells were chosen to obtain a statistically significant number of cells. These digital images were used for calculation of cell area. Image analysis software, Image, developed at the National Institutes of Health, Bethesda, MD, was applied for this purpose [Tanzer et al., 1993]. At least 150 cells were analyzed for each type of substrate used for the spreading assays. Mean cell area and standard error were determined for cells of each experimental protocol. Cells were photographed using an inverted microscope and attached camera (Nikon, Japan). All attachment assays, including numbers of cells bound and quantitation of cell areas, were carried out in seven independent experiments.

### Myoblast Growth and Differentiation Assay

Eight-well slide culture chambers (Nunc, Naperville, IL) were coated with GLn or ULn at a concentration of 10 μg/200 μl/well in PBS and dried overnight under UV light for sterilization. Nontreated wells with 200 μl of PBS per well were used as a control. Wells were rinsed with DMEM and C2C12 or L6 myoblasts were plated at 10,000 cells per well in 400 μl of DMEM/2% horse serum/1% antibiotic/antimycotic mixture (Gibco). Cells were allowed to grow for 3–7 days in a $CO_2$ incubator. Myoblasts were photographed, washed at 4°C in PBS, and fixed with 100% methanol for 15 min in a freezer. Differentiation was studied using immunofluorescence staining for MHC. Immunofluorescence assays were done according to standard methods. Spent culture medium from the MF 20 hybridoma was used as a source of primary antibody. Fluorescein isothiocyanate-conjugated goat anti-mouse IgG (Sigma) or Texas Red-conjugated donkey anti-mouse antibodies (Jackson Immunoresearch Laboratories, Inc., West Grove, PA) were used for immunodetection.

## RESULTS

### Myoblast Binding and Spreading on Glycosylated and Unglycosylated Laminin Substrates

C2C12 myoblasts attached and spread equally well on both types of laminin substrates (Fig. 1). After 30 min cells were well spread and showed typical myoblast morphology (Fig. 1A,B). The cells responded equally well when laminin was used at 1 μg/chamber instead of 5 μg/chamber (Fig. 1C,D). In contrast, in control wells with uncoated plastic surfaces the cells were round and did not spread (Fig. 1E). The same number of cells were bound to glycosylated and unglycosylated Ln substrates, as well as to plastic (data not shown). Cells did not adhere to wells coated only with 1% BSA (used in our experiments as a blocking agent for nonspecific binding). The same experiments were done in parallel with L6 rat myoblasts and these cells behaved similarly to the mouse myoblasts (not shown). The experiments using Con A lectin showed that lectin in the medium and lectin immobilized on the plastic or Ln substrates had different effects on C2C12 myoblast cell binding (Fig. 2). While

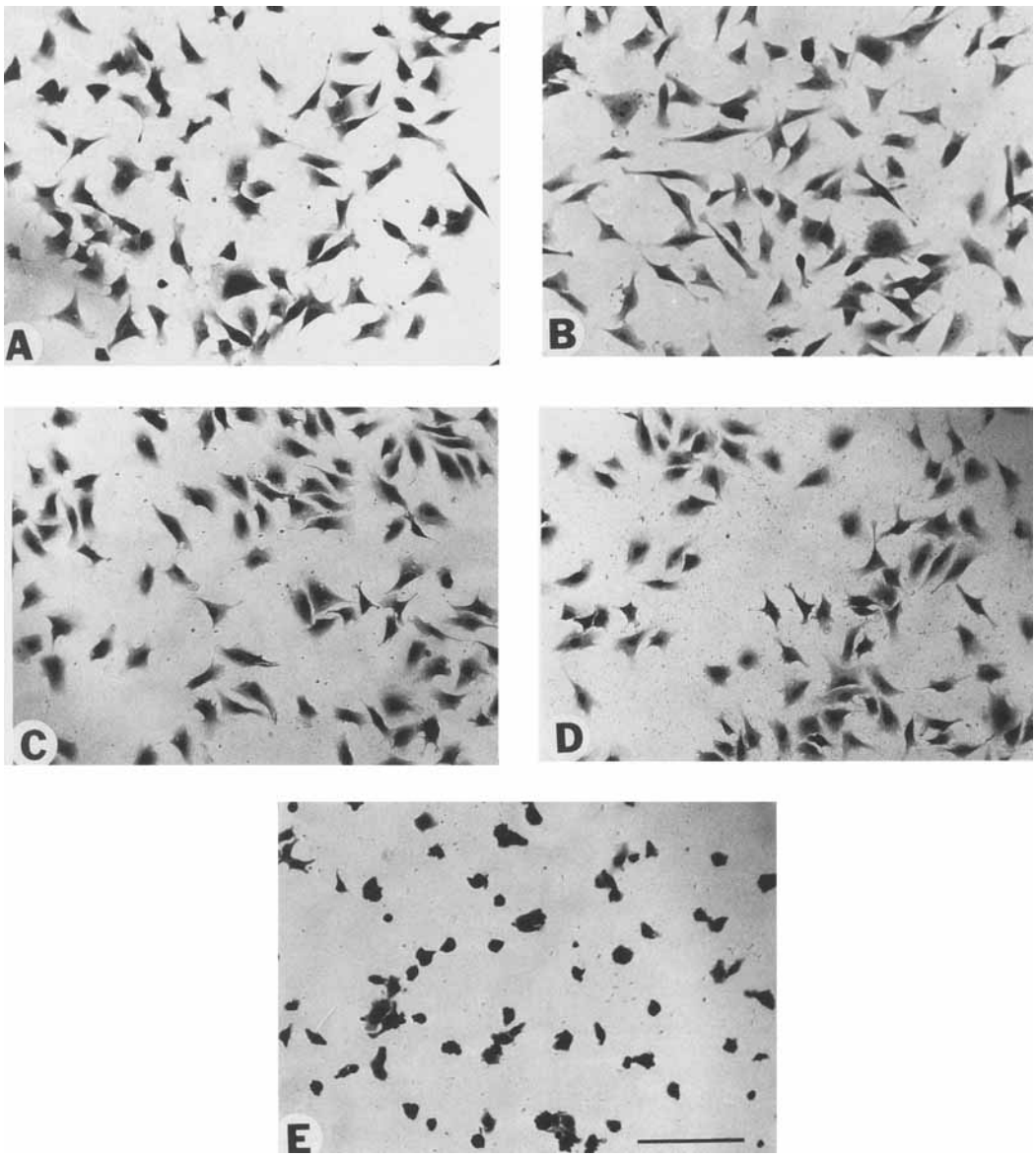


**Fig. 1.** Binding and spreading of C2C12 myoblasts on GLn, ULn substrates, and plastic. Cells were seeded into wells containing either 1 or 5 μg GLn/well (**A, C**), 1 or 5 μg ULn/well (**B, D**), or untreated wells (**E**). One hour after seeding the cells were fixed, stained, and micrographs were taken. The bar represents 75 μm.

soluble Con A lectin decreased myoblast binding and spreading on both GLn and ULn substrates (Fig. 2A and B, respectively) the same lectin immobilized on Ln substrates did not alter cell binding or spreading (Fig. 2C vs. D). Comparison of cell areas on different substrates was made (Fig. 3). The control area corresponded to cells attached to plastic. As shown, cells spread to a greater extent on both types of Ln than did cells bound to plastic. There was no significant difference between cells spread on GLn or ULn. Because the C2C12 myoblasts show variable morphology (cells vary from round to elongated) in some experiments the cell areas for both morphological groups were separately estimated. Cell areas, comparing the two morphological groups on the same Ln substrate, as well as between substrates, did not significantly differ (not shown).

### Myoblast Growth and Differentiation on GLn and ULn Substrates

Under suitable conditions, myoblasts undergo differentiation in cell culture (Fig. 4A,B). The

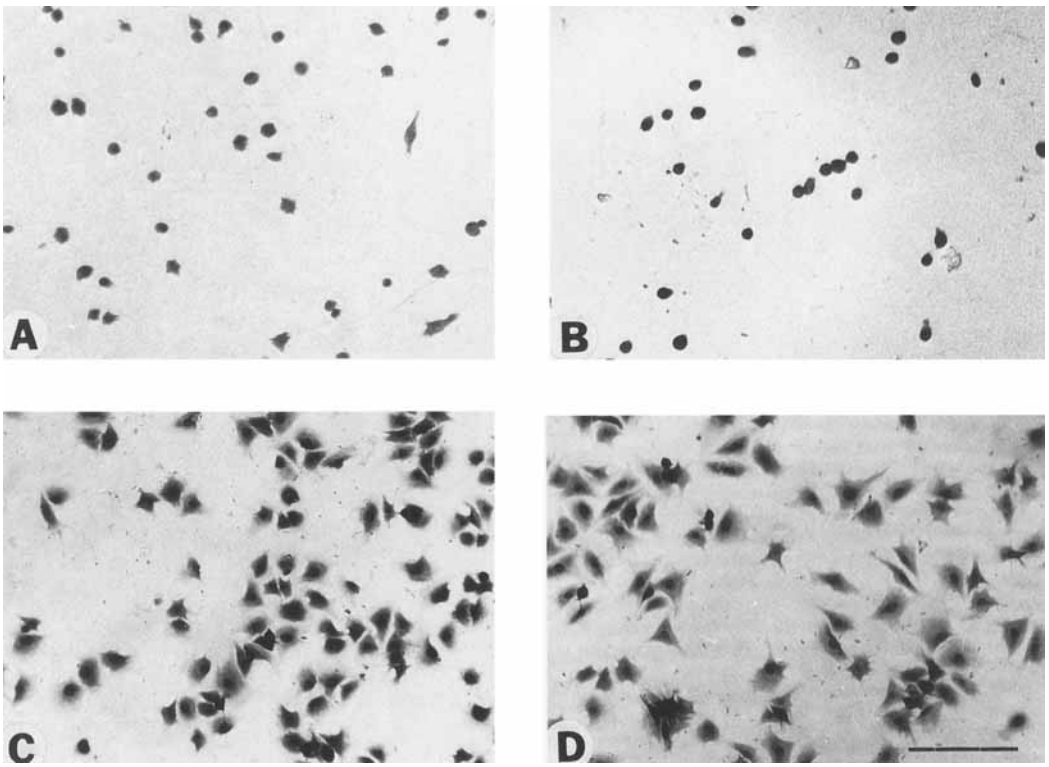


**Fig. 2.** Effect of Con A lectin on C2C12 myoblast spreading. Five micrograms of GLn (**A, C**) or ULn (**B, D**) were immobilized on plastic wells. Con A lectin was immobilized on laminin-coated plastic (**C, D**) or added with the cells during the experiment (**A, B**) as described in Materials and Methods. The bar represents 75 μm.

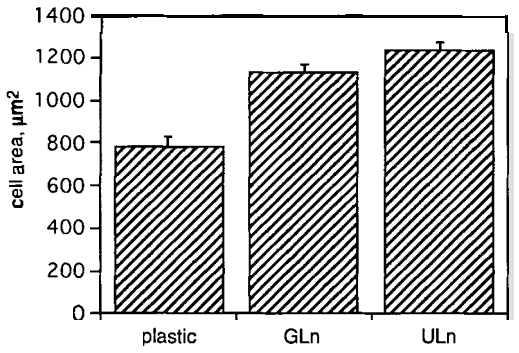


**Fig. 3.** C2C12 myoblast spreading on GLn and ULn substrates. The conditions were as described for Figures 1 and 2. Cell areas were estimated as described in Materials and Methods. Five micrograms of GLn or ULn per well was used. Untreated plastic surfaces were used as a control. At least 100 cells were analyzed in each case. The data from one (of 7 representative experiments) are summarized. The bars indicate standard error.

appearance of myosin heavy chain, as an index of differentiation, is shown in Figure 4D and it is not seen prior to differentiation (Fig. 4C). C2C12 myoblasts adhered to and differentiated on Gln and Uln surfaces equally well but failed to differentiate on uncoated plastic (Fig. 5). Cells adherent to the Ln-coated surfaces grew quickly and formed monolayers on the third day of culture (Fig. 5A,B). In contrast, cells adherent to plastic grew slowly, formed clumps, and never produced monolayers (Fig. 5C). MHC began to appear in cells on the 4th to 5th day after their adhesion to wells coated with either type of Ln (Fig. 6A,C). Cells adherent to plastic failed to show MHC immunostaining (Fig. 6E). MHC immunostaining progressively increased in cells adherent to either laminin surface (Fig. 6B,D) but did not appear in the control wells (Fig. 6F). Growth and differentiation on GLn and ULn were similar for the L6 myoblasts (not shown).

## DISCUSSION

Ln plays an important role in myoblast growth and differentiation [Foster et al., 1987; von der Mark and Ocalan, 1989]. Ocalan et al. [1988] and Goodman et al. [1989a] showed that laminin stimulated DNA synthesis, cell motility, and the characteristic bipolar morphology of MM14 mouse myoblasts. Ln substrates enhanced myo-

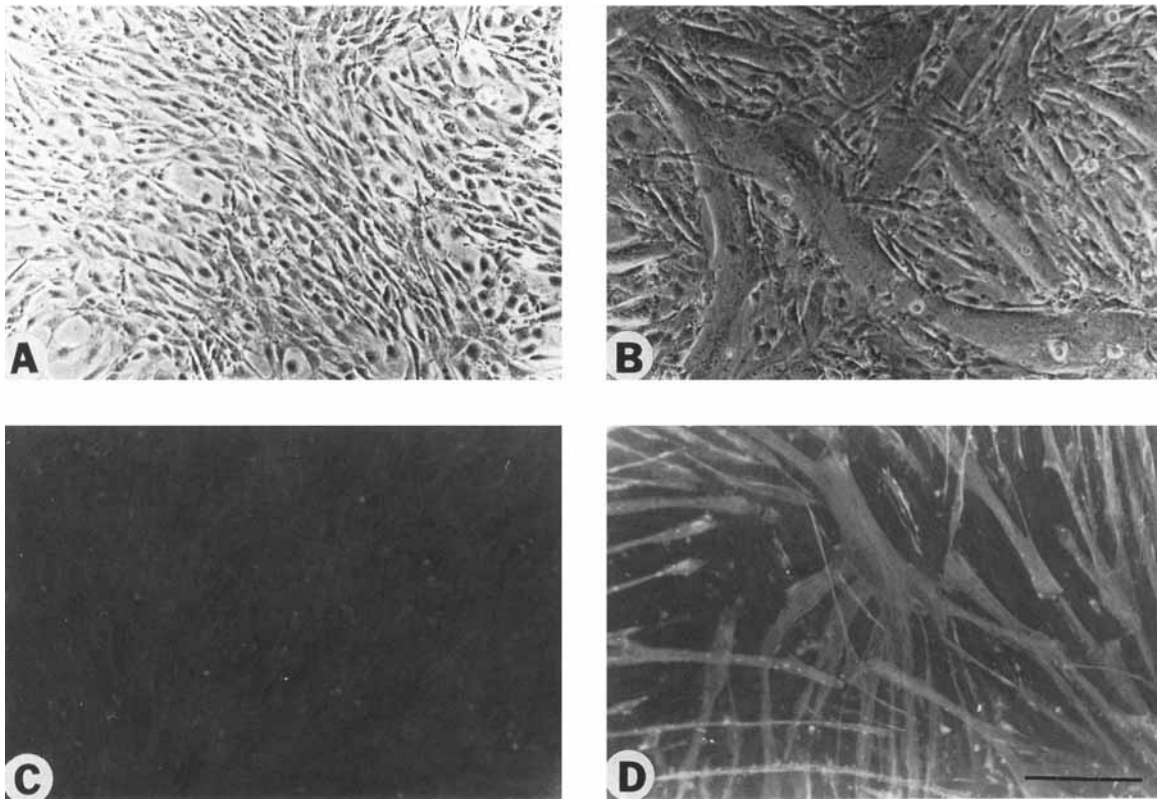


**Fig. 4.** Growth and differentiation of C2C12 myoblasts. Cells were initially grown in medium containing 15% fetal serum (FBS) until they formed a monolayer. Subsequently, 2% horse serum was substituted for FBS. Micrographs of cells during growth (**A, C**) or differentiation (**B, D**; day 5, following the use of horse serum) were taken by light microscopy or fluorescence microscopy. MF 20 monoclonal antibodies against MHC were used for immunolabelling of myoblasts (**C, D**). The bar represents 240 μm.

genesis and stimulated expression of desmin and myosin in differentiating myoblast cultures [von Der Mark and Ocalan, 1989]. Ln seems most important at early stages of myoblast differentiation. At that time, myoblasts secrete abundant amounts of Ln into the extracellular matrix and the myoblasts become spread [Olwin and Hall, 1985; Goodman et al., 1989a].

Ln glycosylation is an important factor in the behavior of some types of cells on Ln substrates [Runyan et al., 1986; Dean et al., 1988, 1990; Chandrasekaran et al., 1991; Tanzer et al., 1993]. Our results show that for mouse C2C12 and rat L6 myoblasts, Ln glycosylation is not essential for cell adhesion, spreading, and differentiation. Myoblasts bound and spread equally well on GLn as well as on ULn substrates. On both substrates cells were spread within 30 min after binding. The rate of cell growth and differentiation was the same on GLn and ULn. One of the possible explanations for the different behavior that various cell types show on GLn and ULn substrates may be differences in their Ln receptors.

Several types of integrins are known to function as Ln receptors. Integrins α3β1 [Gehlsen et al., 1989] and α6β1 [Hall et al., 1990; Sonnenberg et al., 1990] bind primarily to the E8 fragment of laminin, while integrin α1β1 [Hall et al., 1990] binds to the E1 fragment. Depending upon cell type, the same integrins may have different ligand specificities. Thus, VLA-2 (α2β1) integrin is a laminin/collagen receptor of melanoma cells, while in fibroblasts and platelets the same integrin functions only as a collagen receptor [Elices and Helmer, 1989]. For adhesion and migration on Ln, myoblasts utilize a domain in the E8 fragment [Goodman et al., 1989b]. Many cells use integrin α6β1 to bind to the E8 fragment, including mouse B16, Swiss 3T3, 3T6, HT1080, F9, and F19 cells [von der Mark et al., 1991]. However, myoblasts do not use α6β1 integrin for interactions with Ln but use a less common, closely related α7β1 integrin [von der Mark et

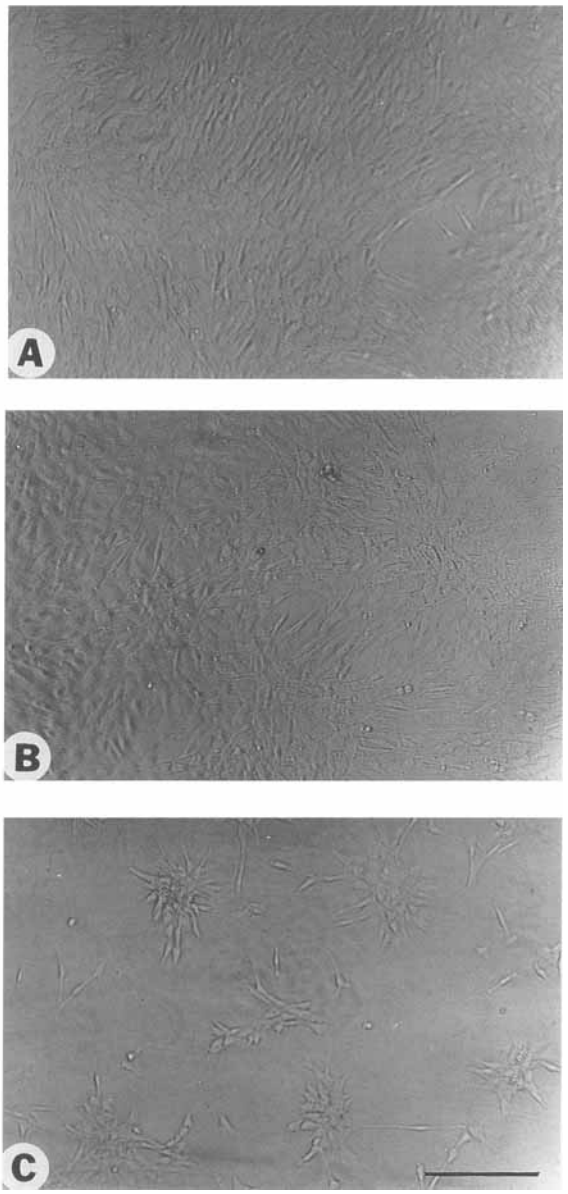


Fig. 5. Growth of C2C12 myoblasts on Ln substrates. Five micrograms of GLn (**A**) or ULn (**B**) were immobilized on plastic. Cells were seeded into untreated wells (**C**) or wells with Ln substrates in medium containing 2% horse serum and allowed to grow. Micrographs were taken on the third day of growth. The bar represents 360 μm.

al., 1990, 1991; Song et al., 1992; Collo et al., 1993]. Two isoforms of α7 subunits with alternative cytoplasmic domains exist. At the replicating myoblast stage only the α7B isoform is present, while at the stage of differentiation and fusion myoblasts also express the α7A isoform [Collo et al., 1993]. Antibodies to the α6 subunit inhibit B16 cell adhesion to Ln or its E8 fragment [Kramer et al., 1991] but do not inhibit myoblast binding to these substrates [von der Mark et al., 1991]. The myoblast-binding site of the E8 domain seems to be distinct from the site which promotes neurite elongation [Deutzmann et al., 1990; von der Mark et al., 1991].

Adhesion and spreading are complex processes that involve different cell structures and diverse types of cell-ECM interactions. Compared to adhesion, cell spreading necessarily invokes greater involvement of cell-matrix interactions. Cell surface receptors of a non-integrin category were shown to participate in the process of cell spreading on an Ln substrate. For example, binding of cell surface GalTase to Ln oligosaccharides was shown to be important for B16-F10 melanoma spreading but not for initial cell attachment to Ln [Runyan et al., 1988]. Anti-GalTase IgG, as well as the GalTase modifier protein, α-lactalbumin, both block GalTase activity and inhibit B16-F10 melanoma cell spreading on Ln but not initial cell attachment. Cell surface GalTase also participates in the initiation of neurite outgrowth from PC 12 cells on LN substrates [Begovac and Shur, 1990]. Cooper et al. [1991] reported that blocking surface GalTase with α-lactalbumin does not decrease C2C12 myoblast spreading on Ln. Thus, myoblast spreading on Ln seems to require components other than surface GalTase or Ln carbohydrates. The ectoenzyme, 5′-nucleotidase, which hydrolyzes AMP into adenosine and inorganic phosphate, was shown to be involved in the spreading of chick embryo fibroblasts and myoblasts on a Ln substrate. Mehul et al. [1992], using immunofluorescent labeling techniques, observed that 5′-nucleotidase codistributes with laminin during the development of chicken striated muscle. Antibodies directed against 5′-nucleotidase inhibit the spreading of chicken fibroblasts and chicken myoblasts, after their initial attachment to Ln. Intact Ln and its E8 fragment, consisting of the distal half of the long arm, stimulates activity of ecto-5′-nucleotidase in chick myoblasts. Interestingly, the E1 fragment of Ln, derived from the short arms, inhibits ecto-5′-nucleotidase activity [Mehul et al., 1993].

Lectin binding to a GLn substrate inhibits spreading of two different cell types, murine B16 melanoma cells and rat PC12 pheochromocytoma cells [Dean et al., 1988, 1990]. In our experiments, pretreatment of Ln substrates with Con A lectin did not significantly decrease cell spreading, but the same lectins, used in a solu-

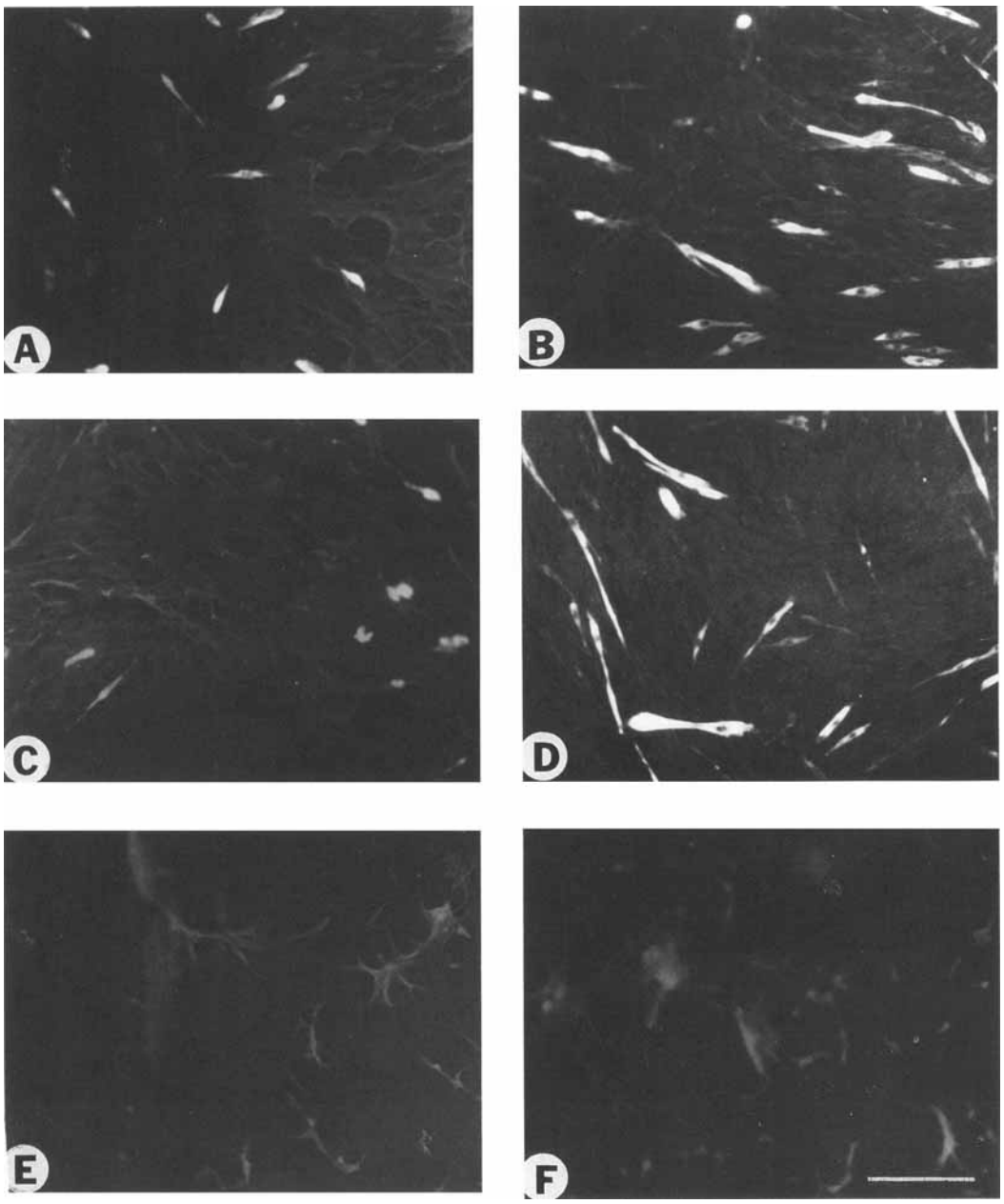


**Fig. 6.** Effect of glycosylation of Ln substrate on C2C12 myoblast differentiation. Five micrograms of GLn (**A, B**) or ULn (**C, D**) were immobilized on wells. Untreated wells served as a control (**E, F**). Cells were seeded and allowed to grow. In contrast to Figure 4, cells were initially plated in medium containing 2% horse serum, to allow for slow growth and differentiation in order to observe any subtle discrepancies in differentiation. On the fifth (**A, C, E**) and seventh (**B, D, F**) days of growth, myoblasts were fixed, immunolabelled with anti-MHC monoclonal antibody, and photographed. The bar represents 360 μm.

tion during binding experiments, impaired cell adhesion and spreading. These composite results imply that myoblast surface carbohydrates are more critical than carbohydrates of the substratum. Since integrins are glycoproteins they may be one of the cell surface components which are affected by lectins. There are 14 and 13 potential asparagine-linked potential glycosylation sites on the α and β integrin subunits, respectively [Argraves et al., 1987]. Binding of laminin-nidogen complex to gp 120/140, an Ln-binding integrin of B16-F10 melanoma cells immunologically related to the α6β1 integrin, depends on asparagine-linked oligosaccharides

from both ligand and receptor [Chammas et al., 1991]. Treatment of gp120/140 with α-galactosidase abolishes its LN-binding activity. On the other hand, β-chain complex antennary structures, whose synthesis could be inhibited by swainsonine (SW), were associated with cell spreading rather than cell adhesion. SW-treated B16-F10 cells maintain both their ability to bind Ln and their adhesiveness to Ln-coated plates, but cell spreading on Ln is considerably impaired [Chammas et al., 1993]. Other lectin-sensitive sites may be cell surface Ln receptors resembling the 72 kDa ConA-binding protein (cnbg) of chick embryo fibroblasts (CEF) [Moutsita et al., 1991]. Isolated cnbg 72 specifically inhibited CEF spreading on Ln after initial cell attachment, whereas cnbg 72 did not impair the spreading of CEF on fibronectin. Cell surface 5′-nucleotidase, involved in the chick myoblast spreading on Ln, also can bind Con A. This lectin rapidly and specifically inhibits 5′-nucleotidase in intact cells, in isolated membranes, or the purified enzyme [reviewed by Carraway et al., 1979].

It is well known that Ln substrates stimulate myogenesis in culture [von Der Mark and Ocalan, 1989]. Under our experimental conditions, when medium containing low amounts of growth factors and extracellular matrix components was used for myoblast growth and differentiation, cells on plastic did not form monolayers and did not differentiate. On the contrary, both forms of Ln (GLn and ULn) supported growth and differentiation of myoblasts equally well. We can't exclude the possibility that after several days in culture myoblasts synthesize and secrete Ln and this may influence the results of the experiment. But the low rate of growth and absence of differentiation in myoblast cultures plated on plastic surfaces makes this an unlikely possibility.

In summary, although Ln oligosaccharides have been implicated in the spreading of some types of rodent cells, they are not important for the spreading, growth, and differentiation of rodent myoblasts on laminin. However, lectin blocking of cells spreading on Ln indicates that myoblast surface glycoconjugates do participate in the myoblast response to laminin. It is this latter observation which requires further exploration and elucidation of the cell surface participants.

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## REFERENCES

Argraves WS, Suzuki S, Arai H, Thompson K, Pierschbacher MD, Ruoslahti E (1987): Amino acid sequence of the human fibronectin receptor. J Cell Biol 105:1183–1190.

Arumugham RG, Hsieh TC, Tanzer ML, Laine RA (1986): Structures of the asparagine-linked sugar chains of laminin. Biochem Biophys Acta 883:112–126.

Begovac PC, Huang Q (1993): Altered expression of cell surface galactosyltransferase increases PC 12 cell neurite outgrowth on laminin. J Cell Biochem (Suppl) 17E:167.

Begovac PC, Shur BD (1990): Cell surface galactosyltransferase mediates the initiation of neurite outgrowth from PC 12 cells on laminin. J Cell Biol 110:461–470.

Carraway KL, Doss RS, Huggins JW, Chesnut RW, Carothers-Carraway CA (1979): Effect of cytoskeletal perturbant drugs on ecto-5′-nucleotidase, a Concanavalin A receptor. J Cell Biol 83:529–543.

Chammas R, Veiga SS, Line S, Potocnjak P, Brentani RR (1991): Asn-linked oligosaccharide-dependent interaction between laminin and gp 120/140. J Biol Chem 266:3349–3355.

Chammas R, Veiga SS, Travassos LR, Brentani RR (1993): Functionally distinct roles for glycosylation of α and β integrin chains in cell-matrix interactions. Proc Natl Acad Sci USA 90:1795–1799.

Chandrasekaran S, Dean III JW, Giniger MS, Tanzer ML (1991): Laminin carbohydrates are implicated in cell signaling. J Cell Biochem 46:115–124.

Chandrasekaran S, Tanzer ML, Giniger M (1994a): Oligomannosides initiate cell spreading of laminin-adherent murine melanoma cells. J Biol Chem 269:3356–3366.

Chandrasekaran S, Tanzer ML, Giniger M (1994b): Characterization of oligomannoside binding to the surface of murine melanoma cells. J Biol Chem 269:3367–3373.

Chung AE, Jaffe R, Freeman IL, Vergnes JP, Braginsk JE, Carlin B (1979): Properties of a basement membrane-related glycoprotein synthesized in culture. Cell 16:277–287.

Collo G, Starr L, Quaranta V (1993): A new isoform of the laminin receptor integrin α7β1 is developmentally regulated in skeletal muscle. J Biol Chem 268:19019–19024.

Cooper DW, Massa SM, Barondes SH (1991): Endogenous muscle lectin inhibits myoblast adhesion to laminin. J Cell Biol 115:1437–1448.

Dean JW, Chandrasekaran S, Tanzer ML (1988): Lectins inhibit cell binding and spreading on a laminin substrate. Biochem Biophys Res Commun 156:411–416.

Dean JW, Chandrasekaran S, Tanzer ML (1990): A biological role of the carbohydrate moieties of laminin. J Biol Chem 265:12553–12562.

Deutzmann R, Aumailley M, Wiedemann H, Pysny W, Timpl R, Edgar D (1990): Cell adhesion, spreading and neurite stimulation by laminin fragment E8 depends on maintenance of secondary and tertiary structure in its rod and globular domain. Eur J Biochem 191:513–522.

Eckstein DJ, Shur BD (1989): Laminin induces the stable expression of surface galactosyltransferase on lamellipodia of migrating cells. J Cell Biol 108:2507–2517.

Elices MJ, Helmer ME (1989): The human integrin VLA-2 is a collagen receptor on some cells and a collagen/laminin receptor on others. Proc Natl Acad Sci USA 86:9906–9910.

Foster R, Thompson J, Kaufman J (1987): A laminin substrate promotes myogenesis in rat skeletal muscle cultures: Analysis of replication and development using antidesmin and anti-BrdUrd monoclonal antibodies. Dev Biol 122:11–20.

Fujiwara S, Shinkai H, Deutzmann R, Paulsson M, Timpl R (1988): Structure and distribution of N-linked oligosaccharide chains on various domains of mouse tumour laminin. Biochem J 252:453–461.

Gehlsen KR, Dickson K, Agraves WS, Engvall E, Ruoslahti E (1989): Subunit structure of a laminin binding integrin and localization of its binding site on laminin. J Biol Chem 264:19034–19038.

Goodman SL, Deutzmann R, Nurcombe V (1989a): Locomotory competence and laminin-specific cell surface sites are lost during myoblast differentiation. Development 106: 795–802.

Goodman SL, Risse G, von der Mark K (1989b): The E8 subfragment of laminin promotes locomotion of myoblasts over extracellular matrix. J Cell Biol 109:799–809.

Hall DE, Reichardt LF, Crowley E, Holley B, Moezzi H, Sonnenberg A, Damsky CH (1990): The α1/β1 and α6/β1 integrin heterodimers mediate cell attachment to distinct sites on laminin. J Cell Biol 110:2175–2184.

Knibbs RN, Perini F, Goldstein IJ (1989): Structure of the major concanavalin A reactive oligosaccharides of the extracellular matrix component laminin. Biochemistry 28: 6379–6392.

Kramer RH, Vu MP, Cheng Y-F, Ramos DM, Timpl R, Waleh N (1991): Laminin-binding integrin α7β1: Functional characterization and expression in normal and malignant melanocytes. Cell Regul 2:805–817.

Mehul B, Doyennette-Moyne M-A, Aubery M, Codogno P, Mannherz HG (1992): Enzymatic activity and in vivo distribution of 5′-nucleotidase, an extracellular matrix binding glycoprotein, during the development of chicken striated muscle. Exp Cell Res 203:62–71.

Mehul B, Aubery M, Mannherz HG, Codogno P (1993): Dual mechanism of laminin modulation of ecto-5′-nucleotidase activity. J Cell Biochem 52:266–274.

Moutsita R, Aubery M, Codogno P (1991): An Mr 72 cell surface Concanavalin A binding glycoprotein is specifically involved in the spreading of chick embryo fibroblasts onto laminin substrate. Exp Cell Res 192:236–242.

Ocalan M, Goodman SL, Kuhl U, Hauschka SD, von der Mark K (1988): Laminin alters cell-shape and stimulates motility and proliferation of murine skeletal myoblasts. Dev Biol 125:158–167.

Olwin BB, Hall ZW (1985): Developmental regulation of laminin accumulation in the extracellular matrix of a mouse cell line. Dev Biol 112:359–367.

Runyan RB, Maxwell GD, Shur BD (1986): Evidence for a novel enzymatic mechanism of neural crest cell migration on extracellular glycoconjugate matrices. J Cell Biol 102: 432–441.

Runyan R, Versalovic J, Shur B (1988): Functionally distinct laminin receptors mediate cell adhesion and spreading: The requirement for surface galactosyltransferase in cell spreading. J Cell Biol 107:1863–1871.

Sasaki M, Kato S, Kohno K, Martin GR, Yamada Y (1987a): Sequence of the cDNA encoding the laminin B1 chain reveals a multidomain protein containing cysteine-rich repeats. Proc Natl Acad Sci USA 84:935–939.

Sasaki M, Yamada Y (1987b): The laminin B2 chain has a multidomain structure homologous to the B1 chain. J Biol Chem 262:17111–17117.

Sasaki M, Kleinman HK, Huber H, Deutzmann R, Yamada Y (1988): Laminin, a multidomain protein. The A chain has a unique globular domain and homology with the basement membrane proteoglycan and the laminin B chains. J Biol Chem 263:16536–16544.

Song WK, Wang W, Foster RF, Bielser DA, Kaufman SJ (1992): H36-α7 is a novel integrin alpha chain that is developmentally regulated during skeletal myogenesis. J Cell Biol 117:643–657.

Sonnenberg A, Linders CJT, Modderman PW, Damsky CH, Aumailley M, Timpl R (1990): Integrin recognition of different cell binding fragments of laminin (P1, E3, E8) and evidence that α6β1 but not α6β4 functions as a major receptor for fragment E8. J Cell Biol 110:2145–2155.

Tanzer ML, Giniger MS, Chandrasekaran S (1993): Laminin oligosaccharides play a pivotal role in cell spreading. In Jones G, Wigley C, Warn R (eds): "Cell Behaviour: Adhesion and Motility." Cambridge: The Company of Biologists Limited, pp. 147–154.

Timpl R, Rohde H, Robey PG, Rennard SI, Foidart J-M, Martin GR (1979): Laminin-A glycoprotein from basement membranes. J Biol Chem 254:9933–9937.

von der Mark K, Ocalan M (1989): Antagonistic effects of laminin and fibronectin on the expression of the myogenic phenotype. Differentiation 40:150–157.

von der Mark K, von der Mark H, Goodman SL (1990): The role of laminin in myoblast differentiation. Cell Biol Int Rep 14:36.

von der Mark H, Durr J, Sonnenberg A, von der Mark K, Deutzmann R, Goodman SL (1991): Skeletal myoblasts utilize a novel β1-series integrin and not α6β1 for binding to the E8 and T8 fragments of laminin. J Biol Chem 266:23593–23601.

Wu C, Friedman R, Chung AE (1988): Analysis of assembly of laminin and laminin-entactin complex with laminin chain specific monoclonal and polyclonal antibodies. Biochemistry 27:8780–8787.