

Neural Cell Adhesion Molecule Expression Is Regulated by Schwann Cell–Neuron Interactions in Culture

Bernd Seilheimer, Elke Persohn, and Melitta Schachner

Department of Neurobiology, University of Heidelberg, 6900 Heidelberg 1, Federal Republic of Germany

Abstract. To investigate the cellular and molecular signals underlying regulation of cell adhesion molecule expression, the influence of interactions between dorsal root ganglion neurons and Schwann cells on their expression of L1 and N-CAM was quantitated by immunogold electronmicroscopy. The numbers of antibody binding sites on cell surfaces of neurons and glia were compared between pure populations and co-cultures. After 3 d of co-culture, expression of L1 was reduced by 91% on Schwann cells and 36% on neurons, with expression in pure cultures being taken as 100%. N-CAM expression was unchanged on neurons and reduced by 43% on Schwann cells. Within 3 d after removal of neurons from Schwann cell–neuron co-

cultures by immunocytolysis, expression of L1 and N-CAM on Schwann cell surfaces increased by 69 and 84%, respectively. Cell surface antigens recognized by an antibody to mouse liver membranes were unchanged in co-cultures. Furthermore, in co-cultures of neurons and sciatic nerve fibroblasts neither of the three antibodies detected any changes in expression of antigens when pure and co-cultures were compared. These observations suggest that adhesion molecules are not only involved in neuron–Schwann cell recognition and neurite outgrowth on Schwann cells (Seilheimer, B., and M. Schachner. 1988. *J. Cell Biol.* 107:341–351), but that cell interactions, in turn, modulate the extent of adhesion molecule expression.

CELL surface molecules, operationally termed adhesion molecules, play important roles in cell–cell and cell–substrate interactions during development of the nervous system (Edelman and Thiery, 1985, 1987). These molecules are thought to mediate in vitro adhesion and aggregation between different cell types, migration of granule neurons (Lindner et al., 1983), neurite outgrowth on glia (Bixby et al., 1988; Seilheimer and Schachner, 1988; Tomaselli et al., 1987), formation of orderly retinotectal projections (Fraser et al., 1984; Thanos et al., 1984), development of the laminar cytoarchitecture in the retina (Buskirk et al., 1980; Rütishauser et al., 1985), and neurite outgrowth on other neurites (Chang et al., 1987; Lagenaur and Lemmon, 1987). For the regulation of specific cell interactions, a precisely timed appearance and disappearance in expression of adhesion molecules would appear necessary. It is, therefore, important to study the cellular and molecular signals that underlie adhesion molecule expression in the developing, adult, and regenerating nervous systems. Cell cultures offer the advantage to study such interactions under controlled conditions by confronting homogeneous populations of defined cell types with each other. The peripheral nervous system of mammals offers the unique opportunity to study the cellular and molecular signals underlying neuron–glia interactions, since adhesion molecule expression undergoes considerable changes not only during development, but also during regeneration (Daniloff et al., 1986; Martini and Schachner, 1986, 1988; Nieke and Schachner, 1985). Schwann cells have

been shown to undergo changes in adhesion molecule expression after deprivation of axons by transection or crushing (Daniloff et al., 1986; Martini and Schachner, 1988). Furthermore, neurons have been observed to alter adhesion molecule expression depending on the particular type of interactions they engage in: axons express L1 when fasciculating, but when oriented perpendicularly to the fasciculating axons, when myelinated, or when nonfasciculating, they do not express immunohistologically detectable levels of L1 (Bartsch, U., F. Kirchhoff, and M. Schachner, manuscript submitted for publication; Dodd et al., 1988; Hekmat et al., 1988; Holley, J., and M. Schachner, unpublished observations; Martini and Schachner, 1986; Persohn and Schachner, 1987). In contrast, expression of N-CAM is not as impressively regulated by different cell contacts. However, even this more broadly distributed adhesion molecule appears restricted in its expression to particular types of cell contact and mediates neuron–neuron, glia–glia, and neuron–glia contacts (Keilhauer et al., 1985; Pollerberg et al., 1986, 1987).

In the peripheral nervous system, L1 and N-CAM have been found to be present on both neurons and Schwann cells (Faissner et al., 1984b; Martini and Schachner, 1986; Mirsky et al., 1986; Sanes et al., 1986; Seilheimer and Schachner, 1987, 1988). The two molecules are expressed on axons and Schwann cells at the onset of myelination, but are reduced in their expression on both cell types, when the glial processes have turned approximately one and a half loops around

the axon (Martini and Schachner, 1986, 1988). Thereafter, neither axons nor Schwann cells express detectable levels of L1 antigen, whereas N-CAM remains expressed periaxonally and, more weakly, in compact myelin. At the nodes of Ranvier, the axolemma is always L1 and N-CAM negative. Nonmyelinated, fasciculating axons and nonmyelinating Schwann cells express L1 and N-CAM even in the adult animal.

After transection of the adult mouse sciatic nerve, L1 and N-CAM reappear within several days in the distal part of the transected nerve (Martini and Schachner, 1988; Nieke and Schachner, 1985), during which time Schwann cells undergo a high proliferative response (Bradley and Asbury, 1970). The reduction of L1 and N-CAM expression to its normal adult level takes more than a year, thus recapitulating normal development, although at an apparently more protracted, less coordinated time scale (Nieke and Schachner, 1985).

On the basis of these observations we set out to study the cellular and molecular signals that regulate the expression of cell adhesion molecules on Schwann cells. We used pure cultures of Schwann cells and fibroblasts from the developing sciatic nerve and dorsal root ganglion neurons from mouse (Seilheimer and Schachner, 1988) to study whether neurons and Schwann cells influence each other in their expression of adhesion molecules.

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.

Antibodies

Polyclonal antibodies to mouse L1, N-CAM, and mouse liver membranes, and monoclonal antibodies to Thy-1.2 and MESA-1 have been described (Faissner et al., 1984a; Ghandour et al., 1982; Goridis et al., 1983; Hämmerling et al., 1978; Pollerberg et al., 1986; Rathjen and Schachner, 1984).

Schwann Cell Cultures

Schwann cells were prepared from sciatic nerves of ~40 1-d-old mice as described in detail (Seilheimer and Schachner, 1987). In brief, sciatic nerves were incubated three times for 15 min at 37°C with 0.25% trypsin and 0.03% collagenase. Nerves were mechanically dissociated by three cycles of suction through an injection needle and released cells collected by centrifugation. The cell pellet was resuspended in culture medium (50% [vol/vol] DME, 50% Ham's F12 medium, 10 mM Hepes, 100 IU/ml penicillin, 72 IU/ml streptomycin, and 0.2% FCS) containing 10 µg/ml laminin from EHS sarcoma (Bethesda Research Laboratories, Gaithersburg, MD).

After 3 d in culture, fibroblasts were removed by complement dependent immunocytolysis with monoclonal antibodies Thy-1.2 (Hämmerling et al., 1978) and MESA-1 (Ghandour et al., 1982). Debris from immunocytolyzed cells was cleared by incubation for 10 s at room temperature with trypsin/EDTA solution and gentle washing. Cells were then incubated for 4 min at 37°C with the trypsin/EDTA solution, collected by centrifugation and resuspended for subculture in Eagle's basal medium (BME)¹ containing 10% horse serum and antibiotics (Seilheimer and Schachner, 1988). Cells had a purity of 99.5 ± 0.5% 1 d after the subculture step as determined by morphology and expression of S-100 (Seilheimer and Schachner, 1987) and will be referred to hereafter as pure Schwann cell cultures. Schwann cells were plated at a density of 2 × 10⁴ cells/50 µl BME containing 10% horse serum per coverslip (15 mm in diameter).

1. Abbreviations used in this paper: BME, Eagle's basal medium; DPBS, Dulbecco's modified phosphate buffered saline; NGF, nerve growth factor.

Cultures of Dorsal Root Ganglion Neurons

Cultures of dorsal root ganglion neurons were established from 1-d-old mice. Ganglia (~100) were dissected from the animals and incubated for 45 min at 37°C in 0.25% trypsin (Cooper Biomedical Inc., Frankfurt, FRG) and 0.03% collagenase (Worthington Biomedical Corp., Berlin, FRG) in 2 ml DME containing 10 mM Hepes. After removal of 1.5 ml supernatant, 0.5 ml culture medium containing 0.2% FCS and 0.01% DNase I (Boehringer Mannheim Diagnostics, Inc., Houston, TX) was added and ganglia mechanically dissociated in this medium by five cycles of pipetting up and down through a fire-polished Pasteur pipette. Released cells were collected by centrifugation (5 min at 4°C and 80 g) and resuspended in 200 µl BME containing 10 mM Hepes, 10 IU/ml penicillin and 10 IU/ml streptomycin, 10% horse serum, and 100 ng/ml beta nerve growth factor (β-NGF) from mouse submaxillary glands (culture medium). The cell suspension (~3.5 × 10⁶ cells in total) was transferred onto a cushion of 35% Percoll (1 ml; Pharmacia Fine Chemicals, Piscataway, NJ) and centrifuged for 15 min at 4°C and 200 g. The cell pellet (~3 × 10⁵ cells of which ~90–95% are neurons) was washed once with 5 ml culture medium and plated in culture medium on a double layer of rat tail collagen (Bornstein, 1958; Ehrmann and Gey, 1956) at densities of 1 × 10⁴ cells/50 µl on glass coverslips (15 mm in diameter). After 12–16 h in vitro, cytosine arabinoside (10⁻⁵ M; Sigma Chemical Co., St. Louis, MO) was added to the culture and removed after 3 d by replacement with culture medium. Cultures were maintained for seven further days without change of culture medium.

Preparation of Fibroblasts

Fibronectin-positive fibroblasts or fibroblast-like cells were prepared from 1-d-old mice by a procedure that followed exactly the preparation of Schwann cells up to the immunocytolysis step (see above, and Seilheimer and Schachner, 1987). Instead of immunocytolysis, the cultures were washed two times with DME and then incubated for 1 min at room temperature in DME containing 1 µg/ml trypsin and 0.02% EDTA. Fibroblasts were then lifted off the layer of Schwann cells by gentle pipetting, collected by centrifugation, and resuspended and seeded onto rat-tail collagen-coated coverslips in BME containing 10% horse serum and antibiotics (Seilheimer and Schachner, 1988). Yields were ~1.5 × 10⁶ cells from ~40 mice and purity was between 98.3 and 99% by the criteria of L1 and S-100 negativity, fibronectin positivity, and epithelioid morphology (Seilheimer and Schachner, 1987).

Co-Cultures of Schwann Cells, Neurons, and Fibroblasts

For co-culture of dorsal root ganglion neurons and Schwann cells, single cell suspensions of neurons were prepared, plated on rat-tail collagen (1 × 10⁴ cells/50 µl per coverslip), and treated with cytosine arabinoside as described above. Cells were maintained for 10 d in vitro before Schwann cells (2 × 10⁴ cells, 3 d after the subculture step) or fibroblasts (1 × 10⁴ cells) were added. The relative densities of the two partner cell types were such that each Schwann cell or fibroblast was contacted by at least one dorsal root ganglion neurite as seen by phase contrast microscopy. Cells were co-cultured for 3 d in BME containing 10% horse serum and antibiotics (Seilheimer and Schachner, 1988). In some experiments β-NGF (100 ng/ml) was added to the co-cultures to estimate whether L1 expression on cell surfaces differed in the presence or absence of NGF. When purified cell populations were cultured individually for control, culture conditions, and manipulations were identical to those for co-cultures.

Table I. Expression of L1 and N-CAM in Pure Cultures of Schwann Cells, Dorsal Root Ganglion Neurons, and Fibroblasts from Early Postnatal Mice

Cells	Cell surface antigen		
	L1	N-CAM	Liver membrane
Schwann cell	+	+	+
Neuron	+	+	+
Fibroblast	—	+	+

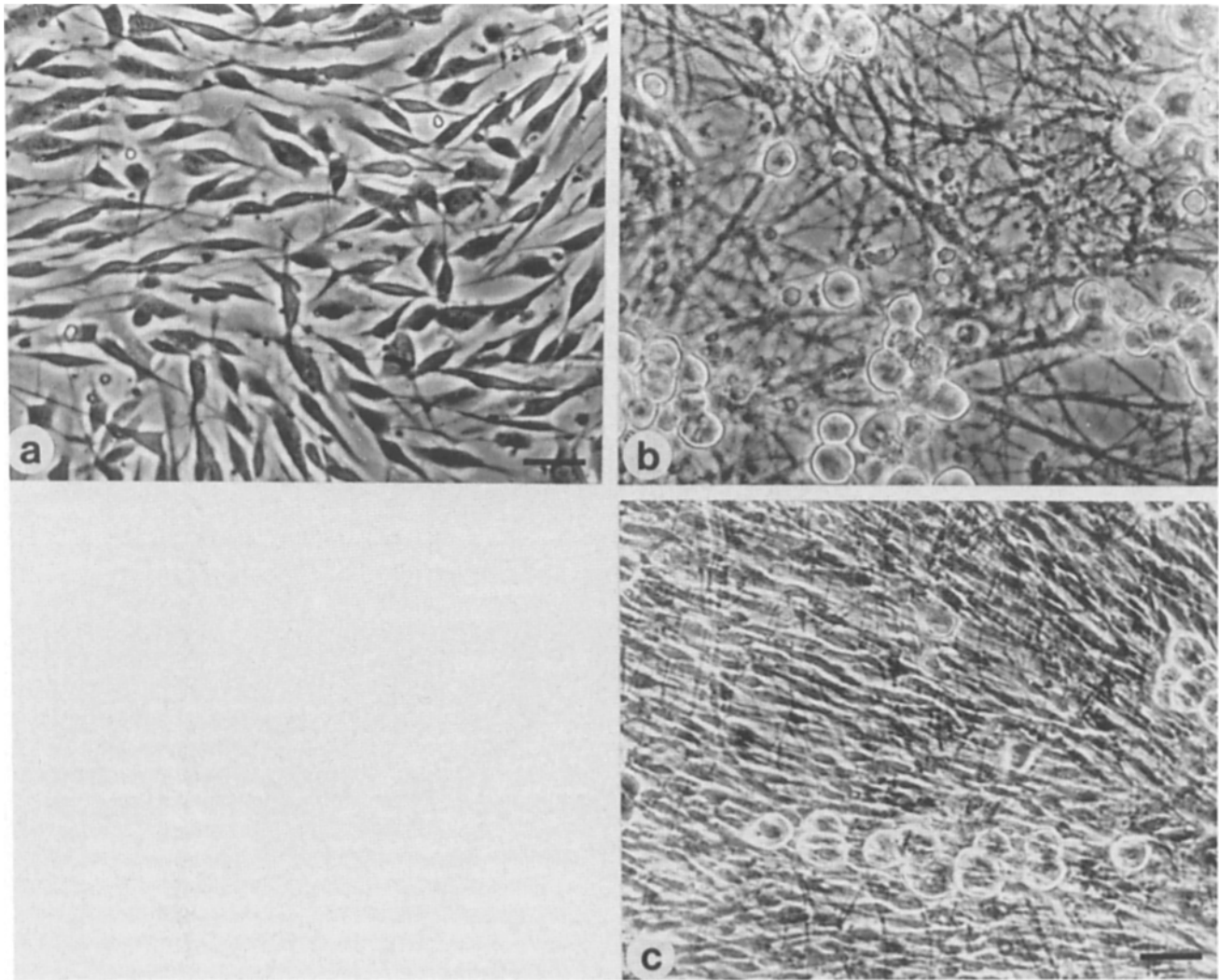


Figure 1. Phase contrast microscopic images of pure cultures of Schwann cells (a) and dorsal root ganglion neurons (b) 3 and 7 d after plating in vitro, respectively. Co-cultures of dorsal root ganglion neurons and Schwann cells after 3 d in vitro (c). For this co-culture, dorsal root ganglion neurons were cultured for 4 d and Schwann cells were plated directly onto them after preparation by immunoselection. Individual densities of Schwann cells and dorsal root ganglion neurons were the same in co-culture as in the individual cultures. Bars: (a) 12 μm ; (b and c) 8 μm .

Complement-dependent Immunocytolysis in Co-Culture

After 3 d in Schwann cell–neuron co-culture, cells were washed once with DME and incubated for 2 h at 37°C with monoclonal antibody Thy-1.2 (Hämmerling et al., 1978) in DME at a concentration of $\sim 1 \mu\text{g/ml}$. After the incubation, cultures were washed gently in DME followed by incubation with guinea pig complement (final dilution 1:10) in DME for 2 h at 37°C. The immunocytolyzed neuronal network was removed by three gentle washing steps with DME. To reduce stickiness of some remaining axonal debris to Schwann cells, cells were incubated in BME containing only 0.2% horse serum. After 24 h cells were washed once in DME and maintained for an additional 2 d in BME containing 10% horse serum. For control, pure cultures of Schwann cells were mock treated in the same manner as co-cultures and maintained under identical conditions and the same amount of time as the immunocytolyzed co-cultures.

Immunoelectron Microscopy

Cell cultures were washed once in culture medium at 37°C and immediately fixed with 1.25% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, containing 0.03 M sucrose for 1 h at room temperature. Cultures were washed at room temperature in Dulbecco's modified phosphate buffered saline

(DPBS), pH 7.3, for 1 h and blocked for 15 min DPBS containing 10% horse serum and 0.01% BSA (blocking buffer). Primary antibodies were then incubated overnight at 4°C in blocking buffer. Antibodies were all used at saturating levels; i.e., at least three serial dilution steps lower than the titer endpoint amounting to a final concentration of at least 1 $\mu\text{g/ml}$. For control, cultures were immersed without antibody in blocking buffer. Cultures were washed in DPBS for at least 1 h and then incubated with gold-coupled antibodies to rabbit IgG (Janssen Pharmaceutica, Beerse, Belgium) in DPBS for 1.5 h, all at room temperature. After a further washing step the cultures were postfixed with 2% OsO_4 in 0.1 M phosphate buffer, pH 7.3, for 40 min, dehydrated in an ascending series of ethanol and propylene oxide, and embedded in Epon 812 (Agar Aids, Stansted, Essex, UK). After polymerization (48 h, 60°C), glass coverslips were removed from the Epon-embedded cultures by adding hydrofluoric acid (E. Merck, Darmstadt, FRG) for 20 min at room temperature and blocks were rinsed with water overnight. Representative parts of the cultures were cut out and mounted on an Epon block for sectioning. Ultrathin cross sections were mounted on Formvar-coated copper grids (E. Merck), counterstained with uranyl acetate and lead citrate, and examined with a Zeiss EM 10 C.

Quantitative Measurements of Immunogold Labeling Densities

The length of cut cell surfaces was measured with a computer (Olivetti

M24; AI Tektron GmbH, Meerbusch) equipped with cursor and graphic tablet, TV camera, color monitor, and an image analysis program (VIDS III; AI Tektron). For identification of cell types in co-cultures the ultrastructural criteria of Bunge and Bunge were used (1984). In the few cases, when an unequivocal identification was not possible, the structures were not scored. When regions of tight apposition of surface membranes were present (see Figs. 2, b, e, and f) the total cell surface, including that of apposed membranes, was scored. Since the size of these regions was the same in pure as in co-cultures, the potential underestimate of antigenic sites due to decreased penetration of antibodies into more tightly packed areas can be considered to be similar under both culture conditions.

Results

To investigate whether the expression of the two adhesion molecules, L1 and N-CAM, on Schwann cells, neurons, or fibroblasts (Table I) was subject to regulatory influences from partner cells, pure populations of Schwann cells and dorsal root ganglion neurons from early postnatal mice were cultured individually or together. Expression of the two molecules was measured after 3 d of co-culture by immunogold labeling of the fixed cultures and counting the number of gold particles per length of cut cell surface. In pure cultures of Schwann cells (Fig. 1 a) and dorsal root ganglion neurons (Fig. 1 b), the densities of gold particles were one gold particle per 690 ± 31 nm and $2,010 \pm 51$ nm, respectively, when L1 antibodies were used as primary antibodies. No gold particles were observed on fibroblasts from sciatic nerve (not shown). Immunogold labeling intensities were measured on both cell bodies (Fig. 2, a and d) and processes (Fig. 2, b and c). When N-CAM antibodies were used, one gold particle was found per $1,727 \pm 34$ nm on Schwann cell surfaces, per $1,668 \pm 35$ nm on dorsal root ganglion neurons, and per $11,719 \pm 169$ nm on fibroblasts. Antibodies to mouse liver membranes labeled Schwann cells with one gold particle per 840 ± 27 nm, dorsal root ganglion neurons with one gold particle per 965 ± 36 nm, and fibroblasts with one gold particle per $1,009 \pm 40$ nm. The labeling densities were found to be constant over a range of cell densities and saturating antibody concentrations: plating one fourth or two times as many Schwann cells, dorsal root ganglion neurons, or fibroblasts per coverslip as under standard conditions and using three different saturating antibody concentrations did not result in any significant differences in immunogold labeling densities.

When neurons were cultured together with Schwann cells (Fig. 1 c) or fibroblasts (not shown), cell surface labeling intensities were measured after 3 d of co-culture and compared to those of pure cultures. Identification of somata and processes in co-cultures followed the ultrastructural criteria described by Bunge and Bunge (1984) which we were able to confirm in the pure cultures. In few cases, when processes were difficult to identify unequivocally as derived either from Schwann cell, dorsal root ganglion neuron, or fibroblast, these were omitted from scoring. In co-cultures of Schwann cells with dorsal root ganglion neurons the detectability of antigenic sites labeled with polyclonal L1 antibodies was reduced by $91 \pm 3\%$ when compared to pure cultures of Schwann cells (Table II). On neurons cell surface labeling of L1 was also reduced, but only by $36 \pm 2\%$. Cell surface labeling of N-CAM was reduced on Schwann cells by $43 \pm 4\%$ and remained essentially unchanged on neurons ($9 \pm 2\%$). No differences in cell surface staining was seen with antibodies to mouse liver membranes neither on Schwann

cells nor on neurons. In co-cultures of fibroblasts with dorsal root ganglion neurons no differences were seen in immunogold labeling intensities for L1, N-CAM, or liver membrane antigens, when assayed on fibroblasts (except for L1, which is not expressed by these cells) or neurons. Densities of L1 on Schwann cell and neuronal surfaces were similar when co-cultures were maintained in the presence or absence of NGF.

When neurons were removed by immunocytolysis from Schwann cell–neuron co-cultures and maintained afterwards for 3 d, *in vitro* densities of immunolabeling were one gold particle per 473 ± 51 nm and $1,456 \pm 51$ nm for L1 and N-CAM, respectively. When these values are compared to those obtained from pure Schwann cell cultures treated and maintained under identical conditions as the immunocytolyzed co-cultures, L1 and N-CAM expression amounted to 69 and 84%, respectively, with expression of each antigen being taken as 100%. Addition of supernatants from cultures of dorsal root ganglion neurons (maintained *in vitro* for 2 d without NGF) to cultures of pure Schwann cells did not significantly affect the expression of L1 when measured 3 d after addition.

Discussion

In this study we have used pure populations of Schwann cells, dorsal root ganglion neurons, and fibroblasts from early postnatal sciatic nerve to investigate the influence of cell interactions in co-culture on expression of the adhesion molecules L1 and N-CAM. We could show that in co-cultures of dorsal root ganglion neurons with Schwann cells, L1 and N-CAM, but not other antigens, are modified in their extent of expression. The decrease in expression of L1 on Schwann cells is drastic, abolishing it almost completely ($\sim 90\%$). This decrease is not observed when supernatants of cultures of dorsal root ganglion neurons are added to pure cultures of Schwann cells, suggesting that cell contact may regulate expression of L1. N-CAM is also decreased on Schwann cells in co-cultures with neurons, but not as drastically as on Schwann cells. When neurons are removed from co-cultures and expression of the two glycoproteins compared to pure cultures of Schwann cells, values are significantly increased over those in co-cultures, indicating a high degree of plasticity in regulation of adhesion molecule expression by Schwann cells. Neurons appear to be less susceptible to down-regulation in that only L1 is decreased in expression (by 36%), but N-CAM remains essentially unchanged. Fibroblasts, important partner cells for neurite outgrowth in the fibroblastic cap of regenerating nerve stumps (Daniloff et al., 1986; Martini and Schachner, 1988; Nieke and Schachner, 1985) do not exert any comparable regulatory influences on neurons. The modulatory influence of partner cells on the extent of adhesion molecule expression, therefore, appears to be cell type specific. Also, adhesion molecule expression on neurons is differentially influenced by Schwann cells: neuronal expression is decreased for L1, but not for N-CAM. Thus, a remarkably specific regulation with regard to cell type and cell surface molecule is observed.

At present, it is difficult to correlate the changes in L1 and N-CAM expression *in vitro* to those observed during development and regeneration of the sciatic nerve *in situ*. Since adhesion molecule expression has yet to be studied *in situ* be-

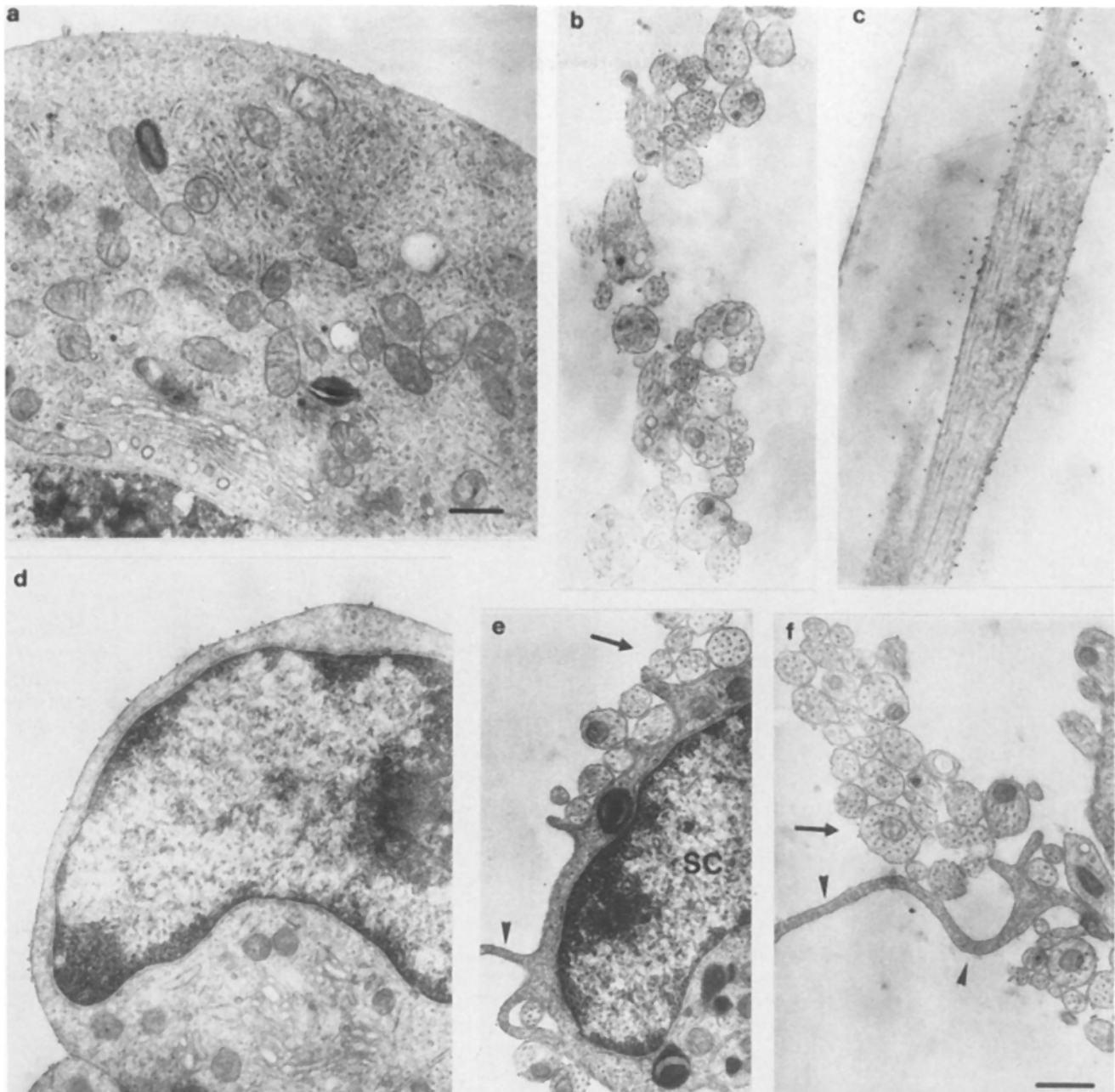


Figure 2. Immunogold electronmicroscopic localization of L1 antigenic sites on the surfaces of pure cultures of dorsal root ganglion neurons (*a* and *b*) and Schwann cells (*c* and *d*), and co-cultures between the two cell types (*e* and *f*) after 3 d in vitro. Cell somata (*a* and *d*) and cell processes (*b* and *c*) are shown. In co-cultures, Schwann cell process (*arrowheads*), cross sections of neurites (*large arrows*), and cell body of Schwann cell (*SC*) are shown. Bars, 0.5 μ m.

fore neuron-glia contact in the early embryo, comparisons can only be made in relation to regeneration in the adult animal. It is noteworthy that both Schwann cells and neurons are L1 and N-CAM positive when in contact with each other in early embryonic development (Martini, R., personal communication) and at birth before the onset of myelination (Martini and Schachner, 1986). With the onset of ensheathment and myelination, Schwann cells and axons both down-regulate expression of L1 and N-CAM (Martini and Schachner, 1986, 1988). After transection of adult sciatic nerve, a drastic increase in L1 and N-CAM expression on Schwann

cells is seen within several days after removal of axonal contact (Daniloff et al., 1986; Jessen et al., 1987; Martini and Schachner, 1988; Nieke and Schachner, 1985). A reexpression of L1 and N-CAM is thus seen on Schwann cells upon removal of axonal contact. A persistence of N-CAM and L1 expression has been found in neuron-free Schwann cell culture even after prolonged time periods (Noble et al., 1985; Faissner et al., 1984b; Seilheimer and Schachner, 1987), while other cell surface markers cease to be expressed in the absence of neurons (Mirsky et al., 1980; Sommer and Schachner, 1981). Other regulatory signals, such as nerve

Table II. Reduction of Cell Surface Densities of L1 and N-CAM in Co-Cultures of Neurons and Nonneuronal Cells as Compared to Pure Cultures

Co-cultures	L1	N-CAM	Liver membrane
	%	%	%
Schwann cell	91 ± 3	43 ± 4	-6 ± 4
Dorsal root ganglion neurons	36 ± 2	9 ± 2	5 ± 3
Fibroblast	—	7 ± 5	-5 ± 3
Dorsal root ganglion neurons	4 ± 3	4 ± 3	9 ± 6

Numbers are mean values of percentages of decrease (±SD) in densities of gold particles on cell surfaces in co-cultures as compared to pure cultures (100%). Indirect immunogold labeling with polyclonal antibodies to L1, N-CAM, and mouse liver membranes was performed after 3 d of co-culture. For each value 7,000 mm of surface membrane were measured at 10,000× from three independent experiments.

growth factor are involved in regulating adhesion molecule expression (Seilheimer and Schachner, 1987). It is, therefore, interesting that synthesis of nerve growth factor is up-regulated in lesioned adult sciatic nerve (Heumann et al., 1987). Since NGF receptors on Schwann cells and NGF synthesis by nonneuronal cells are differentially regulated during development (Davies et al., 1987; Rohrer and Sommer, 1983) and regeneration (Korsching and Thoenen, 1985; Taniuchi et al., 1986), effects of NGF have to be taken into account. Superior cervical ganglion neurons and neural membrane fractions down-regulated NGF receptor binding on cultured Schwann cells. Furthermore, the possibility that adhesion molecule expression in situ may be influenced by degradation products of myelin (Yoshino et al., 1987) have to be considered. Thus, multiple regulatory signals on adhesion molecule expression in situ are expected to exist and to be discovered. It is, therefore, likely that the culture situation may not reflect the balance of all possible influences. Further dissection of the quality and quantity of molecular signals involved in Schwann cell–neuron relationships will be of crucial importance in our understanding of axon–glia interactions not only in the peripheral, but also central nervous system. Our studies have made steps in this direction and have shown for the first time that cell adhesion molecules are not only involved in neuron–Schwann cell interactions (Bixby et al., 1988; Seilheimer and Schachner, 1988), but that cell interactions themselves induce changes in adhesion molecule expression.

The authors are grateful to Andreas Faissner, Gerhard Keilhauer, and Rudolf Martini for helpful discussions; Ludgar Niehörster for help with the preparation of dorsal root ganglion neurons; Bernd Gehrig and Manfred Griesheimer for antibodies; Wilhelm Kriz for the use of his morphometric equipment; Bruni Hähnel for her assistance in its use; and Bundesministerium für Forschung und Technologie for support.

Received for publication 13 July 1988 and in revised form 5 January 1989.

References

Bixby, J. L., J. Lilien, and L. F. Reichardt. 1988. Identification of the major proteins that promote neuronal process outgrowth on Schwann cells in vitro. *J. Cell Biol.* 107:353–361.
 Bornstein, M. B. 1958. Reconstituted rat-tail collagen used as substrate for tissue cultures on coverslips in Maximow slides and Roller-tubes. *Lab. Invest.* 7:134–137.

Bradley, W. G., and A. K. Asbury. 1970. Duration of synthesis phase in neurilemma cells in mouse sciatic nerve during degeneration. *Exp. Neurol.* 26:275–282.
 Bunge, R. P., and M. B. Bunge. 1984. Tissue culture observations relating to peripheral nerve development, regeneration, and disease. In *Peripheral Neuropathy*. Vol. 1. P. J. Dyck, P. K. Thomas, and E. Lambert, editors. W. B. Saunders Company, Philadelphia, PA. 378–399.
 Buskirk, D. R., J.-P. Thiery, U. Rutishauser, and G. M. Edelman. 1980. Antibodies to a neural cell adhesion molecule disrupt histogenesis in cultured chick retinae. *Nature (Lond.)*. 285:488–489.
 Chang, S., F. G. Rathjen, and J. A. Raper. 1987. Extension of neurites on axons is impaired by antibodies against specific neural cell surface glycoproteins. *J. Cell Biol.* 104:355–362.
 Daniloff, J. K., G. Levi, M. Grumet, F. Rieger, and G. M. Edelman. 1986. Altered expression of neuronal cell adhesion molecules induced by nerve injury and repair. *J. Cell Biol.* 103:929–945.
 Davies, A. M., C. Bandtlow, R. Heumann, S. Korsching, H. Rohrer, and H. Thoenen. 1987. Timing and site of nerve growth factor synthesis in developing skin in relation to innervation and expression of the receptor. *Nature (Lond.)*. 326:353–358.
 Dodd, J., S. B. Morton, D. Karagogeos, M. Yamamoto, and T. M. Jessell. 1988. Spatial regulation of axonal glycoprotein expression on subsets of embryonic spinal neurons. *Neuron Cell press.* 1:105–116.
 Edelman, G. M., and J.-P. Thiery. 1985. *The Cell in Contact*. Vol. I. John Wiley & Sons, Inc., New York.
 Edelman, G. M., and J.-P. Thiery. 1987. *The Cell in Contact: Structure and Function of Adhesion Molecules*. Vol. II. John Wiley & Sons, Inc., New York. In press.
 Ehrmann, R. L., and G. O. Gey. 1956. The growth of cells on a transparent gel of reconstituted rat-tail collagen. *J. Natl. Cancer Inst.* 16:1375–1403.
 Faissner, A., J. Kruse, C. Goridis, E. Bock, and M. Schachner. 1984a. The neural cell adhesion molecule L1 is distinct from the N-CAM-related group of surface antigens BSP-2 and D2. *EMBO (Eur. Mol. Biol. Organ.) J.* 3:733–737.
 Faissner, A., J. Kruse, J. Nieke, and M. Schachner. 1984b. Expression of neural cell adhesion molecule L1 during development in neurological mutants and in the peripheral nervous system. *Dev. Brain Res.* 15:69–82.
 Fraser, S. E., B. A. Murray, Ch.-M. Chuong, and G. M. Edelman. 1984. Alteration of the retinectal map in *Xenopus* by antibodies to neural cell adhesion molecules. *Proc. Natl. Acad. Sci. USA.* 81:4222–4226.
 Ghandour, S., K. Langley, G. Gombos, M. Hirn, M.-R. Hirsch, and C. Goridis. 1982. A surface marker for murine vascular endothelial cells defined by monoclonal antibody. *J. Histochem. Cytochem.* 30:165–170.
 Goridis, C., H. Deagostini-Bazin, M. Hirn, M.-R. Hirsch, G. Rougon, R. Sadoul, O. K. Langley, G. Gombos, and J. Finne. 1983. Neural surface antigens during nervous system development. *Cold Spring Harbor Symp. Quant. Biol.* XLVIII:527–537.
 Hämmerling, G. J., H. Lemke, U. Hämmerling, C. Höhmann, R. Wallich, and K. Rajewsky. 1978. Monoclonal antibodies against murine cell surface antigens: anti-H2, anti-Ia and anti-T-cell antibodies. *Curr. Top. Microbiol. Immunol.* 81:100–106.
 Hekmat, A., V. Künemund, G. Fischer, and M. Schachner. 1988. Elaboration of a perpendicular orientation of cerebellar basket and stellate cells versus granule cell neurites in culture. *Neuron Cell press.* In press.
 Heumann, R., S. Korsching, C. Bandtlow, and H. Thoenen. 1987. Changes of nerve growth factor synthesis in non-neural cells in response to sciatic nerve transection. *J. Cell Biol.* 104:1623–1631.
 Jessen, K. R., R. Mirsky, and L. Morgan. 1987. Myelinated, but not unmyelinated axons, reversibly down-regulate N-CAM in Schwann cells. *J. Neurocytol.* 16:681–688.
 Keilhauer, G., A. Faissner, and M. Schachner. 1985. Differential inhibition of neurone–neurone, neurone–astrocyte and astrocyte–astrocyte adhesion by L1, L2 and N-CAM antibodies. *Nature (Lond.)*. 316:728–730.
 Korsching, S., and H. Thoenen. 1985. Treatment with 6-hydroxydopamine and colchicine decreases nerve growth factor levels in sympathetic ganglia and increases them in the corresponding target tissues. *J. Neurosci.* 5:1058–1061.
 Lagenaur, C., and V. Lemmon. 1987. An L1-like molecule, the 8D9 antigen is a potent substrate for neurite extension. *Proc. Natl. Acad. Sci. USA.* 84:7753–7757.
 Lindner, J., F. G. Rathjen, and M. Schachner. 1983. L1 mono- and polyclonal antibodies modify cell migration in early postnatal mouse cerebellum. *Nature (Lond.)*. 305:427–430.
 Martini, R., and M. Schachner. 1986. Immunoelectron microscopic localization of neural cell adhesion molecules (L1, N-CAM, MAG) and their shared carbohydrate epitope and myelin basic protein (MBP) in developing sciatic nerve. *J. Cell Biol.* 103:2439–2448.
 Martini, R., and M. Schachner. 1988. Immunoelectron-microscopic localization of neural cell adhesion molecules (L1, N-CAM and myelin-associated glycoprotein) in regenerating adult mouse sciatic nerve. *J. Cell Biol.* 106:1735–1746.
 Mirsky, R., K. R. Jessen, M. Schachner, and C. Goridis. 1986. Distribution of the adhesion molecules N-CAM and L1 on peripheral neurons and glia in adult rats. *J. Neurocytol.* 15:799–815.
 Mirsky, R., J. Winter, E. R. Abney, R. M. Pruss, J. Gavrilovic, and M. C.

- Raff. 1980. Myelin-specific proteins and glycolipids in rat Schwann cells and oligodendrocytes in culture. *J. Cell Biol.* 84:483-494.
- Nieke, J., and M. Schachner. 1985. Expression of the neural cell adhesion molecules L1 and N-CAM and their common carbohydrate epitope L2/HNK-1 during development and after transection of the mouse sciatic nerve. *Differentiation.* 30:141-151.
- Noble, M., M. Albrechtsen, C. Møller, J. Lyles, E. Bock, C. Goridis, M. Watanabe, and U. Rutishauser. 1985. Glial cells express N-CAM/D2-CAM-like polypeptides in vitro. *Nature (Lond.).* 316:725-728.
- Persohn, E., and M. Schachner. 1987. Immunoelectron-microscopic localization of the neural cell adhesion molecules L1 and N-CAM during postnatal development of the mouse cerebellum. *J. Cell Biol.* 105:569-576.
- Pollerberg, E., M. Schachner, and J. Davoust. 1986. Differentiation stage-dependent surface mobilities of the two molecular weight forms of the neural cell adhesion molecule N-CAM. *Nature (Lond.).* 324:462-465.
- Pollerberg, E., K. Burridge, K. Krebs, S. Goodman, and M. Schachner. 1987. The 180 kD component of the neural cell adhesion molecule N-CAM is involved in cell-cell contacts and cytoskeleton-membrane interactions. *Cell Tissue Res.* 250:227-236.
- Rathjen, F. G., and M. Schachner. 1984. Immunocytological and biochemical characterization of a new neuronal cell surface component (L1 antigen) which is involved in cell adhesion. *EMBO (Eur. Mol. Biol. Organ.) J.* 3:1-10.
- Rohrer, H., and I. Sommer. 1983. Simultaneous expression of neuronal and glial properties by chick ciliary ganglion cells during development. *J. Neurosci.* 3:1683-1693.
- Rutishauser, U., M. Watanabe, J. Silver, F. A. Troy, and E. R. Vimr. 1985. Specific alteration of NCAM-mediated cell adhesion by an endoneuraminidase. *J. Cell Biol.* 101:1842-1849.
- Sanes, J. R., M. Schachner, and J. Covault. 1986. Expression of several adhesive macromolecules (N-CAM, L1, J1, NILE, uvomorulin, laminin, fibronectin, and a heparan sulfate proteoglycan) in embryonic, adult, and denervated adult skeletal muscle. *J. Cell Biol.* 102:420-431.
- Seilheimer, B., and M. Schachner. 1987. Regulation of neural cell adhesion molecule expression on cultured mouse Schwann cells by nerve growth factor. *EMBO (Eur. Mol. Biol. Organ.) J.* 6:1611-1616.
- Seilheimer, B., and M. Schachner. 1988. Studies of adhesion molecules mediating interactions between cells of peripheral nervous system indicate a major role for L1 in mediating sensory neuron growth on Schwann cells in culture. *J. Cell Biol.* 107:341-351.
- Sommer, I., and M. Schachner. 1981. Monoclonal antibodies (01 to 04) to oligodendrocyte cell surfaces: an immunocytological study in the central nervous system. *Dev. Biol.* 83:311-327.
- Taniuchi, M., J. B. Schweitzer, and E. M. Johnson, Jr. 1986. Nerve growth factor receptor molecules in rat brain. *Proc. Natl. Acad. Sci. USA.* 83:1950-1954.
- Thanos, S., F. Bonhoeffer, and U. Rutishauser. 1984. Fiber-fiber interaction and tectal cues influence the development of the chicken retinotectal projection. *Proc. Natl. Acad. Sci. USA.* 81:1906-1910.
- Tomaselli, K. J., C. H. Damsky, and L. F. Reichardt. 1987. Interactions of a neuronal cell line (PC12) with laminin, collagen IV, and fibronectin: identification of integrin-related glycoproteins involved in attachment and process outgrowth. *J. Cell Biol.* 105:2347-2358.
- Yoshino, J. E., P. W. Mason, and G. H. DeVries. 1987. Developmental changes in myelin-induced proliferation of cultured Schwann cells. *J. Cell Biol.* 104:655-660.