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ARTICLES

# Laminin Carbohydrates Are Implicated in Cell Signaling

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**Abstract** We have examined how laminin carbohydrates participate in cellular responses and have focused upon cell spreading and neurite outgrowth. Our earlier studies showed that unglycosylated laminin fully supported cell adhesion but did not promote subsequent spreading of mouse melanoma cells or neurite outgrowth of rat pheochromocytoma cells (Dean et al. (1990): *J Biol Chem* 265:12553–12562). In the present experiments, we determined whether those cellular responses could be restored to adherent cells. When a mixture of unglycosylated and glycosylated laminins was used as a substratum for mouse melanoma cells, some cells began to spread when 30% glycosylated laminin was present. At least 65% glycosylated laminin was required to elicit a maximal spreading response by the majority of the cells. In separate experiments, we found that cell spreading was fully restored by a pronase digest of glycosylated laminin; a similar digest of unglycosylated laminin had no effect. These results indicate that laminin carbohydrates, rather than polypeptide sequences, were responsible for cell spreading. We also conclude that substrate attachment of the carbohydrate moieties was not essential. In other experiments, laminins containing immature oligosaccharides were produced using two glycosylation pathway inhibitors, swainsonine or castanospermine. When such laminins were used to study cell spreading or neurite outgrowth, laminin containing immature oligosaccharides was as effective as laminin which contains fully processed oligosaccharides. In contrast, laminin with partially processed oligosaccharides had incomplete activity. These composite reconstitution experiments show that laminin carbohydrates provide essential information to responsive cells, enabling them to progress from an adherent state to a spread form or to extend neurite processes.

**Key words:** basement membrane, laminin, oligosaccharides, cell spreading, neurite outgrowth

Basement membrane proteins have multiple biological functions (Timpl, 1989). Laminin, a glycosylated basement membrane protein, promotes cell adhesion, growth, migration, differentiation, and neurite outgrowth (Beck et al., 1990). These biological responses have been primarily attributed to the peptide sequences of laminin. Recent studies suggest that laminin carbohydrates may also participate in such cellular responses (Dean et al., 1988; 1990; Bouzon et al., 1990). Laminin carbohydrates are recognized by non-integrin cell surface components (Mercurio, 1990). Among these components are a 67 kDa protein which has both a peptide recognition site and a lectin-like galactoside binding site (Mecham et al., 1989). Two S-type lectins, the Mac-2 surface antigen of macrophages (Woo et al., 1990) and one obtained from cardiac tissue (Zhou et al., 1990) bind to laminin. The former lectin recognizes galactosyl moieties while the latter primarily recognizes poly-N-acetyllactosamine units. Cell surface galactosyltransferase also plays an important role in mediating biological responses to laminin (Shur, 1989).

Previously, we found that Concanavalin A selectively interfered with laminin-induced cell spreading or neurite outgrowth (Dean et al., 1988). Subsequently we showed that unglycosylated laminin, purified from tunicamycin-treated cultures of mouse cells which constitutively produce laminin, allowed cell attachment but did not support spreading of cells or neurite outgrowth (Dean et al., 1990). Independently, Bouzon et al. (1990) have found that some lectins could inhibit the spreading of mouse melanoma cells on laminin. Thus, the recent literature consistently shows that cell surface–associated proteins can respond to the carbohydrate moieties of laminin when such carbohy-

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drates are available. We are interested in deciphering the mechanism(s) of the responses.

In the present investigation we carried out several types of reconstitution experiments. In one approach we sought the threshold level of response to intact glycosylated laminin. In another approach we tested laminin glycopeptides for their ability to restore biological activity and quantitated that response. In a third approach we altered the chemical nature of laminin carbohydrates in two different ways and tested the cellular responses to each of these substrata. The composite results implicate laminin carbohydrates as major response elements in cell signaling.

## MATERIALS AND METHODS

### Materials

A mouse embryonal carcinoma derived cell line, M1536 B3, was the generous gift of Dr. Albert Chung, University of Pittsburgh, Pittsburgh, PA. B16 F1 mouse melanoma cells were provided by Dr. I. Fidler, University of Texas Cancer Center, Houston, TX. PC12 rat pheochromocytoma cells were donated by Dr. K. J. Tomaselli, Howard Hughes Medical Institute, San Francisco, CA. A monoclonal antibody producing hybridoma cell line was the kind gift of Dr. Dale Abrahamson, University of Alabama, Birmingham, AL. Cell culture medium, DMEM (Dulbecco's modified Eagle's medium), and antibiotic/antimycotic cocktail were purchased from Gibco-BRL, Gaithersburg, MD. Fetal bovine serum was from HyClone Laboratories Inc., Logan, UT. Cell counting was done using a Coulter Cell Counter supplied by Coulter Electronics, Hialeah, FL. Castanospermine, swainsonine, and Endo H (Endo β-acetylglucosaminidase) were products of Boehringer-Mannheim, Indianapolis, IN. CHAPS (3-[(3-cholamidopropyl)dimethylammonio]-1-propane sulfonate) and BCA (bicinchoninic acid) protein assay reagent were purchased from Pierce Chemical Co., Rockford, IL. Cell culture dishes (100 mm) and nontissue culture-treated 96-well assay plates were supplied by Beckton Dickinson, Lincoln Park, NJ. Purified pronase was obtained from Calbiochem, San Diego, CA. Pfanstiehl Labs, Waukegan, IL, was the supplier of mono- and disaccharides, N-acetyl-D-mannosamine, N-acetyl-D-glucosamine, D-galactose, D-mannose, D-glucose, fucose, N-acetylneuraminic acid, sucrose, and lactose. $^3$H-mannose (25 mCi/mM) was obtained from New England Nuclear, Wilmington, DE. Biogel P-2 gel filtration material was the product of BioRad Laboratories, Richmond, CA. All other reagents were of analytical grade.

### Cell Culture

M1536 B3 cells were used for the production of various laminin preparations. Cells were grown in culture as described previously (Dean et al., 1990) in DMEM supplemented with 15% fetal bovine serum and antibiotic/antimycotic cocktail. For the production of unglycosylated laminin, tunicamycin (0.5 μg/ml) was added to culture medium and 48 hours later cells were harvested and lysed with 0.05 M Tris buffer, pH 7.4, containing 0.15 M NaCl and 1% (w/v) CHAPS. Cell lysates were stored at −20°C in the presence of 2 mM PMSF (phenylmethylsulfonyl fluoride) until used. In separate experiments M1536 B3 cells were cultured in the presence of castanospermine or swainsonine to produce laminin containing immature oligosaccharides. Through dose response studies, the optimum levels for castanospermine and swainsonine were found to be 20 μg and 5 μg per ml culture medium, respectively. M1536 B3 cells were plated at a density of approximately $2 \times 10^6$ cells per 100 mm culture dish. When the cells were approaching early confluence, castanospermine or swainsonine was added in fresh medium. After an additional eight hours this medium was replaced with new medium containing fresh drug. Cells continued to grow in this medium and 48 hours later the spent medium was collected, centrifuged for 5 minutes at 1000*g*, and stored at −20°C in the presence of 2 mM PMSF.

### Laminin Purification

Purification of unglycosylated and glycosylated laminin from tunicamycin-treated lysates of M1536 B3 cells was carried out using an anti-laminin monoclonal antibody immunoaffinity column as described previously (Dean et al., 1990). For the purification of castanospermine- or swainsonine-derived laminin a similar protocol was followed. Cell culture medium was passed through the immunoaffinity gel and the unbound material was washed with 0.05 M Tris, pH 7.4, containing 0.15 M NaCl, 1% CHAPS (w/v), and 2 mM PMSF followed by elution of bound protein using 0.1 M diethylamine, pH 11.0, containing 1% CHAPS (w/v) and 2 mM PMSF. All purified laminin preparations were concentrated and dialyzed against 0.05 M Tris

buffer (pH 7.4) containing 0.15 M NaCl and 2 mM PMSF and stored at −20°C until used.

### Endoglycosidase H Treatment of Castanospermine- and Swainsonine-Derived Laminin

Cells were cultured in the presence of castanospermine or swainsonine as described above. After 8 hours of treatment with either castanospermine or swainsonine (or untreated controls), fresh medium containing the same level of drug plus $^3$H-mannose (30 μCi/ml) was added. The medium was removed 24 hours later and to 10 μl of centrifuged medium 30 μl of 0.1 M sodium citrate buffer (pH 5.6), 4 μl of 20% SDS, and 2 μl of β-mercaptoethanol were added. This mixture was then placed in a boiling water bath for 3 minutes, removed, and incubated at 37°C for 18 hours following the addition of 2 μl of 2 mM PMSF and 7 μl of Endo H. In the case of controls 50 mM sodium phosphate pH 7.0 was added in place of the enzyme. SDS polyacrylamide gel electrophoresis was carried out in a 3–10% gradient gel by the method of Laemmli (Laemmli 1970) under reducing conditions. The gel was then fluorographed and an identical gel run in parallel was stained by Coomassie Brilliant Blue R-250 to visualize the protein bands.

### Preparation of a Pronase Digest of Laminin

Laminin was isolated from a mouse EHS (Engelbreth-Holm-Swarm sarcoma) tumor as described previously (Timpl et al., 1979) and dialyzed against pronase digestion buffer (0.1 M Tris, pH 8.0, containing 2 mM $CaCl_2$). Pronase (1 mg/ml) dissolved in the same buffer was preincubated for one hour at 50°C and 200 μl of this solution was added to 10 ml of dialyzed laminin (1.1 mg/ml) under a toluene layer. The solution was then incubated in a shaking water bath at 50°C for 72 hours with the addition of 200 μl of preincubated enzyme at 24 and 48 hours. The solution was then boiled to stop the reaction and centrifuged at 25,000*g* for one hour. The supernatant was then lyophilized, dissolved in 1 ml $H_2O$, and then desalted on a Biogel P-2 column equilibrated in PBS (phosphate buffered saline, pH 7.4). Unglycosylated laminin was also digested by pronase in order to be used as a carbohydrate-free control to test for activity of the free amino acids and small polypeptides that remain after digestion. SDS polyacrylamide gel electrophoresis of all active digests, including the 5 fold concentrate, on 3–10% gradient gels followed by silver staining showed no visible bands indicating the complete digestion of laminin (data not shown).

### Cell Spreading and Neurite Outgrowth Assays

Various laminins dialyzed against PBS were allowed to dry overnight, at 37°C, in the wells of nontissue culture-treated 96-well plates. In experiments where a mixture of unglycosylated/glycosylated laminin was used, a solution of glycosylated laminin was added to a solution of unglycosylated laminin and the mixtures, varying from 0 to 100 percent, were dried on assay plates. In all the cases the total protein concentration was maintained at 5 μg/well. The laminin coated wells were then rinsed twice with PBS and used for cell binding and spreading or neurite outgrowth assays.

The B16 F1 mouse melanoma cells were seeded in triplicate at 5,000 cells per well in serum-free DMEM. In the pronase digest experiments, cells were plated onto substrates of unglycosylated laminin and varying amounts of pronase digest were immediately added. PBS alone was added to the control wells. In an attempt to ascertain which specific sugar might be responsible for cell spreading, up to 100 mM of N-acetyl-D-mannosamine, N-acetyl-D-glucosamine, D-galactose, D-mannose, D-glucose, fucose, N-acetyl-neuraminic acid, sucrose, or lactose dissolved in PBS was added to cells plated on unglycosylated laminin. Culture plates were placed in the incubator for one hour under standard culture conditions. Medium was then dumped out of the plates and all wells were washed twice with PBS. Cell viability was routinely assessed on the basis of trypan blue exclusion and was found to be greater than 85%. The adherent cells were then fixed, stained, and counted by a modification of the method of Ruoslahti et al. (1982). Briefly, the cells were fixed overnight at 4°C with 3% formaldehyde in PBS; cells were then stained with 1% toluidine blue in the same solution for 2 hours at room temperature and rinsed three times with PBS. The cells were visually counted and spreading was assessed by microscopic examination.

For experiments involving binding and neurite outgrowth of the PC12 line, cells that had been primed for 10 to 14 days were released with 0.025% trypsin in Earle's basic salt solution, triturated vigorously in a long narrow bore pipette to remove any neurite processes, and seeded at 3,000 cells per well onto the various

laminin substrates described above. The 96-well plates were incubated for 24 hours under standard culture conditions, the fluid was then dumped out, and the wells were rinsed twice with PBS. The cells were then fixed, stained with toluidine blue, and counted visually. Neurite outgrowth was assessed as the number of cells exhibiting axonal process outgrowth longer than two cell diameters, compared to the total number of cells counted (Tomaselli et al., 1987). At least 300 cells were scored per well.

### Analytical Procedures

All protein assays were carried out with the BCA reagent by the method of Smith et al. (1985) using bovine serum albumin as the standard protein. The carbohydrate content was assayed by the phenol-sulfuric acid reducing sugar analysis as described by Dubois et al. (1956) using D-glucose as the standard sugar.

## RESULTS

### Adhesion and Spreading of B16 F1 Mouse Melanoma Cells on Mixtures of Unglycosylated and Glycosylated Laminin

We used a mixture of varying proportions of unglycosylated and glycosylated laminin to determine the threshold for cell spreading. Figure 1 shows that some cells began to spread when 30% glycosylated laminin was present in the mixture. A gradual increase in the amount of glycosylated laminin in the mixture was reflected in an increase in the number of spread cells. A minimum of about 65% glycosylated laminin (Panel D) was required to show cell spreading equivalent to that found on 100% glycosylated laminin (Panel E).

### Effect of a Pronase Digest of Glycosylated Laminin on Cell Spreading

In order to determine if the signal(s) for initiating cell spreading could be restored by laminin glycopeptides, we carried out cell spreading assays on unglycosylated laminin after the addition of a pronase digest of either unglycosylated or glycosylated laminin to the culture medium. The exhaustive pronase digestion of laminin yields a mixture of laminin glycopeptides (Arumugham et al., 1986). When cells were plated on unglycosylated laminin in the presence of varying amounts of pronase digest, we could titrate the cell spreading response and a maximal response occurred at the highest concentrations (Fig. 2). We found that 5 times the amount of carbohydrate present in 5 μg of intact laminin maximally stimulated the spreading of cells plated on unglycosylated laminin. A pronase digest of unglycosylated laminin, at the same concentration, had no effect on cells plated on unglycosylated laminin. Addition of various monosaccharides or disaccharides, described under Materials and Methods, to cells plated on unglycosylated laminin failed to elicit spreading. Thus, while glycosyl groups of laminin oligosaccharides evoke cell spreading, many individual sugars themselves do not emulate that effect. When these experiments were repeated using glycosylated laminin as a substratum no difference in spreading response was observed (data not shown).

### Production of Laminin Containing Immature Oligosaccharides

To further define which carbohydrate determinants might be important for the biological responses, laminin molecules containing oligosaccharides at two levels of immaturity were produced and used in the assay systems. The most immature forms were those of castanospermine-derived laminin while swainsonine-derived laminin contains oligosaccharides closer to full maturity. Endo H, an endoglycosidase which recognizes primarily high mannose oligosaccharide chains (Trimble and Tarentino, 1991), was used to cleave the oligosaccharide moieties of laminin produced by the cells grown in castanospermine or swainsonine. Figure 3 shows that Endo H removes the $^{3}$H-mannose labeled oligosaccharides from these laminin molecules, also indicating that they may contain a uniform population of oligosaccharide moieties. An identical gel stained with Coomassie Brilliant Blue R-250 showed staining of protein bands in all of the lanes, including the lanes corresponding to 4 and 6 in Figure 3, where no radioactive bands were observed.

### Adhesion and Spreading of B16 F1 Mouse Melanoma Cells on Laminin Containing Immature Oligosaccharides

The inhibitor-derived laminins were purified as described under Materials and Methods and employed as a substrate in the test systems. B16 F1 cells were plated onto substrata of either unglycosylated, glycosylated, castanospermine-derived, or swainsonine-derived laminin in our standard assay procedure. We found that about

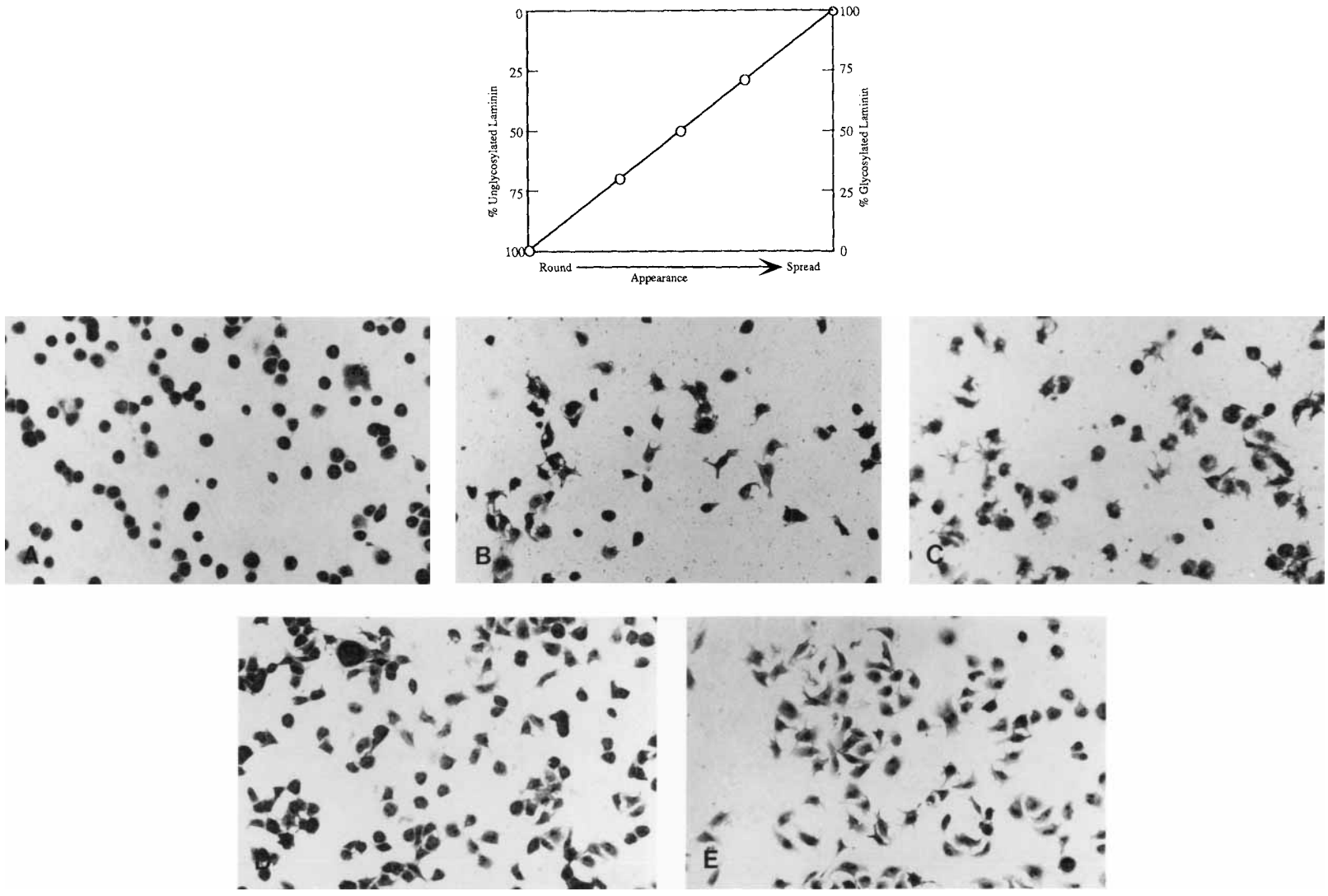


**Fig. 1.** Spreading of B16 F1 mouse melanoma cells on substrate-bound unglycosylated and glycosylated laminin. Varying proportions of unglycosylated and glycosylated laminin (5 μg/ml total) were mixed and dried onto 96-well assay plates. This figure summarizes the experimental protocol and its results, and shows the results in photomicrographs of B16 F1 mouse melanoma cells on 100% unglycosylated laminin (**Panel A**), 70% unglycosylated and 30% glycosylated laminin (**Panel B**), 50% unglycosylated and 50% glycosylated (**Panel C**), 35% unglycosylated and 65% glycosylated laminin (**Panel D**), and 100% glycosylated laminin (**Panel E**).

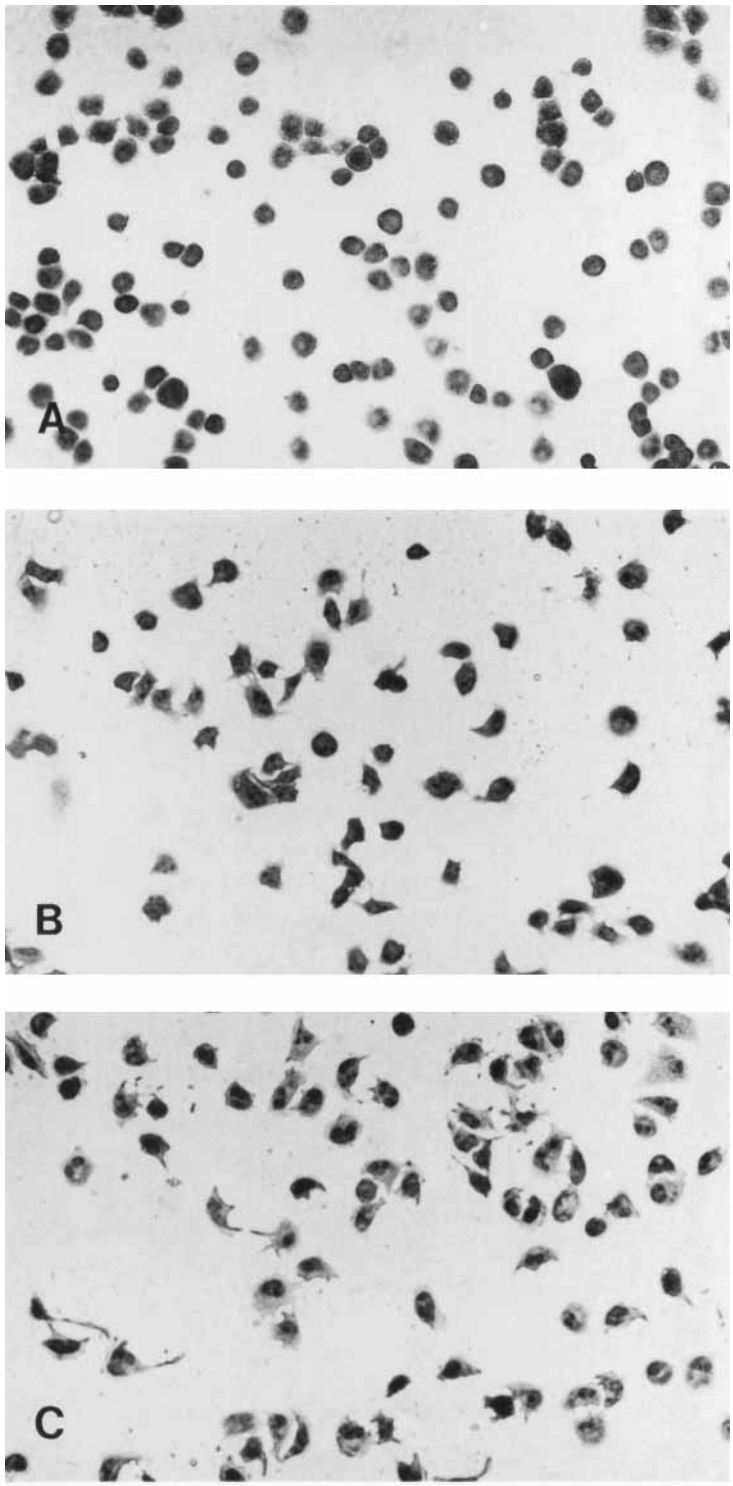


**Fig. 2.** Effect of glycopeptides of laminin on spreading of B16 F1 mouse melanoma cells on unglycosylated laminin. B16 F1 cells were added to substrate-bound unglycosylated laminin (5 μg/well) in culture medium immediately followed by pronase digest or buffer; control (**Panel A**); pronase digest containing either three times (**Panel B**) or five *times* (***Panel C***) the amount of carbohydrate present in 5 μg glycosylated laminin. No spreading occurred when equivalent amounts of a pronase digest of unglycosylated laminin was used, in parallel experiments. When the cells were plated on glycosylated laminin (5 μg/well) in the presence of either pronase digest no apparent change in their spreading behavior was noticed; the cells appeared as shown in Fig. 1, Panel E.

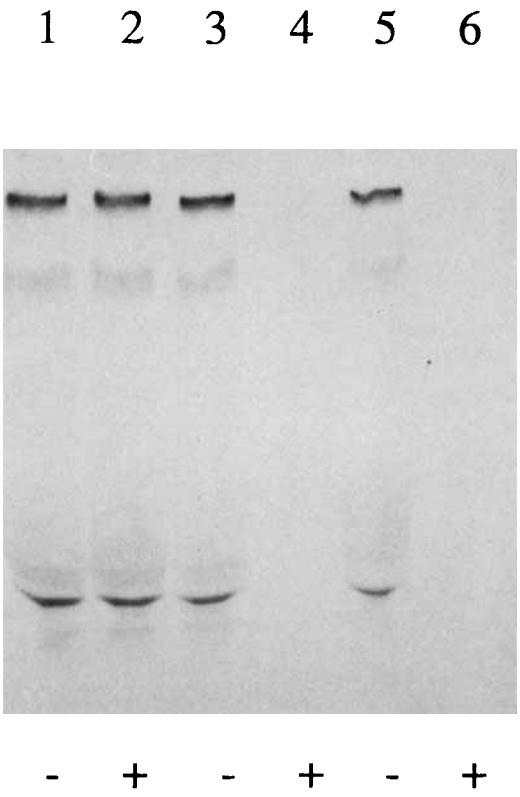


**Fig. 3.** Endo H treatment of laminin containing immature oligosaccharides produced by castanospermine- or swainsonine-treated cultures of a mouse embryonal carcinoma derived cell line (M1536 B3). Cells were cultured in the presence of castanospermine or swainsonine and $^3$H-mannose. The spent medium, containing laminin, was reduced with β-mercaptoethanol and treated with Endo H for 18 hours. Samples were then boiled in electrophoresis sample buffer and polyacrylamide gel electrophoresis was carried out in a 3–10% gradient gel. Enzyme-treated (+) and untreated (−) reaction mixtures containing control medium (**lanes 1,2**), castanospermine-derived medium (**lanes 3,4**), and swainsonine-derived medium (**lanes 5,6**) are shown in this fluorogram.

the same number of cells adhered and spread on both glycosylated laminin and castanospermine-derived laminin (Fig. 4). However, only about half as many cells spread on swainsonine-derived laminin, although adhesion was equivalent to the other laminin preparations.

### Adhesion and Neurite Outgrowth Assay of PC12 Rat Pheochromocytoma Cells on Laminin Containing Immature Oligosaccharides

The results obtained for PC12 rat pheochromocytoma cells plated on differentially glycosylated laminins (Fig. 5) were very similar to those described for B16 F1 cells. In the case of PC12 cells quantitation supported the visual data. Table I summarizes the results of cells extending neurites on different laminin substrates. The number of cells extending neurite processes was equivalent for glycosylated and immature (castanospermine-derived) laminins. Neurite extension was significantly reduced on a more mature (swainsonine-derived) laminin.

## DISCUSSION

Laminin is a glycosylated molecule which contains as much as 30% carbohydrate by weight (Arumugham et al., 1986; Knibbs et al., 1989). Our earlier studies showed that the unavailability or absence of the carbohydrate moieties prevented cell spreading and neurite outgrowth on laminin (Dean et al., 1988; Dean et al., 1990). In the present study we sought to restore the responses of the cells on unglycosylated laminin by providing laminin carbohydrates, in either substrate-bound form or as soluble fragments. The mixed glycosylated and unglycosylated laminin substrate experiments reveal that a threshold amount of glycosylated substrate is required to initiate spreading of the melanoma cells. A maximal response occurred at a concentration of 65% glycosylated laminin. Careful examination of the panels in Figure 1 suggests that while all cells may initiate the response at 30% glycosylated laminin, they are spread to varying degrees. This phenomenon persists to the highest concentration (100%) of glycosylated laminin.

Further evidence of the importance of laminin carbohydrates in the phenomenon of cell spreading is provided by the response elicited by a pronase digest of glycosylated laminin. These studies show a partial spreading response at lower concentrations of this digest which resembles the response seen when low proportions of glycosylated laminin were used in the mixed substrate experiments. Since pronase digests of unglycosylated laminin had no effect in these experiments it must be the oligosaccharide chains which signal cell spreading rather than polypeptide sequences. These results also imply that a substrate-attached carbohydrate moiety is unnecessary for cell spreading and that carbohydrate-related conformational effects on the laminin protein itself may be unimportant for cellular responses.

We were able to produce laminin containing defined, immature oligosaccharides by growing cells (which constitutively synthesize laminin) in the presence of either castanospermine or swainsonine, glycosylation inhibitors which specifically arrest oligosaccharide processing at two different stages in the pathway (Elbein, 1987). Castanospermine inhibits the glycosylation path-

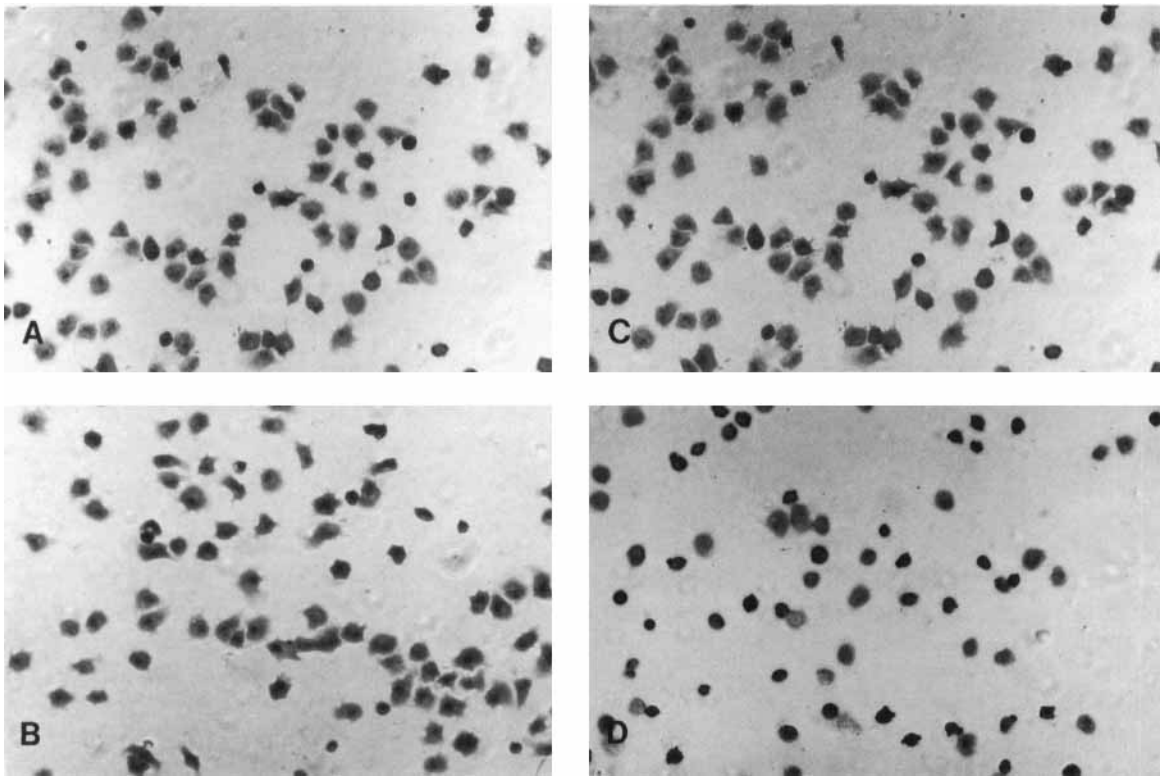


**Fig. 4.** Binding and spreading of B16 F1 mouse melanoma cells on differentially glycosylated laminin substrates. The different laminins (5 μg/well) were individually coated overnight onto 96-well assay plates in triplicate. B16 F1 cells were plated onto glycosylated laminin (**Panel A**), castanospermine-derived laminin (**Panel B**), swainsonine-derived laminin (**Panel C**), and unglycosylated laminin (**Panel D**). Cells were maintained on these substrates for 1 hour, fixed, stained, and photographed.

way at an early stage yielding the most immature form of oligosaccharides while swainsonine acts at a later stage resulting in a more mature form of oligosaccharides. The two forms of immature laminin differ in that the castanospermine-derived oligosaccharide contains a larger structure, $Glc_3$-$Man_9$-$(GlcNac)_2$, than the swainsonine-derived oligosaccharide which has the structure GlcNac-$Man_5$-$(GlcNac)_2$ (Elbein, 1987). The laminin molecules thus produced were treated with Endo H, an endoglycosidase which primarily recognizes and cleaves high mannose oligosaccharide chains (Trimble and Tarentino, 1991). The carbohydrate moieties of laminin produced under the influence of either inhibitor should be fully susceptible to Endo H, showing that each inhibitor potentially yields a uniform population of immature oligosaccharides; the results presented in Figure 3 support this point. We suggest that large, high mannose oligosaccharide chains impart the same information for cellular responses as do laminin molecules that contain mature sugar chains of similar size. Interestingly, the swainsonine-derived laminin, which contains truncated high mannose chains, yields partial responses. These results imply that the size of the oligosaccharide chain may be an important factor in the cellular response. The signal may not reside in one specific sugar moiety since we tested a variety of candidate mono- and disaccharides (see Materials and Methods) for their ability to elicit a spreading or neurite outgrowth response on unglycosylated laminin. These saccharides had no effect.

Our results establish that at least two different cell types which attach to laminin must recognize the carbohydrates of that glycoprotein in order to progress further in their biological responses. We had previously shown, through circular dichroism spectral studies, that the absence of glycosyl groups did not affect laminin conformation (Dean et al., 1990); the current data support that observation and show that cellular responses can be restored by soluble

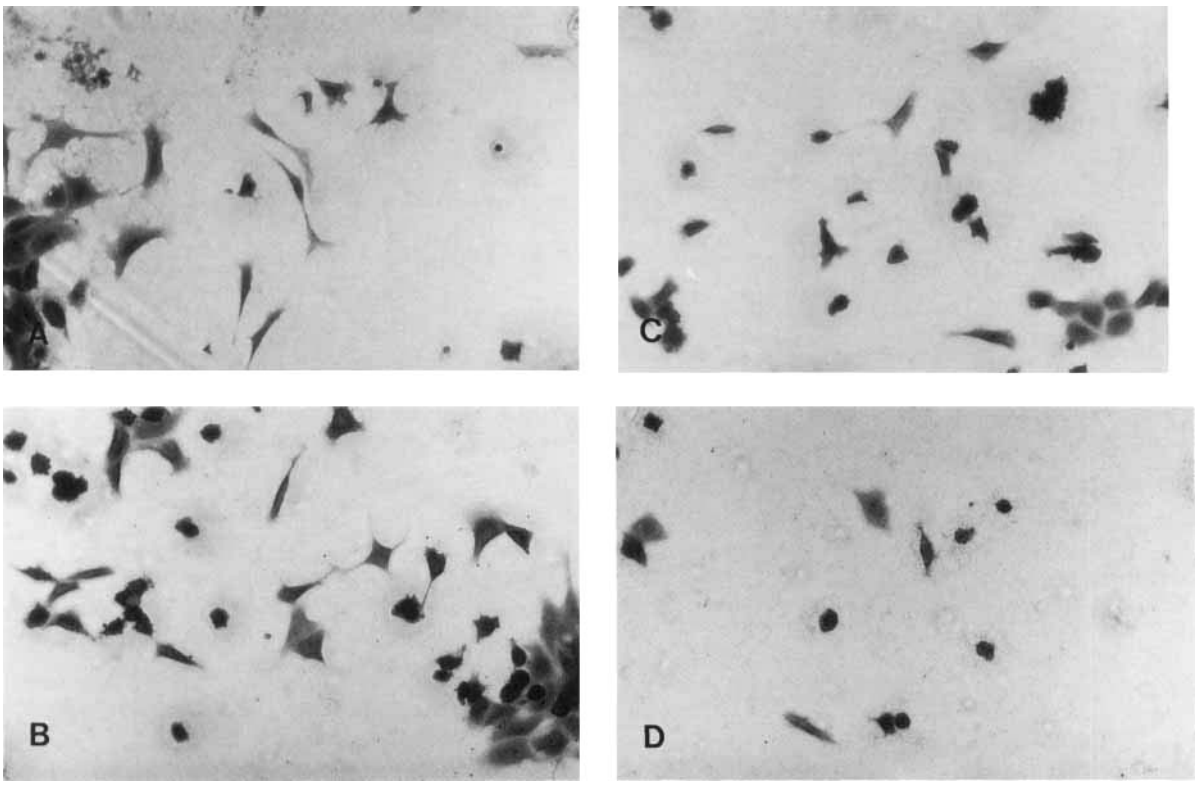


**Fig. 5.** Assay of neurite outgrowth of PC12 rat pheochromocytoma cells on differentially glycosylated laminin substrates. PC12 cells were seeded onto glycosylated laminin (**Panel A**), castanospermine-derived laminin (**Panel B**), swainsonine-derived laminin (**Panel C**), and unglycosylated laminin (**Panel D**). All experiments were conducted in triplicate for 24 hours using 5 μg/well laminin substrates.

**TABLE I. Percentages of Rat Pheochromocytoma (PC12) Cells Exhibiting Neurite Outgrowth on Differentially Glycosylated Laminin Substrates***

| | Substrate | |
|---|---|---|
| Laminin | 3 μg/well | 5 μg/well |
| Glycosylated | 49 ± 6% | 48 ± 4% |
| Castanospermine-derived | 47 ± 3% | 55 ± 5% |
| Swainsonine-derived | 21 ± 6% | 23 ± 3% |
| Unglycosylated | 4 ± 2% | 2 ± 2% |

*PC 12 rat pheochromocytoma cells were grown in culture as described previously (Dean et al., 1990). Neurite outgrowth on different forms of laminin substrata, each in triplicate wells, was visually assessed as indicated in the methods section. Results are expressed as percentage ± SD.

laminin glycopeptides. The data also indicate that the restoration of cell spreading or neurite outgrowth are due solely to the presence of laminin carbohydrates. An excess of soluble laminin glycopeptides, compared to intact, glycosylated laminin, was required to obtain the responses. In other systems, substantially higher amounts of either peptides (Pierschbacher et al., 1983; Tashiro et al., 1989) or oligosaccharides (Sarkar et al., 1979) are also needed to elicit biological responses, compared to the intact parent molecule. Indeed, characterization of such moieties has shown that, on a molar basis, they are orders of magnitude less active compared to the original macromolecule. Use of glycosyl inhibitors to generate immature laminin carbohydrates has provided some insight into which oligosaccharide determinants are being recognized by the responding cells. We should now be able to determine the minimal structural determinants required for response as well as to elucidate the cellular responding elements themselves.

## NOTE ADDED IN PROOF

Chammas et al. (R. Chammas, S. S. Veiga, S. Line, P. Potocnjak and R. R. Brentani. J. Biol. Chem. 266:3349–3355, 1991) have recently reported that carbohydrates from both laminin

and α6/β1 integrin play a role in their mutual interaction.

## ACKNOWLEDGMENTS

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