Core 1 Glycans on α-Dystroglycan Mediate Laminin-induced Acetylcholine Receptor Clustering but Not Laminin Binding*

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Although unique O-linked oligosaccharides on α-dystroglycan are important for binding to a variety of extracellular ligands, the function(s) of more generic carbohydrate structures on α-dystroglycan remain unclear. Recent studies suggest a role for glycoconjugates bearing the core 1 disaccharide Galβ(1–3)GalNAc in acetylcholine receptor (AChR) clustering on the surface of muscle cells. Here, we report experiments demonstrating that the core 1-specific lectin jacalin almost completely abrogated laminin-induced AChR clustering in C2C12 myotubes and that α-dystroglycan was the predominant jacalin-binding protein detected in C2C12 myotube lysates. Although jacalin likely inhibited laminin-induced AChR clustering by directly binding to α-dystroglycan, jacalin had no effect on laminin binding to the myotube surface or to α-dystroglycan. Like jacalin, peanut agglutinin lectin also binds the core 1 disaccharide but not when it is terminally sialylated as expressed on α-dystroglycan. We show that C2C12 α-dystroglycan bound to peanut agglutinin only after digestion with neuraminidase. Simultaneous treatment of myotubes with neuraminidase and endo-O-glycosidase diminished α-dystroglycan binding to peanut agglutinin and inhibited neuraminidase-induced AChR clustering. We conclude that sialylated core 1 oligosaccharides of α-dystroglycan are important for laminin-induced AChR clustering and that their function in this process is distinct from the established role of α-dystroglycan oligosaccharides in laminin binding.

The dystroglycan complex was originally identified as a component of the skeletal muscle dystrophin-glycoprotein complex, which spans the sarcolemma of muscle cells and physically couples the actin cytoskeleton with the extracellular matrix (1–3). The dystroglycan complex consists of α-dystroglycan, a highly glycosylated extracellular protein that binds to several extracellular ligands, and β-dystroglycan, a single-pass transmembrane protein that links cytoplasmic dystrophin with α-dystroglycan (3). Both dystroglycan subunits are encoded by a single highly conserved pro-peptide that is proteolytically processed into α- and β-dystroglycan, which remain stably associated through non-covalent interactions (3). Like muscle deficient in dystrophin or other core components in the dystrophin-glycoprotein complex (1, 2), deficiency of the dystroglycan complex in skeletal muscle results in compromised sarcolemmal integrity (4, 5). Thus, it is generally thought that one important function of the dystroglycan complex in skeletal muscle is to mechanically protect the sarcolemma against shear stresses imposed during muscle contraction. However, the dystroglycan complex has also been linked with more dynamic developmental or pathological processes (6). In skeletal muscle, several studies have implicated the dystroglycan complex in either the formation or maintenance of acetylcholine receptor (AChR)1 and acetylcholineesterase dense specializations within the motor endplate of the neuromuscular junction (4, 7–10).

O-linked oligosaccharides of unknown structure are clearly important for α-dystroglycan binding to its extracellular ligands as well as its function in vivo because mutations in glycosyltransferases that post-translationally modify α-dystroglycan result in loss of extracellular ligand binding activity and muscular dystrophy (3). α-Dystroglycan is also modified by more generic N-linked glycans and sialylated core 1 oligosaccharides with the structure Siaα2–3Galβ(1–3)GalNAc (11). However, enzymatic removal of these glycans has no effect on α-dystroglycan binding to laminins (12), agrin (13), or neurexins (14). Recently, Martin and colleagues (15, 16) have reported data suggesting that the common core 1 disaccharide Galβ(1–3)GalNAc may play a role in AChR clustering. Most relevant to the current study, the core 1-reactive lectin peanut agglutinin was demonstrated to completely inhibit AChR clustering induced by neuraminidase treatment of myotubes (15, 16). However, the core 1-modified glycoconjugate(s) important for neuraminidase-induced AChR clustering and blocked by peanut agglutinin were not identified. Here, we report that the core 1-specific lectin jacalin bound predominantly to α-dystroglycan in C2C12 myotubes and inhibited laminin-induced AChR clustering. We further demonstrate that α-dystroglycan was the predominant peanut agglutinin-binding protein detected in neuraminidase-treated C2C12 myotubes and that both α-dystroglycan binding to peanut agglutinin and neuraminidase-induced AChR clustering were dramatically inhibited by endo-O-glycosidase digestion. Additional experiments indicate that the sialylated core 1 oligosaccharides of α-dystroglycan play a role in laminin-induced AChR clustering through a mechanism independent from oligosaccharide-mediated binding of α-dystroglycan to laminin.

EXPERIMENTAL PROCEDURES

Cell Culture—The C2C12 cell line was obtained from ATCC and used between 3 and 7 passages. Proliferating cells were grown in 10 cm

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‡ The abbreviations used are: AChR, acetylcholine receptor; PBS, phosphate-buffered saline; HRP, horseradish peroxidase; ANOVA, analysis of variance; MuSK, muscle-specific kinase.
dishes or on poly-L-lysine-coated coverslips with Dulbecco’s modified Eagle’s medium (Collgro, Fisher Scientific) containing 10% fetal bovine serum (Hyclone, Logan, UT) plus 1% antibiotic/antimycotic (Sigma) at 37 °C in a humid atmosphere of 5–10% CO2. After reaching confluency, the media was switched to Dulbecco’s modified Eagle’s medium containing 2% equine serum (Hyclone) plus 1% antibiotic/antimycotic. Cells were cultured until full differentiation to multi-nucleate myotubes was observed morphologically (4 days) with fresh media exchanged every 2 days.

Analysis of AChR Clustering—3-day old C2C12 myotubes grown on poly-L-lysine-coated glass coverslips were incubated with 120 nM laminin-1, 50 μg/ml jacalin lectin (EY Labs, San Mateo, CA), or both diluted in media for 18 h at 37 °C. Cells were rinsed with 50 mM Tris-HCl, pH 7.4, 1 mM CaCl2, and fixed in 4% paraformaldehyde for 10 min at room temperature. Fixed cells were rinsed in PBS and mounted on slides for immunofluorescence analysis. In addition, 4-day-old C2C12 myotubes were treated with 0.1 unit/ml endo-β-galactosidase for 21 h at 37 °C. Cells were rinsed twice with media and fixed and stained as described above. Fluorescence images were collected with a Bio-Rad MRC 1000 confocal microscope (Kear® Center for Biological Imaging), or Zeiss CCF 25 fluorescence microscope using a 40× oil immersion objective and the number of AChR clusters per field for each treatment (Fig. 1). Immunofluorescence images were collected with a Bio-Rad MRC 1000 confocal microscope.

**Myotube Solubilization and Lectin Chromatography**—Differentially C2C12 myotubes were rinsed twice with 37 °C PBS and scraped off each dish into 5 ml of ice-cold PBS containing the following protease inhibitors: 100 μg/ml benzamidine, 40 μg/ml phenylmethylsulfonyl fluoride, 0.5 μg/ml leupeptin, 0.5 μg/ml aprotinin, 1 mM iodoacetamide, and 0.5 μg/ml pepstatin A. Cells were pelleted by brief centrifugation and solubilized in 0.5 ml/dish of 1% Triton X-100 (50 mM Tris-HCl, pH 7.4, 1 mM CaCl2, 1 mM MgCl2, 100 mM benzamidine, 40 μg/ml phenylmethylsulfonyl fluoride, 0.5 μg/ml leupeptin, 0.5 μg/ml aprotinin, 1 mM iodoacetamide, and 0.5 μg/ml pepstatin A). Cells were resuspended and cropped using Adobe Photoshop 5.0 and imported into CorelDraw 10 for figure preparation.

**Myotube Solubilization and Lectin Chromatography**—Differentially C2C12 myotubes were rinsed twice with 37 °C PBS and scraped off each dish into 5 ml of ice-cold PBS containing the following protease inhibitors: 100 μg/ml benzamidine, 40 μg/ml phenylmethylsulfonyl fluoride, 0.5 μg/ml leupeptin, 0.5 μg/ml aprotinin, 1 mM iodoacetamide, and 0.5 μg/ml pepstatin A. Cells were pelleted by brief centrifugation and solubilized in 0.5 ml/dish of 1% Triton X-100 (50 mM Tris-HCl, pH 7.4, 1 mM CaCl2, 1 mM MgCl2, 100 mM benzamidine, 40 μg/ml phenylmethylsulfonyl fluoride, 0.5 μg/ml leupeptin, 0.5 μg/ml aprotinin, 1 mM iodoacetamide, and 0.5 μg/ml pepstatin A). Cells were pelleted by brief centrifugation and the void volumes retained for Western blot analysis. Lectin beads were then washed extensively with wash buffer and bound proteins eluted by incubation with Laemmli sample buffer (LSB, 3% SDS, 5% 2-mercaptoethanol, 65 mM Tris-HCl, pH 6.8, and 1% β-mercaptoethanol) containing 0.1% lactose.

To determine the effects of neuraminidase and endo-O-glycosidase treatment on the peanut agglutinin-reactivity of α-dystroglycan, C2C12 myotubes were incubated for 2 h at 37 °C with 0.1 units/ml C. perfringens neuraminidase (Roche Applied Science) in the absence or presence of 15 milliunits/ml endo-O-glycosidase (Prozyme, San Leandro, CA). Cells were rinsed twice with 37 °C PBS, harvested, and solubilized for peanut agglutinin-agarose chromatography as described above.

**Immunofluorescence Analysis of α-Dystroglycan Distribution and Laminin-1 Binding to C2C12 Myotubes—3-day-old C2C12 myotubes were incubated for 18 h at 37 °C in the absence or presence of 50 μg/ml jacalin lectin or 50 μg/ml serotonin plus laminin-1 during incubation with Dulbecco’s modified Eagle’s medium containing 2% equine serum. After reaching confluency, the media was switched to Dulbecco’s modified Eagle’s medium containing 2% equine serum (Hyclone) plus 1% antibiotic/antimycotic. Cells were cultured until full differentiation to multi-nucleate myotubes was observed morphologically (4 days) with fresh media exchanged every 2 days.

**Western Blot Analysis of Laminin-1 Binding to C2C12 Myotubes—** Control, laminin-1-treated, and laminin-1 plus jacalin-treated myotubes were rinsed with both 50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM CaCl2, and solubilized with Laemmli sample buffer. The relative laminin-1 chain immunoreactivity present in equal amounts of total myotube protein was assessed by SDS-polyacrylamide gel electrophoresis and Western blotting using an affinity-purified rabbit polyclonal antibody raised against mouse laminin-1.

**Analysis of α-Dystroglycan**—The effect of jacalin on laminin-1 binding to α-dystroglycan was also assessed by two methods. First, polystyrene microtiter wells coated overnight at 4 °C with 0.1 μg of α-dystroglycan purified from rabbit skeletal muscle were blocked with 3% bovine serum albumin in 50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM CaCl2, 1 mM MgCl2, and incubated with 4 μg laminin-1 in the presence of increasing concentrations of jacalin. Laminin-1 binding was detected with affinity-purified rabbit polyclonal antibodies against laminin-1, followed by HRP-conjugated anti-rabbit secondary and color development with 3,3’,5,5’-tetramethylbenzidine (Bio-Rad Laboratories, Hercules, CA). Color development was quantitated kinetically at 655 nm using a Molecular Dynamics Spectramax 340UV/Vis microplate reader for inhibition, a parallel assay was performed in the presence of increasing concentrations of purified monoclonal antibody I116 (the kind gift of Dr. Kevin Campbell), which was previously shown to inhibit laminin-1 binding to α-dystroglycan (12). In the second assay, C2C12 myotubes were solubilized in 50 mM Tris-HCl, pH 7.4, 1 mM CaCl2, 1 mM MgCl2, 100 μg/ml benzamidine, 40 μg/ml phenylmethylsulfonyl fluoride, 1% Triton X-100, and 50 mM NaCl to facilitate laminin binding to α-dystroglycan in the presence of detergent (12), and lysates were incubated overnight at 4 °C with jacalin-agarose either alone or in the presence of 0.25 μg/ml or 0.5 μg/ml purified laminin-1. Beads were washed extensively, and bound proteins were eluted with Laemmli sample buffer. To determine whether laminin-1 bound directly to jacalin-agarose, purified laminin-1 was diluted to 0.5 μg/ml in 50 mM Tris, pH 7.4, 150 mM NaCl, and 1 mM CaCl2, and 100 μl was incubated overnight with 20 μl of jacalin-agarose at 4 °C. Jacalin-agarose was washed and bound proteins eluted as described above. The amount of laminin-1 chain and α-dystroglycan present in the starting material, voids, and lectin-bound fractions was assessed by SDS-polyacrylamide gel electrophoresis and stained with Coomassie blue or transferred to nitrocellulose as previously described (11). Western blotting was performed with either monoclonal antibody I116 to α-dystroglycan (11), or affinity-purified rabbit polyclonal antibodies against laminin-1 followed by HRP-conjugated secondary antibody. Lectin blotting was performed by blocking nitrocellulose transfers with PBS containing 0.05% Tween 20 followed by incubation with 1 μg/ml HRP-conjugated jacalin or peanut agglutinin (EY Labs) in blocking buffer for 1 h at room temperature and extensive washing with PBS. All antibody and lectin staining was detected by enhanced chemiluminescence using Pierce Supersignal Peroxidase substrate. Gels and chemiluminescence films were imaged using a Bio-Rad GS670 imaging densitometer and the images processed using Bio-Rad Molecular Analyst software (version 1.4) before importation into CorelDraw 10 for figure preparation.

**RESULTS**

Several laminin isoforms bind α-dystroglycan (12, 18–21) and induce AChR clustering in myotubes through a mechanism dependent on laminin binding to α-dystroglycan (9, 22–25). Recent studies (15, 16) further suggest a role for glycoconjugates bearing the core 1 disaccharide Galβ1–3GalNAc in AChR clustering on the surface of muscle cells. The lectin jacalin shows specificity for Galβ1–3GalNAc but can also bind avidly to Siaα2→3Galβ1–3GalNAc (26). Because α-dystroglycan is modified by sialylated core 1 disaccharides (see Fig. 2A), we treated C2C12 myotubes with 120 nM laminin-1, 50 μg/ml jacalin, or both and quantitated the number of AChR clusters per field for each treatment (Fig. 1, A and B). Although jacalin alone had no effect on the number of AChR clusters observed relative to untreated control myotubes and laminin-1...
alone induced an ~4-fold increase in the number of AChR clusters, jacalin almost completely abrogated laminin-1-induced AChR clustering (Fig. 1B).

To begin to elucidate the mechanism by which jacalin may inhibit laminin-induced AChR clustering, Triton lysates of C2C12 myotubes were resolved by SDS-PAGE, transferred to nitrocellulose and stained with peroxidase-conjugated jacalin. Surprisingly, jacalin detected only a single diffuse band that stained with a monoclonal antibody (DG) and integrin detected only a single diffuse band that stained with a polyclonal antibody raised against α-dystroglycan (α-DG). Molecular weight standards (x10^3) are indicated on the left. Shown in C are the relative amounts of α-dystroglycan (α-DG) and β1 integrin detected on Western blots loaded with equivalent volumes of Triton-solubilized C2C12 lysate (TOT), the jacalin-agarose flow-through (Jac VOID), and jacalin-bound fraction (Jac-Agarose).

Because jacalin is bivalent and the number of sialylated core 1 oligosaccharides present on α-dystroglycan is unknown, we considered the possibility that jacalin inhibited laminin-1-induced AChR clustering by sequestering dystroglycan into cluster-incompetent aggregates on the myotube surface. However, we found that prolonged incubation of C2C12 myotubes with 50 μg/ml jacalin had no effect on the normally diffuse distribution of β-dystroglycan (Fig. 3A).

The carbohydrate moieties present on α-dystroglycan are clearly necessary for its binding to laminins, agrin, and neuramins whereas the specific oligosaccharides on α-dystroglycan responsible for its binding to extracellular ligands have not been identified (3). Therefore, jacalin could inhibit laminin-1-induced AChR clustering by blocking laminin-1 binding to α-dystroglycan. To test this possibility, C2C12 myotubes were either left untreated or incubated with 120 nM exogenous laminin-1 in the absence or presence of 50 μg/ml jacalin, and bound laminin-1 was assessed by two methods. In the first, laminin-1 bound to the myotube surface was assessed qualitatively by immunofluorescence microscopy using an affinity-purified polyclonal antibody raised against laminin-1 (Fig. 3B). Consistent with a previous study (29), we detected virtually no laminin-1 immunoreactivity on the surface of control myotubes, whereas the surfaces of laminin-1-treated myotubes were strongly stained (Fig. 3B). However, surface staining of myotubes incubated with both laminin-1 and jacalin was indistinguishable from that observed for myotubes incubated with laminin-1 alone (Fig. 3B). In the second method, control myotubes and myotubes incubated with laminin-1 in the absence or presence of jacalin were rinsed and solubilized with 1% SDS to extract all protein retained on the culture dish. Western blot analysis of equal amounts of the SDS myotube extracts obtained after each treatment regime revealed an equivalent amount of laminin-1 α1 chain immunoreactivity retained by...
cultures incubated with laminin-1 regardless of whether jacalin was present during the incubation period (Fig. 3C).

Although the results presented in Fig. 3, B and C, suggest that jacalin did not inhibit laminin-1-induced AChR clustering by preventing laminin-1 binding to the myotube surface, it remained possible that laminin-1 predominantly binds to myotubes via interaction with β1 integrin but that jacalin subsequently blocks laminin-1 interaction with α-dystroglycan on the myotube surface. However, we performed two experiments indicating that jacalin was unable to directly block laminin-1 binding to α-dystroglycan. First, we measured laminin-1 binding to purified α-dystroglycan immobilized in microtiter wells and measured no effect of jacalin even at concentrations as high as 100 µg/ml (Fig. 4A). As a control, we performed a parallel experiment with monoclonal antibody IIH6, which has previously been shown to block the laminin/α-dystroglycan interaction (12). As expected, IIH6 completely inhibited laminin-1 binding to immobilized α-dystroglycan (Fig. 4A) when present at a concentration of 50 µg/ml (63 nM). The great molecular weight disparity between jacalin (50,000) and IIH6 IgM (800,000), the data in Fig. 4A indicate that jacalin was unable to inhibit laminin-1 binding to α-dystroglycan even when present at 500-fold molar excess over laminin-1 and ∼30-fold molar excess over the concentration of IIH6 that completely inhibited binding. Finally, we tested whether exogenous laminin-1 could inhibit the binding of C2C12 myotube α-dystroglycan to jacalin-agarose. Triton-solubilized myotube lysates were pre-incubated with 0, 0.25, or 0.5 µg/ml of purified laminin-1 followed by incubation with jacalin-agarose. We observed similar amounts of α-dystroglycan retained by jacalin-agarose in the absence or presence of added laminin-1 (Fig. 4B). Staining of an identical blot with affinity-purified polyclonal antibodies to laminin-1 revealed that equivalent amounts of laminin-1 chain were retained by jacalin-agarose (Fig. 4B) even though purified laminin-1 alone exhibited no binding to jacalin-agarose (Fig. 4C). The results of Fig. 4, B and C, indicate that laminin-1 was retained on jacalin-agarose through interaction with α-dystroglycan in the myotube lysate and that we had saturated all laminin-1 binding sites on α-dystroglycan. We conclude that laminin-1 and jacalin do not compete for the same or overlapping binding sites on α-dystroglycan and that the three proteins may form a ternary complex.

Previous studies (15, 16) demonstrated that the enzyme neuraminidase can induce AChR clustering in C2C12 myotubes and that neuraminidase-induced AChR clustering is blocked by the lectin peanut agglutinin. Like jacalin, peanut agglutinin lectin also binds specifically to the core 1 disaccharide but cannot when modified by a terminal sialic acid as expressed on α-dystroglycan (Fig. 2A). Given the data in Figs. 1 and 2 suggested that jacalin inhibited laminin-1-induced AChR clustering by binding to sialylated core 1 oligosaccharides on α-dystroglycan, we hypothesized that peanut agglutinin may also inhibit neuraminidase-induced AChR clustering by binding to desialylated core 1 disaccharides of neuraminidase-digested α-dystroglycan. Peroxidase-conjugated peanut agglutinin lectin stained none of the proteins present in control myotube lysates but stained a single diffuse band with an electrophoretic migration that matched that of α-dystroglycan immunoreactivity in neuraminidase-treated myotubes (Fig. 5A). Peanut agglutinin staining of this single band was substantially decreased when neuraminidase-treated myotubes were simultaneously digested with endo-O-glycosidase (Fig. 5A), which specifically cleaves unmodified core 1 disaccharides from the underlying polypeptide backbone (Fig. 2A). To confirm that the peanut agglutinin-reactive protein in neuraminidase-treated myotubes was indeed α-dystroglycan, we performed peanut agglutinin-agarose chromatography on control myotube lysates as well as lysates prepared from myotubes digested with neuraminidase in the absence or presence of endo-O-glycosidase. Although α-dystroglycan in neuraminidase-treated myotubes bound to peanut agglutinin-agarose, no α-dystroglycan in control lysates was retained by the lectin matrix (Fig. 5B). Moreover, the amount of α-dystroglycan bound to peanut agglutinin-agarose was dramatically reduced.
When myotubes were simultaneously incubated with neuraminidase and endo-O-glycosidase (Figs. 5B). These results suggest that peanut agglutinin inhibits neuraminidase-induced AChR clustering by binding to desialylated core 1 disaccharides present on α-dystroglycan.

If core 1 disaccharides are important for AChR clustering, we reasoned that endo-O-glycosidase treatment should also inhibit neuraminidase-induced AChR clustering. Consistent with previous studies from several labs (15, 17, 30, 31), neuraminidase treatment of C2C12 myotubes caused an ~4-fold increase in the number of clusters observed in comparison with untreated myotubes (Fig. 6). More interestingly, endo-O-glycosidase dramatically inhibited neuraminidase-induced AChR clustering (Fig. 6). When taken together with the results of Martin and colleagues (15, 16), the data in Figs. 5 and 6 indicate that either blocking (by peanut agglutinin) or specific enzymatic removal of core 1 disaccharides from α-dystroglycan largely abrogates neuraminidase-induced AChR clustering. Although the relevance of neuraminidase-induced AChR clustering in vitro is unclear, these data further support an important role for α-dystroglycan core 1 disaccharides in AChR clustering, regardless of the mode by which cluster formation is triggered.

**DISCUSSION**

Distinct oligosaccharide structures have long been implicated in the process of neuromuscular synaptogenesis based on unique labeling of adult neuromuscular junctions with carbohydrate-specific lectins and antibodies as well as perturbation of AChR clustering in myotube cultures by lectins and glycosidases (15–17, 30–34). With regard to the identity of glycoconjugates bearing sialylated oligosaccharides important in AChR cluster formation, recent data suggests that agrin and MuSK both bind to and are modified by several oligosaccharides that are potentially the site of action by glycosidases and lectins that have been shown to significantly alter AChR clustering in vitro (15, 16). Modulation of agrin and MuSK activity by glycosylation is likely of great physiological relevance because they, along with rapsyn and AChRs, are the core components necessary for AChR cluster formation in vivo (35). Existing evidence suggests that neuraminidase induces AChR clustering in myotubes at least in part by removing sialic acid residues from oligosaccharides present on MuSK (31, 36). Although peanut agglutinin could conceivably inhibit neuraminidase-induced AChR clustering by directly binding to MuSK, Watty and Burden (36) showed that the sialic acid moieties present on MuSK were entirely contained within N-linked oligosaccharides. Moreover, this study observed no binding of peanut agglutinin to MuSK either before or after treatment with neuraminidase (36). These results indicate that neuraminidase does not stimulate AChR clustering by exposing core 1 disaccharides on MuSK, nor does peanut agglutinin block clustering through binding to core 1 disaccharides on MuSK.

In addition to inducing AChR clustering through its action on sialylated N-linked oligosaccharides on MuSK (36), our results demonstrate that neuraminidase treatment also exposes peanut agglutinin-reactive core 1 disaccharides detected predominantly on α-dystroglycan (Figs. 5 and 6), which suggests that peanut agglutinin exerts its inhibitory effect by binding directly to desialylated α-dystroglycan. Our experiments with endo-O-glycosidase (Figs. 5 and 6) further indicate that removal of core 1 disaccharides from desialylated α-dystroglycan is as effective in abrogating neuraminidase-induced AChR clustering as was blocking core 1 disaccharides by peanut agglutinin (15, 16). More importantly, we demonstrated that jaclin also binds predominantly to α-dystroglycan and effectively inhibits laminin-induced AChR clustering (Figs. 1 and 2) independent of treatment by any glycosidase. These results suggest that the sialylated core 1 oligosaccharides present on native α-dystroglycan may participate in the formation or sta-
abrogate the putative protein-oligosaccharide interaction and can (35). In this model, either blocking (by lectin binding) or Molecules interacting with the sialylated core 1 oligosaccharides may collapse an extended conformation of the underlying polypeptide backbone (37), and both the extensive glycosylation (38) and electron microscopic evidence (38) support the idea that this may hold true for α-dystroglycan as well. Therefore, it is conceivable that enzymatic removal of sialylated core 1 oligosaccharides may collapse an extended conformation of α-dystroglycan that is necessary to transduce laminin binding into some form of downstream signaling. This mechanism is difficult to reconcile with our (Fig. 1) and others’ observations (15, 16) that lectin binding to core 1 oligosaccharides also effectively inhibited neuraminidase- and laminin-induced AChR clustering. We instead favor the hypothesis that the sialylated core 1 oligosaccharides of α-dystroglycan mediate its lateral association with another cell surface or extracellular molecule that is important for AChR cluster formation. Molecules interacting with the sialylated core 1 oligosaccharides of α-dystroglycan could either stimulate AChR clustering upon binding to α-dystroglycan, or they may constitutively inhibit clustering until repressed when bound to α-dystroglycan (35). In this model, either blocking (by lectin binding) or enzymatic removal of the oligosaccharide would be sufficient to abrogate the putative protein-oligosaccharide interaction and disrupt AChR clustering.

Although an obvious candidate for lateral interaction with α-dystroglycan is α1β1 integrin (27), we have not been able to detect a biochemical interaction between α-dystroglycan and α1β1 integrin even after prolonged incubation of C2C12 myotubes with laminin. Based on its direct binding to and activation by the isolated core 1 disaccharide (15), MuSK can be postulated as a candidate for lateral interaction with α-dystroglycan. However, the disaccharide Galβ1(1–4)GlcNAc also binds to and activates MuSK with the same efficacy as the isolated core 1 disaccharide whereas SiaA(2–3)Galβ1(1–4)GlcNAc exhibits neither activity (15). Therefore, the lack of specificity of MuSK for core 1 disaccharide, the absence of activity upon sialylation, and the ability of laminin to induce AChR clustering in MuSK−/−myotubes (22) all argue against this possibility and suggests that α-dystroglycan may laterally associate with a novel cell surface molecule. Fortunately, the results and methodologies described herein provide both the basis and means to identify sialylated core 1 interacting proteins in future experiments.

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