Constitutive Release of α4 Type V Collagen N-terminal Domain by Schwann Cells and Binding to Cell Surface and Extracellular Matrix Heparan Sulfate Proteoglycans*

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During peripheral nerve development, Schwann cells synthesize collagen type V molecules that contain α4(V) chains. This collagen subunit possesses an N-terminal domain (NTD) that contains a unique high affinity heparin binding site. The α4(V)-NTD is adhesive for Schwann cells and sensory neurons and is an excellent substrate for Schwann cell and axonal migration. Here we show that the α4(V)-NTD is released constitutively by Schwann cells both in culture and in vivo. In cultures of neonatal rat Schwann cells, α4(V)-NTD release is increased significantly by ascorbate treatment, which facilitates collagen post-translational modification and collagen trimer assembly. In peripheral nerve tissue, the α4(V)-NTD is localized to the region of the outer Schwann cell membrane and associated extracellular matrix. The released α4(V)-NTD binds to the cell surface and extracellular matrix heparan sulfate proteoglycans of Schwann cells. Pull-down assays and immunofluorescent staining showed that the major α4(V)-NTD-binding proteins are glypican-1 and perlecain. α4(V)-NTD binding occurs via a mechanism that requires the high affinity heparin binding site and that is blocked by soluble heparin, demonstrating that binding to proteoglycans is mediated by their heparan sulfate chains.

The development of the peripheral nervous system is critically dependent on the migration and proliferation of Schwann cells (1–3). These processes are needed to provide sufficient numbers of glial cells to ensheathe and myelinate peripheral axons. Schwann cell proliferation and migration are regulated by molecular signals derived from peripheral axons as well as the extracellular matrix (ECM)1 that invests peripheral nerve tracts. A major source of the peripheral nerve ECM is Schwann cells (4).

The structure and composition of the ECM are modified during nerve development. These modifications contribute to the modulation of cellular functions that are regulated by ECM contact. During the late embryonic period when Schwann cell migration and proliferation are most active, the ECM consists mainly of a fibrillar matrix component (4). During the transition to axonal ensheathment and myelination, Schwann cell basal laminae appear. Previous studies (5, 6) have shown that contact with the basal lamina triggers myelination of axons by Schwann cells.

A prominent component of the developing nerve ECM is α4 type V collagen (7). A high level of expression of the α4(V) polypeptide is restricted to only a few tissues, including developing peripheral nerve, suggesting that this collagen chain carries out a unique function (8). Native α4(V) polypeptides are secreted by Schwann cells as triple helical collagen heterotrimers that also contain the ubiquitously expressed α1(V) and α2(V) collagen chains (8). The α4(V) collagen polypeptide consists of a large central collagen domain that is flanked by a noncollagenous N-terminal domain (NTD) of 475 amino acids (including a signal peptide of 29 amino acids) and a C-terminal domain of 251 amino acids. The α4(V)-NTD contains a high affinity heparin binding site that is not present in other type V collagen polypeptides (9). The heparin binding site consists of multiple repeats of the consensus heparin binding sequence FYXY (where Y is lysine or arginine).

Studies (9–11) of the function of α4(V) collagen molecules have revealed diverse effects on Schwann cell adhesion and axonal migration, which are mediated by distinct protein domains. The collagen domain inhibits axonal outgrowth and Schwann cell migration and blocks the adhesion and migration-promoting activities of other ECM proteins, such as collagen type IV (10). The α4(V)-NTD, in contrast, promotes axonal outgrowth and Schwann cell migration and is an excellent substrate for Schwann cell adhesion and spreading. These effects of the NTD are dependent on its heparin binding activity and are blocked by soluble heparin or heparan sulfate but not by function-blocking anti-integrin antibodies (9). These findings suggest that Schwann cell interaction with α4(V)-NTD is mediated by heparan sulfate proteoglycans. This conclusion is supported by the observation that syndecan-3, a Schwann cell transmembrane heparan sulfate proteoglycan, binds in vitro to α4(V)-NTD by a heparan sulfate-dependent mechanism (11).

This study extends these findings and presents data on the processing of the α4(V) collagen chain in Schwann cell cultures and in developing peripheral nerve tissue. The α4(V)-NTD is released constitutively by a proteolytic mechanism that is enhanced by collagen assembly. The released α4(V)-NTD accumulates in Schwann cell cultures and rat peripheral nerve tissue. α4(V)-NTD binds to the Schwann cell plasma membrane and ECM via binding to the heparan sulfate proteoglycans, glypican-1 and perlecain.

MATERIALS AND METHODS

Schwann Cell Cultures—Schwann cells were isolated from newborn rat sciatic nerves as described previously (12). The cells were cultured on poly-l-lysine-coated culture dishes in Dulbecco’s modified Eagle’s media.

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1 The abbreviations used are: ECM, extracellular matrix; NTD, N-terminal domain; PBS, phosphate-buffered saline; PI-PLC, phosphatidylinositol-specific phospholipase C; P, postnatal day (e.g. P2); ΔHBS, mutated α4(V)-NTD that lacks the heparin binding site.
medium with 10% fetal bovine serum and 2 µM forskolin. To investigate the effects of ascorbate on α4(V) collagen, Schwann cells were grown to confluence and then 1-ascorbic acid (Sigma) was added at a final concentration of 50 µM. Ascorbate was replenished after 24 h. After 48 h, the medium was harvested, centrifuged to remove unattached cells, and stored at −80 °C. Cell extracts were prepared by washing the cells twice with PBS (150 mM NaCl, 50 mM sodium phosphate, pH 7.4) and then scraping them into 300 µl of electrophoresis sample buffer with 2% SDS. Extracts were heated at 100 °C for 10 min and stored at −80 °C.

Preparation of Anti-collagen Type V Antibodies—The preparation of antibodies against the α4(V) collagen chain and α1(V) N-terminal domain was described previously (8, 11). Anti-α4(V)-NTD antibodies were also generated. The recombinant His-tagged α4(V)-NTD was expressed in BL23-plys-S Escherichia coli cells (Novagen) that were transformed with pET-30a(+) vector containing the NTD coding sequence. The protein was purified by chromatography on His-Bind resin (Novagen) followed by heparin-agarose chromatography (Sigma). The purified protein was used to immunize New Zealand White rabbits as described previously (11). Antibodies were affinity-purified on a column of α4(V)-NTD coupled to Affi-Gel-15 (Bio-Rad) by elution with 100 mM glycine, pH 2.5.

Expression of Myc-tagged α4(V) Collagen—A Myc epitope tag was inserted into rat α4(V) collagen cDNA (NCBI accession number AF272661) at nucleotide position 1163 by overlapping PCR. Amplification conditions were 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min. The resulting products were gel-purified and used as templates in a subsequent PCR reaction with pET-30a (+) vector containing the NTD coding sequence. The protein was expressed in Escherichia coli (Novagen) cells transformed with pET-30a(+) vector. The purity of the recombinant His-tagged α4(V)-NTD was assessed by SDS-PAGE and Coomassie blue staining.

Immunocytochemistry—Immunostaining of Schwann cells was performed first by rinsing the cells with PBS and incubating them on ice with primary antibodies diluted in blocking buffer for 1 h. Next, the cells were rinsed with PBS and incubated with Alexa-conjugated secondary antibodies (Molecular Probes) diluted in blocking buffer for 1 h at room temperature. Finally, the cells were rinsed with PBS, overlaid with cover slips using MOWIOL mounting solution and imaged using a Leica Microsystems scanning confocal microscope.

Sciatic nerves were isolated, embedded in Tissue-Tek freezing medium, and frozen at −20 °C. Cryosections (7 µm thick) were cut using a Reichert-Jung cryostat, placed on glass microscope slides, and fixed with 3% paraformaldehyde in PBS for 30 min. After fixing, sections were rinsed with PBS and incubated with primary antibodies diluted in blocking buffer for 1 h at room temperature. Following a rinse with PBS, sections were incubated with Alexa-conjugated secondary antibodies (Molecular Probes) diluted in blocking buffer for 1 h. After a final rinsing with PBS, the sections were overlaid with cover slips using MOWIOL mounting solution and imaged using a Leica Microsystems confocal microscope.

SC α4(V)-NTD Binding Assays—Schwann cells were plated in glass slide chambers (Nunc) coated with collagen type IV (2.5 µg/cm²) (BD Biosciences) and cultured for 3 days in Schwann cell growth medium. The cells were then placed on ice, rinsed with PBS, and incubated for 20 min in serum-free medium (50% Dulbecco’s modified Eagle’s medium, 50% Ham’s F-12 medium, 100 µg/ml apotransferrin, 100 ng/ml insulin) plus 1% bovine serum albumin with or without purified recombinant α4(V)-NTD (10 ng/mL). Next, the cells were rinsed with PBS and prepared for immunocytochemistry as described above. To investigate the effect of removing glycosphosphatidylinositol-anchored proteins, the cells were rinsed with PBS and incubated with heparitinase (4.5 milliunits/ml) (Seikagaku) for 18 h at 37 °C. α4(V)-NTD binding was assayed as described above.

Pull-down Assays of α4(V)-NTD Binding—Detergent extracts of cultured Schwann cells were clarified by centrifugation, mixed with purified recombinant α4(V)-NTD bound covalently to cyanogen bromide-activated Sepharose 4B (Sigma), and rocked gently at 4 °C for 3 h. The beads were harvested by a brief centrifugation, and supernatant solutions containing unbound proteins were removed. The beads were rinsed three times with PBS, and bound proteins were eluted with 1.5 mM sodium chloride and 10 mM Tris-HCl, pH 7.5. Eluted proteins were digested with trypsin in the presence of trypsin inhibitor (4.5 milliunits/ml) (Seikagaku) for 18 h at 37 °C.
Release of α4 Type V Collagen N-terminal Domain

RESULTS

Constitutive Release of α4(V)-NTD in Schwann Cell Cultures—The domain structure of the α4(V) collagen polypeptide is shown in Fig. IA. We have shown previously (8) that the addition of ascorbic acid to Schwann cell cultures stimulates the assembly of α4(V) polypeptides into triple helical collagen molecules that also contain α1(V) polypeptides.

As shown in Fig. 2, ascorbic acid also stimulated the release of the NTD from the α4(V) collagen polypeptide. This was demonstrated by immunoblot analysis of Schwann cell-conditioned medium from cultures grown without or with ascorbic acid. When the medium from cultures grown in the absence of ascorbic acid was analyzed, antibodies to α4(V) collagen or the α4(V)-NTD revealed a single immunoreactive band of ϳ200 kDa. Similar results were obtained when polypeptides from the medium of cultures transfected with α4(V) collagen cDNA containing a Myc epitope tag inserted into the NTD were stained with anti-Myc antibodies (Fig. 2) (also see Fig. 1 for the location of the Myc epitope tag). Based on immunoreactivity with anti-α4(V)-NTD and anti-Myc antibodies, the 200-kDa polypeptide contained the α4(V)-NTD but not the noncollagenous C-terminal domain (see Fig. 1). The latter conclusion is based on the observation that this polypeptide was not detected by anti-V5 antibodies when medium from cells transfected with α4(V) cDNA containing a V5 epitope tag at the C terminus was analyzed (data not shown).

Conditioned medium of Schwann cells incubated with ascorbic acid contained the 200-kDa immunoreactive band plus a more prominent band of ϳ95 kDa that was stained by anti-α4(V) and anti-NTD antibodies (Fig. 2). Ascorbic acid produced a slight reduction in mobility of the 200-kDa polypeptide, consistent with increased post-translational modification. An apparently identical Myc-reactive polypeptide of 95 kDa was observed in medium from ascorbate-treated Schwann cells transfected with Myc-tagged α4(V) collagen cDNA. From these results, we conclude that the 95-kDa polypeptide is the intact released α4(V)-NTD. After 48 h of ascorbate treatment, the ratio of released α4(V)-NTD to full-length 200-kDa α4(V) polypeptide in Schwann cell-conditioned medium was ϳ10:1. The released α4(V)-NTD was not detected in Schwann cell lysates (data not shown), suggesting that the release occurred after secretion of the α4(V) collagen polypeptide. Together, these results demonstrate that the α4(V)-NTD is released constitutively from type V collagen molecules and that it then accumulates in conditioned medium of cultured Schwann cells. The α4(V)-NTD release is accelerated greatly by ascorbate treatment, which promotes the assembly of collagen type V trimers.

Type V collagen molecules secreted by Schwann cells are heterotrimers that contain both α4(V) and α1(V) collagen polypeptides. The α1(V) collagen polypeptide also contains a noncollagenous N-terminal domain that shows a high degree of sequence homology with the N-terminal half of the α4(V)-NTD. As shown in Fig. 2, conditioned medium of Schwann cells cultured without ascorbate contained a 200-kDa polypeptide that was stained with antibodies raised against the α1(V)-NTD. Analysis of the medium from cells cultured with ascorbic acid showed the 200-kDa band plus additional bands of ϳ180 kDa and a doublet of ϳ45 kDa. The lower molecular mass of the doublet polypeptides was approximately half the size of the released α4(V)-NTD. These results suggest that the α1(V)-NTD contains two protease cleavage sites, the presence of which results in the release of two nonoverlapping NTD fragments.

α4(V)-NTD Release in Vivo—α4(V) collagen is expressed by Schwann cells in newborn rat peripheral nerves and at significantly reduced levels in adult nerves (7). To investigate the fate of the α4(V) NTD in vivo, rat sciatic nerves were harvested...
on postnatal day 2 (P2) and at 3 months of age. Nerve extracts were subjected to immunoblot analysis with anti-\(\alpha_4\) collagen and anti-\(\alpha_4\)-NTD antibodies. As shown in Fig. 3, extracts of P2 nerves contained the 200-kDa form of \(\alpha_4\) collagen and a much greater amount of the released \(\alpha_4\)-NTD. In extracts of nerves from adult animals, the 200-kDa \(\alpha_4\) collagen form was barely detectable, whereas the released \(\alpha_4\)-NTD was detectable but present at lower levels than in extracts of P2 nerves. These results demonstrate that \(\alpha_4\)-NTD is released and then accumulates in peripheral nerves in vivo. The highest levels of \(\alpha_4\)-NTD are observed in developing nerves.

The localization of \(\alpha_4\)-NTD in peripheral nerve tissue was investigated by confocal microscopy. Fig. 4 shows images of postnatal day 10 sciatic nerve sections stained with anti-\(\alpha_4\)-NTD antibodies (A and C) or fluorescent phalloidin to stain actin filaments (B and D). E–J, cells were incubated in medium with recombinant \(\alpha_4\)-NTD without (E and F) or with (G and H) 10 \(\mu\)g/ml heparin or in medium with recombinant \(\alpha_4\)-NTD-\(\Delta\)HBS (I and J). The cells were stained with anti-\(\alpha_4\)-NTD antibodies (E, G, and I) or fluorescent phalloidin (F, H, and J). All cells were viewed by laser scanning confocal microscopy.
and Schwann cells. The addition of soluble results suggest that the treated Schwann cell cultures (see below). Together, these acid-treated cultures, endogenous and the heparan sulfate proteoglycan perlecan. In ascorbic samples. We believe the 200-kDa protein is a perlecan-derived nase-digested proteins with anti-heparan sulfate stub antibodies or specific anticoagulin antibodies. α4(V)-NTD-bound proteoglycans included perlecan (core protein >250 kDa), a 200-kDa core protein, and glypican-1 (core protein ~64 kDa) (Fig. 6). Syndecan-3 was not detected in the α4(V)-NTD-bound samples. We believe the 200-kDa protein is a perlecan-derived fragment that does not react with the anti-perlecan antibody. No binding of heparan sulfate proteoglycans to NTD-HBS-conjugated beads was detected (Fig. 6), demonstrating that proteoglycan binding was mediated by the α4(V)-NTD high affinity heparin binding site. Similar results were obtained when extracts of P7 rat sciatic nerve were subjected to α4(V)-NTD pull-down assays (data not shown).

Glypican-1 is a lipid-anchored proteoglycan of the plasma membrane, and perlecan is a secreted ECM proteoglycan. To confirm that these proteoglycans are major binding sites for α4(V)-NTD, the localization of bound α4(V)-NTD and these proteoglycans was visualized by immunofluorescence microscopy. Fig. 7, A–C, shows micrographs of cultures that were incubated with soluble α4(V)-NTD and then stained with anti-

NTD, anti-laminin, and anti-neurofilament antibodies. Laminin is a major component of the basal lamina sheets that are apposed closely to the outer Schwann cell membrane (4). Antilaminin antibodies produced linear staining of the region outlining the outer Schwann cell membrane, corresponding to the location of the Schwann cell ECM. Anti-α4(V)-NTD antibodies produced discontinuous and slightly more diffuse staining of the same region, suggesting that α4(V)-NTD is localized to the Schwann cell ECM. This was confirmed by dual staining of nerve sections with anti-α4(V)-NTD and anti-laminin antibodies (Fig. 4, C–F).

α4(V)-NTD Binding to Schwann Cells—Experiments were carried out to investigate the interaction between α4(V)-NTD and Schwann cells. The addition of soluble α4(V)-NTD to Schwann cells that were incubated in medium without ascorbic acid resulted in α4(V)-NTD binding to the Schwann cell surface, which was visualized readily by immunofluorescence microscopy (Fig. 5).

LC extract

FIG. 6. Identification of α4(V)-NTD-binding proteoglycans. Schwann cell detergent extracts were mixed with α4(V)-NTD or α4(V)-NTD-ΔHBS immobilized on beads. Bound proteins were isolated and digested with heparitinase, as described under “Materials and Methods.” The proteins were subjected to immunoblot analysis and stained with anti-heparan sulfate Neo-antigen antibody 3G10 (anti-HS), or anti-perlecan, anti-glypican-1, or anti-syndecan-3 antibodies. The first lane on left shows the results of immunoblot analysis of heparitinase-digested Schwann cell extract stained with anti-heparan sulfate Neo-antigen antibody. Numbers indicate positions of migration of molecular mass markers (in kDa).

The role of the α4(V)-NTD high affinity heparin binding site in binding to Schwann cells was investigated. As shown in Fig. 5, the addition of soluble heparin completely blocked the binding of α4(V)-NTD to Schwann cells. In addition, α4(V)-NTD that was mutated to remove the heparin binding site (ΔHBS) (9) failed to bind to Schwann cells. These results suggest that α4(V)-NTD binds via its heparin binding site to heparan sulfate proteoglycans on the Schwann cell surface.

Ascorbic acid stimulates the assembly in Schwann cell cultures of fibrillar ECM that contains collagen types I, IV, and V and the heparan sulfate proteoglycan perlecan. In ascorbic acid-treated cultures, endogenous α4(V)-NTD was also associated with the fibrillar extracellular matrix (not shown). Exogenous α4(V)-NTD bound to the fibrillar ECM in ascorbate-treated Schwann cell cultures (see below). Together, these results suggest that the α4(V)-NTD binds to both the cell surface and ECM heparan sulfate proteoglycans of Schwann cells.

Identification of α4(V)-NTD-binding Proteoglycans in Schwann Cells—Schwann cell proteoglycans that bind to α4(V)-NTD were isolated by pull-down assays using α4(V)-NTD or α4(V)-NTD-ΔHBS immobilized on beads. Schwann cell lysates contained several heparan sulfate proteoglycan species with core proteins ranging in size from ~50 to >250 kDa, as revealed by heparitinase digestion and staining with an antibody that recognized the heparitinase digestion product on core proteins (Fig. 6). Heparan sulfate proteoglycans that bound to α4(V)-NTD-coated beads were identified by staining hepariti-
\(\alpha 4(V)\)-NTD (green) and anti-glypican-1 (red) antibodies. Both antibodies produced a punctate staining pattern on the Schwann cell surface. Superimposition of \(\alpha 4(V)\)-NTD and glypican-1 images (Fig. 7C) shows a substantial overlap of the two proteins. To provide additional evidence that \(\alpha 4(V)\)-NTD bound to glypican-1, the effects of digestion with PI-PLC were investigated. As shown in Fig. 8, incubation with PI-PLC abolished cell surface glypican-1 staining. Incubation with PI-PLC also substantially reduced the binding of soluble \(\alpha 4(V)\)-NTD. These data also demonstrate that glypican-1 and surface-bound \(\alpha 4(V)\)-NTD are enriched in the numerous filopodia that extend from the Schwann cell surface.

Fig. 7, D–F, shows micrographs of cultures that were incubated in medium with ascorbate to induce ECM assembly and then incubated with soluble \(\alpha 4(V)\)-NTD and stained with anti-\(\alpha 4(V)\)-NTD (red) and anti-perlecan (green) antibodies. Both antibodies stained prominent fibrillar structures that correspond to the Schwann cell ECM. Superimposition of \(\alpha 4(V)\)-NTD and perlecan images (Fig. 7F) shows a substantial overlap of the two proteins. Together, these data support the conclusion that the major Schwann cell binding sites for \(\alpha 4(V)\)-NTD are glypican-1 and perlecan.

**DISCUSSION**

In this study we investigated the processing of the \(\alpha 4(V)\) collagen chain by Schwann cells. This collagen chain is expressed abundantly by Schwann cells during peripheral nerve development (14). An unusual feature of the structure of \(\alpha 4(V)\) collagen is the presence of a large noncollagenous N-terminal domain that contains a unique high affinity heparin/heparan sulfate binding site (8, 9). Here we show that the \(\alpha 4(V)\)-NTD is released constitutively by Schwann cells both in culture and in vivo and that it accumulates in conditioned culture medium or the endoneurium ECM in vivo. The released \(\alpha 4(V)\)-NTD binds to the cell surface and to ECM heparan sulfate proteoglycans, especially glypican-1 and perlecan. This binding occurs via a mechanism that requires the high affinity heparin binding site and appears to be mediated by binding of the \(\alpha 4(V)\)-NTD to heparan sulfate chains. This process is summarized in Fig. 9.

The protease responsible for \(\alpha 4(V)\)-NTD release is not known. The proteolytic cleavage that results in \(\alpha 4(V)\)-NTD release appears to occur after secretion of the polypeptide because \(\alpha 4(V)\)-NTD is not detected in Schwann cell lysates. Release is increased dramatically by ascorbate treatment of the cells, which promotes assembly of collagen type V heterotrimers as a result of increased post-translational modification of amino acid residues in the collagen domain. After 48 h of ascorbate treatment, ~90% of the \(\alpha 4(V)\) collagen chains that accumulate in Schwann cell medium have undergone \(\alpha 4(V)\)-NTD release. Several protease inhibitors that have been reported to block proteolytic collagen processing events in other systems (15) were tested for their ability to block Schwann cell-mediated \(\alpha 4(V)\)-NTD release. Furin inhibitor I (10 \(\mu M\)), 1,10-phenanthroline (15 \(mM\)), arginine (25 \(mM\)), and the matrix...
metalloproteinase inhibitor BB-94 (50 μM) failed to produce demonstrable inhibition (data not shown).

Although the protease cleavage site was not identified directly, several observations suggest that the site is located within the second collagen GXX sequence interruption. Expression of α4(V) collagen with a Myc epitope tag near the C-terminal end of the noncollagenous NTD (see Fig. 1) resulted in the accumulation of Myc-tagged α4(V)-NTD in Schwann cell medium. Expression of α4(V) collagen with a Myc epitope tag located between the second and third GXX repeat of the uninterrupted collagen domain (amino acid 369) resulted in the accumulation of Myc-tagged α4(V) collagen domain (data not shown). When the Myc epitope tag was inserted within the second GXX interruption (amino acid 472), the epitope was destroyed. This region contains the sequence AQAQA, which has been suggested as the site for cleavage of the α1(XI) collagen chain (16). This putative cleavage site sequence is conserved in other members of this collagen gene family, including α1(V) and α4(V) collagen (Fig. 1). It is also of interest that α1(V) and α1(XI) collagens each contain an additional AQAQ motif near the middle of the noncollagenous N-terminal domain that is not present in the α4(V)-NTD (Fig. 1). This could explain the observation that the α4(V)-NTD is released as a single large fragment, whereas the α1(V) NTD is released as two smaller fragments of approximately equal size (Fig. 9).

We also investigated the fate of released α4(V)-NTD. In peripheral nerve tissue, anti-α4(V)-NTD immunoreactivity was associated with the region corresponding to the Schwann cell outer membrane and the closely apposed Schwann cell ECM (Fig. 4). The immunostaining reflects primarily the localization of released α4(V)-NTD, because immunoblot analysis of nerve tissue revealed a strong preponderance of released α4(V)-NTD compared with α4(V) collagen chains containing full-length NTD (Fig. 3). It is noteworthy that a similar localization is observed for glypican-1 and perlecan in peripheral nerve tissue (12).

Experiments with cultured Schwann cells revealed that soluble α4(V)-NTD binds to the Schwann cell plasma membrane primarily through binding to glypican-1. Evidence for this is provided by the co-localization of bound α4(V)-NTD and surface glypican-1, the significant reduction in cell surface α4(V)-NTD binding by pretreatment with PL-PLC, and pull-down assays that demonstrated glypican-1 binding to α4(V)-NTD. α4(V)-NTD binds to heparan sulfate chains on glypican-1, based on the observations that the binding requires the high affinity heparin binding site in α4(V)-NTD and that the binding is blocked by low concentrations of exogenous soluble heparin. α4(V)-NTD also binds to the heparan sulfate proteoglycan perlecan in Schwann cell cultures. The association of α4(V)-NTD with perlecan in the ECM of Schwann cell cultures incubated in medium with ascorbic acid was observed.

In the experiments reported here, we failed to detect significant binding of α4(V)-NTD to the transmembrane heparan sulfate proteoglycan syndecan-3. We have shown previously (11) that α4(V)-NTD binds syndecan-3. It was this observation, in fact, that led to the initial identification and isolation of α4(V) collagen in our laboratory. The explanation for this apparent contradiction lies in the fact that the steady state concentration of cell surface-associated syndecan-3 is very low in Schwann cells. This is a result of constitutive matrix metalloproteinase-mediated shedding of the syndecan-3 extracellular domain from the plasma membrane (17). The ability to detect binding of α4(V)-NTD to glypican-1 and perlecan but not syndecan-3 likely reflects the relative steady state levels of these heparan sulfate proteoglycans on the Schwann cell surface and not the inherent specificity of the binding reaction.

Immobile α4(V)-NTD is adhesive for Schwann cells and sensory neurons and is an excellent substrate for Schwann cell and axonal migration (9, 10). In contrast, α4(V)-NTD is a poor substrate for fibroblast adhesion. The adhesive property of α4(V)-NTD for Schwann cells appears to be dependent on heparan sulfate-mediated binding, because adhesion is blocked by soluble heparin and abolished by deletion of the high affinity heparin binding site. The findings reported here suggest strongly that glypican-1 is the primary cell surface α4(V)-NTD-binding protein on Schwann cells.

The physiological function of α4(V)-NTD release is not known. A potential function of α4(V)-NTD release may be to terminate the adhesive activity of the molecule by untethering it from the ECM. It is also possible that the α4(V)-NTD possesses additional functional activity of an unknown nature and that heparan sulfate-mediated binding to the Schwann cell surface and nerve ECM provides a mechanism to immobilize the protein. Such immobilization could be used to target α4(V)-NTD to a specific subcellular localization. As revealed by the Schwann cell binding experiments, α4(V)-NTD bound to glypican-1 is enriched in the numerous filopodia that decorate the Schwann cell surface. Alternatively, α4(V)-NTD immobilization could provide a reservoir of protein to be released in response to a specific physiological stimulus. Such a mechanism has been proposed (18, 19) for other heparan sulfate-binding proteins, including polypeptide growth factors and secreted proteases.

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