Biochemical and Functional Characterization of a Novel Neuron-Glia Adhesion Molecule that Is Involved in Neuronal Migration

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Abstract. Adhesion molecule on glia (AMOG) is a novel neural cell adhesion molecule that mediates neuron–astrocyte interaction in vitro. In situ AMOG is expressed in the cerebellum by glial cells at the critical developmental stages of granule neuron migration. Granule neuron migration that is guided by surface contacts between migrating neurons and astroglial processes is inhibited by monoclonal AMOG antibody, probably by disturbing neuron–glia adhesion. AMOG is an integral cell surface glycoprotein of 45–50-kD molecular weight with a carbohydrate content of at least 30%. It does not belong to the L2/HNK-1 family of neural cell adhesion molecules but expresses another carbohydrate epitope that is shared with the adhesion molecules L1 and myelin-associated glycoprotein, but is not present on N-CAM or J1.

To build a complex organ such as the nervous system, a number of cellular and molecular mechanisms would seem necessary. There is substantial evidence that cell surface molecules play important roles in the specification of cell contacts not only between neighboring partner cells, but also between the cell surface and the extracellular matrix (for reviews, see Edelman and Thiery, 1985). These cell surface molecules have been operationally defined as cell adhesion molecules since they mediate adhesion of cells in in vitro assays. The question is, how many molecules specify the development of the nervous system at distinct, yet coordinated morphogenetic steps: neural induction, cell proliferation, migration, aggregation, cytodifferentiation, synapse formation, cell death, and synapse elimination (Cowan, 1982).

Four cell surface glycoproteins have now been recognized to mediate adhesion among different neural cell types at different developmental stages. These are the neural cell adhesion molecule (N-CAM)[1] (for review, see Edelman, 1985), the neuron-glia adhesion molecule J1 (Kruse et al., 1985), myelin-associated glycoprotein (for review, see Quarles, 1984; Poltorak, M., R. Sadoul, G. Keilhauer, C. Landa, T. Fahrig, and M. Schachner, manuscript submitted for publication) and L1 (for review, see Schachner et al., 1985), which is immunochemically identical to the nerve growth factor–inducible large external glycoprotein (NILE) (Bock et al., 1985), which, in turn, has been shown to be the mouse equivalent of chicken Ng-CAM (Friedlander et al., 1986). It is likely that these molecules do not act independently of each other in the formation of cell contacts. For instance, N-CAM and L1 cooperate synergistically in aggregation of early postnatal cerebellar and neuroblastoma cells (Faissner et al., 1984; Rathjen and Rutishauser, 1984), but act in a less than additive manner in cell–cell adhesion and migration of granule cell neurons (Keilhauer et al., 1985; Lindner et al., 1986b). Furthermore, L1 is closely associated with the 180-kD component of N-CAM since the two molecules co-redistribute with each other in the surface membrane (Thor et al., 1986). These observations point to modulatory effects of one adhesion molecule on the other and would endow a particular cell with combinatory possibilities in regulating the quality and strength of cell surface contacts. It is important in this regard that fasciculating axons express both L1 and N-CAM, whereas nonfasciculating axons or neurites only express N-CAM (Holley, J., and M. Schachner, manuscript submitted for publication; Persohn and Schachner, 1987). Accordingly, antibodies to L1 and N-CAM both disturb fasciculation of neurites in vitro (Fischer et al., 1986; Friedlander et al., 1986; Rutishauser et al., 1978; Stallcup and Beasley, 1985). Migration of granule neurons from the external to the internal granular layer in the developing cerebellum also appears to depend on the activity of both L1 and N-CAM (Lindner et al., 1986b).

Neuronal migration is a complex set of individual steps. These steps require that granule cells become postmitotic, adhere to preformed granule cell axons to form fascicles, and appose their cell body to the radial processes of Bergmann glia with a leading process guiding the migration of the cell body along the glial surface until the granule cell has reached the internal granular layer to take up its final position for synaptogenesis (for review, see Rakic, 1982). We have previously suggested that the involvement of L1 in granule cell migration concerns the sorting out of postmitotic, premigratory granule cell bodies from the proliferating granule cell

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1. *Abbreviations used in this paper:* AMOG, adhesion molecule on glia; N-CAM, neural cell adhesion molecule.

precursors, and/or fasciculation of granule cell axons since L1 is apparently not involved in neuron-glia interaction (Keilhauer et al., 1985; see also Fushiki and Schachner, 1986; Persohn and Schachner, 1987). N-CAM, on the other hand, might well be involved in the apposition of premigratory neurons to Bergmann glial processes (Lindner et al., 1986b) since it mediates not only neuron-neuron but also neuron-astrocyte adhesion (Keilhauer et al., 1985).

The present study was initiated with the aim to determine whether other cell surface molecules are involved in granule cell migration. Here we describe a novel adhesion molecule on glia (AMOG) that in the cerebellum is expressed by glia and involved in neuron-glia interaction and granule cell migration. Its developmental appearance and disappearance coincides temporally with granule cell migration.

Materials and Methods

Animals

C57BL/6J mice were used for the migration assay. NMRI mice were used for all other experiments. Fl hybrids between the inbred rat strains Lou x Sprague Dawley were used for immunization. Nude mice on the NMRI background were obtained from Zentrale Tierzuchtanlage Hannover, Hannover, FRG.

Antibodies and Lectins

Monoclonal antibody to AMOG was obtained from female Lou x Sprague Dawley Fl hybrid rats (4-6-wk old) immunized with 80 μg of a glycoprotein fraction from a detergent-solubilized enriched plasma membrane fraction from early postnatal mouse cerebellum isolated by affinity chromatography with lens lentil lectin as described (Rathjen and Schachner, 1984). Splenocytes of animals with high titers were chosen for fusion with the mouse myeloma clone Ag8-653 (Kearney et al., 1979) according to de St. Groth and Scheidegger (1980). Fusions were first screened by the immunospot-binding test (see below) (Hawkes et al., 1982). Positive hybridoma supernatants were then screened immediately for cell surface reactivity on viable monolayer cultures of early postnatal mouse cerebellar cells by indirect immunofluorescence (Schnitzer and Schachner, 1981). Cloning of hybridoma cells was performed according to Lagenaur et al. (1980). The antibody is an IgG as determined by gel filtration or SDS PAGE or the Ouchterlony test using subclass-specific antibodies (Miles Laboratories Ltd., Slough, UK).

Polyclonal AMOG antibodies to immunoaffinity-purified AMOG (see below) and electroeluted bands (Hunkapiller et al., 1983) were prepared in New Zealand white rabbits by subcutaneous and intraperitoneal injections as described (Rathjen and Schachner, 1984). Polyclonal antibodies to laminin from EHS sarcoma (Bethesda Research Laboratories, Gaithersberg, MD) were also prepared in rabbits by the same procedure. Polyclonal antibodies to the cell adhesion molecule J1 and mouse liver membranes have been described (Kruse et al., 1985; Lindner et al., 1983; Pollerberg et al., 1986).

Antibodies to glial fibrillary acidic protein, vimentin, cell adhesion molecule L1, 04 antigen, BSP-3 (Na/K ATPase), MESA-1, laminin, and fibronectin were kind gifts of Drs. L. F. Eng, W. W. Franke, A. Faissner, J. Trotter, C. Goridis, and W. Werz, respectively, and were used as described (Gorvel et al., 1984; Schnitzer and Schachner, 1981; Sommer and Schachner, 1981; Schnitzer et al., 1981; Rathjen and Schachner, 1984; Ghandour et al., 1982).

IgG fraction from rabbit and rat antisera or ascites were obtained according to Fahey and Terry (1979). Fab fragments of poly- and monoclonal IgG fractions were prepared with mercuripapain (Sigma Chemical GmbH Munich, FRG) according to Porter (1959).

Plant lectins wheat germ agglutinin and concanavalin A were purchased from Sigma Chemical GmbH.

Isolation of AMOG by Immunoaffinity Chromatography

Monoclonal AMOG antibody was purified from ascites of nude mice and coupled to Sepharose 4B according to Stoeckl et al. (1976) at a concentration of 4.6 mg protein/ml of gel matrix. For isolation of AMOG, crude membranes were prepared from adult mouse brain by homogenization in hypotonic buffer containing 1 mM NaHCO3, 1 mM spermidin, 0.2 mM MgCl2, 0.2 mM CaCl2 and 5 mM phenylmethylsulfonylfluoride, 5 mM iodoacetamide, and 40 U/ml aprotinin, pH 7.9, and then subjected to centrifugation for 20 min at 4°C and 2,000 g (Hermle Z365-K; Hermle KG, GoBheim, FRG). The pellet was washed three times in hypotonic buffer. Combined supernatants were centrifuged for 45 min at 4°C and 30,000 g (Sorvall RG-5B; DuPont Instruments, Bad Nauheim, FRG). The pellet was resuspended in solubilization buffer (150 mM NaCl, 20 mM Tris, 1 mM EDTA, 1 mM EGTA, 0.5% NP-40 and 40 U aprotinin/ml, pH 7.2). After 1-h stirring on ice the detergent extract was cleared by centrifugation for 1 h at 4°C and 100,000 g (Koutron TGA; Koutron Instruments, Munich, FRG). The supernatant was added first to an antibody column containing immobilized IgG from nonimmune rats. The flow-through of this column was connected directly to the monoclonal AMOG antibody-containing column (5 ml of gel matrix volume). The column was then washed with 250 ml of solubilization buffer, subsequently treated with 250 ml solubilization buffer containing 0.4 M NaCl, and finally washed with 250 ml solubilization buffer containing 0.1% Na deoxycholate instead of NP-40. Bound material was eluted with an alkaline buffer containing 0.1 M diethylamine, 0.1% Na deoxycholate, pH 11.5. Five column volumes were collected and immediately neutralized by addition of 1 M Tris buffer, pH 6.8.

Analytical Procedures

SDS PAGE analysis was performed with linear (7-15%) or without linear (7%, 10%) acrylamide concentration gradients in slab gels according to Laemmli (1970). Staining of gels by reduction of silver ions was carried out according to Oakley et al. (1980). Protein standards (Sigma Chemical GmbH) were myosin (205 kD), β-galactosidase (116 kD), phosphorylase B (97 kD), BSA (66 kD), ovalbumin (45 kD), and carbonic anhydrase (29 kD). ^{14}C-methylated molecular weight standard proteins (0.833 μCi per each protein/ml, total 5 μCi/ml; Amersham Buchler GmbH, Braunschweig, FRG) were myosin, phorphorylase B, bovine serum albumin, ovalbumin, carbonic anhydrase, and lysozyme (14.3 kD). Protein determinations were carried out according to Lowry et al. (1951) or with a micromethod according to Neuhoff et al. (1979). BSA served as standard.

Western blot analysis of proteins isolated by SDS PAGE was carrried out according to Towbin et al. (1979) as modified by Faissner et al. (1985).

Radioactive Isotopes

[^{35}S]Methionine (1,000 mCi/mM, Amersham Buchler GmbH) was aliquoted in pulse medium at 1 mCi/ml (see below) and stored until use at −70°C. Na^{125}I (carrier-free, 17 Ci/ml; New England Nuclear) was used upon arrival. [^{3}H]fucose (27 Ci/mM; Amersham Buchler GmbH) was stored at 4°C.

Biosynthetic Labeling of Glycoproteins in Cell Culture

Cultures of cerebellar cells were labeled with [^{35}S]methionine and [^{3}H]fucose as described (Faissner et al., 1985).

Cell Surface Labeling with ^{125}I

Lactoperoxidase-catalyzed radioiodination of cell surface proteins in cerebellar monolayer cultures was carried out according to Goridis et al. (1978a) with the following modifications: cultures were washed three times with Hank's balanced salt solution (HBSS) and two times with HBSS containing 10 mM Hepes, pH 7.7 (buffer A). Iodination was carried out at room temperature in 1 ml buffer A containing 1.8 mCi carrier-free Na^{125}I, 12.5 μg lactoperoxidase (Sigma Chemical GmbH), and 10 μl buffer B (1:1,000 dilution of a 30% solution of H$_2$O$_2$). Buffer B (10 μl) was added another two times within 20 min. Cells were then washed three times with HBSS containing 10 mM Hepes, 5 mM NaI, approtinin, soybean trypsin inhibitor, turkey egg white trypsin inhibitor (all at 10 μg/ml), phenylmethylsulfonylfluoride and iodoacetamide (both at 5 mM), and scraped off the petri dish. Cells were collected by centrifugation (10 minutes at 4°C and 60 g) and solubilized in buffer (see above) for 1 h on ice. Supernatants were obtained by two centrifugation steps (see above) and then either stored at −70°C or immediately used for immunoprecipitation.

Immunoprecipitation

Immunoprecipitations were performed as described (Faissner et al., 1985).

Immunospot-binding Test

This test was carried out as described previously (Hawkes et al., 1982).

Cell Culture

Monolayer cultures of trypsin-dissociated cerebella from 6-d-old mice were prepared and maintained as previously described (Schnitzer and Schachner, 1981).

Explant cultures from 6-d-old mouse cerebellum were prepared according to Fischer et al. (1986).

Organotypic explants of cerebellar folia from 10-d-old mice, labeling of premigratory neurons with [³H]thymidine, and histological and autoradiographic procedures we used have been described (Lindner et al., 1983, 1986a, b). In brief, pieces of folia from the cerebellar cortex were labeled in vitro with [³H]thymidine (6.7 Ci/mM; Amersham Buchler GmbH) at 1 μCi/ml for 90 min at 37°C in serum-free defined culture medium. The tissue pieces were then washed thoroughly with defined medium and four to six pieces were maintained for 3 d in 3 ml of defined medium with or without addition of antibodies. Frozen sections (10-μm thick) were cut in sagittal orientation from these folia, fixed, dried, and prepared for autoradiography.

Image Analysis of Autoradiographies from [³H]thymidine-labeled Cerebellar Folium Explants

The positional distribution of [³H]thymidine-labeled cells was determined by automatic image analysis as described (Lindner et al., 1986a). Microscopic images of autoradiograms of frozen sections of cerebellar folia were recorded with a video camera. Labeled cells were recognized by means of their optical density using a locally adoptive threshold algorithm (Komitowsky et al., 1983). For quantitative comparison of the distribution patterns of labeled cells under various experimental conditions the mean value (μm from pial surface) of every histogram was calculated and designated as migratory index (MI). Inhibition of cell migration was calculated as follows: % inhibition = [MI (control) − MI (experimental)]/[MI (control)] × 100. For each experimental value several independent experiments were carried out in duplicates.

Preparation of Neurons and Astrocytes

Purification of small neurons and astrocytes from 6-d-old mouse cerebellum was carried out as described (Keilhauer et al., 1985). Small cerebellar neurons were obtained by centrifugation through a Percoll gradient. Astrocytes were obtained by complement-dependent immunocytolysis with cell surface reactive monoclonal M5 antibody directed against all cerebellar neurons (Keilhauer et al., 1985), O4 antibody directed against more- and less-differentiated oligodendrocytes (Sommer and Schachner, 1981), and MESA-1 antibodies directed against endothelial cells (Ghandour et al., 1982).

Cell Adhesion Assay

The adhesion assay was carried out as previously described (Keilhauer et al., 1985). Probe cells were obtained from monolayer cultures of enriched neurons and astrocytes 2 d after seeding (neurons) or immunocytolysis (astrocytes). For removal from the petri dish cultures, cells were treated with 5 μg/ml trypsin and 1 mM EDTA in HBSS for 20 min at room temperature. For vital staining of probe cells, fluorescein diacetate (10⁻⁶ M) was included during the trypsinization step. Adhering cells were scored by counting ~1,000 cells in 5-10 microscopic fields per well (Costar, Data Packaging Corp., Cambridge, MA) at 100× magnification with an ICM 405 Zeiss inverted fluorescence microscope in blind tests. Monolayer cultures of astrocytes were used as target cells only when they had formed a continuous monolayer so that probe cells bound exclusively to cells and not to the poly-L-lysine substrate. Each value was run in duplicate or triplicate. Inhibition of adhesion in the presence of antibodies was calculated by: % inhibition = [adhesion (control) − adhesion (antibody)]/[adhesion (control)] × 100.

Immunocytological Procedures

Indirect immunofluorescence on viable and paraformaldehydeacetone-treated cell cultures was performed as described by Schnitzer and Schachner (1981). Cryostat sections from fresh frozen tissue were stained by indirect immunofluorescence according to Goridis et al. (1978b). Double immunofluorescence procedures were carried out (Schnitzer and Schachner, 1981) using tetramethylrhodamine (TRITC)- and fluorescein (FITC)-conjugated

goat anti-rat, anti-mouse, or anti-rabbit IgG antibodies (Cappel Laboratories, Cochranville, PA; and Miles Laboratories, Ltd.). They were used at dilutions of 1:200 or 1:400.

Indirect immunoelectronmicroscopy was carried out on midsagittal sections of 5- and 21-d-old cerebella by the pre- and postembedding (Lowicryl) staining procedures as described (Martini and Schachner, 1986) with the following modifications: after perfusion brains were left overnight at 4°C in 4% paraformaldehyde, sections were incubated with first antibody overnight at 4°C, and Epon-Araldite was used for embedding.

Results

Monoclonal AMOG antibody was obtained by an immunization protocol similar to the one that gave rise to monoclonal antibodies L1 and L2. A glycoprotein fraction from enriched plasma membranes of early postnatal mouse cerebella was used for immunization. Since the antibody did not react with the cell adhesion molecules L1, N-CAM, J1, myelin-associated glycoprotein, or other members of the L2/HNK-1 family of adhesion molecules (Kruse et al., 1984) we wondered whether it recognized another surface molecule involved in cell-cell interactions. In vitro assay systems were therefore used initially to probe for the antibody's capability to interfere with neuronal migration and neural cell adhesion.

Granule Cell Migration

An assay system was used that monitors the migration of granule neurons in cerebellar tissue pieces in vitro (Moonen et al., 1982; Lindner et al., 1983). In this assay system the migration of [³H]thymidine-labeled granule cells is quantitated by image analysis (Lindner et al., 1986a, b). Granule cell migration was inhibited in the presence of mono- and polyclonal AMOG antibodies (Table I; Figs. 1 and 2). Inhibition of 36% by monoclonal AMOG antibody is the highest observed until present. Polyclonal L1 antibodies were previously found to inhibit migration by 30% and polyclonal N-CAM antibodies by 10% (Lindner et al., 1986b). No differences in the extent of inhibition was observed when Fab fragments or total IgG were used (see also Lindner et al., 1983). Polyclonal AMOG antibody that was prepared against

Table I. Migration of [³H]thymidine-labeled Cells in Cerebellar Folium Explants in the Presence of Various Antibodies and Lectins

Antibodies	Concentration	Inhibition of granule cell migration
	mg/ml	%
Mono-AMOG, IgG	1	35.7 ± 1.9 (7)
Mono-AMOG, IgG	0.5	24.1 ± 4.3 (3)
Mono-AMOG, Fab	0.5	23.8 ± 5.1 (2)
Poly-AMOG, IgG	1	15.5 ± 3.5 (2)
Poly-laminin, IgG	1	5.7 ± 3.4* (3)
Poly-laminin, IgG	0.5	2.3 ± 7.4 (2)
Poly-laminin, Fab	1	0.7 ± 2.4 (4)
Poly-laminin, Fab	0.5	1.8 ± 4.4 (2)
Poly-J1, IgG	1	0.4 ± 5.3 (4)
Poly-J1, IgG	0.7	−3.5 ± 4.2 (4)
Wheat germ agglutinin	0.01	5.9 ± 2.3* (3)
Concanavalin A	0.01	22.9 ± 4.1 (3)

* The differences to medium control explants are not significant (t-test). Numbers are mean values ± SD. Number of experiments are indicated in parentheses. Mono, monoclonal antibody; poly, polyclonal antibody.
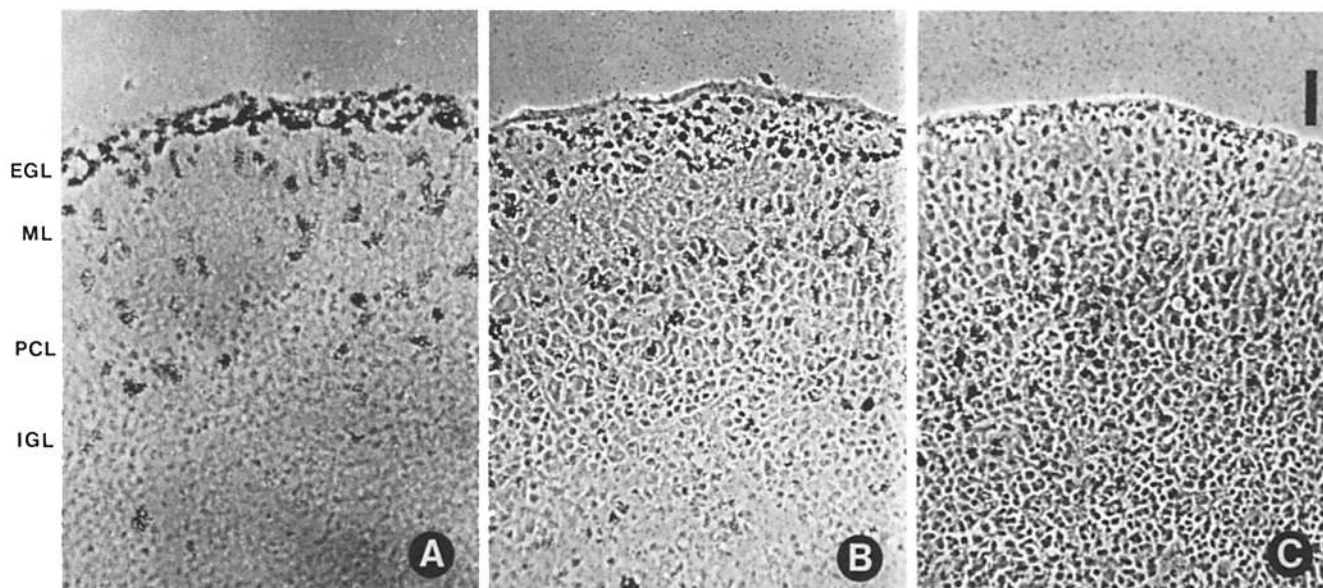
Figure 1. Effects of mono- and polyclonal AMOG antibodies on the migration of cerebellar granule cells. Autoradiography and distribution of [³H]thymidine pulse–labeled cells in sagittal sections of cerebellar folium explants of 10-d-old C57BL/6J mice is shown after maintenance for 3 d in vitro. Explant maintained in the presence of monoclonal AMOG antibody (IgG, 1 mg/ml) (A), or polyclonal AMOG antibody (IgG, 1 mg/ml) (B), and without antibodies (C). EGL, external granular layer; ML, molecular layer; PCL, Purkinje cell layer; IGL, internal granular layer. Bar, 50 µm.

AMOG isolated from adult mouse brain by immunoaffinity chromatography on a monoclonal AMOG antibody column inhibited only by 16%. This lower percentage of inhibition could be due to several reasons and was not investigated further. The numbers of dead cells as measured by propidium iodide incorporation (Lindner et al., 1983) and thymidine-labeling indices were not different between cerebellar explants in the absence or presence of AMOG antibodies (not shown).

Several other antibodies that bind to the cell surface of neurons, glia or both did not modify migration. These included polyclonal antibodies to mouse liver membranes, monoclonal M2 antibody (not shown; see Lindner et al., 1986b), and the neuron-glia adhesion molecule J1, all of which did not significantly reduce migration (Table I). It has also been suggested that the extracellular matrix protein laminin plays a role in granule cell migration in the cerebellar cortex (Liesi, 1985). Polyclonal antibodies to laminin from EHS sarcoma, however, did not affect migration of neurons in vitro (Table I), whereas they significantly inhibited adhesion of neurons to a laminin substrate (Werz, 1986). The plant lectin wheat germ agglutinin did not interfere with migration, whereas concanavalin A did (Table I). Both lectins react with all cerebellar cell types (Sack et al., 1983). At concentrations of 10 µg/ml both lectins did not reduce cell survival in explant or monolayer cultures.

It should be mentioned that AMOG antibodies did not significantly modify outgrowth patterns of neurites on poly-lysine in explant cultures of early postnatal mouse cerebellum (Fischer et al., 1986) (not shown).

These experiments show that AMOG antibodies are able to specifically interfere with granule cell migration.

Cell Type Specificity of AMOG-mediated Cell Adhesion

To investigate which cerebellar cell types engage in AMOG-mediated cell adhesion, homogeneous populations of astro-

cytes and neurons from early postnatal mouse cerebellum were tested in a Ca⁺⁺-independent adhesion assay (Keilhauer et al., 1985). This procedure allows the isolation of astrocytes and small neurons in vitro at developmental stages equivalent to those of cerebellar granule cell migration in vivo. Under assay conditions, where ∼90% of the input probe cells (single cell suspension) adhere to the target cells (monolayer culture), neurons did not express AMOG and
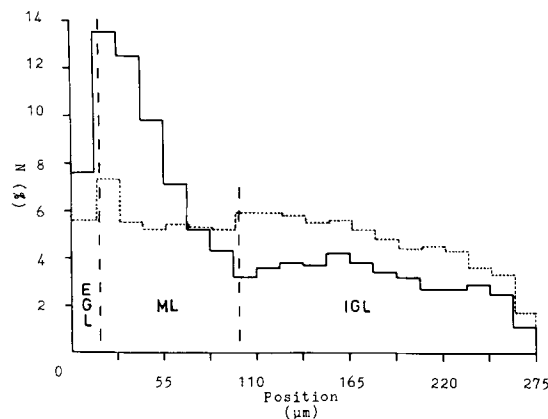

Figure 2. Relative distribution of [³H]thymidine-labeled cells with respect to their distance from the pial surface. Cerebellar folium explants were maintained for 3 d in vitro in the absence (. . .) or presence (−) of monoclonal AMOG antibodies (IgG, 1 mg/ml). The position of thymidine-labeled cells is plotted as a function of distance from the pial surface (in µm). In the presence of antibodies, 28% of thymidine-labeled cells remained in the EGL and ML compared with controls. The histogram was made on the basis of five independent experiments and calculated from the evaluation of 15 images per experiment. EGL, external granular layer; ML, molecular layer; IGL, internal granular layer; N, number of labeled cells expressed in percentage.

Table II. Adhesion between Neurons and Astrocytes from Early Postnatal Mouse Cerebellum in the Presence of Various Antibodies

Antibodies	Concentration	Inhibition of adhesion	
		Neuron‖ to astrocyte§	Astrocyte‖ to astrocyte§
	mg/ml	*%*	*%*
Mono-AMOG, IgG	1	25 ± 3.2 (6)	4 ± 4 (3)
Mono-AMOG, Fab	1	26 ± 1.8 (3)	4 ± 2.1 (4)
Poly-AMOG, IgG	1	16 ± 5.7 (4)	1 ± 6.7 (3)
Poly-J1*	1	47 ± 7 (3)	−7 ± 12 (2)
Poly-N-CAM‡	1	29 ± 7 (8)	24 ± 2 (4)
Poly-liver*	1	−3 ± 2 (3)	1 ± 1 (2)

* Taken from Kruse et al., 1985.
‡ Taken from Keilhauer et al., 1985.
‖, Probe cell; §, target cell.
Numbers are mean values ± SD. Number of experiments are given in parentheses.
Mono, monoclonal antibody; poly, polyclonal antibody.

>95% of all astrocytes expressed it. To verify that during preparation of probe cells trypsin does not affect AMOG on the cell surface, [^{35}S]methionine-labeled cultures were subjected to the preparation procedure for probe cells and AMOG was immunoprecipitated from these cells and analyzed by SDS PAGE. AMOG was immunoprecipitated in undegraded form as 50-kD component (see below) and detected only in the cell pellet, but not in the supernatant (not shown).

Adhesion of neurons to astrocytes was inhibited by ∼25% with monoclonal AMOG antibodies (IgG fraction or Fab fragments at 1 mg/ml) (Table II). Polyclonal AMOG antibodies showed a smaller percentage of inhibition (16%). No inhibition of adhesion was observed when astrocytes were allowed to adhere to astrocytes. Polyclonal antibodies to mouse liver membranes that react with neurons and astrocytes had previously been shown not to interfere with adhesion, whereas polyclonal antibodies to the adhesion molecule J1 reduced neuron to astrocyte adhesion by ∼50% (Kruse et al., 1985) and N-CAM antibodies by ∼30% (Keilhauer et al., 1985). Since neurons did not express AMOG under these conditions, neuron to neuron adhesion in the presence of AMOG antibodies was not measured.

These experiments show that AMOG is specifically involved in Ca^{++}-independent neuron to astrocyte adhesion.

Immunocytological Analysis of AMOG-Positive Cell Types

To further investigate which cell types express AMOG and to characterize the developmental appearance of AMOG on

Table III. Immunofluorescence Labeling Intensities with AMOG Antibodies in Different Layers of the Cerebellar Cortex as a Function of Age

Age	P2	P8	P15	P24
External granular layer	±	+	±	*
Molecular layer	+	+++	++	+
Internal granular layer	±	++	+++	++

Indirect immunohistology of midsagittal sections of mouse cerebellar cortex was performed with monoclonal AMOG antibodies at several postnatal ages. P, postnatal day; ± , hardly detectable; +, weakly detectable; ++, detectable; +++, strongly detectable; * the external granular layer no longer exists at this age.

astrocytes, immunocytological studies were carried out on cultured cells and in histological sections of the early postnatal mouse cerebellum.

In monolayer cultures of cerebella from 6-d-old mice after 3 d of maintenance in vitro, all glial fibrillary acidic protein-positive astrocytes (Bignami et al., 1972) expressed AMOG as seen by immunodoublefluorescence using monoclonal AMOG antibody (not shown). Cells with astrocytic morphology that expressed vimentin but not glial fibrillary acidic protein were also AMOG positive. Fibronectin-positive fibroblasts and MESA-1-positive endothelial cells with fibroblast morphology did not express AMOG. L1-positive neurons were AMOG-negative in cultures maintained for 2 d, whereas after 3–4 d AMOG immunoreactivity was also observed on neurons, with cell bodies more intensely immunofluorescent than processes. O4 antigen-positive oligodendrocytes were found to express AMOG after 6 d in vitro. Less than 50% of all O4 antigen–positive oligodendrocytes expressed AMOG at this stage.

In fresh frozen sections of 2-d-old mouse cerebellum AMOG was weakly detectable by indirect immunofluorescence. At this stage the antigen was hardly detectable in the external and internal granular layers, and weakly detectable in the molecular layer (Table III). At postnatal days 5 and 8 AMOG was most strongly detectable in the molecular layer, easily detectable in the internal granular layer and more weakly, but significantly detectable in the external granular layer. At postnatal day 15 the antigen was hardly detectable in the external granular layer, detectable in the molecular layer, and most strongly detectable in the internal granular layer. At postnatal days 21 and 24 antigen expression was again reduced in the molecular and internal granular layers.

The developmental appearance and disappearance of AMOG as observed at the light microscopic level was also seen by electronmicroscopy. During times of granule cell migration Bergmann glial processes expressed AMOG (Fig. 3 *a*). Granule cells did not express the antigen on cell body or processes, which is particularly obvious when they are not in contact with Bergmann glial cells. At sites of granule cell body–Bergmann glial cell contact it was not possible to discern whether the antigen was localized on the astrocyte or the neuron. Endfeet of Bergmann glial processes abutting onto the pia mater were also strongly AMOG-positive (Fig. 3 *b*). The basement membrane between glial endfeet and pia
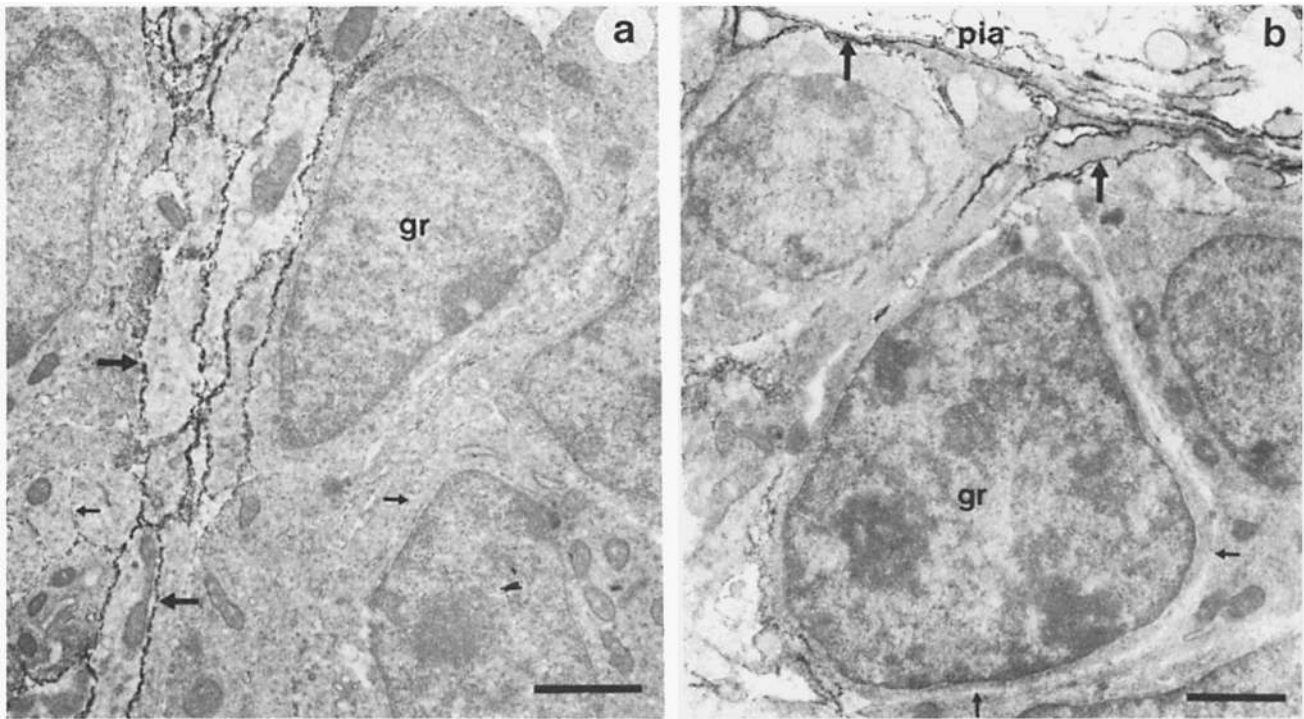
Figure 3. Immunoelectron microscopic localization of monoclonal AMOG antibody in the cerebellar cortex of 5-d-old mice. Indirect immunolabeling was performed by preembedding staining procedures using peroxidase-coupled second antibody. Sections were not counterstained. (*a*) Migrating granule cell in contact with Bergmann glial processes in the inner part of the external granular layer. Note the peroxidase reaction product on glial processes (*large arrows*). The migrating granule cell bodies (*small arrow, right*) and granule cell axons (parallel fibers, *small arrow, left*) are antigen negative. The contact site between Bergmann glial process and migrating granule cell is antigen positive. (*b*) Pial surface and outer part of the external granule layer. The glial endfeet are strongly antigen positive at contact sites between granule cells and pial basement membrane (*large arrows*). No peroxidase reaction product is seen on granule cell bodies (*small arrows*). The peroxidase reaction product in the pia mater appears to be nonspecific since it is also seen in control sections treated with antibodies to human hemoglobin or without first antibody (not shown). *gr*, Granule cell body. Bars, 1 μm.

mater was AMOG-negative. No specific staining was observed in the pia mater. Results were the same by pre- and postembedding staining procedures.

These observations show that AMOG expression is highest at times of granule cell migration (between day 4 and 15, with a peak at day 8). During the phase of migration the antigen appears to be most strongly associated with the radial processes of Bergmann glial cells in the molecular layer, whereas after the completion of migration the antigen seems to be most strongly associated with astrocytes in the internal granular layer, the final destination of granule cell migration.

Biochemical Analysis of AMOG by Immunoaffinity Chromatography and Immunoprecipitation

Monoclonal AMOG antibody columns retained from detergent solubilized crude membrane fractions of adult mouse brain several components as analyzed by SDS PAGE (Fig. 4). The major component was a broad band migrating with an apparent molecular weight of 45–50 kD (Fig. 4, lane *1*). This component will be referred to as 50-kD component. Other bands were also detectable in most eluates of the monoclonal antibody column: a smear of bands between 100 and 140 kD, and minor bands at ∼60 kD, and in higher molecular weight ranges. Often, some material was observed at the interface between the stacking and running gels. None of the bands could be released from membranes when these were treated with high or low salt, high or low pH, guanidi-

nium hydrochloride or urea (Faissner et al., 1985), indicating that AMOG is an integral membrane protein. The relative proportion of the higher molecular weight bands did not change drastically when the antibody column was loaded with detergent extract and washed with 2.4 M NaCl in saponin-containing buffer instead of 0.4 M NaCl in saponin-free buffer (Fig. 4, lane *2*). In some immunoaffinity preparations only the 50-kD component was observed (Fig. 4, lane *3*). The reason for this variation in antigen purity isolated under apparently identical conditions is presently unknown. When SDS PAGE was performed under nonreducing conditions the 50-kD band was again the predominant one whereas other bands changed their apparent molecular weights (Fig. 4, lane *4*). Several higher molecular weight components were observed under these conditions (Fig. 4, lane *4*). Antibodies prepared against the total eluate from the monoclonal AMOG antibody column reacted by Western blot analysis with several bands (Fig. 4, lane *5*). These polyclonal AMOG antibodies were used in the migration and adhesion assays. Monoclonal AMOG antibodies were not active in Western blots.

To investigate whether the different bands are immunochemically related and which of these components is the cell surface component AMOG that is expressed by astrocytes, several experiments were performed. First, polyclonal antibodies were prepared against the individual bands isolated by SDS PAGE and electroeluted from the gel (Hunkapiller
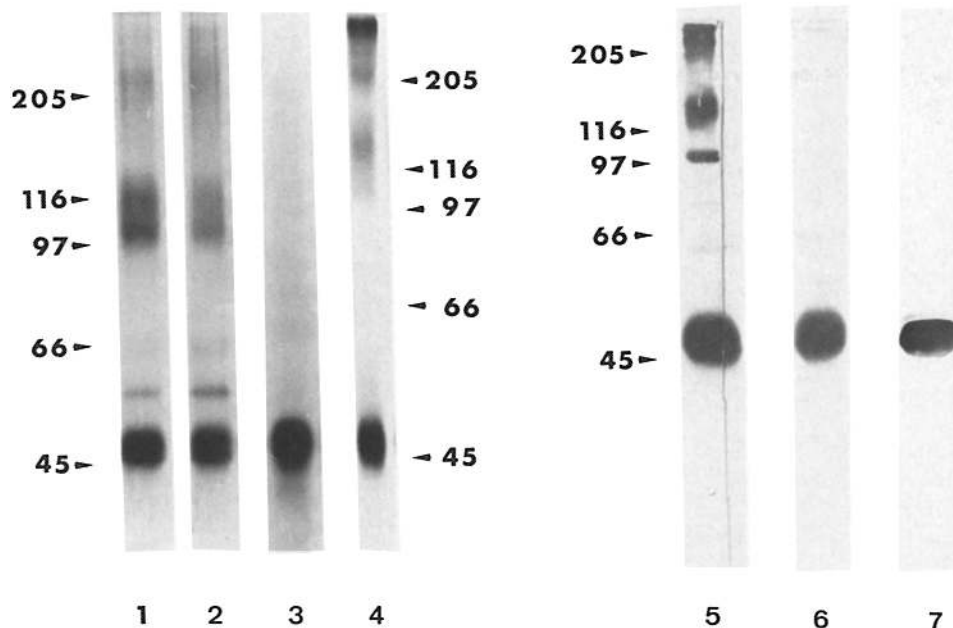
Figure 4. Characterization of immunoaffinity-purified AMOG by SDS PAGE and Western blot analysis. Lane *1*, AMOG immunoaffinity purified over a monoclonal AMOG antibody column from adult mouse brain (7% gel, silver stain). Lane *2*, AMOG immunoaffinity purified as in lane *1*, except the monoclonal antibody column was washed with 2.4 M NaCl in 20 mM Tris buffer containing 0.5% saponin, pH 6.8 before antigen elution at pH 11.5 (7% gel, silver stain). Lane *3*, preparation of immunoaffinity-purified AMOG that only contains the 50-kD band (7% gel, silver stain). Lane *4*, immunoaffinity-purified AMOG (as in lane *1*) under nonreducing SDS PAGE conditions (10% gel, silver stain). Lane *5*, Western blot of immunoaffinity-purified AMOG using polyclonal antibodies prepared against immunoaffinity-purified AMOG (as shown in lane *1*). Lane *6*, Western blot of immunoaffinity-purified AMOG with polyclonal antibody prepared against the electroeluted 50-kD band of immunoaffinity-purified AMOG. Lane *7*, Western blot of immunoaffinity-purified AMOG with monoclonal L3 antibody.

et al., 1983). Antibodies to the 50-kD component reacted only with this component and not with the higher molecular weight ones (Fig. 4, lane *6*). Monoclonal L3 antibodies that recognize a carbohydrate epitope (Kücherer et al., 1987) also reacted only with the 50-kD component (Fig. 4, lane *7*). Immunoaffinity-purified AMOG was not recognized by mono-

clonal BSP-3 antibodies (Gorvel et al., 1984) that recognize a Na/K ATPase with apparent molecular weights of 50 and 100 kD (not shown). Unfortunately, immunocytological analysis of these polyclonal antibodies showed that the antibodies to the 50-kD component did not react in monolayer cultures of live or permeabilized cerebellar cells, suggesting that the antibodies only recognize the antigen in a SDS-denatured state.

The surface localization of AMOG was then further investigated by radioiodinating live cerebellar monolayer cultures with lactoperoxidase exclusively on the cell surface and subsequent immunoprecipitation with monoclonal AMOG antibodies. Only the 50-kD was immunoprecipitated (Fig. 5, lane *5*), indicating that the 50-kD component is exposed at the cell surface. After biosynthetic labeling of monolayer cultures with [³H]fucose, the 50-kD component appeared in immunoprecipitates, whereas the 100–140-kD and the higher molecular weight components were again not seen (Fig. 5, lane *3*). When immunoprecipitations were performed on cultures biosynthetically labeled with [³⁵S]methionine, the 50-kD band was again the predominant one (Fig. 5, lane *1*). Immunoprecipitates with polyclonal antibodies prepared against the total eluate from the monoclonal AMOG affinity column (Fig. 4, lane *4*) showed that the other bands isolated from adult mouse brain by affinity chromatography are also present in the cerebellar cultures after labeling with [³⁵S]methionine (Fig. 5, lane *2*). The high molecular weight bands were not immunoprecipitated when cultures were labeled with [³H]fucose (Fig. 5, lane *4*) or cell surface radioiodinated (Fig. 5, lane *6*), suggesting that these bands are not exposed on the cell surface. When [³⁵S]methionine labeling was performed in the presence of tunicamycin (Heifetz et al., 1979; Keller et al., 1979) a shift from 50 to 32 kD was observed (not shown) indicating that AMOG contains N-glycosidically-linked carbohydrate chains that amount to approximately one-third of the molecule's molecular weight. AMOG
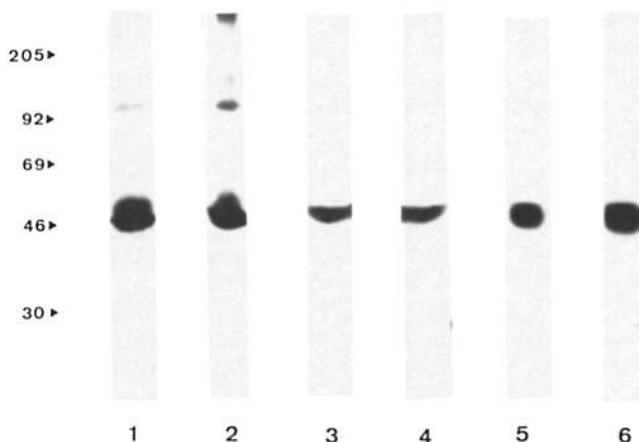

Figure 5. Biosynthesis and cell surface labeling of AMOG antigen in primary monolayer cultures of cerebella from 6-d-old mice maintained in vitro for 3–6 d. Fluorographs of immunoprecipitates obtained with monoclonal (lanes *1*, *3*, and *5*) and polyclonal (lanes *2*, *4*, and *6*) AMOG antibodies after SDS PAGE (7–15% linear gradient gels). Polyclonal antibodies used were prepared against AMOG preparations containing the higher molecular weight bands. Lanes *1* and *2*, immunoprecipitates after biosynthetic labeling with [³⁵S]methionine. Lanes *3* and *4*, immunoprecipitates from detergent-solubilized cells after biosynthetic labeling with [³H]fucose. Lanes *5* and *6*, immunoprecipitates from solubilized cells after lactoperoxidase-catalyzed radioiodination of live monolayer cultures.

was never found in the culture supernatants by immunoprecipitation.

These experiments indicate that the 50-kD component is the cell surface–exposed AMOG.

Discussion

AMOG, an adhesion molecule on glia, is a novel neural cell adhesion molecule that is distinct from the previously characterized ones: L1, N-CAM, J1, and the myelin-associated glycoprotein. Under the stringent conditions of immunoprecipitation AMOG appears by SDS PAGE as a broad band with a molecular weight of ∼50 kD. It is an integral membrane glycoprotein with a high degree of glycosylation. Expressed by astrocytes, it is involved in neuron to astrocyte, but not astrocyte to astrocyte, adhesion. It is also expressed by oligodendrocytes and likely to subserve an adhesive function on these glial cells also. Our observations suggest that AMOG is an adhesion molecule that mediates cerebellar granule cell migration by specifying the direct contact between the migrating neuron and the "contact-guiding" astroglial process, the Bergmann glial fiber. AMOG is detectable shortly before the onset of granule cell migration and, unlike L1 and N-CAM that remain expressed also in adulthood, ceases to be detectable on Bergmann glial cells at the end of the migratory period. It is interesting that at the end of granule cell migration AMOG is more strongly expressed by astrocytes in the internal granular layer than on Bergmann glial processes, possibly indicating an involvement of AMOG in the arrest of granule cell movement in the internal granular layer by neuron-glia contact.

Two other molecules that have been suggested to be mediators of granule cell migration by neuron-glia contact, the adhesion molecule J1 (Kruse et al., 1985) and the extracellular matrix glycoprotein laminin (Liesi, 1985), are apparently not involved in this process. It cannot be excluded from our studies, however, that the antibodies used do not recognize the functionally decisive epitopes for migration. Thus, an epitope linked to a heparan sulfate proteoglycan laminin complex has been suggested to be the responsible site for neurite outgrowth (Lander et al., 1985). However, the laminin and J1 antibodies used in this study were both shown to inhibit neuron to laminin and neuron to astrocyte adhesion, respectively (Kruse et al., 1985; Werz, 1986).

The observation that monoclonal AMOG antibodies bind only to glial surfaces during the time of granule cell migration excludes a "homophilic" binding mechanism of AMOG on glial cells to AMOG on neurons. This view is supported by the finding that AMOG antibodies interfere with astrocyte-neuron but not with astrocyte–astrocyte adhesion in vitro. The fact that other molecules copurify with AMOG by immunoaffinity chromatography under less stringent conditions than those used for immunoprecipitation, but nevertheless involving high salt and detergent, is interesting from the point of view that the neuronal-binding molecule for AMOG must be different from AMOG and could therefore be one of those copurifying molecules. Furthermore, as an integral membrane protein AMOG could associate with intracellular partners in the cell surface–cytoplasm signalling process. Preliminary experiments to relate the 100–140-kD bands to cytoskeleton-membrane linker proteins, such as the 100-kD protein α-actinin, have failed so far (unpublished observations). The identity of the copurifying bands thus remains to be determined.

The observation that AMOG is expressed by neurons after maintenance for 3–4 d in vitro, particularly on cell bodies and less on neurites, is difficult to explain at present. Since AMOG has never been found in a soluble or secreted form, it is unlikely that the appearance of AMOG on neurons is due to its deposition on the neuronal-binding molecule. Since AMOG has never been detected in the cerebellum on neurons in situ at any developmental stage, it is possible that neurons start to express AMOG when released from certain intercellular constraints in vitro.

AMOG does not express the L2/HNK-1 carbohydrate epitope (Kruse et al., 1984; Noronha et al., 1986; Chou et al., 1985) that is shared by the neural cell adhesion molecules L1, N-CAM, J1, and myelin-associated glycoprotein (Kruse et al., 1984, 1985). AMOG expresses, however, a carbohydrate epitope recognized by the monoclonal antibody L3 that is present on several glycoproteins from mouse brain including L1 and the myelin-associated glycoprotein, but, interestingly, not J1 and N-CAM. The occurrence of this epitope thus introduces AMOG into another family of cell adhesion molecules that is characterized by a carbohydrate structure common to members within this family, but shared also by some but not all members of another family. The functional significance of these cross-reactivities and of the L3 carbohydrate epitope on AMOG remains to be established.

References

Bazin, H. 1982. Production of rat monoclonal antibodies with LOU rat non-secreting IR9835 myeloma cells. Protides Biol. Fluids Proc. Colloq. 29: 615–618.

Bignami, A., L. F. Eng, D. Dahl, and C. T. Uyeda. 1972. Localisation of the glial fibrillary acidic protein in astrocytes by immunofluorescence. Brain Res. 43:429–435.

Bock, E., C. Richter-Landsberg, A. Faissner, and M. Schachner. 1985. Demonstration of immunochemical identity between the nerve growth factor inducible large external (NILE) glycoprotein and the cell adhesion molecule L1. EMBO (Eur. Mol. Biol. Organ.) J. 4:2765–2768.

Chou, K. H., A. A. Ilyas, J. E. Evans, R. H. Quarles, and F. B. Jungalwala. 1985. Structure of a glycolipid reacting with monoclonal IgM in neuropathy and with HNK-1. Biochem. Biophys. Res. Commun. 128:383–389.

Cowan, W. M. 1982. A synoptic view of the development of the vertebrate central nervous system. Life Sci. Res. Rep. 24:7–24.

de St. Groth, S. F., and D. Scheidegger. 1980. Production of monoclonal antibodies: strategy and tactic. J. Immunol. Methods. 35:1–21.

Edelman, G. M. 1985. Cell adhesion and the molecular processes of morphogenesis. Ann. Rev. Biochem. 54:135–169.

Edelman, G. M., and J. P. Thiery. 1985. The Cell in Contact. John Wiley & Sons, Inc., New York, NY. pp. 507.

Fahey, J. L., and E. W. Terry. 1979. Ion Exchange Chromatography and Gel Filtration. Handbook of Experimental Immunology. In D. M. Weir, editor. Blackwell Scientific Publications Ltd., Oxford, UK. 19–43.

Faissner, A., J. Kruse, C. Goridis, E. Bock, and M. Schachner. 1984. 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., D. B. Teplow, D. Kübler, G. Keilhauer, V. Kinzel, and M. Schachner. 1985. Biosynthesis and membrane topography of the neural cell adhesion molecule L1. EMBO (Eur. Mol. Biol. Organ.) J. 4:3105–3113.

Fischer, G., V. Künemund, and M. Schachner. 1986. Neurite outgrowth patterns in cerebellar microexplant cultures are affected by antibodies to the cell surface glycoprotein L1. J. Neurosci. 6:605–612.

Friedlander, D. R., M. Grumet, and G. M. Edelman. 1986. Nerve growth factor enhances expression of neuron-glia cell adhesion molecule in PC12 cells. *J. Cell Biol.* 102:413–419.

Fushiki, S., and M. Schachner. 1986. Immunocytological localization of cell adhesion molecules L1 and N-CAM and the shared carbohydrate epitope L2 during development of the mouse neocortex. *Dev. Brain Res.* 289:153–167.

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., M. A. Joher, M. Hirsch, and M. Schachner. 1978a. Cell surface proteins of cultured brain cells and their recognition by anti-cerebellum (anti-NS-4) antiserum. *J. Neurochem.* 31:531–539.

Goridis, C., J. Martin, and M. Schachner. 1978b. Characterization of an antiserum to synaptic glomeruli from rat cerebellum. *Brain Res. Bull.* 3:45–52.

Gorvel, G. P., A. Liabeuf, D. Massey, D. Liot, C. Goridis, and S. Maroux. 1984. (Na⁺ + K⁺)-ATPase recognition in mouse organs by a monoclonal antibody. *Cell Tissue Res.* 238:253–261.

Hawkes, R. B., E. Niday, and G. Gordon. 1982. A dot-immunobinding assay for monoclonal and other antibodies. *Anal. Biochem.* 119:142–147.

Heifetz, A., R. W. Keenan, and A. D. Elbein. 1979. Mechanism of action of tunicamycin on the UDP-GLcNAc-dolichyl phosphate GLc NAc-1-phosphate transferase. *Biochemistry.* 18:2186–2192.

Hunkapiller, M., E. Lujan, F. Ostrander, and L. E. Hood. 1983. Isolation of microgram quantities of proteins from polyacrylamide gels for amino acid sequence analysis. *Methods Enzymol.* 91:227–236.

Kearney, J. F., A. Radbruch, B. Liesegang, and K. Rajewsky. 1979. A new mouse myeloma cell line that has lost immunoglobulin expression but permits the construction of antibody-secreting hybrid cell lines. *J. Immunol.* 123:1548–1550.

Keilhauer, G., A. Faissner, and M. Schachner. 1985. Differential inhibition of neurone-neurone, neurone-astrocyte and a astrocyte-astrocyte adhesion of L1, L2 and N-CAM antibodies. *Nature (Lond.).* 316:728–730.

Keller, R. K., D. J. Boon, and F. C. Crum. 1979. N-acetylglucosamin-1-phosphate transferase from hen oviduct: solubilization, characterization, and inhibition by tunicamycin. *Biochemistry.* 18:3947–3952.

Komitowsky, D., G. Zinser, and C. Stute. 1983. Digital picture analysis as an integral part of the information system for experimental oncopathology of the German Cancer Research Center. *Methods. Inf. Med.* 22:69–74.

Kruse, J., G. Keilhauer, A. Faissner, R. Timpl, and M. Schachner. 1985. The J1 glycoprotein—a novel nervous system cell adhesion molecule of the L2/HNK-1 family. *Nature (Lond.).* 316:146–148.

Kruse, J., R. Mailhammer, H. Wernecke, A. Faissner, I. Sommer, and M. Schachner. 1984. Neural cell adhesion molecules and myelin-associated glycoprotein share a common carbohydrate moiety recognized by monoclonal antibodies L2 and HNK-1. *Nature (Lond.).* 311:153–155.

Kücherer, A., A. Faissner, and M. Schachner. 1987. The novel carbohydrate epitope L3 is shared by some neural cell adhesion molecules. *J. Cell Biol.* 104:1597–1602.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.).* 227:680–685.

Lagenaur, C., I. Sommer, and M. Schachner. 1980. Subclass of astroglia recognized in mouse cerebellum by monoclonal antibody. *Dev. Biol.* 79:367–378.

Lander, A. D., D. K. Fujii, and L. F. Reichardt. 1985. Laminin is associated with the "neurite outgrowth-promoting factors" found in conditioned media. *Proc. Natl. Acad. Sci. USA.* 82:2183–2187.

Liesi, P. 1985. Do neurons in the vertebrate CNS migrate on laminin? *EMBO (Eur. Mol. Biol. Organ.) J.* 4:1163–1170.

Lindner, J., J. Guenther, H. Nick, G. Zinser, H. Antonicek, M. Schachner, and D. Monard. 1986a. Modulation of granule cell migration by a glia-derived protein. *Proc. Natl. Acad. Sci. USA.* 83:4568–4571.

Lindner, J., F. G. Rathjen, and M. Schachner. 1983. L1 mono- and polyclonal antibodies modify cell migration in early postnatal mouse cerebellum. *Nature (Lond.).* 305:85–90.

Lindner, J., G. Zinser, W. Werz, C. Goridis, B. Bizzini, and M. Schachner. 1986b. Experimental modification of postnatal cerebellar granule cell migration in vitro. *Brain Res.* 377:298–304.

Lowry, O. H., N. I. Rosebrough, A. C. Farr, and R. I. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193:265–275.

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.

Moonen, G., M. P. Grau-Wagemans, and I. Selak. 1982. Plasminogen activator-plasmin system and neuronal migration. *Nature (Lond.).* 298:753–755.

Neuhoff, V., K. Philipp, H. G. Zimmer, and S. Mesecke. 1979. A simple, versatile, sensitive and volume-independent method for quantitative protein determination which is independent of other external influences. *Hoppe-Seyler's Z. Physiol. Chem.* 360:1657–1670.

Noronha, A., A. Ilyas, R. H. Quarles, H. Antonicek, and M. Schachner. 1986. Molecular specificity of L2 monoclonal antibodies that bind to carbohydrate determinants of neural cell adhesion molecules and resemble other monoclonal antibodies recognizing the myelin-associated glycoprotein. *Brain Res.* 385:237–244.

Oakley, B. R., D. R. Kirsch, and N. R. Morris. 1980. A simplified ultrasensitive silver stain for detecting proteins in polyacrylamide gels. *Anal. Biochem.* 105:361–363.

Persohn, E., and M. Schachner. 1987. Immunoelectron microscopic localization of the neural cell adhesion molecules L1 and N-CAM during postnatal development of mouse cerebellum. *J. Cell Biol.* In press.

Pollerberg, G. E., M. Schachner, and J. Davoust. 1986. Surface mobilities of two molecular weight forms of the neural cell adhesion molecule N-CAM are characteristic of distinct differentiation stages. *Nature (Lond.).* 324:462–465.

Porter, R. R. 1959. The hydrolysis of rabbit gamma globulin and antibodies with crystalline papain. *Biochem. J.* 73:119–131.

Quarles, R. H. 1984. Myelin-associated glycoprotein in development and disease. *Dev. Neurosci.* 6:285–303.

Rakic, P. 1982. The role of neuronal-glia cell interaction during brain development. *Life Sci. Res. Rep.* 20:25–38.

Rathjen, F. G., and U. Rutishauser. 1984. Comparison of two cell surface molecules involved in neural cell adhesion. *EMBO (Eur. Mol. Biol. Organ.) J.* 3:461–465.

Rathjen, F., 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.

Rutishauser, U., W. E. Gall, and G. M. Edelman. 1978. Adhesion among neural cells of the chick embryo. IV. Role of the cell surface molecules CAM in the formation of neurite bundles in cultures of spinal ganglia. *J. Cell Biol.* 79:382–393.

Sack, H.-J., M. Stöhr, and M. Schachner. 1983. Cell type-specific binding of Ricinus lectin to murine cerebellar cell surfaces in vitro. *Cell Tissue Res.* 228:183–204.

Schachner, M., A. Faissner, G. Fischer, G. Keilhauer, J. Kruse, V. Künemund, J. Lindner, and H. Wernecke. 1985. Functional and structural aspects of the cell surface in mammalian nervous system development. *In* The Cell in Contact. G. M. Edelman and J. P. Thiery, editors. John Wiley & Sons, Inc., New York, NY. 257–276.

Schnitzer, J., and M. Schachner. 1981. Expression of Thy-1, H-2 and NS-4 cell surface antigens and tetanus toxin receptors in early post-natal and adult mouse cerebellum. *J. Neuroimmunol.* 1:429–456.

Schnitzer, J., W. W. Franke, and M. Schachner. 1981. Immunocytochemical demonstration of vimentin in astrocytes and ependymal cells of developing and adult mouse nervous system. *J. Cell Biol.* 90:435–447.

Sommer, I., and M. Schachner. 1981. Monoclonal antibodies (O1 to O4) to oligodendrocyte cell surfaces: an immunocytological study in the central nervous system. *Dev. Biol.* 83:311–327.

Stallcup, W. B., and L. Beasley. 1985. Involvement of the nerve-growth factor inducible large external glycoprotein (NILE) in neurite fasciculation in primary cultures of rat brain. *Proc. Natl. Acad. Sci. USA.* 82:1276–1280.

Stoeckl, K., C. Gagnon, G. Guroff, and H. Thoenen. 1976. Purification of nerve growth factor antibodies by affinity chromatography. *J. Neurochem.* 26:1207–1211.

Thor, G., E. Pollerberg, and M. Schachner. 1986. Molecular association of two neural cell adhesion molecules, L1 antigen and the 180 kD component of N-CAM within the surface membrane of cultured neuroblastoma cells. *Neurosci. Lett.* 66:121–126.

Towbin, H., T. Staehelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. *Proc. Natl. Acad. Sci. USA.* 76:4350–4354.

Werz, W. 1986. PhD Thesis, University of Heidelberg.