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Regulation of neural cell adhesion molecule expression on cultured mouse Schwann cells by nerve growth factor

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Schwann cells from early postnatal mouse sciatic nerve were obtained as a homogeneous population and shown by indirect immunofluorescence to express the neural cell adhesion molecules L1, N-CAM and J1 and their common carbohydrate epitope L2/HNK-1. L1 and N-CAM are synthesized in molecular forms that are slightly different from those expressed by small cerebellar neurons or astrocytes. As in astrocytes, the J1 antigen is expressed by Schwann cells in multiple forms generally ranging from 160 to 230 kd in the reduced state. J1 is secreted by Schwann cells in a 230-kd mol. wt form. Expression of L1 by Schwann cells can be regulated by nerve growth factor (NGF). L1 expression on the cell surface is increased 1.6-fold in the presence of NGF after 3 days of maintenance *in vitro* and 3-fold after 16 days. NGF does not change expression of N-CAM. The glia-derived neurite-promoting factor (GdNPF) increases L1 expression by a factor of 1.9 and decreases N-CAM expression by a factor of 0.4 after 3 days *in vitro*. J1 expression on Schwann cell surfaces remains unchanged in the presence of NGF or GdNPF. Antibodies to NGF abolish the influence of NGF on L1 expression. Addition of NGF antibodies to the Schwann cell cultures without exogenously added NGF decreases L1 expression, indicating that Schwann cells secrete NGF that may influence L1 expression by an autocrine mechanism. Our experiments show for the first time that cell adhesion molecule expression on a non-neuronal cell, the Schwann cell, can be directly regulated by the neurotrophic factor NGF. These observations indicate a considerable degree of 'plasticity' of peripheral glia in regulating cell adhesion molecule expression.

Key words: adhesion/cell culture/L1/N-CAM/nerve growth factor/Schwann cell

Introduction

Since the pioneering work of Ramon y Cajal (1928) Schwann cells have been recognized as the uniquely endowed substrate that allows regrowth and regeneration not only of peripheral, but also central nervous system neurons (for review, see Aguayo, 1985). This capability sustained in adulthood distinguishes peripheral from central nervous system glia. From these observations it is plausible to assume that Schwann cells alone or in combination with their mesenchymal environment either synthesize substances that support axon regrowth or cease to produce inhibitory factors. It is likely that cell adhesion molecules play important roles in neuron-Schwann cell interactions during development and regeneration.

We have recently shown that Schwann cells recapitulate expression of the neural cell adhesion molecules L1 and N-CAM after transection of the adult mouse sciatic nerve in a temporal sequence similar to the one observed during normal development (Nieke and Schachner, 1985; Martini and Schachner, 1986). During development, both Schwann cells and axons are L1- and N-CAM-positive when they first contact each other. As myelination proceeds L1 ceases to be detectable, while N-CAM is greatly reduced on both partner cells when the Schwann cell processes have turned ~1.5 loops around the axon. At this stage the myelin-associated glycoprotein (MAG) becomes detectable periaxonally and in the non-compacted myelin loops. In myelinated fibres L1 expression is absent from myelin and axon, whereas N-CAM remains weakly detectable. However, non-myelinating Schwann cells and unmyelinated axons remain L1- and N-CAM-positive throughout adulthood. After transection of adult sciatic nerve L1 and N-CAM become re-expressed by Schwann cells within only a few days. As in development, expression of both adhesion molecules ceases at the onset of myelination and continues on non-myelinating Schwann cells and unmyelinated axons.

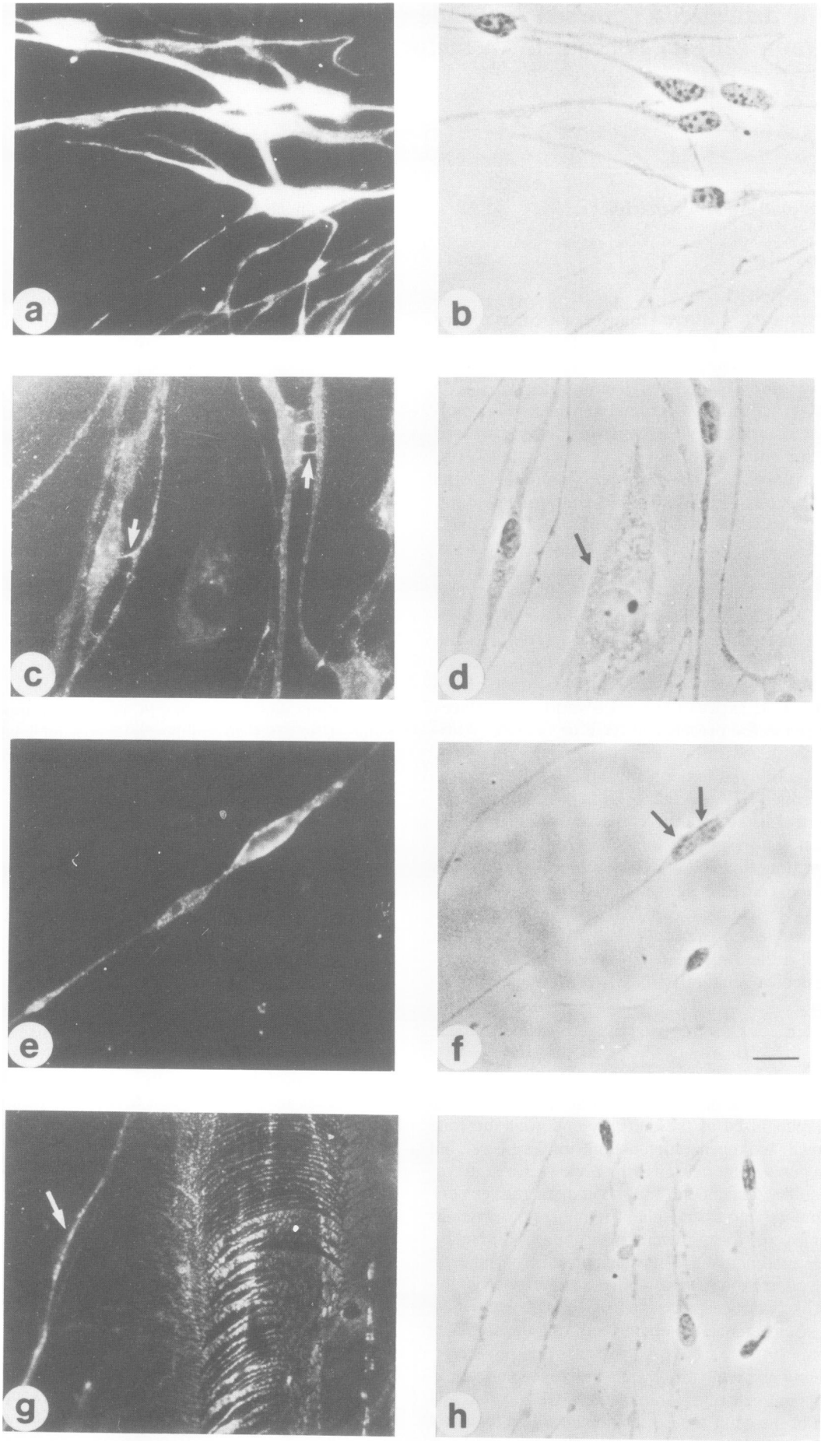
Upon denervation nerve growth factor (NGF) appears to be detectable in Schwann cells (Rush, 1984). Developing Schwann cells express NGF receptors (Rohrer, 1985; Rohrer and Sommer, 1983) and these are re-expressed upon axon removal under regenerative conditions (Taniuchi *et al.*, 1986). Based on these observations and the fact that PC12 pheochromocytoma cells are induced by NGF to synthesize increased levels of L1/NILE (McGuire *et al.*, 1978; Lee *et al.*, 1981; Bock *et al.*, 1985) we became interested in the question whether NGF can regulate L1 and N-CAM expression on Schwann cells.

Results

Preparation of pure Schwann cell cultures from mouse sciatic nerve

Since purification procedures for Schwann cells, previously designed for the rat (Brockes *et al.*, 1979), did not yield satisfactory results for Schwann cells of the mouse, a novel method had to be designed. Its major features are an immunocytolysis using not only Thy-1, but also MESA-1 antibodies to remove fibroblasts or fibroblast-like cells, and plating and maintaining cells in a laminin-rich culture medium to differentially facilitate attachment and survival of Schwann cells and, finally, reduction of serum concentrations down to 0.2% fetal calf serum to decrease proliferation of fibroblasts. Elimination of fibroblast growth by one or two cycles of cytosine arabinoside treatment (1-10 μM; 6-48 h) was not feasible, since viability of Schwann cells was severely affected.

To verify the purity of Schwann cell cultures, morphological and immunocytochemical criteria were used. Cells with the typical bi-polar shape were generally seen and by their expression of S-100 identified immunocytochemically (Figure 1a and b) as Schwann cells. When cultured in 10% fetal calf serum, Schwann cells assumed a more epithelioid shape (Figure 1i-l). Residual epithelioid fibroblasts or fibroblast-like cells always had a more flattened and triangular form (Figure 1c and d) that was clearly different from the epithelioid form of Schwann cells. Schwann cells never expressed the fibroblastic or endothelial cell markers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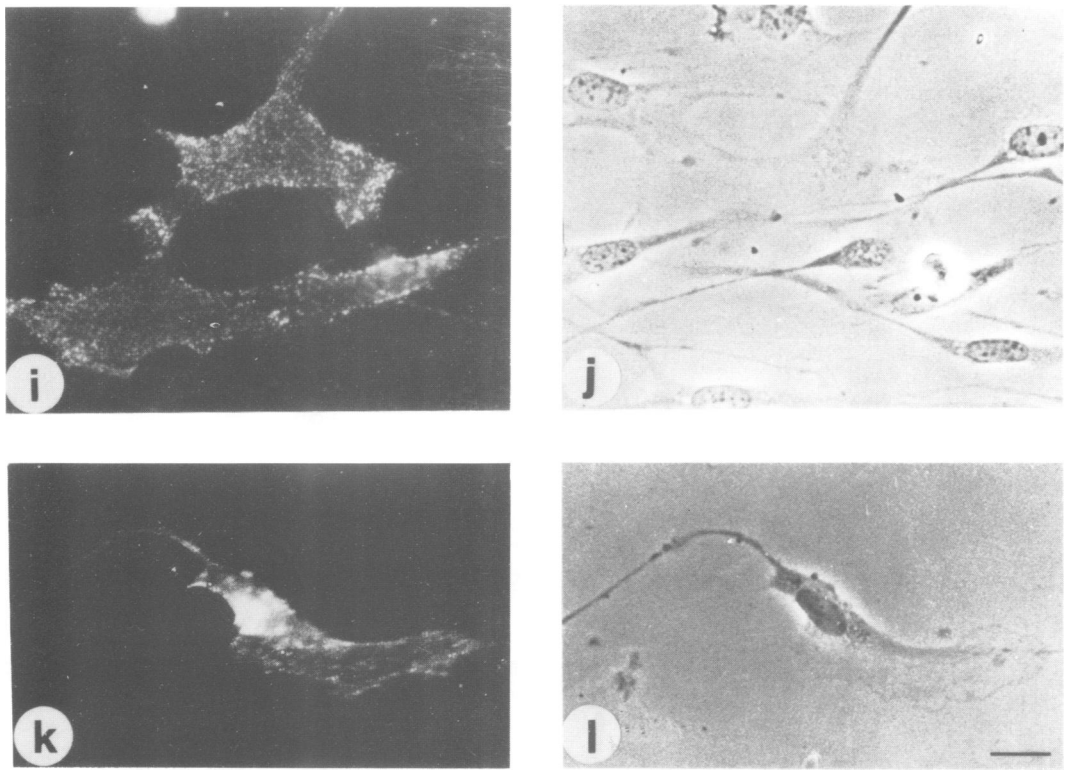

Fig. 1. Indirect immunofluorescence localization of S-100, L1, N-CAM and the L2/HNK-1 epitope on pure populations of live cultured Schwann cells. **(a)** Polyclonal S-100 antibody; culture maintained for 11 days in culture medium containing 0.2% FCS. Note the spindle-shaped morphology of S-100-positive cells **(b)**. **(c)** Monoclonal L1 antibody; culture maintained for 11 days in culture medium containing 0.2% FCS. Note the more pronounced localization of L1 antigen on filopodia touching other Schwann cells (arrows, **c**). Epithelioid cell with a fibroblast-like appearance is L1 antigen-negative (arrow, **d**). **(e)** Monoclonal HNK-1 antibody; culture maintained for 11 days in culture medium containing 0.2% FCS. Note the expression of the HNK-1 epitope on a Schwann cell with two nuclei (arrows, **f**). Other Schwann cells are HNK-1 epitope-negative. **(g)** Polyclonal J1 antibody; culture maintained for 3 days in culture medium containing 0.2% FCS. Note the strong staining of Schwann cell processes on the left (arrow). The plane of focus on the right was adjusted such that curiously arranged J1 antigen deposits on the coverslip could be seen. **(i–k)** Polyclonal N-CAM antibodies; culture was maintained for 11 days *in vitro* in culture medium containing 10% FCS, instead of only 0.2% FCS as in **(a–h)**. Note the epithelioid morphology of N-CAM-positive Schwann cells that are characteristic for cultures in 10% FCS (**i** and **j**). The percentage of N-CAM-positive Schwann cells in this field is not representative. Schwann cell with a fine process and a more epithelioid-shaped extension (**k** and **l**). **(b,d,f,h,j** and **l)** Corresponding phase contrast micrographs to fluorescence images **(a,c,e,g,i** and **k)** respectively. Bars = 3.8 μm.

Table I. Marker profile of morphologically identified cells in Schwann cell cultures

Criteria of identification	Schwann cells (% positive cells)	Fibroblasts or fibroblast-like cells (% positive cells)
Morphology	99.5 ± 0.05	0.5 ± 0.05
S-100	100 ± 0.6	0 ± 0.03
Thy-1.2	0 ± 0	0.15 ± 0.04
MESA-1	0 ± 0	0.2 ± 0.04
Fibronectin	0 ± 0	0.18 ± 0.04
Sum of Thy-1.2 and MESA-1	0 ± 0	0.3 ± 0.04
Sum of Thy-1.2, MESA-1 and fibronectin	0 ± 0	0.45 ± 0.05
L1	98 ± 0.9[a]	0 ± 0[a]
N-CAM	65 ± 0.8[a]	0.5 ± 0.09[a]
J1	100 ± 0.5	Negative, n.q.
L2/HNK-1 epitope	14 ± 0.8	Negative, n.q.

Antigen-positive cells were determined by indirect immunofluorescence using the antigen-specific antibodies (see Table III). Values are expressed as percentages of antigen-positive cells within a cell population classified by morphological criteria as Schwann cells or fibroblasts or fibroblast-like cells. Cells were taken 3 days after immunocytolysis and subculture.
[a]Polyclonal antibodies were used.
n.q. = Not quantified.

Thy-1, MESA-1 or fibronectin (Table I). Fibroblasts remaining after immunocytolysis were never found to express L1, J1 or the L2/HNK-1 epitope, but all of them expressed N-CAM (Table I). Schwann cells expressed L1, N-CAM, J1 and the L2/HNK-1 epitope (Figure 1, Table I). The Schwann cell cultures had a purity of 99.5 ± 0.5% by morphological and immunocytochemical criteria. No discrepancy was observed between morphological identification and antigen marker profiles.

Biochemical identification of cell adhesion molecules in Schwann cell cultures

To determine the molecular forms of adhesion molecules expressed by Schwann cells, immunoprecipitations were performed on detergent lysates and supernatants of [^{35}S]methionine-labelled cultures (Figure 2). Immunoprecipitates obtained with polyclonal L1, N-CAM (B.Seilheimer, A.Faissner, G.Keilhauer and M. Schachner, in preparation) and J1 antibodies are shown (Figure 2). L1 was detectable as a single band at 210 kd (lane 1) and N-CAM as a broad band between 140 and 200 kd (lane 4) (Seilheimer *et al.*, in preparation). J1 was found to be expressed in (lane 7) that were also seen in cerebellar astrocytes (lane 6). Since the intensities of individual bands varied to some extent between experiments, an exact comparison of J1 expression between cell types was difficult. Secreted J1 antigen appeared as a predomi-

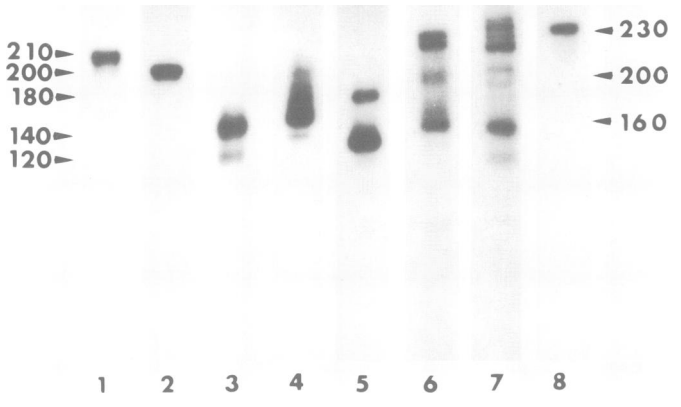

Fig. 2. Autoradiograms of immunoprecipitates from [^{35}S]methionine-labelled cultures of Schwann cells **(lanes 1, 4 and 7)**, small cerebellar neurons **(lanes 2 and 5)** and cerebellar astrocytes **(lanes 3 and 6)** with polyclonal antibodies to L1 **(lanes 1 and 2)**, N-CAM **(lanes 3–5)** and J1 **(lanes 6 and 7)**. Immunoprecipitate from supernatant of Schwann cell culture with polyclonal J1 antibody **(lane 8)**. Apparent mol. wts (M_r) are indicated at the margins.

Table II. Influence of NGF, GdNPF and renin on cell surface expression of L1, N-CAM and J1 in Schwann cell cultures

Protein	Antigen		
	L1	N-CAM	J1
NGF	1.6 ± 0.1	0.9 ± 0.04	1.1 ± 0.1
	(3.0 ± 0.14[a])	(1.1 ± 0.24[a])	
GdNPF	1.9 ± 0.04	0.4 ± 0.1	1.0 ± 0.01
Renin	0.9 ± 0.05	n.d.	1.0 ± 0.01

Schwann cells were cultured for 3 days (or 16 days[a]) in the absence and presence of NGF, GdNPF or renin. Antigen expression was measured by quantifying the amount of polyclonal antibodies bound to live cultures by subsequent application of ^{125}I-labelled protein A. In the case of L1, ^{125}I-labelled monoclonal antibodies were also used for quantification with identical results. Numbers represent multiples or fractions of values obtained in the absence of NGF, GdNPF or renin, i.e. c.p.m. specifically bound to cultures (3×10^4 cells) maintained in the presence of the three compounds divided by c.p.m. specifically bound to cultures (3×10^4 cells) maintained in the absence of compounds. Numbers are mean values from at least eight independent experiments with each value run in quintuplicate ± SD.
N-CAM was measured in two independent experiments after 16 days in culture.
n.d. = Not done.

nant band at 230 kd (lane 8), while astrocytes showed a predominant band at 220 kd (not shown; see Kruse *et al.*, 1985).

Regulation of L1 expression on Schwann cells by nerve growth factor (NGF), glia-derived neurite-promoting factor (GdNPF) and renin

To investigate whether L1 and N-CAM expression on Schwann cells could be modified by NGF, antigen expression was measured by a radioimmunoassay specifically aimed at the quantification of antigen on the cell surface. Binding of polyclonal antibodies to live Schwann cells was quantified by ^{125}I-labelled protein A. In the case of L1, directly radioiodinated monoclonal antibodies were also used. GdNPF (Monard *et al.*, 1973) which, like NGF, promotes neurite outgrowth, was used as a control. Renin was also used as a control, since it is known to be a minor contaminant in NGF preparations from mouse submaxillary glands (Harper *et al.*, 1983). Schwann cells were cultured for 3 days in the presence of NGF added at the time of plating or after 13 days *in vitro*; GdNPF and renin were added at the time of plating for 3 days.

Three days after plating L1 expression on the cell surface was increased by a factor of 1.6 by NGF and 1.9 by GdNPF (Table II). When Schwann cells were cultured for 16 days, NGF increased L1 antigen by a factor of 3.0 as measured by both mono- and polyclonal antibodies. When antibodies to NGF (10 μg/ml) were added to the cultures at the same time as NGF the stimulatory effect of NGF on L1 expression was abolished. Furthermore, addition of NGF antibodies to the cultures without exogenously added NGF decreased L1 expression by a factor of 0.8 ± 0.03, indicating that Schwann cells secrete NGF. Renin did not alter L1 antigen expression after 3 days in culture. NGF did not alter the expression of N-CAM on Schwann cells after 3 or 16 days *in vitro*. However, GdNPF decreased N-CAM expression by a factor of 0.4. For control, J1 antigen expression was measured and found to be similar for NGF, GdNPF and renin. Since J1 is secreted into the culture medium and could be detected by immunofluorescence staining not only on Schwann cell surfaces but also as substrate-attached material, the measured values probably indicate substrate saturation levels.

To estimate cell number, size and viability in the presence of NGF, GdNPF or renin Schwann cells were removed after 3 days *in vitro* from the culture dish by mild trypsin treatment and analysed by a Coulter counter. Number and size of Schwann cells were not different after exposure to the compounds with respect to control values. To further evaluate a mitogenic effect on Schwann cells, thymidine incorporation was measured. GdNPF, but not NGF or renin, increased the percentage of [^{3}H]thymidine pulse-labelled (24 h) cells by approximately 40%. Since cultures appeared healthy and no obvious signs of cell death could be observed, these findings indicate that GdNPF increases DNA synthesis without inducing cell division.

Discussion

We have shown here that Schwann cells respond to NGF by increased cell surface expression of the neural cell adhesion molecule L1. N-CAM expression remains unchanged under the conditions of this study. It could be excluded by Coulter counter analysis that the effect of NGF on L1 expression is due to cell proliferation (see Lillien and Claude, 1985) or changes in cell size and viability. It should be emphasized that our study was geared towards measuring the amount of cell adhesion molecules on the cell surface and not in the intracellular compartment. This seemed particularly pertinent, since only a minor fraction of cell surface glycoproteins usually reaches the plasma membrane and the majority is localized intracellularly (Carbonetto and Fambrough, 1979; Schmidt and Catterall, 1986; Schmidt *et al.*, 1985; Tamkun and Fambrough, 1986) without exerting its physiological role.

Our experiments show for the first time that non-neuronal cells can respond to NGF by a quantitative change in a physiologically significant parameter. Increased expression of the cell adhesion molecule L1 is meaningful during development and regeneration, when Schwann cells engage in cell surface contacts with neurons and promote neurite extension. It is conceivable that an increased presence of L1 on Schwann cells after peripheral nerve transection would promote surface interactions with regrowing axons. When axons have reached their target cells and become myelinated, increased cell adhesion molecule expression would no longer be needed and could therefore be turned off or reduced. NGF production by the target tissue could subserve this stimulatory

Table III. List of antibodies

Antibody	Species of origin	Reference	Dilutions used for		
			Indirect immunofluorescence	Radioisotope quantification	Immunocytolysis
Monoclonal S-100	Mouse	Haan *et al.* (1982)	1:100	–	–
Polyclonal L1	Rabbit	Rathjen and Schachner (1984)	1:200	1:200	–
Monoclonal L1	Rat	Rathjen and Schachner (1984)	1:40	(1×10^6 c.p.m.)	–
Polyclonal N-CAM	Rabbit	Goridis *et al.* (1983)	1:100	1:100	
Monoclonal N-CAM	Rat	Goridis *et al.* (1983)	1:40	–	–
Polyclonal J1	Rabbit	Kruse *et al.* (1985)	1:100	1.100	–
Monoclonal MESA-1	Mouse	Ghandour *et al.* (1982)	1:100	–	1:70
Monoclonal Thy-1.2	Mouse	Hämmerling *et al.* (1978)	1:50	–	1:35
Monoclonal L2	Rat	Kruse *et al.* (1984)	1:40	–	–
Monoclonal HNK-1	Mouse	Kruse *et al.* (1984)	1:100	–	–

effect on L1 expression. In addition, it appears from our study that Schwann cells themselves secrete NGF, since addition of NGF antibodies to Schwann cell cultures reduces L1 expression on Schwann cells. This autocrine mechanism may thus subserve an important regulatory role in L1 expression during development and regeneration. It is interesting, therefore, to investigate whether neurons influence production of NGF and expression of NGF receptors by Schwann cells. The observation that the increase in L1 expression in the presence of NGF is smaller (factor of 1.6) after 3 days *in vitro* and larger after 16 days (factor of 3) raises the possibility that Schwann cells change their responsiveness to NGF or modify their levels of NGF production with time in culture. It is conceivable that an even more pronounced increment in L1 expression by exogenously added NGF would have been observed in our study, if there were no endogenous production of NGF that raises basal levels of NGF and thus also L1 expression.

Combined with previous observations on the regulatory effect of neurons on L1 and N-CAM expression by Schwann cells (Seilheimer *et al.*, in preparation), the present study shows that NGF may represent yet another regulatory signal for adhesion molecule expression by Schwann cells. The balance between these signals and others which probably exist would then control the degree of influence that cell adhesion molecules on Schwann cells could exert on outgrowing neurites. It should be mentioned that an increase in L1 expression is indeed observed *in vivo* in the distal part of a transected adult sciatic nerve, thus correlating with our present observations *in vitro*. On the other hand, N-CAM also increased in the distal nerve stump *in vivo*, but no influence of NGF on N-CAM expression by Schwann cells could be observed *in vitro*. These observations encourage our search for other regulatory signals. An understanding of these signals and the physiological role of L1 in the outgrowth of axons on Schwann cell surfaces will be an important step towards dissecting the cellular and molecular mechanisms of peripheral nerve regeneration.

Materials and methods

Animals

For all experiments NMRI mice were used. They were obtained from Zentrale Tierzuchtanlage Hannover and maintained at the departmental animal facilities.

Factors

Nerve growth factor (NGF) was prepared from submaxillary glands of adult male mice (Bocchini and Angeletti, 1969). GdNPF was prepared from glioma culture supernatants according to Günther *et al.* (1985). This factor was used at concentrations known to optimally stimulate proliferation of astrocytes *in vitro* (10^{-5} M) (G.Fischer, J.Günther, V.Künemund, D.Monard and M.Schachner, unpublished observations). Renin was purified from hog kidney according to Corvol *et al.* (1973, 1977).

Antibodies

Mono- and polyclonal antibodies to mouse L1 and N-CAM, polyclonal antibodies to J1, monoclonal antibodies to the L2/HNK-1 carbohydrate epitope, and monoclonal antibodies to S-100, MESA-1 and Thy-1.2 have been described previously. Production and specificity of these antibodies are listed in Table III. For indirect immunofluorescence and immunocytolysis, antibodies were used as ammonium sulphate cuts of ascites or hybridoma culture supernatants obtained either from serum-free or serum-supplemented culture media. Tetramethylrhodamine (TRITC) or fluorescein (FITC) conjugated antibodies to rat or rabbit immunoglobulins were purchased from Cappel (Philadelphia, USA) or Dakopatts (Glostrup, Denmark) and generally used at dilutions of 1:200 or 1:400.

Preparation of pure Schwann cell cultures

Sciatic nerves were prepared from ~40 1-day-old NMRI mice. They were digested for 15 min at 37°C in 2 ml Dulbecco's modified Eagle's medium (DMEM) with 10 mM Hepes and 100 IU/ml penicillin and 72 IU/ml streptomycin containing 0.25% trypsin (Cooper Biomedical, Frankfurt) and 0.03% collagenase (Worthington CLS IV, Seromed, Berlin). This incubation was repeated twice. After the last incubation 1.5 ml of the supernatant was discarded and replaced by 0.5 ml culture medium [50% (v/v) DMEM and 50% Ham's F12 medium, 10 mM Hepes, 0.2% fetal calf serum (FCS) and antibiotics (see above)] containing 0.01% DNase I (Boehringer, Mannheim). Nerves were mechanically dissociated by three cycles of suction through injection needle nr. 12 (Becton-Dickinson, Heidelberg). The resulting suspension was centrifuged at room temperature for 5 min at 80 *g*. The cell pellet was resuspended in culture medium containing 10 μg/ml laminin (BRL, Karlsruhe). Yields were $4.5-5 \times 10^6$ cells. Less than 5% of the cells were dead by the criterion of trypan blue uptake. Cells were plated in a tissue culture Petri dish, 10 cm in diameter. After 16–20 h cells were washed once with DMEM containing Hepes and antibiotics. Cultures were maintained further in culture medium containing 5 μg/ml laminin.

After 3 days in culture, cells were washed once with DMEM and incubated for 30 min at 37°C with monoclonal antibodies Thy-1.2 (Hämmerling *et al.*, 1978) and MESA-1 (Ghandour *et al.*, 1982) in DMEM. Antibodies Thy-1.2 and MESA-1 were used at dilutions of 1:35 and 1:70 respectively. After the incubation cultures were washed gently in DMEM followed by incubation with guinea pig complement [final dilution 1:10 (v/v)] in DMEM for 1 h at 37°C. Immunocytolysed cells were removed by incubation for 10 s at room temperature with 2 ml trypsin/EDTA solution (0.05% trypsin and 0.02% EDTA in Tris-buffered saline, pH 7.4). Trypsinization was stopped by washing immediately with 2 ml soybean trypsin inhibitor (Serva, Heidelberg) in DMEM (1 mg/ml). The remaining cells were then each washed three times with DMEM to remove dead cells and debris. Cells were removed from the culture dish by a 4-min incubation at 37°C with the trypsin/EDTA solution, collected by centrifugation at room temperature, first for 4 min at 80 *g* and then for 4 min at 140 *g*, and resuspended in culture medium containing 10 μg/ml laminin for subculture in a Petri dish. Approximately 95% of all plated cells attached within 2–3 h.

If necessary, immunocytolysis was repeated after 1 day in subculture by a modified procedure. Incubation with complement was for 30 min instead of 1 h and short-time trypsinization to remove dead cells was prolonged to 30 s. Cells were plated on laminin-coated (33 μg/ml) glass coverslips (1 cm in diameter) at a density of 30 000 cells/20 μl culture medium/coverslip. Coverslips (five per

Petri dish, 3.5 cm in diameter) were flooded after 4–5 h with 1.5 ml culture medium.

For immunoprecipitation, Schwann cells were maintained in culture medium containing 0.2 or 10% FCS, for indirect immunofluorescence in 0.2 or 10% and for culture in the presence of NGF, GdNPF or renin in 0.2% FCS. Except for differences in the morphology of Schwann cells results were the same under all serum conditions. Schwann cells had a purity of 99.5 ± 0.5% after 1 day *in vitro* as determined by morphology and cell type-specific antigen expression (Table I).

Indirect immunofluorescence

Indirect immunofluorescence was carried out as described by Schnitzer and Schachner (1981).

Immunoprecipitation

Immunoprecipitation from detergent lysates of cell pellets and culture supernatants were carried out according to Faissner *et al.* (1985) as modified for Schwann cell cultures ($0.5-1.5 \times 10^6$ cells, 3 days after the subculture step, as described by Seilheimer *et al.*, in preparation). In brief, after [^{35}S]methionine (200 μCi/ml) labelling of cells, supernatants were saved for immunoprecipitation. Cells were washed and solubilized with Tris-buffered saline containing 0.5% NP-40, 1 mM EDTA and protease detergent extract by a 100 000 *g* centrifugation step, immunoprecipitation was carried out using mono- and polyclonal antibodies in a first reaction step followed by incubation with protein A-Sepharose for polyclonal antibodies and MARK-1-Sepharose for monoclonal antibodies. After several washing steps pellets were resuspended in sample buffer and separated by SDS–PAGE. Radioactive bands were visualized by autoradiography on X-ray film.

Immunoprecipitations with L1, N-CAM and J1 antibodies were also carried out on cultures of pure small neurons and astrocytes from cerebella of 6–8-day-old mice as described previously (Keilhauer *et al.*, 1985).

Culture of Schwann cells in the presence of NGF, GdNPF and renin

Pure Schwann cells (30 000 cells/20 μl/coverslip) were maintained in culture medium containing 0.2% FCS for 1 or 13 days and then for 3 days in the absence or presence of NGF (100 ng/ml), GdNPF (1 μg/ml) or renin (1 μg/ml). Compounds were added once. In some cases, immunoaffinity purified antibodies to NGF (10 μg/ml, Korsching and Thoenen, 1983) were added to the culture medium together with NGF. Antibodies to NGF were also added to cultures not supplemented with NGF.

Quantitation of cell surface expression of cell adhesion molecules

To measure quantitatively the levels of cell adhesion molecules accessible at the surface membrane of Schwann cells the following procedure was adopted: Schwann cells (30 000 cells/20 μl/coverslip) from the subculture step were maintained with and without factors for 3 days in culture medium containing 0.2% FCS. Cells were then washed for 1 min in Dulbecco's phosphate-buffered saline (washing solution), then blocked for 5 min with culture medium containing 10% FCS and washed again for 1 min in washing solution. This and the following steps were carried out at room temperature. Cells were then incubated for 20 min with polyclonal L1, N-CAM and J1 antibodies in culture medium containing Hepes and 10% FCS, washed for 2 min and incubated for 25 min with ^{125}I-labelled protein A (15 μl, 30 mCi/μg, 100 mCi/ml; Amersham). Coverslips were then washed twice, 4 min each, with washing solution, wrapped with a Parafilm sheet, broken into pieces and transferred into counting vials. As controls, cells were incubated with culture medium only, instead of the first antibody. L1 antigen was also quantified using ^{125}I-labelled IgG (29 Ci/mM, 50 μCi/ml) of monoclonal L1 antibody. Iodination was carried out with Bolton–Hunter reagent according to the instructions of the supplier (Amersham). The absolute values of bound radioactivity did not vary from experiment to experiment by more than 5–8%, thus showing a significant degree of reproducibility.

Measurement of [^{3}H]thymidine incorporation and cell proliferation

Incorporation of [^{3}H]thymidine was measured by autoradiography of Schwann cell cultures after the subculture step as described previously (Leutz and Schachner, 1981). In brief, Schwann cells were cultured on laminin-coated coverslips and labelled with [^{3}H]thymidine (2 Ci/ml; 6.7 Ci/mM, Amersham) for 24 or 76 h. Cultures were washed and fixed with paraformaldehyde and glutaraldehyde, coated with a solution of gelatine, dried and coated with photoemulsion (Ilford, G5, diluted 1:2 with distilled water). Coverslips were developed and mounted in Entellan (Merck, Darmstadt) and autoradiographies scored by phase contrast microscopy. Cell number and sizes were determined by Coulter counter analysis as described previously (Fischer and Schachner, 1982). Single cells were collected from the culture dish by mild trypsin treatment (0.02% trypsin, 0.02% EDTA in Dulbecco's phosphate-buffered saline for 3 min at 35.5°C) which also removed dead cells and debris. Three independent experiments were performed in triplicate for each experimental value. For autoradiography, at least 1000 cells were counted per coverslip. For Coulter counter analysis, ~50 000 cells were counted per value.

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References

Aguayo,A.J. (1985) In Cotman,C.W. (ed.), *Synaptic Plasticity.* The Guilford Press, New York, pp. 457–484.
Bocchini,V. and Angeletti,P.U. (1969) *Proc. Natl. Acad. Sci. USA*, **64**, 787–794.
Bock,E., Richter-Landsberg,C., Faissner,A. and Schachner,M. (1985) *EMBO J.*, **4**, 2765–2768.
Brockes,J.P., Fields,K.L. and Raff,M.C. (1979) *Brain Res.*, **165**, 105–118.
Carbonetto,S. and Fambrough,D.M. (1979) *J. Cell Biol.*, **81**, 555–569.
Corvol,P., Devaux,C. and Menard,J. (1973) *FEBS Lett.*, **34**, 189–192.
Corvol,P., Devaux,C., Ito,T., Sicard,P., Ducloux,J. and Menard,J. (1977) *Circ. Res.*, **41**, (Suppl. II), 616–622.
Faissner,A., Teplow,D.B., Kübler,D., Keilhauer,G., Kinzel,V. and Schachner,M. (1985) *EMBO J.*, **4**, 3105–3113.
Fischer,G. and Schachner,M. (1982) *Exp. Cell Res.*, **139**, 285–296.
Ghandour,S., Langley,K., Gombos,G., Hirn,M., Hirsch,M.-R. and Goridis,C. (1982) *J. Histochem. Cytochem.*, **30**, 165–170.
Goridis,C., Deagostini-Bazin,H., Hirn,M., Hirsch,M.-R., Rougon,G., Sadoul,R., Langley,O.K., Gombos,G. and Finne,J. (1983) *Cold Spring Harbor Symp. Quant. Biol.*, **48**, 527–537.
Günther,J., Nick,H. and Monard,D. (1985) *EMBO J.*, **4**, 1963–1966.
Hämmerling,G.J., Lemke,H., Hämmerling,U., Höhmann,C., Wallich,R. and Rajewsky,K. (1978) *Curr. Top. Microbiol. Immunol.*, **81**, 100–106.
Harper,G.P., Barde,Y.-A., Edgar,D., Ganten,D., Hefti,F., Heumann,R., Naujoks,K.W., Rohrer,H., Turner,J.E. and Thoenen,H. (1983) *Neurosci.*, **8**, 375–387.
Keilhauer,G., Faissner,A. and Schachner,M. (1985) *Nature*, **316**, 728–730.
Korsching,S. and Thoenen,H. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 3513–3516.
Kruse,J., Mailhammer,R., Wernecke,H., Faissner,A., Sommer,I., Goridis,C. and Schachner,M. (1985) *Nature*, **311**, 153–155.
Kruse,J., Keilhauer,G., Faissner,A., Timpl,R. and Schachner,M. (1984) *Nature*, **316**, 146–148.
Lee,V.M., Greene,L.A. and Shelanski,M.L. (1981) *Neurosci.*, **6**, 2773–2786.
Leutz,A. and Schachner,M. (1981) *Cell Tissue Res.*, **220**, 393–404.
Lillien,L.E. and Claude,P. (1985) *Nature*, **317**, 632–634.
Martini,R. and Schachner,M. (1986) *J. Cell Biol.*, **103**, 2439–2448.
McGuire,J.C., Greene,L.A. and Furano,A.V. (1978) *Cell*, **115**, 357–365.
Monard,D., Solomon,F., Rentsch,M. and Gysin,R. (1973) *Proc. Natl. Acad. Sci. USA*, **70**, 1894–1897.
Nieke,J. and Schachner,M. (1985) *Differentiation*, **30**, 141–151.
Ramon y Cajal (1928) *Degeneration and Regeneration of the Nervous System.* Oxford University Press, London.
Rathjen,F.G. and Schachner,M. (1984) *EMBO J.*, **3**, 1–10.
Rohrer,H. (1985) *Dev. Biol.*, **111**, 95–107.
Rohrer,H. and Sommer,I. (1983) *J. Neurosci.*, **3**, 1683–1693.
Rush,R.A. (1984) *Nature*, **312**, 364–367.
Schmidt,J.W. and Catterall,W.A. (1986) *Cell*, **46**, 437–445.
Schmidt,J.W., Rossie,S. and Catterall,W.A. (1985) *Proc. Natl. Acad. Sci. USA*, **82**, 4847–4851.
Schnitzer,J. and Schachner,M. (1981) *J. Neuroimmunol.*, **1**, 457–470.
Tamkun,M.M. and Fambrough,D.M. (1986) *J. Biol. Chem.*, **261**, 1009–1019.
Taniuchi,M., Clark,H.B. and Johnson,E.M., Jr (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 4094–4098.

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