Schwann Cell Adhesion to a Novel Heparan Sulfate Binding Site in the N-terminal Domain of α4 Type V Collagen Is Mediated by Syndecan-3*

Previously we reported that type V collagen synthesized by Schwann cells inhibits the outgrowth of axons from rat embryo dorsal root ganglion neurons but promotes Schwann cell migration (Chernousov, M. A., Stahl, R. C., and Carey, D. J. (2001) J. Neurosci., 21, 6125–6135). Analysis of Schwann cell adhesion and spreading on dishes coated with various type V collagen domains revealed that Schwann cells adhered effectively only to the non-collagenous N-terminal domain (NTD) of the α4(V) collagen chain. Schwann cell adhesion to α4(V)-NTD induced actin cytoskeleton assembly, tyrosine phosphorylation, and activation of the Erk1/Erk2 protein kinases. Adhesion to α4(V)-NTD is cell type-specific because rat fibroblasts failed to adhere to dishes coated with this polypeptide. Schwann cell adhesion and spreading on α4(V)-NTD was strongly inhibited by soluble heparin (IC50 ~ 30 ng/ml) but not by chondroitin sulfate. Analysis of the heparin binding activities of a series of recombinant α4(V)-NTD fragments and deletion mutants identified a highly basic region (not present in organized actin-cytoskeletal structures. Soluble heparin binding site and failed to spread or assemble with this polypeptide. Schwann cell adhesion and spreading on α4(V)-NTD that lacked the heparin binding site and failed to spread or assemble organized actin-cytoskeletal structures. Soluble α4(V)-NTD polypeptide that contained the heparin binding site inhibited spreading of Schwann cells on dishes coated with α4(V)-NTD. Affinity chromatography of Schwann cell detergent extracts on a column of immobilized α4(V)-NTD resulted in the isolation of syndecan-3, a transmembrane heparan sulfate proteoglycan. Together, these results suggest that Schwann cells bind to collagen type V via syndecan-3-dependent binding to a novel high affinity heparin binding site in the α4(V)-NTD.

Interactions of Schwann cells and neurons with extracellular matrix (ECM) are critical for many aspects of peripheral nervous system development and function. Cell-ECM interactions regulate such processes as Schwann cell and axonal migration (1–3) and Schwann cell terminal differentiation (4, 5). Cellular interactions with ECM are mediated by cell surface receptors that bind specific ECM proteins. The most extensively characterized ECM receptors are the integrins, a family of transmembrane heterodimeric proteins that also bind and regulate cytoplasmic signaling and cytoskeletal complexes (6, 7). Other ECM receptors including membrane heparan sulfate proteoglycans (8) and the collagen-binding discoidin domain receptors (9) have also been described, but their functions are less well understood.

Prior to the onset of myelin formation peripheral nerve ECM is comprised mainly of fibrillar matrix structures that contain collagen types I and V and fibronectin (10). This is a highly dynamic period of nerve development that is characterized by axonal and Schwann cell migration and Schwann cell proliferation. At this time, Schwann cells also elaborate extensive membrane processes that progressively divide axon bundles and eventually segregate individual nerve fibers (11). As nerve development proceeds, changes in ECM structure and composition and ECM receptor expression occur. The most striking alteration is the appearance of the laminin-rich basal lamina structures that encase individual Schwann cells. Schwann cell contact with basal lamina-type ECM is required for myelin assembly (4) and promotes the formation of stable axon-Schwann cell functional units.

Our laboratory identified a novel type V collagen subunit, α4(V), that is expressed abundantly by premyelinating Schwann cells during the period of active Schwann cell migration (12). Collagen type V molecules are synthesized by Schwann cells as heterotrimers that contain α1(V), α2(V), and α4(V) chains. In addition to the collagen triple helical domains, the α1(V) and α4(V) subunits contain non-collagenous N-terminal domains (NTD) that are retained in at least some mature collagen trimers secreted by Schwann cells. α4(V) collagen expression is reduced in terminally differentiated Schwann cells but is induced in response to nerve injury (13). The α4(V) chain displays a high affinity for heparan sulfate and was isolated initially in a search for Schwann cell syndecan-3-binding proteins (14). Syndecan-3 is a member of a family of transmembrane heparan sulfate proteoglycans that have been implicated in cell adhesion (8). Syndecan-3 is expressed by Schwann cells in a temporal pattern that is essentially identical to that of α4(V) collagen (15, 16).

Collagen type V purified from conditioned medium of Schwann cells exhibits distinct adhesive activities for Schwann cells and neurons. Schwann cell type V collagen molecules inhibit axonal migration through a mechanism that is dependent on the collagen triple helical domain (3). In contrast, Schwann cell collagen type V promotes Schwann cell migration via a mechanism that appears to depend on an interaction with the non-collagenous NTD.

The goal of the current study was to characterize the inter-
action between Schwann cells and collagen type V. The results presented demonstrate heparan sulfate-dependent binding of Schwann cells to a high affinity heparin binding region of the α4(V)-NTD.

MATERIALS AND METHODS

Cell Culture—Schwann cells were cultured from neonatal rat sciatic nerves as described previously (17). For routine culture the cells were maintained in Dulbecco’s modified Eagle’s medium with 10% fetal bovine serum and 2 μM forskolin on poly-L-lysine-coated dishes. For the experiments reported in this study Schwann cells were used at the third or fourth passage.

Expression and Purification of Recombinant Proteins—Expression and purification of recombinant rat α1(V)-NTD has been described previously (3). Rat α4(V)-NTD cDNA, corresponding to amino acids 29–269 of the published sequence (12), was cloned into the pET30a+ plasmid vector (Novagen). The plasmid was used to transform competent Escherichia coli BL21pLySs cells. The recombinant His-tagged protein was purified using His-Bind affinity resin (Novagen). To express α4(V)-NTD fragments the cDNA sequence of interest was generated by PCR using appropriate primers with NdeI cDNA as the template. These were cloned into the pET30a+ vector and expressed and purified as described above. A construct containing a deletion of the central basic region was produced by overlapping PCR using cDNA sequences that had been engineered to contain 20 base pairs of overlapping sequence as templates. All expression constructs were sequenced to verify the coding sequence and reading frame. Recombinant protein was assayed by SDS gel electrophoresis and Coomassie Blue staining and by immunoblot analysis with anti-His tag antibodies.

Cell Adhesion Experiments—To test the Schwann cell adhesion activity of the recombinant NTD constructs the proteins were used to coat the wells of 24-well plates (2 μg/cm²). The proteins were diluted in 20 mM Tris-HCl, pH 7.5, 100 mM NaCl to a final concentration of 10 μg/ml and incubated in the wells at 37 °C for 18 h. The solution was removed, and the plates were blocked with a solution of 1% bovine serum albumin in 20 mM Tris-HCl, pH 7.5, 100 mM NaCl at 37 °C for one hour. The blocking solution was removed, and the wells were rinsed with Hank’s balanced salt solution. Schwann cells were removed from the dishes by trypsinization. Trypsin activity was inhibited by the addition of soybean trypsin inhibitor. The Schwann cells were harvested by centrifugation, resuspended in serum-free medium containing 250 ng/ml heregulin peptide, and added to the protein-coated wells. The plates were incubated for 3 h at 37 °C in a humidified incubator. In some experiments inhibitor compounds (glycosaminoglycans or recombinant proteins) were added to the culture medium at the time of plating. Cell attachment and spreading were assessed by phase-contrast microscopy of unfixed cells. For quantitative adhesion assays absorbance of crystal violet-stained formaldehyde-fixed cells was measured as described previously (3).

Heparin Affinity Chromatography—Purified recombinant NTD and NTD fragments were subjected to heparin affinity chromatography on prepacked HiTrap-Heparin HP columns (Amersham Biosciences, Inc.). The columns were equilibrated with 50 mM Tris-HCl, pH 7.4 at a flow rate of 0.5 ml/min. The proteins were diluted in equilibration buffer, and 100 μg of each protein was applied to the column. The column was eluted at a flow rate of 0.5 ml/min with 50 mM Tris-HCl, pH 7.4 (5 ml total) followed by a linear gradient of 0–2 M NaCl in 50 mM Tris-HCl, pH 7.4 (30 ml total) and then 2 M NaCl, 50 mM Tris-HCl, pH 7.4 (5 ml total). Fractions of 1 ml were collected, and aliquots were analyzed by SDS gel electrophoresis. Coomassie Blue-stained gels were quantitated with a laser scanning densitometer.

Receptor Characterization—Purified recombinant α4(V)-NTD was coupled to cyanogen bromide-activated Sepharose. Detergent extracts of cultured Schwann cells were applied to the NTD column. The column was washed with 50 mM Tris-HCl, pH 7.4 and then eluted with buffer containing 5 mM EDTA followed by 1 M NaCl. Aliquots of the column fractions were subjected to immunoblot analysis. Antibodies used in these experiments were affinity-purified rabbit anti-rat syndecan-3 (15), polyclonal anti-integrin β1 (a gift from Dr. Richard Hynes, Howard Hughes Medical Institute), and polyclonal anti-integrin α1 (a gift from Dr. Alexey Belkin, the Holland Laboratory, Amherst, MA). Fluorescence Microscopy—Schwann cells were fixed with 3% paraformaldehyde and stained with fluorescein-phalloidin (Molecular Probes), anti-vinculin (clone hVIN-1, Sigma), or anti-phosphotyrosine (clone 4G10, Upstate Biotechnology) antibodies, as described previously (17).

Immunoblot Analysis—Purified proteins or Schwann cell extracts were resolved on SDS-polyacrylamide gels, transferred to Immobilon-P membranes, and stained as described previously (12). Antibodies used were affinity-purified rabbit polyclonal anti-α4(V)-NTD, anti-α1(V)-NTD (12), and anti-syndecan-3 (15), monoclonal anti-phosphotyrosine, and anti-phosphoErk1/Erk2 (clone MAPR-YT, Sigma). The bound antibody was visualized by enhanced chemiluminescence that was detected and quantitated by means of a Roche Molecular Biochemicals Lumino-Imager.

RESULTS

Schwann Cell Adhesion to the N-terminal Domain of α4(V)-Collagen—We demonstrated previously that Schwann cells adhere to and migrate on dishes coated with collagen type V purified from Schwann cell-conditioned medium (3). To identify sites in collagen type V that mediate adhesive interactions with Schwann cells, neonatal rat Schwann cells were plated on dishes coated with various domains of type V collagen. Schwann cells plated on dishes coated with recombinant α4(V)-NTD attached rapidly and produced numerous processes (Fig. 1A). In contrast, Schwann cells initially adhered to but failed to spread on dishes coated with α1(V)-NTD (Fig. 1B) or the collagen triple helical domain (Fig. 1C) produced by pepsin digestion. The difference in activity between α1(V)-NTD and α4(V)-NTD was especially apparent when the Schwann cells were stained with fluorescein-phalloidin to visualize the actin cytoskeleton. Schwann cells plated on α4(V)-NTD produced numerous actin-containing processes (Fig. 1D), whereas cells plated on α1(V)-NTD failed to extend processes or assemble an organized actin cytoskeleton (Fig. 1E). Schwann cells plated on α1(V)-NTD were similar in appearance to cells plated on bovine serum albumin-coated dishes (Fig. 1F). When Schwann cell adhesion to NTD-coated dishes was quantitated by crystal violet staining of fixed cells, α4(V)-NTD exhibited significantly higher adhesive activity than α1(V)-NTD (Fig. 1I). The adhesive preference displayed by Schwann cells was distinct from that of fibroblasts, which attached avidly and spread on dishes coated with pepsinized collagen type V but not α4(V)-NTD (Fig. 1, G and H).

Schwann Cell Adhesion to α4(V)-NTD Promotes Cytoskeletal Organization and Cell Signaling—Schwann cells that adhered to α4(V)-NTD-coated dishes contained organized cytoskeletal structures that included vinculin-containing focal adhesion-like structures (Fig. 2A), peripheral foci of phosphotyrosine staining (Fig. 2B), and actin-rich filopodia (Fig. 2C). Activation of intracellular signaling pathways in response to α4(V)-NTD adhesion was assayed by immunoblot analysis. For comparison, Schwann cells plated on collagen type IV and the non-physiological substrate poly-L-lysine were also examined. Schwann cell adhesion to α4(V)-NTD or collagen type IV resulted in tyrosine phosphorylation of bands at ~125 and ~200 kDa. These phosphorylated bands were not seen in Schwann cells adhering to poly-L-lysine (Fig. 2D). Similarly, Schwann cell adhesion to α4(V)-NTD or collagen type IV (but not poly-L-lysine) resulted in activation of the mitogen-activated protein kinases Erk1 and Erk2 (Fig. 2E). These results demonstrate that α4(V)-NTD produces a physiological adhesive response in Schwann cells.

Inhibition of Schwann Cell Adhesion and Spreading on NTD by Glycosaminoglycans—The α4(V) collagen chain is characterized by an unusually high affinity for the glycosaminoglycans heparin and heparan sulfate (12). As shown in Fig. 3A, α4(V)-NTD bound to a heparin affinity column and required a sodium chloride concentration of 1 M to be eluted, demonstrating that high affinity heparin binding is a characteristic of the α4(V)-NTD. α1(V)-NTD, in contrast, bound to the heparin column but was eluted at a significantly lower NaCl concentration than α4(V)-NTD.

These results suggest that Schwann cell adhesion to α4(V)-

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NTD might be mediated by cell surface heparan sulfate proteoglycans. This conclusion was supported by the observation that Schwann cell adhesion and spreading on α4(V)-NTD-coated dishes was strongly inhibited by soluble heparin (Fig. 3, B–D). Inhibition by heparin was dose-dependent with half-maximal inhibition achieved at a concentration of 110 ng/ml. In contrast, the glycosaminoglycan chondroitin sulfate failed to inhibit Schwann cell adhesion to α4(V)-NTD even at a concentration of 10 μg/ml (Fig. 3D). The highly sulfated polysaccharide dextran sulfate inhibited Schwann cell adhesion to NTD, but it did so less effectively than heparin (Fig. 3D), suggesting a role for sulfation in mediating the glycosaminoglycan-NTD interaction.

Identification of the High Affinity Heparin Binding Site in α4(V) NTD—A likely candidate for the high affinity heparin binding site within the α4(V)-NTD is a highly basic region of the primary sequence in which 16 of 23 amino acids are arginine or lysine (Fig. 4B). This sequence contains 4 repeats of the heparin binding consensus sequence BBXB (B is a basic amino acid) (18). This basic sequence, which is not present in the α1(V)-NTD, is located near the center of the α4(V)-NTD adjacent to a region that shows a high degree of sequence similarity between the α1(V) and α4(V) chains (Fig. 4A).

A series of recombinant α4(V)-NTD fragments were expressed in bacteria (Fig. 4A). Heparin binding activity was assessed by heparin affinity chromatography. N-terminal (29–268) or C-terminal (226–369, 246–369) fragments of the α4(V)-NTD polypeptide (HBS) was quantitated by crystal violet staining. The cells were fixed 3 h after plating. The values shown are the mean ± S.D. of quadruplicate wells. Cell adhesion to wells coated with bovine serum albumin was subtracted as background.
Schwann Cells Bind to Type V Collagen

Fig. 2. Activation of signaling pathways in Schwann cells spreading on α4(V)-NTD. A–C, Schwann cells were suspended in serum-free medium and plated onto dishes coated with α4(V)-NTD. Three hours after plating the cells were fixed and stained with anti-vinculin (A) or anti-phosphotyrosine (B) antibodies or fluorescein-phalloidin (C) and visualized by fluorescence microscopy. D and E, Schwann cells were suspended in serum-free medium and plated onto dishes coated with α4(V)-NTD, collagen type IV (col IV) or poly-l-lysine (PLL). Three hours after plating the cells were lysed, and aliquots were subjected to immunoblot analysis and stained with antibodies specific for phosphotyrosine (D) or the phosphorylated forms of Erk1/Erk2 mitogen-activated protein kinases (phospho-MAPK) (E). Numbers indicate positions of the migration of molecular mass markers (in kDa).

heparin. Deletion of the basic segment from the N-terminal or C-terminal fragments dramatically reduced (29–245) or abolished (269–369) heparin binding activity. The importance of the basic segment was confirmed by producing a deletion construct that spanned the α4(V)-NTD but lacked the central basic region (Δ246–268). This protein bound to the heparin column but was eluted at a lower NaCl concentration than the full-length NTD protein that contained this segment (Fig. 4, C and D).

Role of the High Affinity Heparin Binding Site in Schwann Cell Adhesion to α4(V)-NTD—To assess the importance of the high affinity heparin binding site in Schwann cell adhesion to α4(V)-NTD, Schwann cells were plated on dishes coated with full-length α4(V)-NTD or the protein that lacked the central basic region (Δ246–268). Schwann cells adhered initially to the Δ246–268 construct, but Schwann cell spreading and process extension were markedly diminished compared with what was observed on full-length α4(V)-NTD (Fig. 5, A and B). Staining of the cells with fluorescein-phalloidin revealed a significant decrease in the production of actin-containing processes and actin cytoskeleton organization in cells plated on Δ246–268 (Fig. 5, E and F). When Schwann cell adhesion was quantitated by crystal violet staining of fixed cells, significantly reduced adhesion was observed to dishes coated with the Δ246–268 protein compared with full-length α4(V)-NTD (Fig. 1F).

Further evidence for an important role of the high affinity heparin binding site was provided by determining the effects of soluble α4(V)-NTD fragments on Schwann cell adhesion to α4(V)-NTD-coated dishes. Schwann cell spreading on α4(V)-NTD was almost completely inhibited by addition to the medium of the 246–369 NTD fragment (Fig. 5P), which contains the C-terminal portion of the α4(V)-NTD including the high affinity heparin binding site. In contrast, the 269–369 NTD fragment, which is identical to the 246–369 fragment except for a lack of the high affinity heparin binding site, had no effect on Schwann cell spreading on α4(V)-NTD-coated dishes (Fig. 5G). The 269–369 NTD fragment was devoid of Schwann cell adhesive activity when it was used to coat culture dishes (data not shown).

Identification of Schwann Cell α4(V)-NTD Receptors—To identify Schwann cell proteins that bind to α4(V)-NTD, detergent extracts of Schwann cells were subjected to affinity chromatography on a column of immobilized α4(V)-NTD. Unbound and bound proteins were identified by immunoblot analysis with specific antibodies. As shown in Fig. 6, the transmembrane heparan sulfate proteoglycan syndecan-3, identified as an immunoreactive high molecular weight smear, bound avidly to the α4(V)-NTD affinity column and was eluted by 1 M NaCl, consistent with charge-dependent binding characteristic of protein-glycosaminoglycan interactions. Syndecan-3 did not bind to unconjugated Sepharose beads (data not shown).

α1β1 is a major collagen-binding integrin expressed by Schwann cells (3, 19). Neither the α1 nor the β1 integrin subunits bound to immobilized α4(V)-NTD, although they were readily detected by immunoblot analysis of Schwann cell detergent extracts (data not shown).

DISCUSSION

The ECM of the developing peripheral nervous system is biochemically and structurally complex, and it contains a number of proteins that are capable of interacting with other matrix proteins and with cells (10). We have investigated a particular peripheral nerve ECM protein, collagen type V, which displays a surprising and somewhat unexpected range of functional activities. Collagen type V molecules are synthesized and secreted by premyelinating Schwann cells (12). These collagen molecules are heterotrimers of α1(V), α2(V), and α4(V) subunits that are incorporated into fibrillar ECM structures where
they co-localize with collagen type I molecules. The α1(V) and α4(V) subunits contain non-collagenous N-terminal domains. In contrast to what occurs for the fibrillar type I and type III collagen molecules, these non-collagenous domains are retained in at least some of the mature collagen trimers (12). In an earlier study we demonstrated that collagen type V trimers purified from Schwann cells (which contained the α1(V) and α4(V) N-terminal domains) promoted the migration of Schwann cells but strongly inhibited the outgrowth of axons from embryonic dorsal root ganglion neurons (3). The triple helical domain of the collagen molecule was shown to account for the axon outgrowth-inhibiting activity but not the Schwann cell adhesive activity. In the present study, we have extended these observations and demonstrate that the α4(V)-NTD contains a novel heparan sulfate binding site that mediates Schwann cell adhesion.

The heparin binding motif identified within the α4N(V)-NTD corresponds to a linear segment of the primary protein sequence that is extremely rich in basic amino acid residues. This segment contains four repeats of the consensus heparin binding sequence BBXB (18). Although many heparin-binding proteins have been shown to contain this consensus sequence, this appears to be the first extracellular protein shown to possess closely spaced multiple repeats of this motif. Analysis of heparin binding activities of synthetic peptides has shown that peptides with multiple repeats of this basic motif display significantly higher binding affinity for heparin than peptides with single copies (20). These findings appear to explain the unusually high affinity of the α4(V)-NTD for heparin and heparan sulfate. Despite similarities in amino acid sequences, this high affinity heparin binding motif is not present in the α1(V)-NTD. This is consistent with the marked difference in Schwann cell adhesive activity of the two domains. Other heparin binding sites have been identified in collagen molecules (21) including collagen type V (22). Some of these are present in the collagen triple helical domains. The functions of these binding sites are not known, but the affinity for heparin is significantly lower than that of the α4(V)-NTD site described here.

When Schwann cell detergent extracts were fractionated on an α4(V)-NTD affinity column the transmembrane heparan sulfate proteoglycan syndecan-3 bound to the column and was displaced by elution with a high concentration of sodium chloride. This suggests that Schwann cells bind to the α4(V)-NTD by means of heparan sulfate chains that are covalently attached to this proteoglycan. Further evidence for an important role of heparan sulfate binding in Schwann cell adhesion to α4(V)-NTD comes from several observations. Schwann cell adhesion to α4(V)-NTD was strongly inhibited by soluble heparin, which presumably acts as a competitive inhibitor of cell-associated heparan sulfates. This inhibition was specific for glycosaminoglycans of the heparin/heparan sulfate family because another sulfated glycosaminoglycan (chondroitin sulfate) had no effect even at high concentrations. Furthermore, a recombinant α4(V)-NTD polypeptide that lacked the high affinity heparin binding site exhibited significantly lower adhesive activity for Schwann cells and failed to elicit formation of actin-containing processes. Finally, an α4(V)-NTD fragment that contained the heparin binding region was an effective inhibitor of Schwann cell spreading on α4(V)-NTD, whereas a fragment that lacked the heparin binding sequence but was otherwise identical had no effect.

Transmembrane heparan sulfate proteoglycans, including...
Fibronectin-dependent engagement of both α5β1 integrins and syndecan-4 has been shown to be required for assembly of focal adhesions and actin-containing stress fibers (26).

Heparan sulfate-mediated adhesion of Schwann cells to α4(V)-NTD promotes actin cytoskeleton assembly and production of actin-containing processes. Association of syndecans with the actin cytoskeleton has been demonstrated previously (27–29). Overexpression of syndecan-1 in Schwann cells increases cell spreading on syndecan-1 binding substrates (e.g., fibronectin) and enhances actin cytoskeleton assembly (27). Clustering of cell surface syndecan-1 induces co-localization with underlying actin filaments (28). This association is not observed for syndecan-1 molecules that lack the central portion of the cytoplasmic domain (29). Syndecan-4, which is expressed by fibroblasts and smooth muscle cells, is localized to focal contacts (30), regions of tight adhesion of cells to ECM, and sites for cytoskeleton-membrane binding and signal transduction. The mechanism of syndecan-actin filament association is not known. Syndecans contain short but highly conserved C-terminal cytoplasmic domains. There is no evidence for direct binding of syndecans to actin. The C-terminal tetrapeptide sequence of syndecans, which is conserved in all known syndecan sequences, binds certain PDZ domain-containing proteins, such as syntenin (31) and CASK (32). The precise function of these proteins is not known, but they are thought to function as membrane scaffold proteins that bind signaling and structural proteins to the plasma membrane. Syndecans have also been implicated in adhesion-dependent signaling (25). The cytoplasmic domain of syndecan-4 binds protein kinase C; binding appears to increase the catalytic activity of the kinase (33, 34). Activation of protein kinase C has been implicated in regulation of focal adhesion assembly and actin cytoskeleton organization.

High level expression of the α4(V) collagen chain is highly restricted and occurs in developing peripheral nerves during the late gestational and early postnatal periods (12–14). During this time the premyelinating Schwann cells and axons undergo a complex morphological transformation in which bundles of “naked” axons are invaded and progressively segregated by Schwann cell processes. This highly dynamic process is dependent on extensive migration of Schwann cells and movement of Schwann cell processes. The results presented here suggest that interactions of Schwann cells with the α4(V)-NTD play a role in promoting Schwann cell morphological plasticity through interactions with cell surface heparan sulfate proteoglycan receptors. This interaction is not needed to maintain the stable and highly ordered structure that characterizes the mature peripheral nervous system because α4(V) collagen expression declines significantly when development is completed. Expression of this collagen chain is rapidly up-regulated, however, following peripheral nerve denervation (13). This finding suggests that α4(V) collagen might participate in peripheral nerve regeneration. It is believed that the inability of the central nervous system to regenerate following injury, in contrast to the peripheral nervous system, is a consequence of the molecular environment. α4(V) collagen, which is expressed in the peripheral but not the central nervous system, displays functional properties that suggest an important role in this process.

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