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A Role of Dystroglycan in Schwannoma Cell Adhesion to Laminin*

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Dystroglycan is encoded by a single gene and cleaved into two proteins α- and β-dystroglycan by posttranslational processing. Recently, α-dystroglycan was demonstrated to be an extracellular laminin-binding protein anchored to the cell membrane by a transmembrane protein β-dystroglycan in striated muscle and Schwann cells. However, the biological functions of the dystroglycan-laminin interaction remain obscure, and in particular, it is still unclear if dystroglycan plays a role in cell adhesion. In the present study, we characterized the role of dystroglycan in the adhesion of schwannoma cells to laminin-1. Immunochemical analysis demonstrated that the dystroglycan complex, comprised of α- and β-dystroglycan, was a major laminin-binding protein complex in the surface membrane of rat schwannoma cell line RT4. It also demonstrated the presence of α-dystroglycan, but not β-dystroglycan, in the culture medium, suggesting secretion of α-dystroglycan by RT4 cells. RT4 cells cultured on dishes coated with laminin-1 became spindle in shape and adhered to the bottom surface tightly. Monoclonal antibody IIH6 against α-dystroglycan was shown previously to inhibit the binding of laminin-1 to α-dystroglycan. In the presence of IIH6, but not several other control antibodies in the culture medium, RT4 cells remained round in shape and did not adhere to the bottom surface. The adhesion of RT4 cells to dishes coated with fibronectin was not affected by IIH6. The known inhibitors of the interaction of α-dystroglycan with laminin-1, including EDTA, sulfatide, fucoidan, dextran sulfate, heparin, and sialic acid, also perturbed the adhesion of RT4 cells to laminin-1, whereas the reagents which do not inhibit the interaction, including dextran, chondroitin sulfate, dermatan sulfate, and GlcNAc, did not. Altogether, these results support a role for dystroglycan as a major cell adhesion molecule in the surface membrane of RT4 cells.

There is now mounting evidence that the intracellular signal transduction pathways activated by the adhesion of cells to other cells or the extracellular matrix (ECM)[1] play crucial roles in cellular differentiation, migration, and proliferation. The prototypical cell adhesion molecules are the cell surface receptors for the ECM glycoproteins. Dystroglycan, originally identified as a member of the sarcolemmal glycoproteins complexed with dystrophin, is encoded by a single gene and cleaved into two proteins α- and β-dystroglycan by posttranslational processing (1, 2). α-Dystroglycan is an extracellular glycoprotein anchored to the cell membrane by a transmembrane glycoprotein β-dystroglycan, and the complex comprised of α- and β-dystroglycan is called the dystroglycan complex (2–4). In striated muscle, α-dystroglycan binds the ECM components laminin-1 and -2 in a Ca^{2+}-dependent manner (3, 5, 6). On the cytoplasmic side of the sarcolemma, β-dystroglycan is anchored to the cytoskeletal proteins dystrophin or its homologues (7, 8). Besides this structural role, β-dystroglycan is also proposed to play a role in signal transduction, based on the finding that its cytoplasmic domain contains a phosphotyrosine consensus sequence and several proline-rich regions that could associate with SH2 and SH3 domains of signaling proteins (1). Dystrophin deficiency causes a drastic reduction of the dystroglycan complex in the sarcolemma and, thus, the loss of the linkage between the subsarcolemmal cytoskeleton and the ECM, eventually leading to muscle cell death in Duchenne muscular dystrophy and its animal model *mdx* mice (for reviews, see Refs. 9 and 10).

The dystroglycan complex is also expressed in non-muscle tissues (1, 3, 11–13). In the peripheral nervous system, it is expressed in the Schwann cell membrane, and the Schwann cell α-dystroglycan binds not only laminin-1 but also laminin-2, a major component of the endoneurium, in a Ca^{2+}-dependent manner (13–15). Recently, laminin-2 was shown to be deficient in congenital muscular dystrophy and its animal model *dy* mice, which are characterized by peripheral dysmyelination as well as muscular dystrophy (16–22). These findings have suggested roles for the dystroglycan-laminin interaction in not only the maintenance of sarcolemmal architecture but also peripheral myelinogenesis.

Despite these recent developments, the biological functions

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[1] The abbreviations used are: ECM, extracellular matrix; BSA, bovine serum albumin; cLSM, confocal laser scanning microscope; WGA, wheat germ agglutinin; PBS, phosphate-buffered saline.

of the dystroglycan complex remain obscure, and in particular, it has not yet been established if the dystroglycan complex is indeed involved in the process of cell adhesion. In the present study, we have identified the dystroglycan complex as a major laminin-binding protein complex in the surface membrane of rat schwannoma cell line RT4 and characterized its role in RT4 cell adhesion to laminin-1.

EXPERIMENTAL PROCEDURES

Cell Culture—Rat schwannoma cell line RT4 was kindly provided by Drs. A. Asai (University of Tokyo) and Y. Kuchino (National Cancer Center, Tokyo) (23). RT4 cells were grown in Dulbecco's modification of Eagle's medium containing 10% fetal calf serum, 16.7 mM glucose, 2 mM glutamine, 100 units/ml penicillin G sodium, and 100 μg/ml streptomycin. Culture medium was changed every 3 days.

Identification of the Dystroglycan Complex in RT4 Cells—For immunocytochemical analysis, RT4 cells were grown on cover glasses, fixed in methanol cooled to −20 °C for 10 min, and then immunostained as described previously (13, 15). Specimens were observed and fluorescent images were obtained on a Zeiss confocal laser scanning microscope (cLSM) model LSM 310, employing an argon ion laser (λ = 488 nm), as described previously (13, 14).

For immunochemical analysis, RT4 cells were grown to confluency on three 10-cm diameter culture dishes, scraped using a rubber policeman, homogenzied in 1.5 ml of phosphate-buffered saline (PBS), and centrifuged at 140,000 × *g* for 30 min at 4 °C. The pellets were homogenized in 1.5 ml of buffer A (50 mM Tris-HCl, pH 7.4, 0.75 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.7 μM pepstatin A, 76.8 nM aprotinin and 1.1 μM leupeptin) containing 1% digitonin and 0.5 M NaCl, extracted for 1 h at 4 °C, and then centrifuged at 140,000 × *g* for 30 min at 4 °C. After the supernatants were diluted 10-fold using buffer A, $CaCl_2$ and $MgCl_2$ were added to the final concentration of 1 mM each. The diluted supernatants were incubated overnight at 4 °C with 100 μl of laminin-1-Sepharose (2.5 mg/ml), which was blocked for 2 h at 4 °C with buffer A containing 0.1% digitonin, 50 mM NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 3% bovine serum albumin (BSA). After washing with buffer A containing 0.1% digitonin, 50 mM NaCl, 1 mM $CaCl_2$, and 1 mM $MgCl_2$, the laminin-1-Sepharose was eluted with 300 μl of buffer A containing 0.1% digitonin, 50 mM NaCl, and 10 mM EDTA.

Identification of α-Dystroglycan in the RT4 Cell Culture Medium—The RT4 cell culture medium was collected by decanting slowly when RT4 cells grew to near confluency on 10-cm diameter culture dishes. After brief centrifugation, the RT4 cell culture medium (80 ml) was circulated over 5 ml of wheat germ agglutinin (WGA)-Sepharose (Pharmacia Biotech Inc.) at 4 °C overnight in the presence of 0.5 M NaCl. The WGA-Sepharose was washed with buffer A containing 0.5 M NaCl and then eluted with 15 ml of buffer A containing 0.3 M *N*-acetylglucosamine (GlcNAc). 100 μl of laminin-1-Sepharose was blocked with buffer A containing 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 3% BSA at 4 °C for 2 h, and then washed with buffer A containing 1 mM $CaCl_2$ and 1 mM $MgCl_2$. The GlcNAc eluates of WGA-Sepharose were incubated with laminin-1-Sepharose at 4 °C overnight in the presence of 1 mM $CaCl_2$ and 1 mM $MgCl_2$. After washing with buffer A containing 1 mM $CaCl_2$ and 1 mM $MgCl_2$, the laminin-1-Sepharose was eluted with 300 μl of buffer A containing 10 mM EDTA.

Assay of Adhesion of RT4 Cells to Laminin-1—As shown in Fig. 3, we have found that RT4 cells synthesize laminin themselves. For cell adhesion assay, however, culture dishes were coated with laminin-1 as follows, to ensure homogeneous distribution of laminin on the dish surface. A solution of mouse EHS sarcoma laminin-1 (Biomedical Technologies) (5 μg/ml in PBS) was added to the 11.3-mm diameter wells of a 48-well plate, incubated at 37 °C overnight, and then aspirated. 200 μl of 3% BSA in PBS were added to the wells, incubated at room temperature for 1 h, and then aspirated. 50,000 RT4 cells were added to the wells and incubated, in the presence or absence of antibodies or inhibitors, at 37 °C in a CO_2 incubator. The shape and adhesion of the cells were observed after 1 h or overnight incubation. For quantification of cell adhesion, the medium, and the unattached cells were removed by pipetting after 1 h of incubation. The number of the attached cells was quantified by means of the endogenous enzyme hexosaminidase as described by Landegren (24): after PBS wash, 120 μl of 3.75 mM *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide (substrate) in 50 mM citrate buffer, pH 5.0, 0.25% Triton X-100 was added to each well and incubated at 37 °C for 1 h. After a suitable interval, the color reaction was developed, and enzyme activity was blocked by the addition of 180 μl of 50 mM glycine, pH 10.4, containing 5 mM EDTA per well. Absorbance was measured at 405 nm.

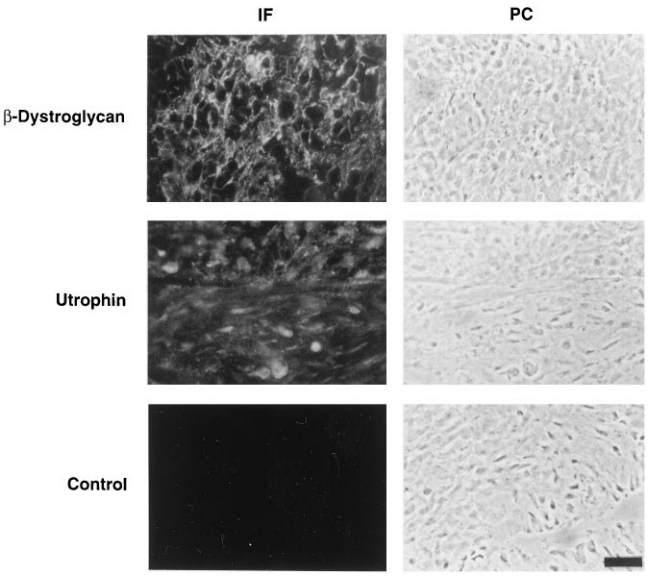

FIG. 1. **Immunohistochemical analysis of dystroglycan and utrophin in a human schwannoma.** Shown are the immunofluorescent (*IF*) and phase contrast (*PC*) images of a human schwannoma. β-Dystroglycan and utrophin were detected by antibodies 8D5 and DRP1, respectively. Control indicates the image obtained by omitting the primary antibody. *Bar*, 25 μm.

Antibodies—Affinity-purified sheep antibody against dystroglycan fusion protein D (Anti-FPD) was characterized previously (1–3). Monoclonal antibodies IIH6 and $VIA4_1$ against α-dystroglycan, 8D5 against the C terminus of β-dystroglycan, A1C and $XIXC_2$ against dystrophin, and DRP1 against utrophin were characterized previously (2, 3, 25, 26). IIH6, $VIA4_1$, and $XIXC_2$ are IgM isotypes, while 8D5 and A1C are IgG isotypes. For inhibition experiments of RT4 cell adhesion to laminin by monoclonal antibodies, hybridoma supernatants containing IIH6, $VIA4_1$, A1C, and $XIXC_2$ were concentrated 10-fold using a Centricon concentrater (Amicon), and one part of the concentrates were added to nine parts of culture medium.

Miscellaneous—Laminin-1-Sepharose was prepared as described previously using mouse EHS sarcoma laminin-1 (13–15). Immunohistochemistry, SDS-polyacrylamide gel electrophoresis, and immunoblotting were performed as described previously (13–15, 26). Laminin blot overlay was performed using mouse EHS sarcoma laminin-1, and the laminin-1 which bound to α-dystroglycan on the nitrocellulose transfer was detected using antibody against mouse EHS sarcoma laminin-1 (Sigma), as described previously (13–15).

RESULTS

Identification of the Dystroglycan Complex as a Major Laminin-binding Protein Complex in the Surface Membrane of RT4 Schwannoma Cells—Previously, we have demonstrated the expression of the dystroglycan complex and utrophin, a dystrophin homologue, in the surface membrane and cytoplasm of Schwann cells, respectively (13, 14). In addition, we have also found the expression of these proteins in human schwannomas by immunohistochemistry (Fig. 1). These findings prompted us to look at the expression of the dystroglycan complex and utrophin in the schwannoma cell line RT4. Fig. 2 shows the results of immunocytochemical analysis using cLSM. Similar to Schwann cells, both α- and β-dystroglycan were localized in the surface membrane of RT4 cells, while utrophin was localized in the cytoplasm diffusely (Fig. 2). To see if these proteins are complexed to function as a laminin receptor, we performed laminin affinity chromatography of the digitonin extracts of RT4 cells (Fig. 3). By immunoblot analysis, α-dystroglycan, with a molecular mass of approximately 160 kDa, was detected in the EDTA eluates of laminin-1-Sepharose (Fig. 3). RT4 cell α-dystroglycan was confirmed to bind laminin-1 in blot overlay (Fig. 3). β-Dystroglycan, with a molecular mass of approximately 40 kDa, was also detected in the EDTA eluates of

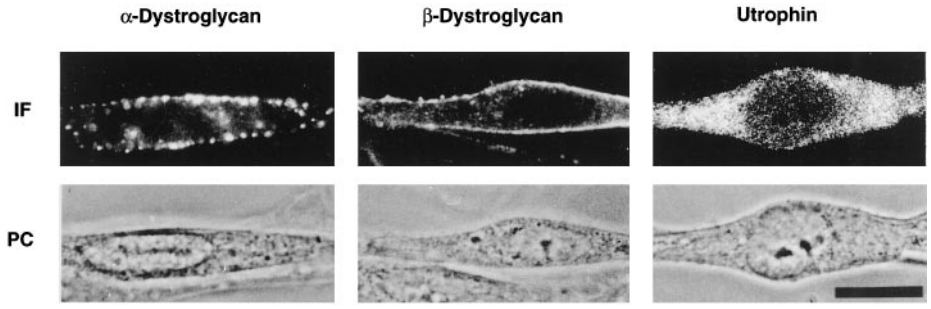

FIG. 2. **Immunocytochemical analysis of the dystroglycan complex and utrophin in RT4 cells.** Shown are the cLSM fluorescent (*IF*) and phase contrast (*PC*) images of RT4 cells. α- and β-dystroglycan and utrophin were detected by antibodies anti-FPD, 8D5, and DRP1, respectively. *Bar*, 2 μm.

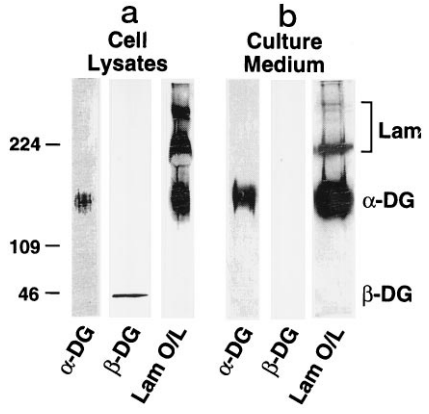

FIG. 3. **Immunochemical characterization of the dystroglycan complex in RT4 cells.** Shown are the results of laminin affinity chromatography of *a*, the digitonin extracts of RT4 cells or *b*, the RT4 cell culture medium. The EDTA eluates of laminin-1-Sepharose were separated by 5–15% SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. α-Dystroglycan (*α-DG*) and β-dystroglycan (*β-DG*) were detected by antibodies IIH6 and 8D5, respectively. *Lam O/L* indicates the identical nitrocellulose transfer overlaid with laminin-1. The bands indicated by *Lam* are the endogenous RT4 laminin which reacted with the antibody against laminin used to detect bound laminin-1 in laminin overlay. Molecular mass standards (Da × 10^{-3}) are shown on the *left*.

laminin-1-Sepharose (Fig. 3). However, RT4 cell β-dystroglycan did not bind laminin-1 in blot overlay (Fig. 3). These results indicate that, similar to striated muscle and Schwann cells, β-dystroglycan is complexed with laminin-binding α-dystroglycan in RT4 cells (3, 14). On the other hand, utrophin was not detected in the EDTA eluates of laminin-1-Sepharose (not shown). In addition, it should also be noted that α-dystroglycan and possibly laminin were the only proteins that bound laminin-1 in blot overlay (Fig. 3). All together, these results indicate that the dystroglycan complex, comprised of α- and β-dystroglycan, is a major laminin-binding protein complex in the surface membrane of RT4 cells. They also indicate that the major fraction of utrophin is localized in the cytoplasm of RT4 cells and not associated with the dystroglycan complex in the cell membrane.

Thus far, secretion of α-dystroglycan by cells has been proposed as an intriguing possibility, but this has never been confirmed (11). By immunoblot analysis, α-dystroglycan, but not β-dystroglycan, was detected in the RT4 cell culture medium after successive WGA and laminin affinity chromatographies (Fig. 3). Taken together with the fact that α-dystroglycan is an extracellular protein anchored to the cell membrane by a transmembrane protein β-dystroglycan (3, 14), these results indicate that a fraction of RT4 cell surface α-dystroglycan is dissociated from the cell membrane β-dystroglycan and released into the culture medium. Although this dissociation may partially be due to lysis of the cells, these findings suggest a possibility of active secretion of α-dystroglycan by RT4 cells.

Characterization of the Role of α-Dystroglycan in Adhesion of RT4 Schwannoma Cells to Laminin-1—Figs. 4*a* to 8*a* show the shape of RT4 cells grown overnight on culture dishes coated with laminin-1 in the presence or absence of various reagents in the culture medium. In the absence of inhibitors, RT4 cells became spindle in shape after 1 h of incubation (not shown) and remained so after overnight incubation (Fig. 4*a*). Spindle cells adhered to the bottom surface of the culture dishes tightly and did not oscillate when the dishes were shaken rigorously by hands. Monoclonal antibody IIH6 against α-dystroglycan was demonstrated previously to inhibit the binding of laminin-1 to α-dystroglycan (3, 27–29). When IIH6 was present in the culture medium, RT4 cells remained round in shape and did not take the characteristic spindle shape, even after overnight incubation (Fig. 4*a*). Round cells oscillated, and many of them drifted when the dishes were shaken rigorously by hand. When the wells were pipetted after 1 h of incubation, the spindle cells in the control wells were not detached, but the round cells in the wells with IIH6 were removed almost completely (not shown). This was confirmed by the quantification procedure described under "Experimental Procedures" (Fig. 4*b*). Monoclonal antibody $VIA4_1$ against α-dystroglycan, which does not inhibit the binding of laminin-1 (3, 29), did not have such effects, neither did monoclonal antibodies A1C and $XIXC_2$ against dystrophin (Fig. 4). We compared the effects of IIH6 on the adhesion of RT4 cells to fibronectin with those to laminin-1. In contrast to dishes coated with laminin-1, the adherence of RT4 cells to dishes coated with fibronectin was not affected by IIH6 (Fig. 5).

Since the interaction of α-dystroglycan with laminin-1 is Ca^{2+}-dependent (1, 3, 11–13), we tested Ca^{2+} dependence of RT4 cell adhesion to laminin-1. When EDTA was added to the culture medium, RT4 cells remained round in shape and were easily detached by pipetting (Fig. 6). Furthermore, the antiadhesive effects of EDTA were reversed by the addition of excess amount of Ca^{2+}, indicating Ca^{2+} dependence of RT4 cell adhesion to laminin-1 (Fig. 6).

Finally, we asked if the known inhibitors of the interaction of α-dystroglycan with laminin-1 would affect RT4 cell adhesion to laminin-1. It was demonstrated previously that sulfatide, fucoidan, and dextran sulfate were potent inhibitors of the interaction of α-dystroglycan with laminin-1, whereas heparin was less potent (30, 31). When sulfatide, fucoidan, or dextran sulfate was added to the culture medium, RT4 cells remained round in shape and were easily detached by pipetting (Fig. 7). The antiadhesive effects of heparin were less than those of sulfatide, fucoidan, or dextran sulfate (Fig. 7). On the other hand, the addition of dextran, chondroitin sulfate, or dermatan sulfate, which were demonstrated previously not to inhibit the interaction of α-dystroglycan with laminin-1 (30), did not affect the shape or adhesion of RT4 cells to laminin-1 (Fig. 7). We have demonstrated previously that the interaction of α-dystroglycan with laminin-1 is inhibited by a relatively high concentration of sialic acid but not GlcNAc (14). In the presence of sialic acid, but not GlcNAc, in the culture medium, RT4 cells remained round in shape and were easily detached by pipetting

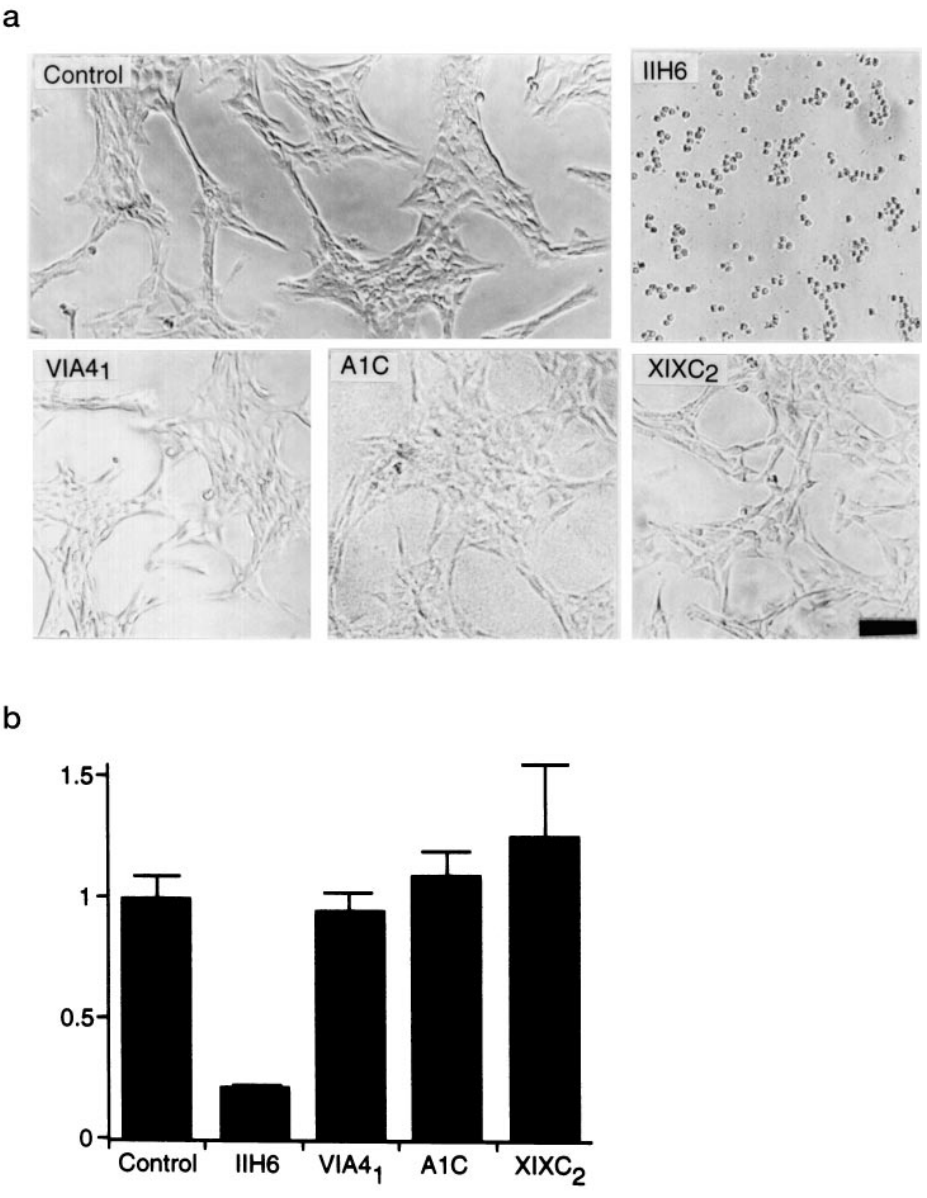

FIG. 4. **Effects of antibodies on the shape and adhesion of RT4 cells to laminin-1.** 50,000 RT4 cells were added to the 11.3-mm diameter wells of a 48-well plate coated with laminin-1 and incubated at 37 °C in a CO_2 incubator, in the absence (*Control*) or presence of monoclonal antibodies IIH6, $VIA4_1$, A1C, or $XIXC_2$. *a*, photographs of the cells were taken after overnight incubation. The shape of the cells was essentially the same after 1 h of incubation. *Bar*, 10 μm. *b*, for quantitative cell adhesion assay, each antibody was applied to three wells. After 1 h of incubation, the unattached cells were removed from the wells by pipetting, and the absorbance was measured as described under "Experimental Procedures." The *bar graph* shows the average absorbance value for the control wells or the wells containing various antibodies. The average value for the control wells is given the arbitrary value of 1. Shown are the results of one experiment representative of three independent experiments. To apply equal amount of force, all wells were pipetted by a single person, who did not know the location of the wells containing various antibodies.

(Fig. 8). These results, altogether, point to a role for α-dystroglycan in the adhesion of RT4 cells to laminin-1.

DISCUSSION

α-Dystroglycan, which is an extracellular peripheral membrane glycoprotein anchored to the cell membrane by a transmembrane glycoprotein β-dystroglycan, binds laminin and agrin in striated muscle, neuromuscular junction, and Schwann cells (1–6, 13–15, 27, 28, 31–34). On the other hand, the cytoplasmic domain of β-dystroglycan contains a phosphotyrosine consensus sequence and several proline-rich regions that could associate with SH2 and SH3 domains of signaling proteins (1, 35). Indeed, Grb2, an adaptor protein in the signal transduction pathways, was recently demonstrated to bind to the cytoplasmic proline-rich regions of β-dystroglycan via the two SH3 domains (35). These findings have suggested a possible role for the dystroglycan complex, comprised of α- and β-dystroglycan, as a signaling receptor involved in the maintenance of sarcolemmal architecture, peripheral synaptogenesis, and myelinogenesis. In addition, the dystroglycan complex has also been implicated in kidney epithelial development, although its ECM ligand in kidney has not yet been identified (29). Despite these recent developments, the precise roles of the interaction of the dystroglycan complex with ECM ligands in these specialized biological processes remain obscure, and in particular, it has not yet been confirmed if the dystroglycan complex plays a role in cell adhesion.

Under these circumstances, identification of cell lines that express the dystroglycan complex in the surface membrane would provide us useful tools for testing the proposed functions of the dystroglycan complex *in vivo*. In the present study, we have demonstrated, by immunochemical analyses, that the dystroglycan complex, comprised of α- and β-dystroglycan, is a major laminin-binding protein complex in the surface membrane of rat schwannoma cell line RT4. Similar to Schwann cells (14), utrophin, which has the binding capacity for the cytoplasmic domain of β-dystroglycan (7, 8, 36), was localized diffusely in the cytoplasm of RT4 cells and not associated with the dystroglycan complex. Thus, the putative membrane-associated cytoskeletal protein anchoring the dystroglycan complex to the underlying submembranous cytoskeleton remains to be elucidated.

In the present study, we have also tested the role of α-dystroglycan in RT4 cell adhesion to laminin-1. When RT4 cells were cultured on laminin-1, they became spindle in shape

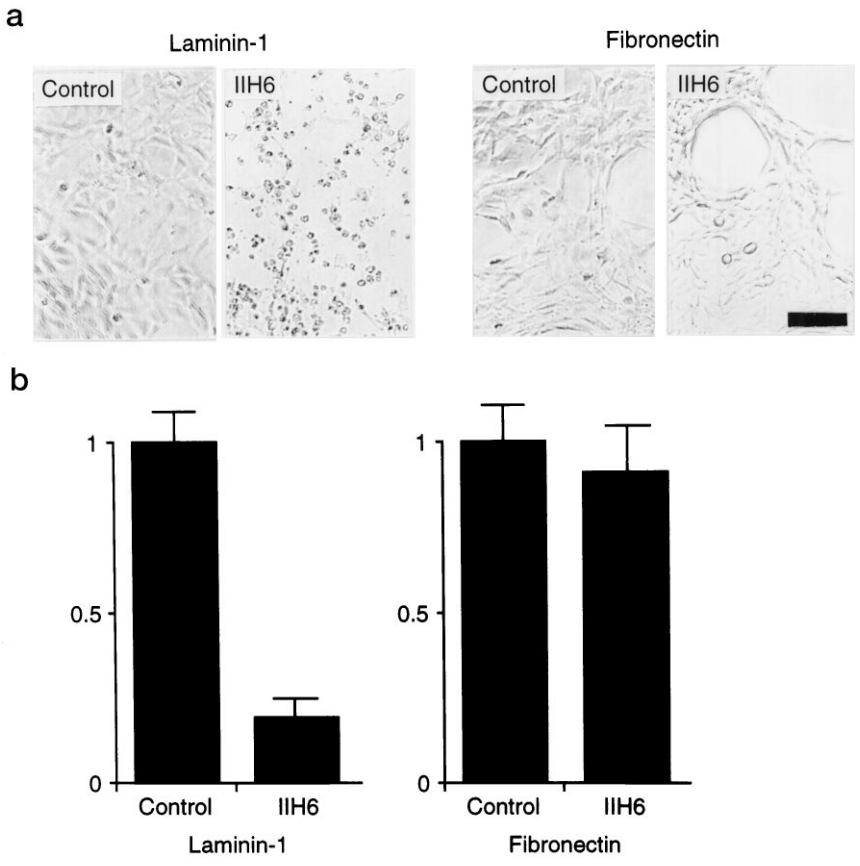

FIG. 5. **Effects of monoclonal antibody IIH6 on the shape and adhesion of RT4 cells to laminin-1 and fibronectin.** Fibronectin adhesion assay was performed in the same way as laminin-1 adhesion assay, except that the wells were coated with bovine plasma fibronectin (Sigma) instead of laminin-1. *a*, photographs of RT4 cells were taken (*bar*, 10 μm) and *b*, quantitative cell adhesion assay was performed as described in the legend to Fig. 4, except that RT4 cells were grown, in the absence (*Control*) or presence of monoclonal antibody IIH6, in the wells coated with laminin-1 or fibronectin.

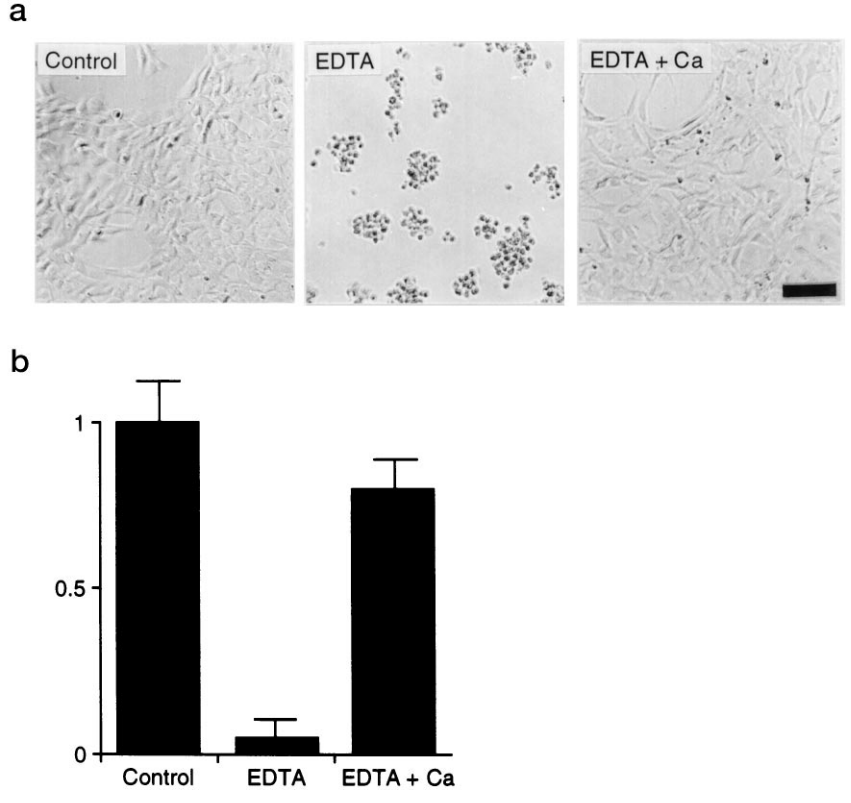

FIG. 6. **Effects of EDTA on the shape and adhesion of RT4 cells to laminin-1.** *a*, photographs of RT4 cells were taken (*bar*, 10 μm) and *b*, quantitative cell adhesion assay was performed as described in the legend to Fig. 4, except that RT4 cells were grown in the absence (*Control*) or presence of 5 mM EDTA (*EDTA*) or 5 mM EDTA plus 10 mM $CaCl_2$ (*EDTA + Ca*).

immediately and adhered to the bottom surface tightly. However, when RT4 cells were cultured on laminin-1 in the presence of the known inhibitors of the interaction of α-dystroglycan with laminin-1, including EDTA, sulfatide, fucoidan, dextran sulfate, heparin, and sialic acid, they remained round in shape and did not adhere to the bottom surface. Because these reagents may also perturb the interaction of laminin-1 with the cell surface adhesion molecules other than α-dystroglycan, such as the members of the integrin family for instance, we have looked at the effects of monoclonal antibody IIH6

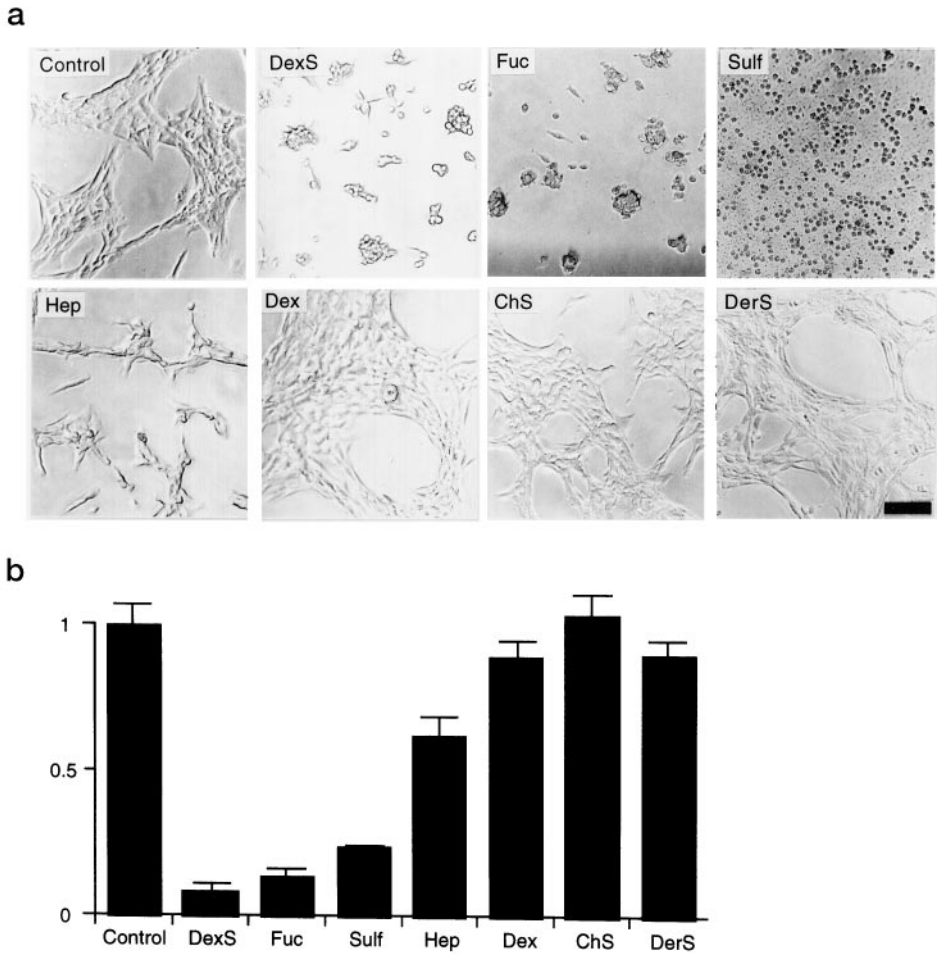

FIG. 7. **Effects of glycoconjugates on the shape and adhesion of RT4 cells to laminin-1.** *a*, photographs of RT4 cells were taken (*bar*, 10 μm) and *b*, quantitative cell adhesion assay was performed as described in the legend to Fig. 4, except that RT4 cells were grown in the absence (*Control*) or presence of 2 mg/ml dextran sulfate (*DexS*), fucoidan (*Fuc*), sulfatide (*Sulf*), heparin (*Hep*), dextran (*Dex*), chondroitin sulfate (*ChS*), or dermatan sulfate (*DerS*).

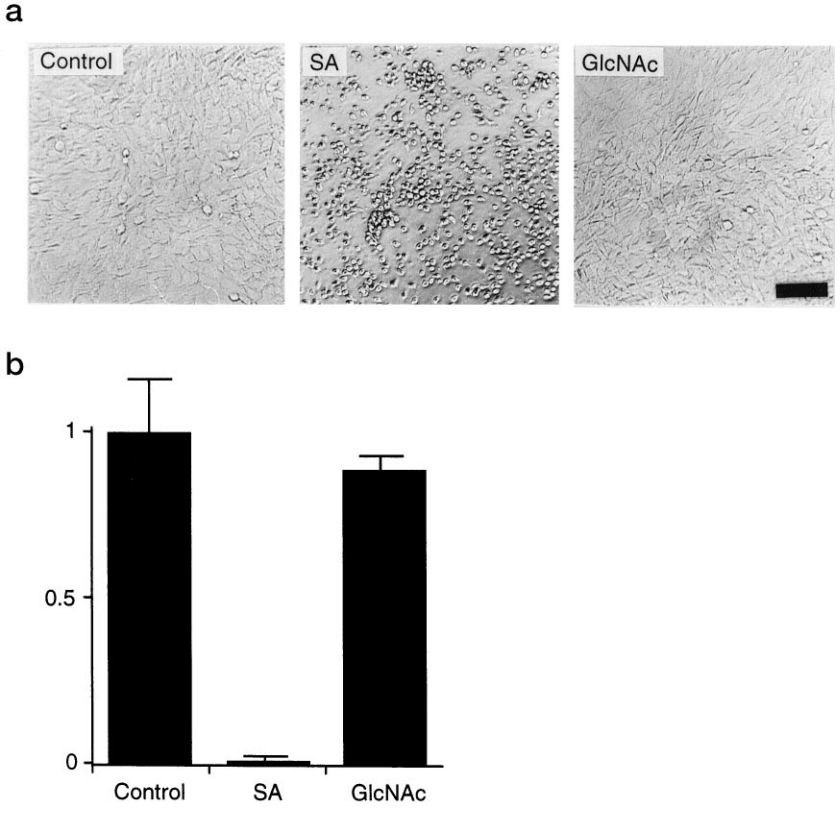

FIG. 8. **Effects of sialic acid on the shape and adhesion of RT4 cells to laminin-1.** *a*, photographs of RT4 cells were taken (*bar*, 10 μm) and *b*, quantitative cell adhesion assay was performed as described in the legend to Fig. 4, except that RT4 cells were grown in the absence (*Control*) or presence of 0.1 M sialic acid (*SA*) or GlcNAc.

against α-dystroglycan, which inhibits the interaction of α-dystroglycan with laminin-1, and found that this antibody drastically reduces the adhesion of RT4 cells to laminin-1. Furthermore, IIH6 did not perturb the adhesion of RT4 cells to fibronectin. Together with the previous demonstration of high affinity binding of laminin-1 to α-dystroglycan (1, 3), these results indicate a role for α-dystroglycan as a major cell adhesion molecule in the surface membrane of RT4 cells and suggest that the dystroglycan complex may play an important role in cell adhesion *in vivo*. Finally, we have demonstrated the results which suggest the secretion of α-dystroglycan by RT4 cells. In the future, it would be interesting to see if α-dystroglycan is secreted *in vivo* and if the secreted α-dystroglycan has inhibitory, and potentially regulatory, effects on the interaction of the cell surface α-dystroglycan with the ECM ligands.

The mechanism by which the dystroglycan complex may mediate such diverse and specific biological processes as sarcolemmal stabilization, epithelial morphogenesis, synaptogenesis, and myelinogenesis remains unclear. For instance, it has been disputed if the dystroglycan complex is actively involved in the acetylcholine receptor clustering in the neuromuscular junction as a signaling receptor of agrin (31, 33, 34, 37–43). Among others, our results seem consistent with at least two possibilities. First, the dystroglycan complex may function as a helper protein in these processes; the initial and high affinity binding of the ECM ligands to the dystroglycan complex may enable the more specific and functional cell surface receptors, such as the members of the integrin family or the putative myotube-associated specificity component (MASC), which was recently proposed to work in concert with the receptor tyrosine kinase MuSK in the neuromuscular junction formation, to interact with these ligands (31, 33, 34, 37, 38, 42). Second, the dystroglycan complex may function as a structural protein in the maturational stages of these processes. In this scenario, it would be intriguing to postulate that the binding of the ECM ligands to the dystroglycan complex may trigger the reorganization of the submembranous dystrophin/utrophin-cytoskeleton and lead to the stabilization of the cell membrane (44). The fact that the binding sites for dystrophin/utrophin and Grb2 overlap in the C terminus of β-dystroglycan raises a possibility that this process may be mediated by Grb2 and other signaling/adaptor proteins (8, 35).

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