REVIEW

# The roles of cell adhesion molecules on the formation of peripheral myelin


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**Abstract. Several cell adhesion molecules of the immunoglobulin superfamily (IGSF) are expressed differently during the development and myelination of the peripheral nervous system. To examine the relationship between the expression of IGSF molecules and Schwann cell differentiation, we established a useful system for myelin formation *in vitro* on collagen gel using primary neuron/Schwann cell co-cultures from neonatal dorsal root ganglions (DRG). At 10 days *in vitro* (DIV), many Schwann cells were found in the areas surrounding aggregates of DRG neurons. After 20 DIV, Schwann cells positioned next to axons and elongated their processes along the axons. Some of them started loosely elaborating a large axon. Under electron microscopy, compact myelin was shown to be formed at 30 DIV. Thus the speed of myelination was much slower *in vitro* than *in vivo*. In co-cultures, L1 and neural cell adhesion molecule (NCAM) were detected at the premyelinating stage, L1 was precisely expressed earlier than NCAM. Expression of myelin associated glycoprotein (MAG) was transiently up-regulated at the early stage of myelination, and then P0 expression was finally increased as myelination proceeded. The change of expression pattern of these molecules in co-cultures was quite similar to that observed in the development *in vivo*. When Schwann cell proliferation was blocked by low serum culture condition, L1 and NCAM expressions were up-regulated. In contrast, the presence of cholera toxin in low serum media markedly increased expressions of P0 and MAG, but decreased the levels of both L1 and NCAM. These results suggest that both L1 and NCAM play roles in the contact and/or recognition between axons and Schwann cells at an early stage of myelination. On the other hand, MAG and P0 are important for axon ensheathment and myelin compaction.** (Keio J Med 50 (4): 240–248, December 2001)



**Key words: Schwann cells, myelination, PNS, cell adhesion molecules**


## Introduction

One of the important developmental events in the nervous system is the differentiation of glial cells to form myelin. Myelin is a highly specialized multilamellar membrane that results from the elaboration of plasma membranes of Schwann cells in the peripheral nervous system (PNS) or oligodendrocytes in the central nervous system. To form and maintain a precise myelin structure is very important in its function as an insulator to increase the velocity of nerve conduction in axons. Disruption of this structure is well known to cause severe neurological diseases such as multiple sclerosis and peripheral neuropathies.[1,2]

In the case of the PNS, the task of the Schwann cell, which originates from a neural crest cell, is to form peripheral myelin.[3] The differentiation of Schwann cells causes a dramatic morphological change in the cells.[4] The process of myelination is largely regulated by protein-protein interactions between the axon and the Schwann cell. It has been suggested that further differentiation of Schwann cells is precisely regulated by a chain expression of myelin specific proteins. Some of these proteins and gangliosides have been identified as


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markers for Schwann cell differentiation. S-100 protein is expressed in immature Schwann cells in the proliferative stage. As Schwann cell differentiation proceeds, O4, glial fibrillary acidic protein and galactocerebroside are expressed successively as markers.[5]

Many reports have demonstrated that cell adhesion molecules are spatio-temporally expressed in the nervous tissues during development and play critically important roles for the proper cell-cell interactions.[6,7] Several characteristic proteins of cell adhesion molecules that belong to the immunoglobulin superfamily (IGSF) have been identified as regulating peripheral myelin formation. P0, having a single V-like external domain, is affiliated with the IGSF.[8] It is exclusively expressed in myelin-forming Schwann cells and is localized in the compacted regions of mature myelin.[9] It has been proposed that P0 exhibits strong homophilic binding and plays a role in the compaction of the multilamellar membrane.[10,11] In contrast, L1 and neural cell adhesion molecules (NCAM) are expressed in both neurons and Schwann cells, suggesting that they play a role in cell-cell contact as recognition molecules.[12] However, it has not been well understood how the expression of these molecules is linked to myelination.

To understand the mechanism of myelination, an important first step is to elucidate the precise relationship between the expression pattern of cell adhesion molecules and the morphological and functional differentiation of Schwann cells to form compact myelin. For this purpose, we established a useful system for myelination on collagen gel by using dorsal root ganglion (DRG) neuron/Schwann cell primary co-cultures. We have carried out high resolution electron microscopy, immunocytochemistry and an enzyme-linked immunosorbent assay (ELISA) to examine changes in the gene expression and morphology of Schwann cells.

## Materials and Methods

### *Materials*

Collagenase/dispase and aprotinin were purchased from Boehringer-Mannheim. Dulbecco's modified Eagle's medium (DMEM), 5-fluoro-5′-deoxyuridine, cytosine arabinoside, cholera toxin, XTT; 2,3-bis (2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)carboxyl]-2H-tetrazolium hydroxide, Menadine; methyl-1,4-naphthoquinone sodium bisulfite, phenylmethylsulfonyl fluoride, pepstatin, and bovine serum albumin (BSA) were purchased from Sigma. Trypsin and poly-L-lysin (PLL) were purchased from Difco laboratories, Dulbecco's phosphate-buffer saline (PBS) was purchased from Nissui, 7S-Nerve growth factor (7S-NGF) was purchased from Chemicon, L-ascorbic acid 2-phosphate were purchased from Wako Pure Chemicals, Type I collagen (Type I-A) was purchased from Nitta gelatin Co., fetal calf serum and horse serum were purchased from Flow laboratories, and goat serum was purchased from Biomakor. Anti-myelin associated glycoprotein (MAG) antiserum and anti-NCAM antiserum were a generous gift from Dr. Sato at Niigata University and Dr. Ono at Shimane Medical College, respectively. Antibodies against L1 and P0 were generated in our laboratory. Other antibodies were purchased from either E-Y Laboratories or Biomakor.

### *Schwann cell culture*

Neonatal Wistar rat sciatic nerves were dissected and treated with 1% collagenase/dispase in DMEM for 1 hour at 37 °C. The reaction was stopped with a 10-fold volume of normal culture media containing 0.5% glucose, 0.045% sodium bicarbonate, 0.002% kanamycin and 10% fetal calf serum (FCS), and then cells were dispersed by pipetting. After centrifugation at $120 \times g$ for 5 min, cells were resuspended with normal culture media. This process was repeated twice to clean the cell suspension. Finally cells were seeded on PLL precoated 35 mm diameter plastic dishes and cultured for 24 hours under normal conditions. On the next day, cultures were treated with $10^{-5}$ M cytosine alabinoside and maintained for a further 1 week to eliminate fibroblast cells. Schwann cells were then allowed to proliferate in response to 20 ng/ml cholera toxin.[13] For the cell growth assay or ELISA, Schwann cells were incubated with 2% FCS-containing media for 2 weeks without cholera toxin.

### *Schwann cell growth assay*

To measure cell growth activity, $1.2 \times 10^4$ Schwann cells were seeded in each well of a 96-well plate. Cells were subsequently allowed to grow for 7 days under four different conditions. The medium was refreshed once at 4 days after the start of incubation. At the end of incubation, cells were treated and lysed with 0.025 mM XTT and 0.2 mM Menadine. The absorption of a generated formazan in each well was measured at 450 nm using a micro-titer plate reader, and the optical density was evaluated as a marker of the density of Schwann cells.[14]

### *DRG neuron and Schwann cell co-cultures: myelin formation in vitro*

DRGs of Wistar rat embryos at 19 days of gestation were isolated by dissociation with collagenase/dispase. The dispersed cells were finally resuspended with a normal culture medium at a density of $1 \times 10^6$ cells/ml and plated onto collagen gel prepared in a 35 mm dish

according to the manufacturer's method. On the next day, cultures were treated with a one 3-day pulse of 1 μM fluoro-deoxyuridine to eliminate non-neuronal cells and then maintained in a normal culture medium containing 100 ng/ml 7S-NGF and 50 μg/ml L-ascorbic acid 2-phosphate for as long as 40 days.

*DRG neuron and Schwann cell co-cultures: immunocytochemistry*

Co-cultures grown on glass coverslips coated with PLL were immunostained for L1, MAG, P0 and laminin using standard protocols. Briefly, the cells were rinsed in PBS several times and fixed by incubation with PLP [0.0375 M phosphate buffer (pH 6.2) containing 0.075 M lysine, 2% paraformaldehyde and 0.01 M sodium metaperiodate] for 10 min at room temperature. Non-specific binding was blocked by pre-incubation with PBS containing 10% BSA and 1% horse serum. All immunostaining was carried out with a 1:500 dilution of polyclonal antibodies. A 1:1,000 dilution of fluorescein-conjugated anti-rabbit goat serum was used as the second antibody. Co-cultures were mounted and were viewed on a Zeiss universal microscope equipped with fluorescence optics.

*Electron microscopy*

The neuron/Schwann cell co-cultures of DRG grown on collagen gel were fixed in buffered 2% glutaraldehyde followed by 2% osmium oxide, dehydrated in ethanol and embedded in Epon 812. Areas to be examined were selected and thin sectioned for electron microscopy. Thin sections were stained with uranyl acetate and lead citrate and viewed under a Hitachi H-700 electron microscope.

For the electron microscopic immunocytochemical examination, the co-cultures were fixed in PLP, treated with a blocking solution, incubated with a primary polyclonal antibody against to P0 overnight at 4 °C, followed by incubation with peroxidase-conjugated secondary antibody for 1 hour at room temperature. After several rinses, co-cultures were further fixed in 2.5% glutaraldehyde for 5 min, and then stained with 3,3′-diaminobenzidine. The ultra thin sections were made by the identical procedure described above.

*ELISA*

(1) Sampling: DRG neuron/Schwann cell co-cultures on collagen gel and Schwann cells grown in T-75 flasks were washed in PBS several times, harvested with a cell scraper and lysed in 1–3 ml lysis buffer (20 mM Tris phosphate buffer pH 8.3, 150 mM sodium chloride, 1 mM EDTA, 0.01 mM pepstatin, 0.2 unit/ml aprotinin, 1 mM phenylmethylsulfonyl fluoride) by sonication. After centrifugation at 100,000 × g for 1 hour at 4 °C, the supernatant was used for ELISA. To examine the developmental change of the expression level of cell adhesion molecules *in vivo*, the ELISA sample was prepared from Wistar rat sciatic nerve tissue at embryonic day 19 (E19), postnatal days 0 (P0), 4 (P4), 7 (P7), and 10 (P10). Dissected sciatic nerves were homogenized with a polytron mixer and centrifuged supernatants were used for the ELISA.

(2) Assay: Each ELISA sample was diluted 3–10 fold with a borate buffer pH 7.6 (50 mM boric acid, 2 mM sodium tetraborate) and 100 μl of each was coated onto each well of 96-well plates. After 1 day incubation at 4 °C, the coated plates were washed with PBS. The wells were then blocked for 1 hour at room temperature with a blocking buffer (described above). After blocking, 100 μl each of primary antibodies was added to the wells and incubated at room temperature for 1 hour. The unbound antibodies were aspirated, followed by three washes with PBS containing 0.05% Tween20. The 100 μl of 1,000-fold diluted secondary antibody (either horseradish peroxidase-conjugated anti-rabbit IgG or horseradish peroxidase-conjugated anti mouse IgG) was added to each well and incubated at room temperature for 1 hour. At the end of incubation, the wells were washed, followed by incubation with 100 μl of phosphate-citrate buffer pH 5.0 (24.3 mM citric acid, 51.4 mM disodium hydrogenphosphate) plus 0.2% O-phenylene diamine and 0.02% hydrogen peroxide to develop color for 30 min at room temperature. At the end of incubation 100 μl of 4.5 M sulfuric acid was added to each well to stop the color reaction. The optical density of each well was determined at 450 nm using a spectrophotometer.

## Results

*Expression of cell adhesion molecules in Schwann cells under different culture condition*

After incubation of neonatal Schwann cells with 2% FCS-containing basal media for 2 weeks, the cells were cultured under 4 different culture conditions for 1 week. When the cells were exposed to 10% FCS-containing media, the density of the cells increased up to 3-fold that of the initial level (Fig. 1). In the presence of cholera toxin in 10% FCS-containing media, the cell density increased additionally up to 6-fold. In contrast, in 2% FCS-containing media the cell density was not affected by the addition of the cholera toxin.

By ELISA analysis, expression levels of L1, NCAM, MAG, P0 and laminin were examined on each lysate of Schwann cells cultured under 4 different conditions. In the cultures exposed to 10% FCS-containing media

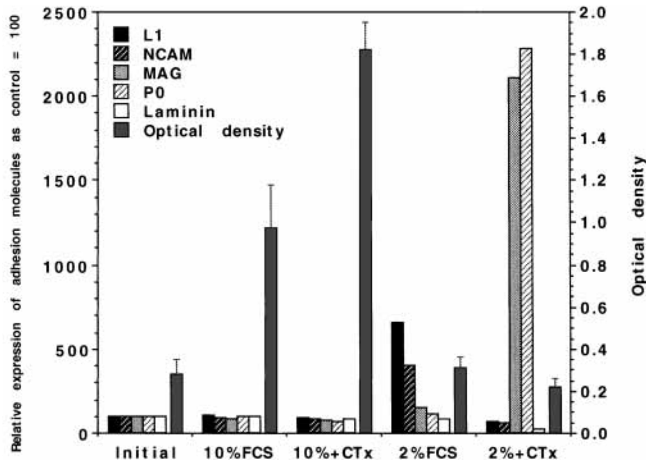


**Fig. 1 Expression of cell adhesion molecules in Schwann cells under different culture condition.** Schwann cells were cultured for 1 week under 4 different conditions, (1) 10% FCS, 10% FCS-containing DMEM, (2) 10% +CTx, 10% FCS-containing DMEM plus 100 ng/ml cholera toxin, (3) 2% FCS, 2% FCS-containing DMEM, (4) 2% +CTx, 2% FCS-containing DMEM plus 100 ng/ml cholera toxin. After a week incubation, samples were lysed. The respective content of L1, NCAM, MAG, P0 and laminin was measured by ELISA and described as a relative expression level to the 'Initial' that is cells before the week incubation. Three hours before the end of culture, XTT and Menadine were added and the cell density of each sample was measured for the optical density of formazan. Each value was calculated as an average for 3 or 4 independent experiments. Bars describe standard deviation (SD) values.

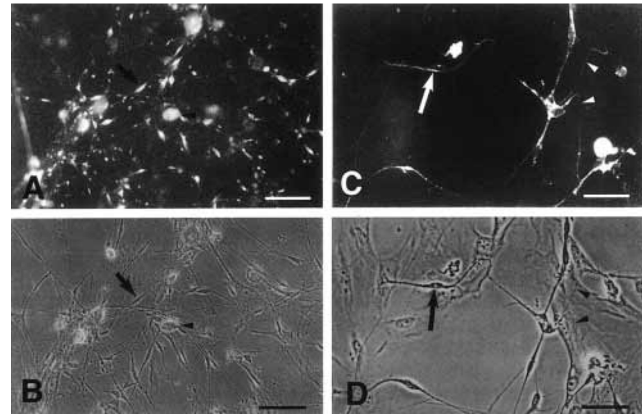


**Fig. 2 Expression of S-100 and L1 in DRG neuron/Schwann cell co-cultures at 4 DIV.** The cells co-cultured on PLL-coated glass coverslips were immunostained for either (A) S-100 or (C) L1, (A) Immuno-microscopic observation of co-cultures at 4 DIV for S-100 like immunostaining, (B) Phase contrast observation of an identical area of A. A short arrow indicates a Schwann cell immunostained with anti-S-100 antibody. Arrow head in panels A and B shows the DRG neuron weakly immunopositive to anti-S-100 antibody, (C) Immuno-microscopic observation of co-culture at 4 DIV for L1 like immunostaining, (D) Phase contrast observation of an identical area of C. The long arrow or arrow head indicates respectively a Schwann cell or DRG neuron immunoreacted with anti-L1 antiserum in panels C and D. It was also shown that fibroblast cells were immuno-negative for antibodies against either S-100 or L1. Bars describe a 200 μm length.

with or without cholera toxin, the expression levels of all five molecules were not significantly changed. In contrast, the levels of L1 and NCAM were increased by about 4–5 fold when the cells were exposed to the 2% serum condition. Additional exposure to cholera toxin in 2% serum medium markedly increased the expression of both MAG and P0 up to 20-fold of the initial level, while the levels of L1, NCAM and laminin were not significantly affected.

### *Immunocytochemical and morphological observations of myelin formation in vitro*

The time course of myelin formation *in vitro* was studied in the co-cultures of DRG neuron and Schwann cell. At 4 days culture *in vitro* (DIV), spindle shaped Schwann cells were markedly immunostained with anti-S-100 antibody (Fig. 2A). The DRG neuron was recognized as a large round cell body in the co-cultures by phase contrast microscopy (Fig. 2B). This large rounded cell body of the DRG neuron was weakly immunostained with anti-S-100 antibody, a marker for the Schwann cells, suggesting its non-specific staining. In contrast, no immunoreactivity was detected in flat shaped fibroblast cells. The expression of L1 was observed in both DRG neurons and Schwann cells, but not in fibroblast cells at the stage of 4 DIV (Fig. 2C). At 10 DIV, many Schwann cells surrounding aggregates of DRG neurons were observed (data not shown).

At 20 DIV, DRG neuron neurites were grown and were attached side by side with Schwann cells (Fig. 3). Most of the Schwann cells at this stage contained elongated nuclei and extended long processes along the axons. Laminin was localized in the extents of the Schwann cell cytoplasm at this stage (Fig. 3A). On the other hand, MAG expression appeared to be up-regulated in some but not in all of the Schwann cell processes (Fig. 3C). Under high resolution electron microscopy, Schwann cells were seen to develop many plasmalemmal domains that processed and/or engulfed large axons (Fig. 4A). In addition, the formation of basal lamina surrounding the Schwann cell/axon complex was also observed. Some of the differentiated Schwann cells formed a mesaxon that spiraled up to 2 turns around an axon at this stage of 20 DIV (Fig. 4B).

Compact myelin was revealed in co-cultures at 30 DIV (Fig. 5). A large diameter axon spiraled with 11 layers of compacted lamellae is shown in figure 5A. A bunch of small rounded microtubules was found inside the axon. Many cases of segregated large axons were

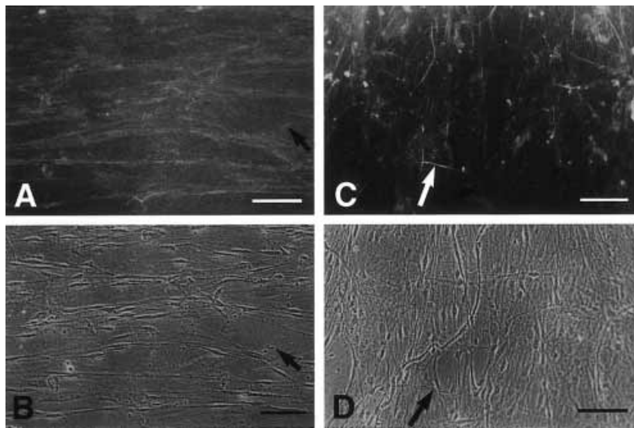


**Fig. 3 Expression of laminin and MAG in DRG neuron/Schwann cell co-cultures at 20 DIV.** The cells co-cultured on PLL-coated glass coverslips were immunostained for either (A) laminin or (C) MAG, (A) Immuno-microscopic observation of co-cultures for laminin at 20 DIV, (B) Phase contrast observation of an identical area of A. Many immuno-positive Schwann cells are shown. A short arrow indicates the weakly immunoreacted fibroblast cell, (C) Immuno-microscopic observation of co-culture for MAG at 20 DIV, (D) Phase contrast observation of an identical area of C. A long arrow indicates the Schwann cell body and its process that were immunoreacted with anti-MAG antiserum. Bars = 200 μm.

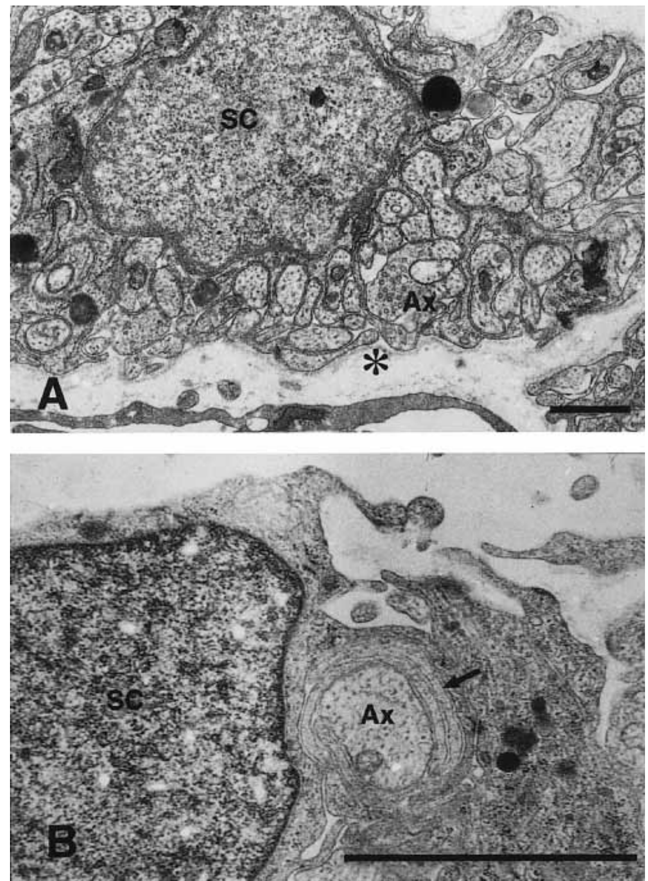


**Fig. 4 Ultrastructural observation of a morphologically changed Schwann cell in co-cultures at 20 DIV.** Co-cultures maintained for 20 DIV on the collagen gels were observed by electron microscopy. Transverse sections through an area of a co-culture that contains few Schwann cells interacting with axons are illustrated, (A) Many cases of Schwann cell membranous protrusions in interspace of the segregated axons are seen. Besides the formation of basal lamina surrounding Schwann cell was evident at this stage. An asterisk indicates a formed basal lamina surrounding Schwann cell/axons complex, (B) Several very thin membranous with no compacted myelin are revealed around the axons. The arrow indicates a mesaxon that spiraled up to 2 turns around an axon. Bars = 1 μm (A), 5 μm (B). Abbreviations: SC, Schwann cell; Ax, axon of DRG neuron.

associated 1:1 with Schwann cells and were wrapped with 10–30 layers of compact myelin sheath. Under pre-embedding immunoelectron microscopy, P0 was shown to be localized in the compacted lamellae (Fig. 5B).

*Temporal expression of cell adhesion molecules during peripheral myelin formation: in vivo and in vitro*

To examine the expression patterns of several cell adhesion molecules in DRG neuron/Schwann cell co-cultures, their contents at different stages *in vitro* were determined by ELISA. Patterns of their expression were additionally compared to those *in vivo* (Fig. 6).

As for L1, the highest content of L1 of 0.9 ng/μg protein was detected in 10 DIV at the time when DRG neuron/Schwann cell interaction started. L1 content was subsequently significantly decreased to one-half the maximum level at 20 DIV and was maintained at this low level till 40 DIV. The L1 content of sciatic nerve at E19 *in vivo* was as high as 0.7 ng/μg protein, following which its expression was developmentally decreased to the level of one-quarter of the maximum at P7 and maintained at this low level till P10 (Fig. 6A).

The NCAM content in co-cultures was 0.77 ng/μg protein at 10 DIV and transiently but not significantly increased in 20 DIV at the time of myelination onset, and then decreased to less than one-half the maximum level at 40 DIV. *In vivo*, the peak (1.0 ng/μg protein) was detected at P4. It was about twice as much as the level at E19. The level of NCAM subsequently quickly reduced at P10 to an eighth of the peak (Fig. 6B).

The MAG content was 0.4 ng/μg protein at 10 DIV and was transiently up-regulated to 1.5-fold at 30 DIV. In *in vivo* development, the expression level of MAG at the embryonic stage was low. It quickly increased after birth, peaked at P7 up to a 6-fold level and slightly decreased at P10 (Fig. 6C).

On the other hand, expression of P0 dramatically increased as co-cultures proceeded *in vitro*. Upregulation of P0 expression coincided with morphological dif-

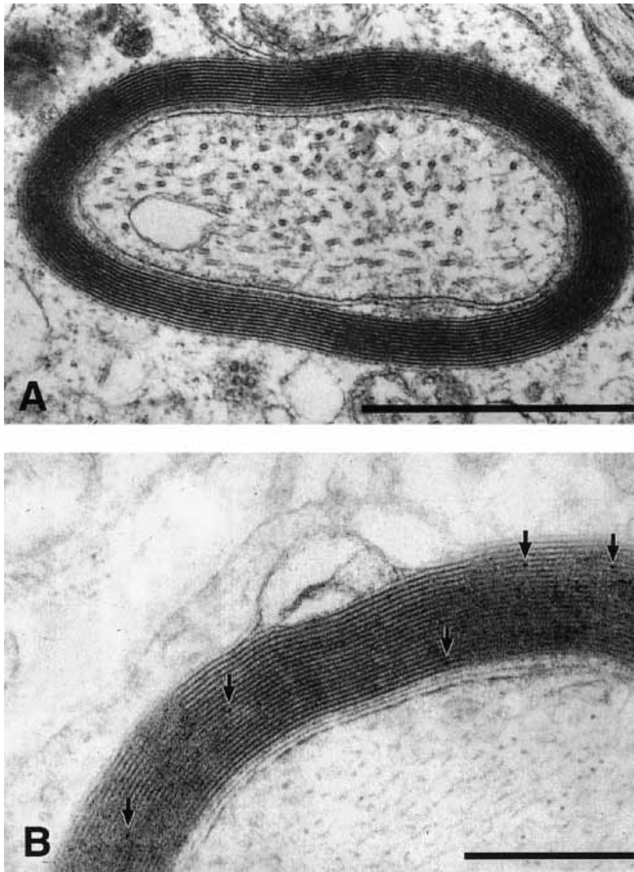


**Fig. 5 Ultrastructural observation of compact myelin in co-cultures at 30 DIV.** Co-cultures maintained for 30 DIV on the collagen gels were observed by electron microscopy, (A) Transverse section through an area of a co-culture that contains an 11-layered compact myelin sheath is shown. Most cases seen in cross-sectional profile of myelin sheaths contained compacted lamellae that are spiraled up between 10–30 layers, (B) P0 localization in compacted myelin *in vitro* shown by immunoelectron microscopic observation. Arrow indicates P0-like immunoreactivity seen as a black dot which is localized in the compacted lamellae. Bars = 1 μm length.

ferentiation of Schwann cells. In the case of sciatic nerve development *in vivo*, P0 was only detectable before P4 and was markedly increased in 50-fold at P7 and P10, suggesting a close relationship with compact myelin formation (Fig. 6D).

## Discussion

The molecular mechanism of myelin formation has not been well understood, while expressions of cell adhesion molecules are regulated spatio-temporally in both axons and myelin-forming cells during myelination.[6,7] In this study, we established a co-cultures system of DRG neurons and Schwann cells on collagen gel and examined the developmental change of adhesion molecule expression during Schwann cell differentiation *in vitro*. In addition, the expression pattern *in vitro* was compared to that *in vivo*.

When Schwann cell proliferation was inhibited in a low serum medium *in vitro*, L1 and NCAM expressions were up-regulated. In the presence of cholera toxin, however, their expression levels were conversely down-regulated and the expression of both MAG and P0 markedly increased. These results suggested that the expression of L1 and NCAM was correlated with the inhibition of Schwann cell proliferation. On the other hand, an increase of intracellular c-AMP at growth arrest potently induced P0 and MAG expression.

Most Schwann cells proliferate actively at the early stage of co-cultures with DRG neurons and at the late stage only the cells surrounding the distal end of the extended axon are still in the proliferative stage.[15] In the case of PNS development *in vivo*, it has been suggested that L1 and NCAM expressions are up-regulated, as proliferation of the Schwann cells is reduced. On the other hand, the effect of intracellular c-AMP content on P0 expression has been complicated. Treatment of Schwann cells from neonatal rat sciatic nerve with folskolin or glial growth factor, both of which activate adenylate cyclase, increases markedly the expression of P0 mRNA and protein.[16,17] On the other hand, no effect of folskolin on P0 expression has been reported.[18] In the latter case, the prepared Schwann cells derived from either neonatal rat sciatic nerves or Waller degenerated nerves in the adult rat were still proliferative. As shown in this study, cholera toxin induced up-regulation of P0 expression when Schwann cells were grown in 2% FCS-containing medium, but not in 10% FCS which usually maintains the proliferation of Schwann cells. Taken together, P0 expression depends on both Schwann cell growth arrest and regulation of intracellular c-AMP content. MAG expression seems to be likely in the case of P0. The expression level of laminin was not affected by Schwann cell growth arrest.

In order to examine myelin formation *in vitro*, co-cultures were carried out on collagen gels instead of PLL-coated plastic dishes. Bunge *et al.* have reported that an extended axon did not attach well to the surface of a PLL-coated dish so that Schwann cells could not adhere to the axon under this condition.[19] When the collagen gel was used as a culture substrate, Schwann cells attached well to the extended axon. Additionally L-ascorbic acid, which is a co-enzyme to prolylhydroxylase for collagen synthesis, is known to be necessary for the formation of myelin *in vitro*.[20] To develop an extracellular matrix structure for myelin formation *in vitro*, the cells have to produce collagen by themselves to interact with laminin. In this study, the L-ascorbic acid 2-biphosphate was used to avoid oxidation instead of ascorbic acid.

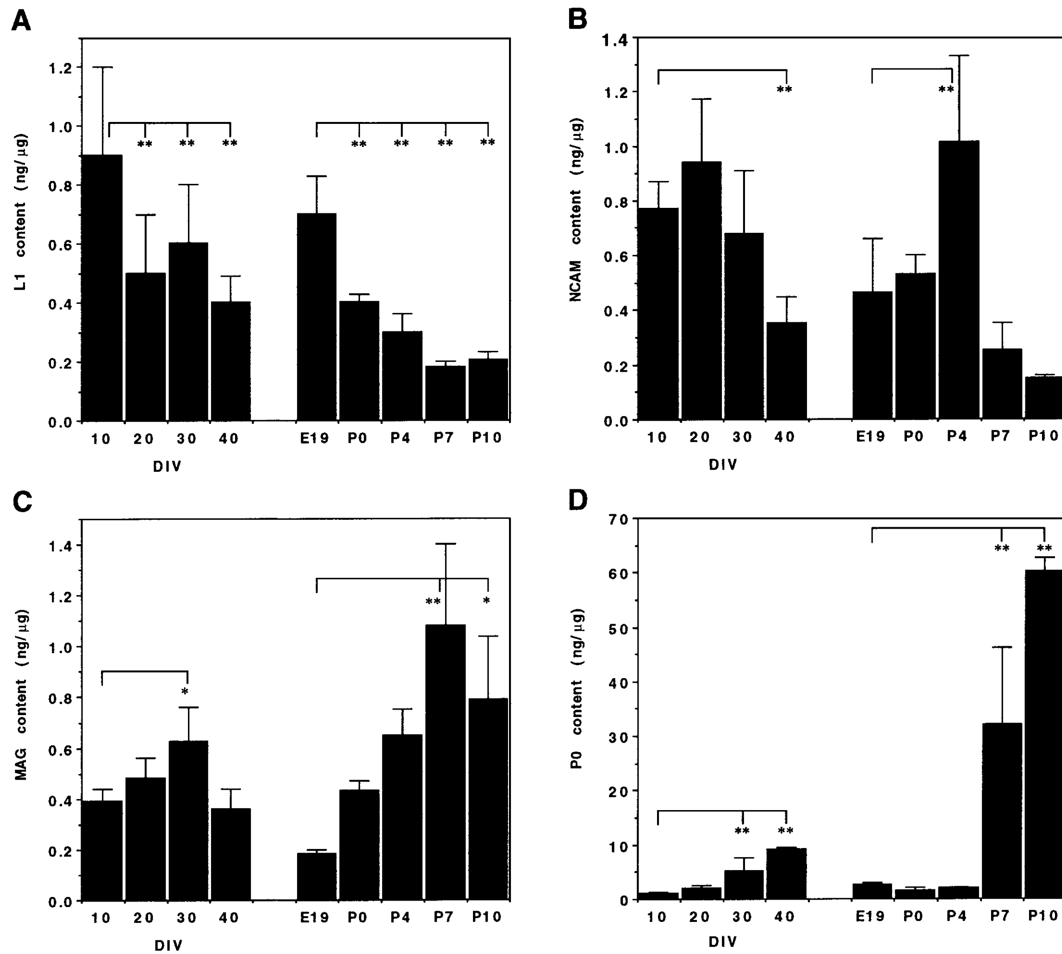


**Fig. 6 Temporal expression patterns of several cell adhesion molecules *in vivo* and *in vitro* during peripheral myelin formation.** Each panel shows a developmental change of either (A) L1, (B) NCAM, (C) MAG or (D) P0 expression in co-cultures *in vitro* compared to that in sciatic nerve *in vivo*. The content of each molecule in samples prepared from either co-cultures or sciatic nerves at the indicated days was measured by ELISA and was calculated as a ng/µg protein content in each preparation. Data are based on four independent experiments. Statistics within figures (mean $\pm$ standard deviation) were compared by Student *t*-test and significantly different statistics are indicated by asterisks (*, $p < 0.05$; **, $p < 0.01$).

Under electron microscopy, we observed two membranous spirals around an axon at 20 DIV and a compacted myelin sheath with more than 10 lamellae at 30 DIV. At the onset of myelination of the sciatic nerve *in vivo*, Schwann cells segregate large axons away from smaller axons, which will remain unmyelinated, and invest the large axons with a single layer of membrane. The Schwann cells initiate a spiral wrapping around a single large axon and wind it up with uncompacted lamellae of 3 or 4 layers at P3. At P7, many compacted lamellae were observed surrounding the axons, which consisted of 17 to 28 layers of membrane. This indicates that each myelination stage at 20 DIV and 30 DIV in co-cultures corresponds to the respective myelination stage at P4 and P7 *in vivo*.

The expression pattern of cell adhesion molecules changed remarkably at either 20 DIV *in vitro* or P4 *in vivo*. At this timing, onsets of myelination are observed. Both L1 and NCAM are highly expressed before the onset, and are subsequently quickly down-regulated. In contrast, the expression of both P0 and MAG are up-regulated as myelin formation proceeds. It seems that the onset of myelination is very important to regulate the expression level of cell adhesion molecules. Sequential expression peaks of each L1, NCAM, MAG and P0 are observed during myelination.

L1 is known to be expressed in both neurons and glial cells. A full length of L1 is expressed in neurons, while a short form that lacks exons 2 and 27 is only expressed in non-neuronal cells.[21,22] Exon 27 encodes for 4 amino acids (RSLE) the sequence of which is important for cell migration and L1 recycling in the growth cone.[22,23] Myelinating Schwann cell membranes that form the initial one and a half spiral wraps around an

axon are also L1-positive.[24] As myelination proceeds, L1 is no longer detected in Schwann cell membranes. L1 antibodies inhibited the myelination of DRG axons maintained *in vitro*,[25,26] suggesting that L1 is necessary for axonal ensheathment and initial spiral wrapping by the myelin membranes. NCAM is also expressed in both DRG neurons and Schwann cells and is suggested to enhance an L1-L1 homophilic adhesion.[27] As myelination proceeds, MAG may function to form uncompacted lamella membranes by possibly either homo- or heterophilic interaction, and finally P0 works for compaction to form a multilamellar membrane structure via P0-P0 adhesion.

Based on previous studies in mice deficient for the corresponding molecules, only P0 plays pivotal roles during peripheral myelin formation. A disruption of the P0 gene in mice leads to a severe dysmyelinating neuropathy with predominantly uncompacted myelin.[28] P0 is the gene responsible for peripheral neuropathies in humans, such as Dejerine-Sottas disease and Charcot-Marie-Tooth disease type 1B.[2,29,30] In contrast to a severe hypomyelination in P0 knockout mice, L1, NCAM and MAG-deficient mice develop normal peripheral myelin.[31–33] However, MAG turned out to be important for the maintenance of the myelin structure.[33] Analyses of P0/NCAM- and P0/MAG-deficient double mutants mice revealed that an absence of NCAM resulted in a transient retardation of Schwann cell spiraling, whereas absence of MAG impaired Schwann cell spiraling for a more extended time period, compared to P0-deficient mutant mice.[34] On the other hand, two lines of L1 null mice showed normal myelination, indicating that L1 is not essential for initial axon ensheathment or myelination.[31,35] Later on, L1-deficient mice showed loss of unmyelinated sensory axons, resulting in reduced sensory function, while sympathetic unmyelinated axons appeared normal.[36] Through nerve transplant studies, adhesion between Schwann cells and axons is disrupted in the case of L1-positive Schwann cells transplanted into L1-deficient nerves, as well as in the case of absence of L1 in both axons and Schwann cells. However, Schwann cell-axon adhesion is maintained when L1-deficient Schwann cells are transplanted into L1-positive nerves.[36] The data suggest that heterophilic axonal-L1 interactions mediate adhesion between unmyelinated sensory axons and Schwann cells. According to this result, there may exist unknown molecule(s) for the initial event of axon ensheathment.

In conclusion, the cell adhesion molecules L1, NCAM, MAG and P0, which all belong to the immunoglobulin superfamily, seem to have a chain expression in the mediation of peripheral myelin formation. L1 probably plays a role co-operatively with NCAM for the recognition and adhesion between axons and Schwann cells at the premyelinating stage. On the other hand, MAG and P0 are important for axon ensheathment and myelin compaction, respectively.

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