

Antibodies to the L1 Adhesion Molecule Inhibit Schwann Cell Ensheathment of Neurons In Vitro

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Abstract. To investigate whether neural adhesion molecules are involved in neuron-induced Schwann cell differentiation, cocultures of pure dorsal root ganglion neurons, and Schwann cells were maintained in the presence of antibodies to evaluate possible perturbing effects. Several parameters characteristic of differentiating Schwann cells were studied, such as transition of spindle-shaped to flattened, i.e., more epithelioid morphology, association with neuronal cell bodies, ensheathment of neurites, production of basal lamina and collagen fibrils, and expression of the myelin associated glycoprotein (MAG). A complete ablation of

Schwann cell differentiation in all features studied was seen with antibodies to the neural adhesion molecule L1. Antibodies to N-CAM did not reduce the association of Schwann cells with neurites but abolished the interdigitation of Schwann cell processes into neurite bundles, while leaving the other parameters studied unaffected. Fab fragments of antibodies to J1, MAG, and mouse liver membranes did not interfere with the manifestation of any of these parameters. None of the antibodies changed incorporation of [³H]thymidine into Schwann cells.

NEURON-GLIA interactions play important roles in the development, maintenance, and regeneration of the nervous system. In the peripheral nervous system, glial cells are thought to mediate structural and functional support of neurons, engage in myelination of axons, and support the regrowth of axons (Aguayo, 1985; Bunge, 1980). Conversely, neurons influence Schwann cells by inducing proliferation (Ratner et al., 1985; Salzer et al., 1980a, b). Neurons also induce Schwann cells to ensheath axons and neuronal cell bodies, leading to the establishment of a one-to-one relationship between the neuronal and glial partner and the production of extracellular matrix components that are secreted and constitute the basal lamina (Bunge et al., 1980, 1982; for review see Bunge et al., 1986). Finally, the decision for myelination and the size of the myelin sheath are determined by particular features of the axon (Aguayo et al., 1976; Duncán, 1934; Weinberg and Spencer, 1975). The molecular signals underlying such interactions are still poorly understood.

Cell surface interactions not only play important roles in transmitting the mitogenic signal of neurons to Schwann cells and allowing neurite extension on Schwann cells (Bixby et al., 1988; Seilheimer and Schachner, 1988), but may also underlie the decision for ensheathment and myelination. Adhesion molecules are the likely candidates for such surface-mediated interactions. The neural adhesion molecules L1 and N-CAM are expressed both by Schwann cells and axons

before the establishment of a one-to-one relationship between them and ensheathment. The two molecules are not only involved in the initial contacts between Schwann cells and axons, but also between Schwann cells and Schwann cells and between fasciculating axons (Martini and Schachner, 1986; 1988; Rieger et al., 1986; Seilheimer and Schachner, 1988). The putative cell adhesion molecule P₀, like L1 and N-CAM a member of the immunoglobulin superfamily (Barthels et al., 1987; Hemperly et al., 1986; Lemke and Axel, 1985; Moos et al., 1988), becomes detectable at the time of a one-to-one association between Schwann cell and axon (Martini et al., 1988). After the myelinating Schwann cell has turned approximately one and a half loops around the axon, L1 and the L2/HNK-1 carbohydrate epitope (Kruse et al., 1984; Künemund et al., 1988) disappear and N-CAM is reduced on both axon and Schwann cell (Martini and Schachner, 1986). At this stage the adhesion molecule myelin-associated glycoprotein (MAG)¹ becomes expressed at the Schwann cell-axon interface and on the turning loops of myelin-forming Schwann cell processes (Martini and Schachner, 1986). From these observations it seemed plausible to assume that adhesion molecules play important roles in the establishment and maintenance of neuron-Schwann cell interactions.

This study was undertaken to investigate whether neural adhesion molecules expressed by Schwann cells or neurons are involved in neuron-Schwann cell interactions. Cultures of pure Schwann cells and dorsal root ganglion neurons were

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1. *Abbreviation used in this paper:* MAG, myelin-associated glycoprotein.

used to determine whether antibodies to several neural adhesion molecules and their shared L2/HNK-1 carbohydrate epitope are able to perturb neuron-induced Schwann cell differentiation.

Materials and Methods

Antibodies

Polyclonal antibodies to mouse L1, N-CAM, J1, and MAG, and mouse liver membranes, and mAb to MAG have been described (Faissner et al., 1984; Goridis et al., 1983; Kruse et al., 1985; Lindner et al., 1983; Pollerberg et al., 1986; Poltorak et al., 1987; Rathjen and Schachner, 1984).

Fluorescein-coupled antibodies to rat, rabbit, or mouse immunoglobulins were purchased from Dakopatts (Hamburg, FRG), Cappel (Denkendorf, FRG) and Miles (Munich, FRG). Colloidal gold (10 nm in diameter)-coupled antibodies to rat, rabbit, or mouse immunoglobulins were purchased from Janssen Pharmaceutica (Beerse, Belgium).

Fab-fragments of polyclonal antibodies were prepared according to Porter (1959).

Cell Cultures

Pure cultures of Schwann cells were prepared by immunocytolysis of contaminating fibroblasts from sciatic nerves of 1-d-old NMRI mice (Seilheimer and Schachner, 1987). These cultures had a purity of 99.5% by morphological and antigenic marker criteria at the time of plating onto neurons.

Pure cultures of dorsal root ganglion neurons from 1-d-old NMRI mice were obtained by Percoll gradient centrifugation and a cycle of cytosine- β -D-arabinofuranoside treatment (Seilheimer and Schachner, 1988; Seilheimer et al., 1989). The purity of these cultures was >99% by morphological and antigenic marker criteria at the time of coculture with Schwann cells.

Cocultures of Schwann cells and dorsal root ganglion neurons were established by plating dorsal root ganglion neurons onto a double-layer of rat tail collagen (Bornstein, 1958) at a density of 10,000 cells/cover slip (15 mm in diameter) and maintained *in vitro* in basal Eagle's medium (BME) containing 10% horse serum and 100 ng/ml NGF (culture medium) for 4–6 wk. 12–16 h after plating, cytosine- β -D-arabinofuranoside (10^{-5} M, Sigma Chemical Co., St. Louis, MO) was added to the culture and removed after 3 d by replacement with culture medium. Before addition of Schwann cells (10,000 cells/cover slip) neurons were washed two times in HBSS. Schwann cells were taken for co-culture after the first subculture step (Seilheimer and Schachner, 1987). Cocultures were maintained in culture medium (without NGF) supplemented with 50 μ g/ml L-ascorbic acid (E. Merck, Darmstadt, FRG) for 2 wk. Ascorbic acid was added to the culture medium every day in a small volume (5 μ l/ml) to a final concentration of 50 μ g/ml. The culture medium was replaced every 2 d. Selective survival of neurons with different cell body sizes was not observed under the culture conditions used (Seilheimer and Schachner, 1988).

Coculture in the Presence of Antibodies

1–2 h after addition of Schwann cells to precultured dorsal root ganglion neurons, Fab fragments or IgG fractions of antibodies were added to a final concentration of 0.3 or 1.0 mg/ml. Antibodies had been dialyzed extensively against culture medium beforehand and were freshly added at feeding intervals of 2 d.

Transmission Electronmicroscopy

Cocultures were fixed at room temperature in 1.25% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, containing 0.03 M sucrose. Cultures were then washed in 0.2 M Palay buffer (Palay and Chan-Palay, 1974) and postfixed in 2% OsO₄ in 0.1 M phosphate buffer, pH 7.2, for 40 min at room temperature. Cultures were dehydrated in an ascending series of ethanol, propylene oxide and embedded in Epon 812 (Agar Aids; Stansted, UK). After polymerization for 48 h at 60°C, glass coverslips were removed from the Epon by etching with hydrofluoric acid (E. Merck) for 20 min at room temperature. Epon blocks were then rinsed overnight in tap water. Central and peripheral parts of the culture on the coverslip were selected, cut away from the Epon block and mounted onto another Epon block for transverse sectioning, such that cells could be observed in contact with their substrate. Ultrathin sections were mounted on Formvar-coated copper grids (E.

Merck) and counterstained with uranyl acetate and lead citrate and examined in a Zeiss EM 10C microscope.

Indirect Immunofluorescence

Indirect immunofluorescence on live cell cultures was carried out as described by Schnitzer and Schachner (1981).

Immunoelectronmicroscopy

Immunogold labeling of cultures maintained in the presence of Fab fragments or IgG fraction of antibodies was performed to check for their activity, survival, and penetration into the cultures. In brief, cells were maintained with antibodies as described above. Excess antibodies were then removed by washing the cells three times at room temperature in culture medium. Gold-labeled secondary antibodies were then applied for 30 min at 37°C. After another series of washing steps cells were fixed in 1.25% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, containing 0.03 M sucrose and processed for electron microscopy as described (Seilheimer et al., 1989). In general, antibodies penetrated well, as seen by immunogold labeling, into the first two layers of cells or cellular processes and into basal lamina and collagen (Fig. 4, *a* and *b*). Neurite fascicles were, however, usually devoid of gold particles in their interior. This immunonegativity is most likely due to the failure of the second antibodies with their adsorbed bulky gold particles to penetrate into the live cell cultures or to the antibody-induced removal of surface antigens by living cells or to virtual absence of cell surface antigen. Survival of neurons and Schwann cells in antibody-treated cultures was estimated by two criteria: actual number of cells in the antibody-treated cultures versus cultures maintained in the absence of antibodies (see next paragraph) and integrity of ultrastructural features.

Immunogold labeling of cultures maintained in the absence of antibodies was carried out as described (Seilheimer et al., 1989).

Determination of [³H]Thymidine Incorporation

Incorporation of [³H]thymidine was measured by autoradiography as described (Leutz and Schachner, 1981). In brief, cells were maintained in coculture for 1–2 h before addition of [³H]thymidine (2 or 6.7 μ Ci/ml; Amersham Corp., Arlington Heights, IL) for 48 h. When thymidine incorporation was determined in the presence of Fab fragments (0.3 or 1.0 mg/ml) of polyclonal antibodies to L1, N-CAM, J1, and mouse liver membranes or IgG fractions of L2 and MAG mAbs (0.3 or 1.0 mg/ml), antibodies were added together with [³H]thymidine, i.e., 1–2 h after addition of Schwann cells to precultured dorsal root ganglion neurons. After the thymidine pulse, cultures were washed with Dulbecco's modified PBS, pH 7.3, and fixed in 4% paraformaldehyde in Dulbecco's PBS. Cultures were then coated with a solution of gelatine, air-dried, and covered with photoemulsion (Ilford G5, diluted 1:2 with distilled water). After the photoemulsion was developed, cell nuclei were stained by bis-benzimide (Fischer et al., 1986). Coverslips were then mounted in Entellan (E. Merck) and labeled cells scored by phase-contrast and fluorescence microscopy. Cells were counted as positive, when the nucleus was stained by bis-benzimide and at least seven grains were detectable over the cell body.

Results

To investigate whether Schwann cell differentiation depends on adhesive interactions with neurons antibody perturbation experiments were performed with pure populations of cultured Schwann cells and dorsal root ganglion neurons from 1-d-old mice. Cultures of dorsal root ganglion neurons were maintained in culture for 4–6 wk before Schwann cells were added and cocultured for 14 d. It is only after maintenance of dorsal root ganglion neurons for a prolonged period of time in culture that Schwann cell ensheathment has been described to occur (Bunge and Bunge, 1978). After 14 d of coculture, almost all Schwann cells assumed a flattened, i.e., more epithelioid morphology which was in contrast to the spindle-shaped morphology of Schwann cells cultured without neurons (Fig. 1). This morphological transformation was seen not only in the center of cultures, where cell bodies

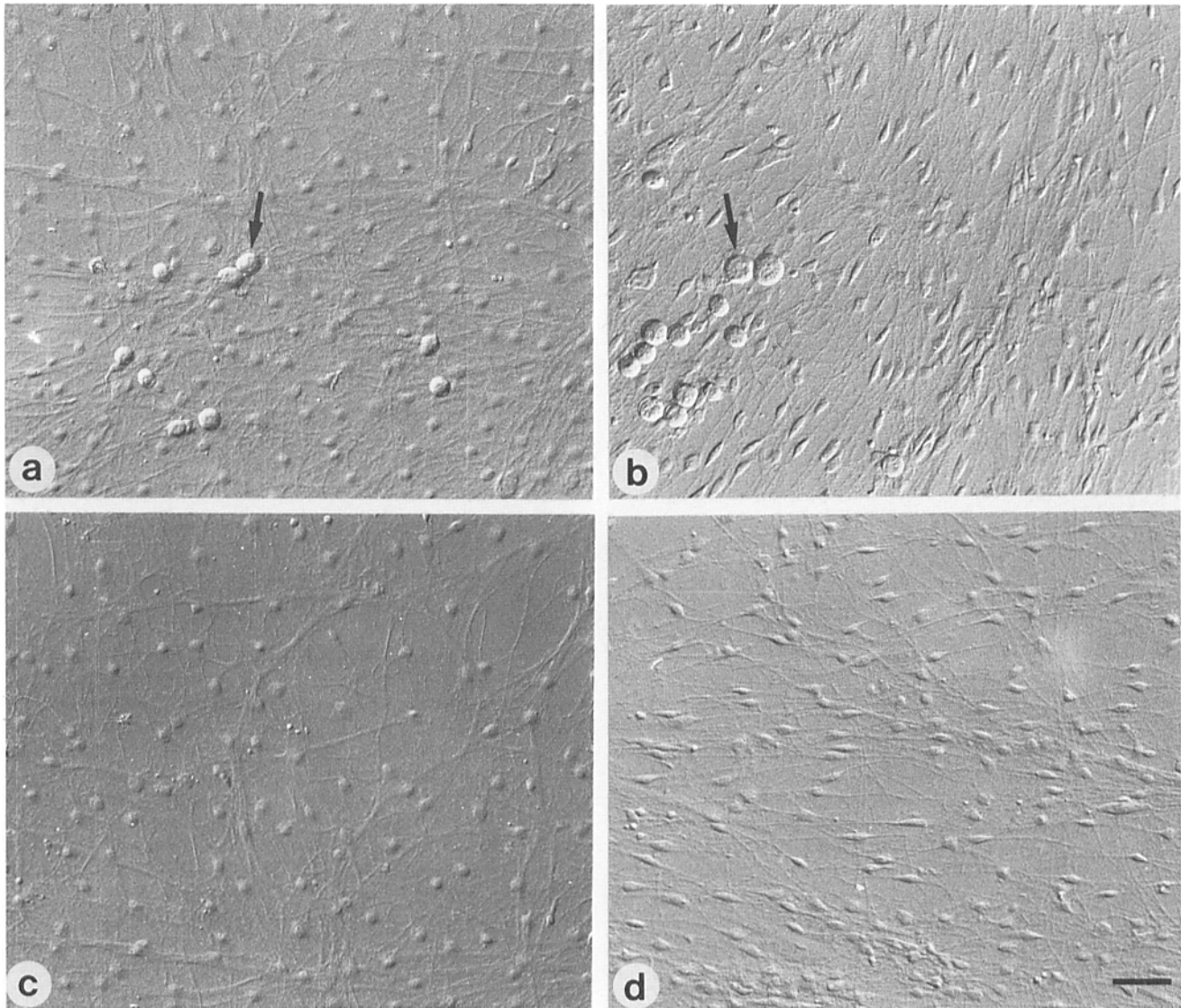


Figure 1. Demonstration of differences in morphology displayed by Schwann cell-neuron cocultures in the absence of antibodies (*a* and *c*) and presence of Fab fragments of polyclonal L1 antibodies (*b* and *d*). Cocultures were maintained for 14 d in the continuous presence of antibodies. Interference contrast microscopy of culture areas in the center (*a* and *b*) and periphery (*c* and *d*) of the coverslip. Dorsal root ganglion neurons were plated into the center of the coverslip and cell bodies are indicated by arrows (*a* and *b*). In the periphery, only neuronal processes are seen (*c* and *d*). Note the epithelioid morphology of Schwann cells in the absence of antibodies (*a* and *c*) and the spindle-shaped morphology in the presence of L1 antibodies (*b* and *d*). Bar, 8 μ m in *a-d*.

were primarily located, but also in the periphery where only neuronal processes were seen (Fig. 1, *a* and *c*). After 14 d of coculture, the MAG was expressed by almost all Schwann cells (Fig. 2, *a* and *b*), whereas in cultures of pure Schwann cells MAG was not detectable after the identical time period in vitro. Schwann cell processes also engulfed neuronal cell bodies and strongly expressed MAG (Fig. 2, *g* and *h*).

Electronmicroscopic observations of these cultures revealed ensheathment of neurites: Schwann cell processes extended into the fascicles of neurites (Fig. 3 *a*). In addition, Schwann cells surrounded neuritic profiles in a somewhat regular turning of spiralling loops of Schwann cell processes (Fig. 3, *b*, *c*, and *d*). Basal lamina was detectable on the surfaces of Schwann cells (Fig. 3, *a* and *b*). Collagen fibrils were seen in the interstitial spaces and could easily be distin-

guished from the rat tail collagen matrix that was used for substrate coating of glass coverslips (Fig. 3 *b*; Fig. 4, *a* and *b*). Basal lamina and collagen fibrils were never detectable in cultures of pure Schwann cells (not shown).

Thymidine incorporation was increased in neuron-Schwann cell cocultures when compared with pure Schwann cell cultures (Table II). In cultures of pure Schwann cells, $29.7 \pm 1.9\%$ of all Schwann cells incorporated thymidine over a pulse period of 48 h, whereas in cocultures $76.5 \pm 2.4\%$ of all Schwann cells had incorporated thymidine during this period, indicating an increment of ~ 2.6 in labeling index.

Coculture in the Presence of Antibodies to J1, MAG, and Mouse Liver Membranes

J1 antibodies react with the cell surfaces of Schwann cells and

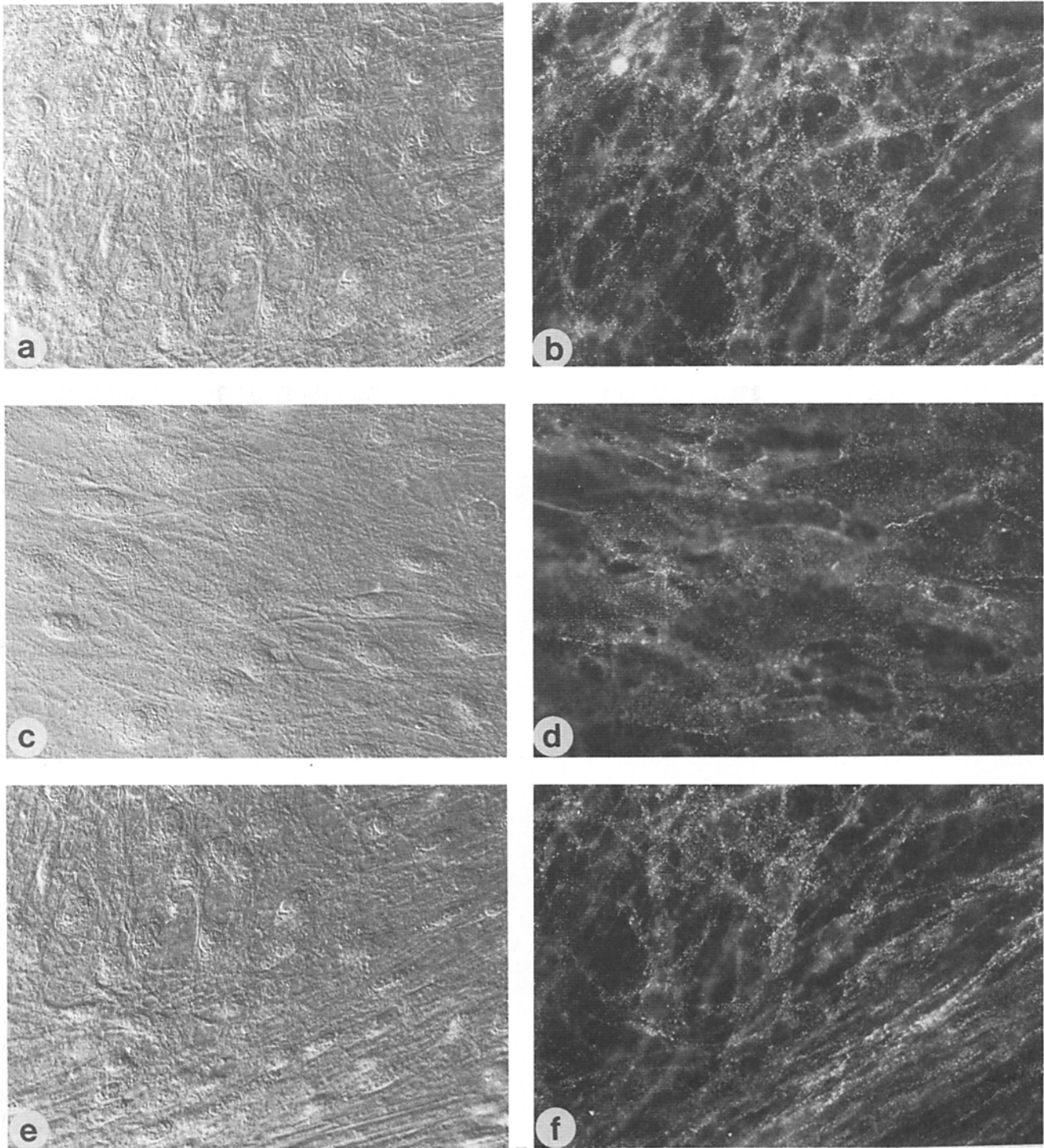


Figure 2. Detection of MAG by indirect immunofluorescence in Schwann cell-neuron cocultures after 14 d *in vitro* in the absence of antibodies (*a*) and presence of Fab fragments of polyclonal N-CAM (*d*), J1 (*f*), liver membrane (*h*), and L1 (*j*) antibodies. *a*, *c*, *e*, *g*, and *i* are the corresponding phase contrast micrographs of fluorescence images *b*, *d*, *f*, *h*, and *j*, respectively. Note the epithelioid morphology of Schwann cells in *a*, *c*, *e*, and *g* and detectability of MAG in *b*, *d*, *f*, and *h* and spindle-shaped morphology of Schwann cells in *i* and absence of MAG immunoreactivity in *j*. The presence of Schwann cells in tight association with the cell body of a dorsal root ganglion neuron and strong MAG immunoreactivity is seen in *g* and *h* (lower right). Bars, 12 μm in *a-h*; 8 μm in *i* and *j*.

associated extracellular matrix in pure and coculture, but not with dorsal root ganglion neurons (Seilheimer and Schachner, 1988). MAG antibodies only react with Schwann cells after ten days of coculture and never with pure Schwann cell cultures. Antibodies to mouse liver membranes react with cell

surfaces of all Schwann cells and neurons in pure and cocultures (Seilheimer and Schachner, 1988).

None of the three antibodies altered the morphology of Schwann cells, induction of MAG expression, association of Schwann cells with neuronal cell bodies, Schwann cell en-

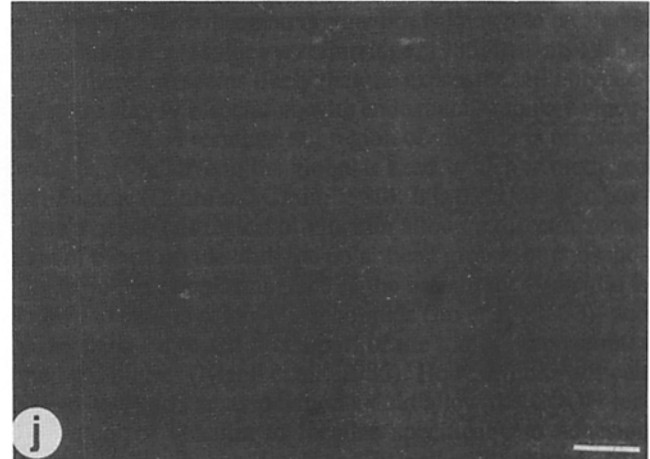
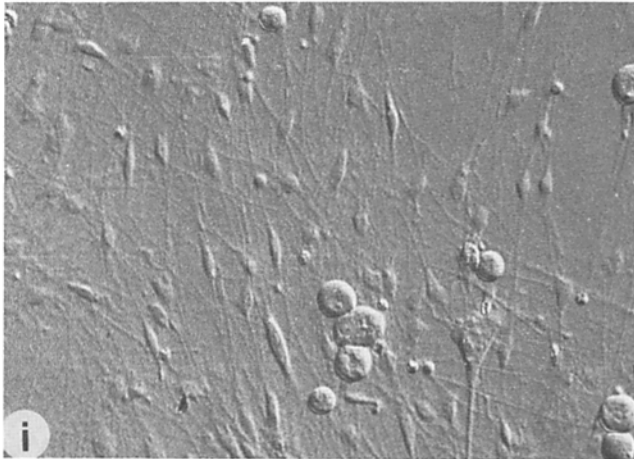
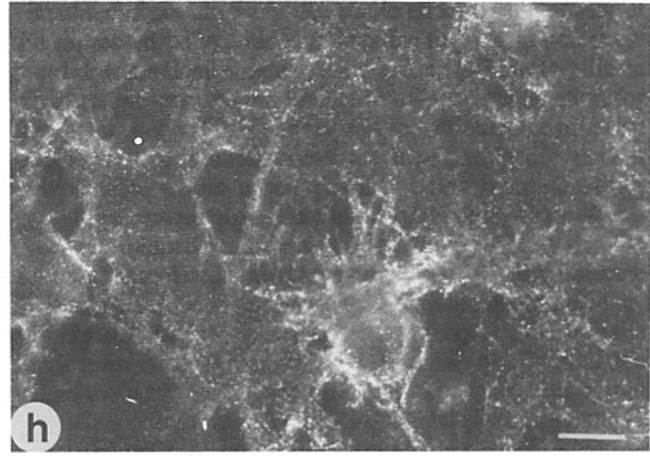


Figure 2.

sheathment of neurons and production of basal lamina and collagen, when compared with cocultures maintained in the absence of antibodies (Table I; for J1 and liver membrane antibodies, see also Figs. 2, *f* and *h* and 3, *f* and *h*). Furthermore, thymidine incorporation was not affected by these antibodies (Table II). For all three antibodies, presence of antibody on the cell surfaces of Schwann cells and, in the case of liver membrane antibodies, also of neurons, was ascertained by immunogold labeling of live cocultures after maintenance for 14 d *in vitro*.

Coculture in the Presence of N-CAM Antibodies

N-CAM antibodies react with both dorsal root ganglion neurons and Schwann cells in pure cultures or cocultures (Seilheimer and Schachner, 1988; Seilheimer et al., 1989).

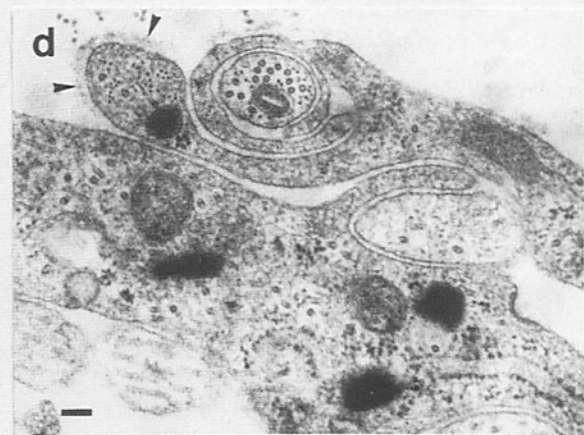
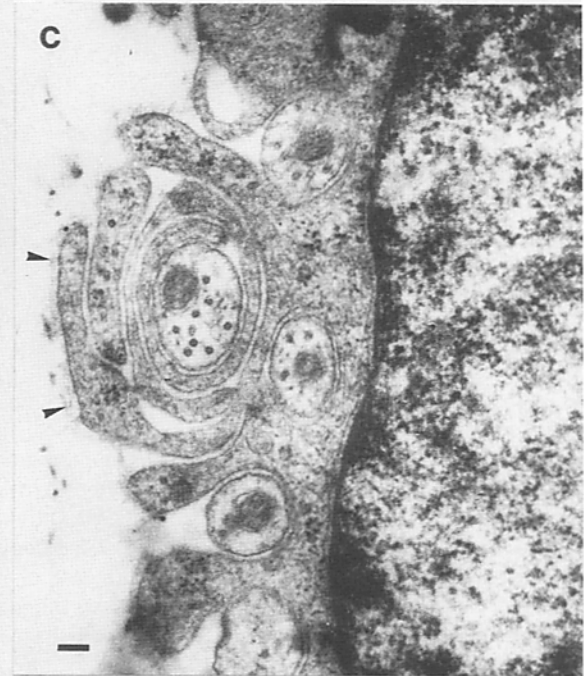
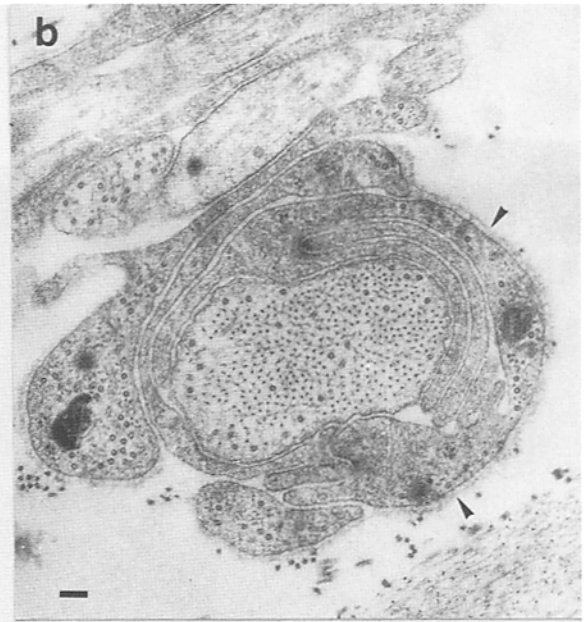
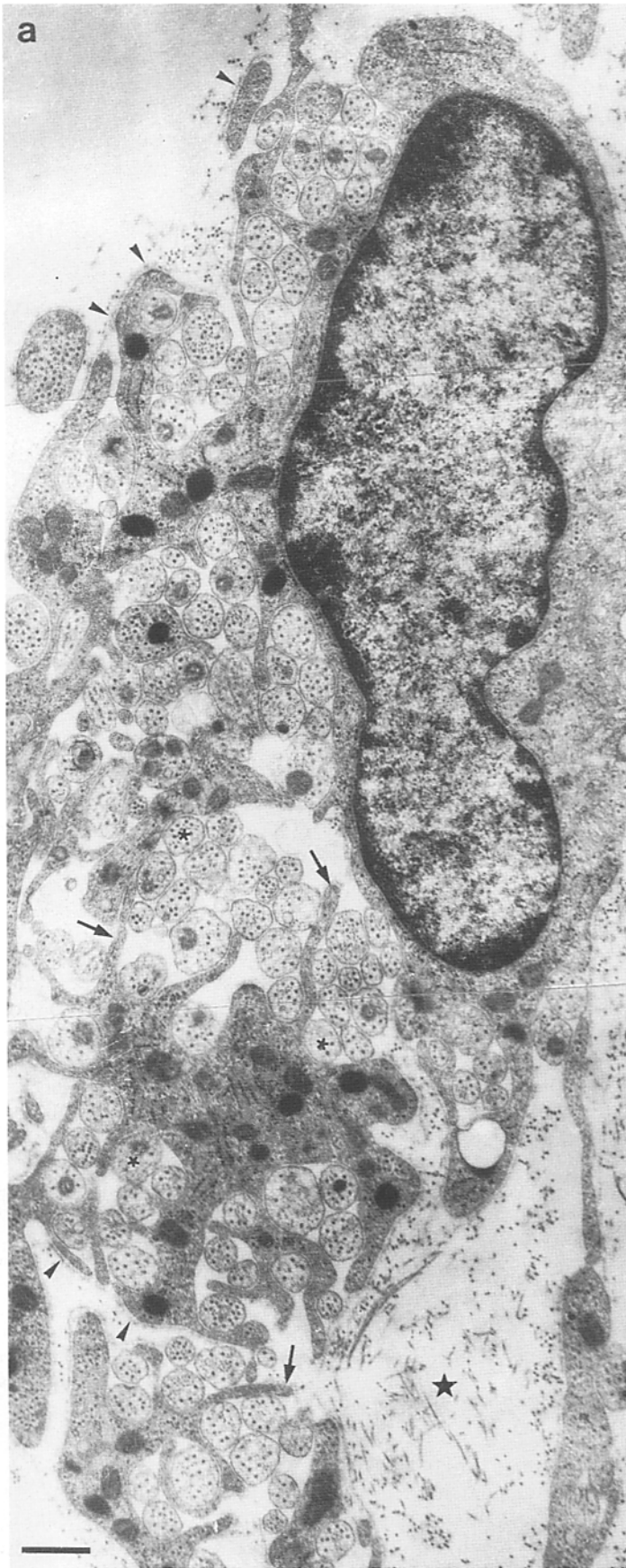
When cocultures were maintained in the presence of Fab fragments of N-CAM antibodies, the parameters under study were differentially influenced. No difference in the appearance of epithelioid morphology and expression of MAG by Schwann cells was seen, when compared with control cultures maintained in the absence of antibodies (Fig. 2 *d*; Table I). Also, basal lamina and collagen were observed by electron microscopy under these conditions (Fig. 4 *c*). However, the percentage of neuronal cell bodies surrounded by Schwann cells was decreased over control cultures (Table I). Also, N-CAM antibodies reduced the extension and interdigitation of Schwann cell processes into neurite bundles

(Fig. 4, *b* and *c*), when compared with control cultures (Fig. 3 *a*). Furthermore, the extent of turning of Schwann cell loops around single neurites was strongly reduced in the presence of N-CAM antibodies (not shown). Very few immunogold particles were observed in the inner layers of the Schwann cell sheaths and the fasciculating neurite bundles (Fig. 4, *b* and *c*). The outer surfaces of Schwann cell bodies and neurites were largely immunonegative (Fig. 4, *b* and *c*). Schwann cell-derived collagen fibrils were amply filled with gold particles on the surface of the immunogold-negative rat tail collagen matrix (Fig. 4 *b*). Basal lamina and collagen fibrils associated with Schwann cell processes were less immunoreactive (Fig. 4, *b* and *c*). Thymidine incorporation was not altered by N-CAM antibodies (Table II). No difference in effects was seen, when Fab fragments or IgG fractions were used in agreement with previous observations in other culture systems (Lindner et al., 1983; Keilhauer et al., 1985).

Coculture in the Presence of L1 Antibodies

L1 antibodies react with both dorsal root ganglion neurons and Schwann cells in pure cultures and cocultures (Seilheimer and Schachner, 1988; Seilheimer et al., 1989). The intensity of immunoreactivity is, however, less pronounced on Schwann cells than on neurons (Seilheimer et al., 1989).

When cocultures were maintained in the presence of Fab fragments of L1 antibodies, a drastic influence was seen on all parameters studied, except for [³H]thymidine incorpora-



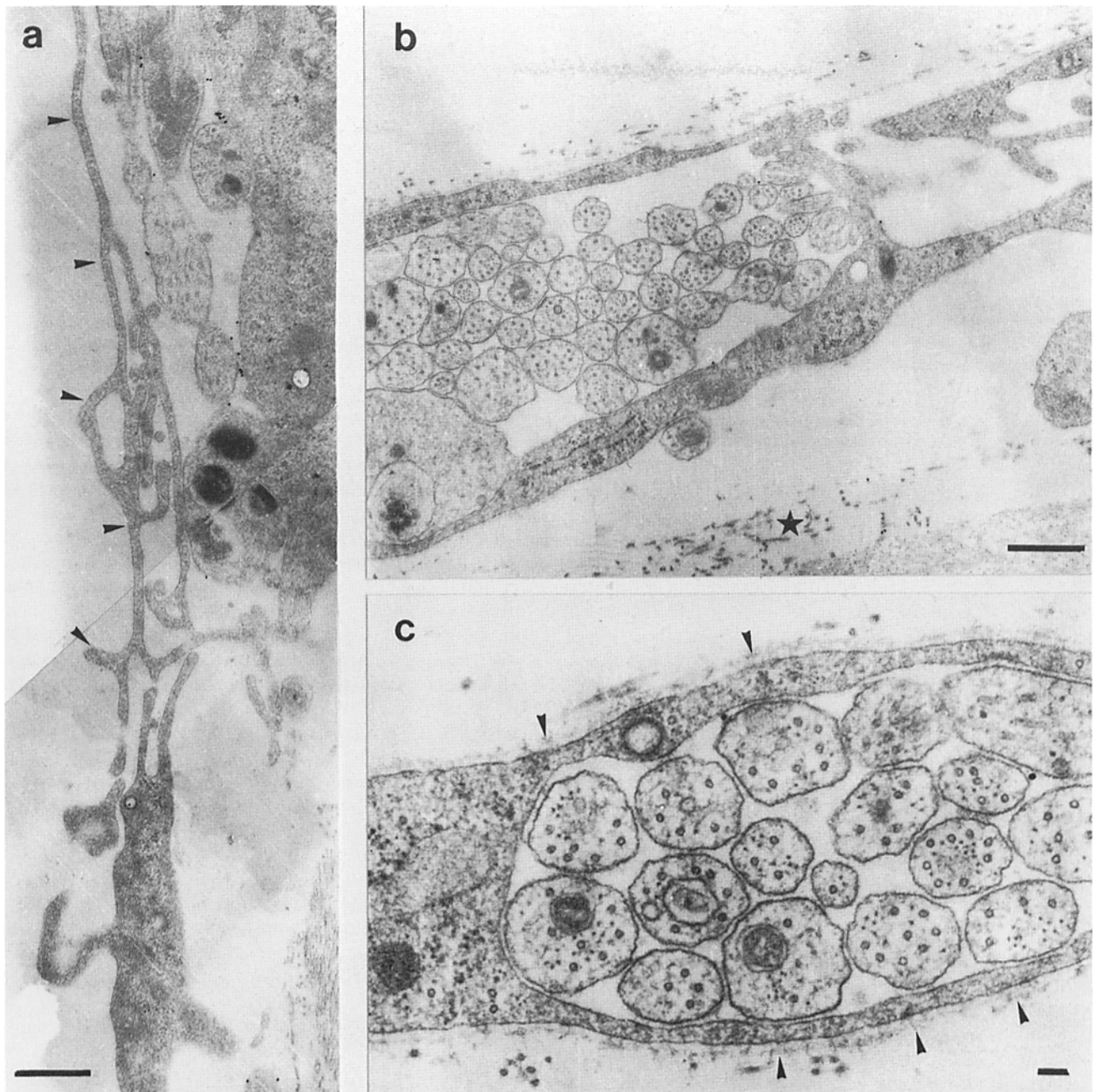


Figure 4. Electron microscopic examination of Schwann cell-neuron cocultures after 14 d in vitro in the presence of Fab fragments of polyclonal L1 (a) and N-CAM (b and c) antibodies. (a) Note the many free Schwann cell processes (arrowheads) not contacting neurites. No basal lamina or collagen fibrils are visible. The extracellular matrix-like deposits in the right lower corner is the rat tail collagen coat used as substrate for cell culture. Gold particles are visible on some neurites and, more frequently, at contact sites between neurites and Schwann cells. Free Schwann cell surfaces were mostly void of gold particles. (b and c) Schwann cells contact neurites, but do not interdigitate into neurite fascicles as well as in the negative control without antibodies or antibodies to J1 or liver membranes (compare with Fig. 3). Note the presence of basal lamina and collagen fibrils (asterisk in b). Rat tail collagen matrix is seen in the lower right corner in b. Few gold particles are visible within the ensheathed bundles of neurite fascicles. Collagen fibrils deposited by Schwann cells are filled with gold particles (see asterisk) on top of the rat tail collagen matrix. Collagen fibrils and basal lamina associated with Schwann cell surfaces do not show immunogold labeling. Bars, 0.5 μm in a and b; 0.1 μm in c.

Figure 3. Electron microscopic examination of Schwann cell-neuron cocultures after 14 d in vitro in the absence of antibodies (a and b) and presence of Fab fragments of polyclonal J1 (c) and liver membrane (d) antibodies. Note the abundance of Schwann cell processes interdigitating (arrows, a) into cross-sectioned neurites, winding of Schwann cell processes around neurites (b, c, and d), and presence of basal lamina (arrowheads in a, b, c, and d) and collagen fibrils (asterisk in a). Rat tail collagen is visible in the lower right hand corner in b. Bars, 0.5 μm in a; 0.1 μm in b, c, and d.

Table I. Determination of Parameters Resulting from Neuron-Schwann Cell Interactions In Vitro

Antibody	Epithelioid Schwann cells	MAG-positive Schwann cells	Schwann cells in association with neuronal cell bodies	Ensheathment	Basal lamina	Collagen fibrils
None	+	+	+	+	+	+
Liver membrane*	+	+	+	+	+	+
L1*	-	-	-	-	-	-
N-CAM*	+	+	±	+§	+	+
J1*	+	+	+	+	+	+
MAG‡	+	+	+	+	+	+

Evaluations are from three independent experiments using cocultures maintained for 14 d in vitro.

* Fab fragments of polyclonal antibodies (0.3 mg/ml).

‡ IgG fraction of MAG mAb (0.3 mg/ml).

§ No separation of neurite bundles by Schwann cell processes.

tion (Table II). The morphological transition from spindle-shaped to epithelioid Schwann cells was not seen (Fig. 1, *b* and *d*). None of the Schwann cells expressed MAG after 14 d (Fig. 2, *i* and *j*). The number of neuronal cell bodies surrounded by Schwann cells was reduced to zero (Table I). Neither basal lamina nor collagen could be detected electron microscopically (Fig. 4 *a*; Table I). A pronounced effect of L1 antibodies was seen also on the ensheathment of neurites. Schwann cell processes did not interdigitate into neurite fascicles to separate individual axons, but remained remote from them (Fig. 4 *a*). Schwann cell processes with long extensions were observed, however, but retracted back onto themselves in a profusely unoriented fashion rather than contacting neurites (Fig. 4 *a*). Immunogold labeling was detectable in the less superficial cell layers of the cultures, whereas free Schwann cell surfaces were generally immunonegative (Fig. 4 *a*).

Discussion

Our study has shown that the neural adhesion molecule L1 plays a pivotal role in the initial phases of Schwann cell differentiation, when ensheathment of neurites by Schwann cell processes occurs. The parameters chosen in this study as indicative of Schwann cell differentiation in culture were transition from spindle-shaped to flattened, i.e., epithelioid appearance of Schwann cells, close cell surface contact of Schwann cells with neuronal cell bodies, ensheathment of neurites by Schwann cell processes, production of basal lamina and collagen, and expression of the MAG, which appears

in culture only after almost 2 wk of coculture with neurons. While in the intact developing peripheral nerve, MAG only appears after cells have turned one and a half times around the axon (Martini and Schachner, 1986), MAG expression in dorsal root ganglion neuron-Schwann cell cocultures appears to precede axon ensheathment (Owens and Bunge, 1989). This difference in results may be due to differences in immunolabeling sensitivities in culture versus the intact tissue or to genuine differences between the in vitro versus in vivo situations. L1 appears to be involved in one of the steps leading to the manifestation of differentiated features, but does not appear to be involved in mediating the mitogenic signal conferred to Schwann cells by neurons and neuronal cell surface components (Ratner et al., 1985) under the conditions of our study. Thus, although a wide spectrum of features in neuron-Schwann cell interactions appear to be perturbed by L1 antibodies, an important consequence of cell surface interactions between neurons and Schwann cells is unaffected. The question is whether L1 mediates a particular key event in the initial contacts of neurons and Schwann cells essential for all other, temporally succeeding events in neuron-Schwann cell interactions or whether L1 engages in different types of interactions at different stages of differentiation, including myelination (Wood et al., manuscript in preparation).

The predominant inhibition of Schwann cell differentiation by L1 antibodies is noteworthy, because Schwann cells and neurons not only express L1, but also other adhesion molecules, such as the neural cell adhesion molecule N-CAM. However, antibodies to the neural cell adhesion molecule N-CAM interfere in a much less pronounced manner with the interaction of Schwann cells with neurons. The only affected feature is the interdigitation of Schwann cell processes into neurite fascicles, which is strongly reduced in the presence of N-CAM antibodies, whereas association of Schwann cells with neurite bundles remains unaffected. All other parameters studied, such as transition of Schwann cells to a more epithelioid morphology, production of basal lamina and collagen, and expression of MAG appear normal under the conditions of this study. These more subtle effects of N-CAM antibodies are noteworthy with regard to previous observations showing that not only L1, but also N-CAM antibodies strongly reduce neuron-Schwann cell adhesion (Seilheimer and Schachner, 1988), while predominantly L1 appears to mediate neurite outgrowth on other neurites (Chang et al., 1987) or Schwann cells (Bixby et al., 1988; Kleitman et al., 1988;

Table II. Effect of Antibodies on Schwann Cell Proliferation

Antibody	[³ H]thymidine-labeled Schwann cells
	%
None	76.5 ± 2.4
Liver membrane*	80.2 ± 1.9
L1*	79.4 ± 2.3
N-CAM*	78.2 ± 2.7
J1*	76.5 ± 1.4
MAG‡	79.2 ± 2.8

* Fab fragments of polyclonal antibodies (0.3 mg/ml).

‡ IgG fractions of MAG mAb (0.3 mg/ml). Values are from three independent experiments ± SD.

Seilheimer and Schachner, 1988). Thus, L1 appears to confer a special feature onto the interacting partner cells that goes beyond the effects of N-CAM alone. It remains to be seen whether L1 acts via homophilic or heterophilic binding mechanisms (Grumet and Edelman, 1988; Keilhauer et al., 1985; Sadoul et al., 1988). In any case, the molecular mechanisms of its actions will be a worthwhile topic for further investigations.

The authors are grateful to Thomas Fahrig, Bernd Gehrig, Manfred Griesheimer, and Frank Kirchhoff for antibodies, and Rudolf Martini and Remy Sadoul for reading the manuscript.

We are also grateful to Friedrich-Ebert-Stiftung for a fellowship (E. Persohn) and Bundesministerium für Forschung und Technologie for support. M. Schachner is a member of the Swiss Federal Institute of Technology, Zürich.

Received for publication 5 June 1989 and in revised form 24 August 1989.

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