Selective Expression of the 180-kD Component of the Neural Cell Adhesion Molecule N-CAM during Development

ELISABETH G. POLLERBERG,* REMY SADOUL,* CHRISTO GORIDIS,† and MELITTA SCHACHNER*  
*Department of Neurobiology, University of Heidelberg, 6900 Heidelberg, Federal Republic of Germany; and †Centre d’Immunologie, Institut National de la Santé et de la Recherche Médicale–Centre National de la Recherche Scientifique de Marseille Luminy, 13288 Marseille Cedex 9, France

ABSTRACT  The rodent neural cell adhesion molecule (N-CAM) consists of three glycoprotein chains of 180, 140, and 120 kD in their adult forms. Although the proportions of the three components are known to change during development and differ between brain regions, their individual distribution and function are unknown. Here we report studies carried out with a monoclonal antibody that specifically recognizes the 180-kD component of mouse N-CAM (N-CAM_{180}) in its highly sialylated embryonic and less glycosylated adult forms. In primary cerebellar cell cultures, N-CAM_{180} antibody reacts intracellularly with all types of neural cells including astrocytes, oligodendrocytes, and neurons. During cerebellar, telencephalic, and retinal development N-CAM_{180} is detectable by indirect immunohistology in differentiated neural cells, but, in contrast to total N-CAM, not in their proliferating precursors in the ventricular zone and primordial and early postnatal external granular layer. In monolayer cultures of C1300 neuroblastoma cells, N-CAM_{180} appears by immunofluorescence more concentrated at contact points between adjacent cells, while N-CAM comprising the 180- and 140-kD component shows a more uniform distribution at the plasma membrane. Treatment of neuroblastoma cells with dimethylsulfoxide, which promotes differentiation, induces a shift toward the predominant expression of N-CAM_{180}. These observations support the notion that N-CAM_{180} is expressed selectively in more differentiated neural cells and suggest a differential role of N-CAM_{180} in the stabilization of cell contacts.

The neural cell adhesion molecule (N-CAM)1 has been implicated in morphogenetic events during formation of the nervous system (for reviews, see references 7 and 30). Antibodies to N-CAM inhibit aggregation of neural cells (42), histotypic deployment in cultured cell aggregates (33) and retinal explants (3), interaction of nerve and muscle cells in vitro (13), and fasciculation of neurites (31, 32). Total N-CAM consists of three glycoproteins of 180, 140, and 120 kD when isolated from adult mouse brain (8, 29). Embryonic mouse brain yields different molecular forms of N-CAM with apparent molecular weights of 180–250 kD (5, 29) and high amounts of sialic acid (11, 16) in an unusual polymeric form (11). Compared with the embryonic form the adult form carries one third of the sialic acid content and is more adhesive both to neuroblastoma cells (34) and to itself (15).

Studies on the subunit composition of the adult form of N-CAM in chick (6) and mouse (12) have mapped the cell binding site and most of the sialic acid residues to proteolytic fragments localized on the outside of the cell. The extracellularly localized amino-terminal ends of the three components share identical amino acid sequences and have nearly identical patterns on peptide maps (6). The 180-, 140-, and 120-kD chains differ only in the length of their carboxy-terminal ends which extend into the cytoplasm. The three protein chains are presumed to be identical in the adult and embryonic forms (12, 29).

While some structural aspects and the membrane disposition of the three components have been investigated, their individual contributions to cell interactions during development of the nervous system have remained elusive. As a first step toward delineating their possible roles specific antibodies
distinguishing the individual components would be valuable. One such antibody was found during the course of characterizing monoclonal antibodies against membrane fractions from neonatal mouse brain. The antibody was shown to react specifically with the 180-kD component of mouse N-CAM (N-CAM180). Here, we report that N-CAM180 is expressed during development at later stages than the other components and is localized differentially at contact sites between cultured neural cells.

**MATERIALS AND METHODS**

**Animals:** C57BL/6J mice were used for membrane preparations, cell cultures, and immunohistological preparations. Their origin and maintenance have been described (37). Mouse embryos were taken from timed pregnancies. Embryonic day 0 was taken as the day a vaginal plug was found. For immunization with membrane preparations 6-wk-old F1 hybrid animals from a cross between Sprague-Dawley and Lou Wistar parents were used.

**Preparation of Crude Membrane Fraction:** 100 neonatal mouse brains were homogenized in 20 ml Tris buffer (25 mM Tris, 5 mM MgCl2, 100 mM KCl, pH 7.4) containing 0.32 M sucrose plus soybean trypsin inhibitor and egg white trypsin inhibitor (10 μg/ml each) (Boehringer Mannheim Co., Mannheim, FRG). The homogenate was centrifuged in 10-ml aliquots through sucrose step gradients (12.5 ml each of 1.2, 0.8, and 0.32 M sucrose in Tris buffer) for 2 h at 16,000 rpm, 4°C in a Beckmann SW 27 rotor (Beckman Instruments, Inc., Munich, FRG). Membrane fractions were harvested from the 0.8–1.2 M sucrose interface and pelleted by centrifugation for 30 min at 40,000 rpm in a Beckmann TI 50 rotor at 4°C. The pellet was resuspended in 10 ml Tris buffer containing 1% saponin and 10% glycerol. The saponin-insoluble fraction was pelleted by centrifugation as described above. The pellet was resuspended in 2 ml 20 mM Tris buffer containing 1% deoxycholate, and the detergent-insoluble fraction was again pelleted. The deoxycholate-soluble components were fractionated according to their molecular weights on a Sephacryl S-400 (Pharmacia Inc., Freiburg, FRG) column (60 ml). The filtrate (90 ml) was collected in 3-ml fractions. A sample of every fifth fraction was used for SDS-PAGE. The fractions were then pooled into three batches containing the high, medium, and low molecular weight proteins. Immunization with the 80-200-kD fraction resulted in monoclonal antibodies O1 and O4 reacting with oligodendrocyte cell surfaces, and immunohistological preparations. Their origin and maintenance between Sprague-Dawley and Lou Wistar parents were used.

**Preparation of Detergent Soluble Membrane Fraction:** The detergent-insoluble fraction was again pelleted. The deoxycholate-soluble material was collected and centrifuged at 40,000 rpm for 1 h at 4°C. The pellet was resuspended in 2 ml 20 mM Tris buffer containing 1% deoxycholate, and the detergent-insoluble fraction was again pelleted. The deoxycholate-soluble components were fractionated according to their molecular weights on a Sephacryl S-400 (Pharmacia Inc., Freiburg, FRG) column (60 ml). The filtrate (90 ml) was collected in 3-ml fractions. A sample of every fifth fraction was used for SDS-PAGE. The fractions were then pooled into three batches containing the high, medium, and low molecular weight proteins. Immunization with the 80-200-kD fraction resulted in monoclonal antibody N-CAM180.

**Monoclonal N-CAM180 Antibody:** F1 hybrid rats were injected subcutaneously and intraperitoneally with 200 μg protein each at days 1, 15, 21, and 40. The first two injections were made in complete, the following in incomplete Freund's adjuvant (Sigma Chemical Co., Munich, FRG). At day 47 the antibody titer was determined by indirect immunofluorescent staining of living cell cultures from early postnatal mouse cerebellum (see below). The rat was boosted on day 50 and 3 d later was killed for preparation of spleen cells. Fusion of spleen cells with the mouse myeloma cell line X3-X6-Ag655 was carried out as described previously (20). Approximately 2,000 hybridoma supernatants were screened by the immunospot binding test (14) using saponin-insoluble, deoxycholate-solubilized material of neonatal mouse brains prepared as described in the previous paragraph. Positive supernatants were then examined by indirect immunofluorescence on sections of fresh frozen cerebellum from 5-d-old mice and on cell cultures of 5-d-old mouse cerebellum maintained for several days in vitro. One of these hybridomas (clone 481) produced a monoclonal antibody that recognized N-CAM180.

**Antibodies:** Monoclonal antibody H28.123 and the polyclonal antibody to N-CAM have been described (12). Peroxidase-conjugated rabbit anti-rat and goat anti-rabbit immunoglobulin antibodies and fluorescein-conjugated rabbit anti-rat and sheep anti-rabbit immunoglobulin antibodies were obtained from Cappel Laboratories (Dynatech, Denkendorf, FRG). Monoclonal antibodies O1 and O4 reacting with oligodendrocyte cell surface antigens, polyclonal antibodies to glial fibrillary acidic protein and vimentin reacting with astrocytes, and L1 antibodies and tetanus toxin reacting with neurons have been described previously (9, 28, 35, 37, 41). Monoclonal antibody N-CAM180, which is an IgG2, was first demonstrated to fluorescein according to Johnson et al. (17). In brief, serum-free hybridoma supernatants containing monoclonal N-CAM180 antibody was concentrated by ammonium sulfate precipitation and dialyzed extensively against 50 mM Na2CO3 buffer, pH 9.5, with 40 μg fluorescein isothiocyanate (Sigma Chemical Co.) at a concentration of 1 mg/ml protein (1.5 mg total) and shaken gently for 3.5 h at 4°C. The fluorescein-conjugated antibodies were purified by gel filtration using a Sephadex G25 (Pharmacia Inc.) column (20 ml).

**Cell Cultures:** Primary cultures of cerebella from 13-d-old mouse embryos and neonatal and 7-d-old mice were prepared and maintained as monolayers on poly-L-lysine-coated coverslips as described (37, 38). The mouse neuroblastoma C1300 N2A clone was cultured in tissue culture flasks using Dulbecco's modified Eagle's medium (high glucose) containing 10% fetal calf serum (Seromed, Munich, FRG) and antibiotics as described (28). For treatment with dimethylsulfoxide (DMSO) N2A cells were seeded in Dulbecco's modified Eagle's medium containing 0.2% fetal calf serum and 2% DMSO. The culture medium was changed every 2 d. For indirect immunofluorescence cells were seeded on glass coverslips coated with poly-L-lysine and maintained for several days in culture.

**Immunocytological Procedures:** Sections, 10-μm thick, were prepared in a cryostat (Jung, Nussloch, FRG), mounted on poly-L-lysine-coated glass coverslips, and air dried for 1 h at room temperature. Indirect immunofluorescent staining of sections was performed as described by Schnitzer and Schachner (37). For indirect immunofluorescence staining of monolayer cultures, cells were maintained in vitro for 3–4 d and examined by single- and double-immunolabeling procedures also as described by Schnitzer and Schachner (37). For permeabilization of cells coverslips were dipped for 30 s in 96% ethanol at −20°C, followed by a 15-min wash with phosphate-buffered saline, pH 7.3 (PBS) and subsequent incubation for 15 min at room temperature with blocking buffer (PBS containing 0.1% bovine serum albumin [BSA] and 1% horse serum) to saturate unspecific binding sites. For double immunolabeling procedures involving cell surface- and intracellularly localized antigens, indirect immunofluorescence was carried out first for the cell surface antigen. Then, cells were permeabilized by treatment with ethanol (see above) and processed for indirect immunofluorescence as described.

**FIGURE 1** Specificity of monoclonal N-CAM180 antibody as analyzed by SDS PAGE and Western blots. (A) N-CAM purified from adult mouse brain by immunoaffinity chromatography using monoclonal antibody H28.123; SDS slab gel (4–15%) was stained by the reducing silver method (24). (B) Western blot of immunoaffinity purified N-CAM (A) using monoclonal N-CAM180 antibody. (C) SDS PAGE of crude membrane preparation from adult mouse brain stained by the reducing silver method. (D) Western blot of membrane preparation (C) using monoclonal N-CAM180 antibody. (E) Western blot of membrane preparation (C) using monoclonal N-CAM180 antibody. (F) Western blot of membrane preparation from neonatal mouse brain using monoclonal N-CAM180 antibody. (G) Western blot of membrane preparation from fetal mouse brain using monoclonal N-CAM180 antibody. (H) Western blot of membrane preparation from neonatal mouse brain using monoclonal N-CAM180 antibody.
Western Blot Analysis of DMSO-treated N2A Cells: N2A cells were maintained in culture in low serum and DMSO (see cell cultures) for various time periods (up to 8 d), harvested at room temperature by gently pipetting in PBS containing 1 mM EDTA, washed once, and sonicated in 10 mM Tris HCl, pH 8 containing 5 mM MgCl₂, 0.2 mM leupeptin, 5 mM iodoacetamide, 0.1 mM phenylmethylsulfonyl fluoride, 20 μg/ml aprotinin, and 0.1 mM 2-deoxy-2,3-dehydro-N-acetyl-neuraminic acid. After addition of Nonidet P-40 (final concentration 1%), the sonicate was left for 15 min on ice and centrifuged for 10 min at 8,000 g and 4°C. Supernatants were adjusted to 10 mg/ml protein (23), and supernatant aliquots were mixed with an equal volume of sample buffer (19) containing 5.6% SDS, 10% beta-mercaptoethanol and boiled for 3 min. SDS PAGE (6.7% slab gels) was performed as described by Laemmli (19). Electrophoretic transfer of proteins from gels to nitrocellulose filters was carried out according to Towbin et al. (43). Polyclonal antibodies to N-CAM were incubated with the nitrocellulose filters and revealed as described by Burnett (2).

Western Blot Analysis of Immunopurified N-CAM and Membrane Fractions of Adult Mouse Brain: N-CAM was purified from adult mouse brain by immunos affinity chromatography using monoclonal antibody H28.123 as described previously (29). Crude membrane fractions from embryonic, neonatal, and adult mice were prepared as described by Rathjen and Schachner (28). SDS PAGE was performed on 4-15% acrylamide slab gels. Proteins fractionated by SDS PAGE were transferred to nitrocellulose filters and processed for Western blot analysis with horseradish peroxidase-conjugated antibodies as described previously (10). Protein determinations were carried out according to Lowry et al. (23).

RESULTS
Monoclonal N-CAM₁₈₀ antibody selectively reacts with the 180-kD component of the neural cell adhesion molecule N-CAM immunopurified from adult mouse brain, but not with the other two components of 140 and 120 kD (Fig. 1, A and B). Also in crude membrane preparations of adult mouse brain (Fig. 1C), in which all three components of N-CAM are recognized by polyclonal antibodies (Fig. 1D), only the 180-kD chain and no additional components are detected by the monoclonal antibody (Fig. 1E). The antibody also reacts in crude membrane preparations from neonatal (Fig. 1G) and 14-d-old fetal (Fig. 1I) mouse brains with the embryonic polysialylated form ranging in apparent molecular weight from 180 to 250 kD.

To evaluate whether N-CAM₁₈₀ is expressed in the same cell types or cells as N-CAM, double immunolabeling experiments were carried out on primary cultures of cerebellum from 13-d-old embryos (after 4 and 8 d in vitro), and neonatal (4 d in vitro) and 7-d-old (4 d in vitro) mice. A complete overlap in staining of antibody N-CAM₁₈₀ with monoclonal N-CAM antibody H28.123 or polyclonal N-CAM antibody is seen at all ages studied (see Fig. 2 for neonatal mouse cerebellum). As has been observed previously for monoclonal and polyclonal N-CAM antibodies, not only neurons but also astrocytes (17a, 21, 25, 40) and oligodendrocytes (17a) are stained by monoclonal N-CAM₁₈₀ antibody (see Fig. 2, b and e for morphologically identified glial cells). N-CAM₁₈₀ is identified on glia by double immunofluorescence with established markers: vimentin and glial fibrillary acidic protein for astrocytes, and O4 and O1 antigens for oligodendrocytes (not shown) (9, 39, 41). The epitope recognized by monoclonal N-CAM₁₈₀ antibody is apparently localized intracellularly, since cultured cells have to be permeabilized by treatment with aldehyde and ethanol to obtain immunolabeling. Treatment of live cultured cells with trypsin (1 mg/ml for 20 min at 37°C) does not reduce immunofluorescence staining with N-
CAM<sub>180</sub> antibodies, but decreases staining intensity for antibody H28.123 (see also reference 12).

To determine whether during development N-CAM<sub>180</sub> is expressed independently of the other two components of N-CAM, three regions of the central nervous system—telencephalon, retina, and cerebellum—were investigated by indirect immunofluorescence using histological sections. Monoclonal antibody H28.123, which recognizes a surface localized epitope on all three components of N-CAM, and polyclonal N-CAM antibodies were used for comparison (29). In all three regions of the developing mouse central nervous system N-CAM<sub>180</sub> is found to be restricted to the more differentiated neural cells, whereas total N-CAM is also detected in the germinative zones containing proliferating neural precursor cells and in their direct postmitotic descendants.

In the developing 3-d-old retina, N-CAM<sub>180</sub> is expressed in the ganglion cell layer, inner plexiform layer, and inner part of the nuclear layer (Fig. 3a). The outer part of the nuclear layer containing proliferating neuronal precursor cells is only weakly stained by N-CAM<sub>180</sub> antibody (Fig. 3a). In contrast, antibody H28.123 reacts with all layers and stains the whole width of the nuclear layer uniformly (Fig. 3b). Similarly, in the telencephalic anlage of 14-d-old embryos, N-CAM<sub>180</sub> is expressed in the marginal zone and developing cortical plate, but not in the intermediate zone and ventricular layer (Fig. 4, a and c) which contain the less differentiated migrating neurons and proliferating neural precursors. Antibody H28.123 reacts with all layers (Fig. 4c). Finally, in the cerebellum of 14-d-old mouse embryos, N-CAM<sub>180</sub> antibodies stain only the intermediate zone, while antibodies to total N-CAM react additionally with the two germinative zones of the cerebellar anlage, the ventricular zone, and the primordial external granular layer (not shown). At postnatal day 7 N-CAM<sub>180</sub> is not detectable in the outer parts of the external

![Image](image_url)

**Figure 3** Immunofluorescence localization of N-CAM<sub>180</sub> in adjacent sections of retina from 3-d-old mice. N-CAM<sub>180</sub> antibody (a) stains predominantly the ganglion cell layer (GCL), inner plexiform layer (IPL), and the inner part of the nuclear layer (NL) (see phase-contrast micrograph [c] for orientation; the dashed line delineates the border between GCL and IPL; arrowheads demarcate the border between IPL and NL). Antibody H28.123 (b) reacts with the same fluorescence intensity throughout all layers. Bar, 30 μm.

![Image](image_url)

**Figure 4** Immunofluorescence localization of N-CAM<sub>180</sub> in coronal sections of the telencephalic anlage of 14-d-old mouse embryos. N-CAM<sub>180</sub> antibody stains predominantly the marginal zone and the developing cortical plate (a and c for higher magnification), whereas antibody H28.123 reacts with the same fluorescence intensity throughout all layers (b). Meninges (M) are negative for N-CAM<sub>180</sub> (c). d is the corresponding phase contrast micrograph to fluorescence image c. V, ventricle. Bars: 50 μm (a and b) and 30 μm (c and d).
granular layer and is only weakly apparent in the inner part of this layer (Fig. 5, a and e), with a characteristic speckled appearance of the immunofluorescence (Fig. 5 e). In contrast, antibodies to total N-CAM stain the full width of the external granular layer (Fig. 5, c and g). Both antibodies react with prospective white matter, Purkinje cells, and nascent molecular layer. In the adult cerebellum both antibodies show a similar staining pattern with label in all layers, but most prominently in the molecular layer of the cerebellum (21). However, the overall staining intensity is markedly reduced with N-CAM180 antibodies.

The apparent absence of N-CAM180 expression in the less

**FIGURE 5** Immunofluorescence localization of N-CAM180 in adjacent sagittal sections of cerebellum from 7-d-old mice. N-CAM180 antibody (a and e) does not stain the outer parts of the external granular layer (EGL), but reacts with the inner part of EGL, molecular layer (ML), and internal granular layer (IGL). Note the dotted appearance of N-CAM180 immunofluorescence in the inner part of EGL (e). Polyclonal antibodies to total N-CAM (c and g) react with the same fluorescence intensity throughout all layers. b, d, f, and g are the corresponding phase contrast micrographs to fluorescence images a, c, e, and g, respectively. The dashed line delineates the meninges. Bars, 80 µm.
differentiated neural cell populations suggested that N-CAM₁₈₀ is involved in more differentiated functions, such as neurite outgrowth and formation of particular cell contacts. We therefore investigated the expression of N-CAM₁₈₀ in the clonal cell line N2A of the mouse C1300 neuroblastoma in culture. N-CAM₁₈₀ is concentrated at contact points between most, but not all cells. The expression of N-CAM₁₈₀ is much reduced at the surface membrane devoid of contact points (Fig. 6 b). This phenomenon is not apparent with the H28.123 antibody (Fig. 6 d). The more uniform distribution seen with the H28.123 antibody is observed whether cells are labeled live or after fixation with ethanol, thus excluding the possibility that ethanol treatment induces a redistribution of N-CAM.

The independent regulation of the expression of the individual N-CAM components seen by immunofluorescence is demonstrated immunochemically in N2A cells induced to differentiate morphologically in the presence of DMSO and low serum concentrations (1, 18). Over a time course of several days after seeding the cells in DMSO and low serum, the N-CAM₁₄₀ band gradually disappears, whereas the concentration of N-CAM₁₈₀ changes only slightly (Fig. 7). N2A

![Figure 6](image-url) Immunofluorescence localization of N-CAM₁₈₀ in monolayer cultures of the mouse C1300 neuroblastoma clone N2A. Cells were permeabilized with ethanol and stained with N-CAM₁₈₀ antibody (b) and antibody H28.123 (d and f). In f, cultures were maintained in low serum and DMSO for 6 d to induce morphological differentiation. N-CAM₁₈₀ is predominantly localized at contact sites between neighboring cells (b), whereas antibody H28.123 shows a more uniform staining over the surface membrane (d). Note the striking restriction of N-CAM immunostaining with antibody H28.123 at contact sites between cells grown in low serum and DMSO (f). a, c, and e are the corresponding phase contrast micrographs to fluorescence images b, d, and f, respectively. Bars, 50 μm.
cells express very little, if any, of the 120-kD component. This shift is also seen when neuraminidase-treated material is analyzed, showing that the 180-kD band is not a hypersialylated form of N-CAM140 in this case. When N-CAM is visualized on DMSO-treated N2A cells by immunofluorescence staining with the H28.123 antibody, a striking concentration of N-CAM at the sites of cell to cell contact is also seen (Fig. 6, e and f). In this instance the H28.123 antibody localizes mainly the 180-kD component, the predominant form of N-CAM expressed in these differentiated cells.

N-CAM180 antibody reacts less strongly than does the H28.123 antibody with shorter, filopodia-like processes protruding from the cell bodies of N2A cells (Fig. 8, b and f). However, once neurite-like processes have extended over longer distances from the cell bodies, N-CAM180 antibody also stains these (Fig. 8, d and h).

FIGURE 7 Expression of the 140- and 180-kD components of N-CAM in mouse C1300 neuroblastoma clone N2A as a function of culture time in low serum and DMSO. Western blot analysis of Nonidet P-40 detergent extracts of cells was carried out with polyclonal N-CAM antibodies. (A) 0 h, (B) 15 h, (C) 1 d, (D) 2 d, (E) 3 d, (F) 4 d, (G) 6 d, (H) 7 d, (I) 8 d.

DISCUSSION

We have shown that the 180-kD component of N-CAM (N-CAM180) is not detectable by immunohistology in neural precursor cells in mouse cerebellum, retina, and telencephalon and appears developmentally later than either N-CAM140 or N-CAM120. In particular, the dividing precursors of granule cells in the external granular layer of the developing cerebellum do not express N-CAM180, whereas their progeny, the postmitotic granule cells of the inner zone of the external granular layer, is clearly labeled by anti-N-CAM180. This indicates that the expression of the individual N-CAM components may be independently regulated at distinct stages of the neuronal cell lineage. In line with these findings we could show by Western blot analysis that the expression of the 180- and 140-kD components is regulated according to the stage of differentiation in the N2A clone of mouse C1300 neuroblastoma. Compared with the undifferentiated cells, which express predominantly the 140-kD component, DMSO-induced differentiation leads to almost exclusive expression of N-CAM180.

Our study cannot rigorously exclude the possibility that during nervous tissue development the antigenic site of antibody N-CAM180 is inaccessible or modified in such a way that it cannot be recognized by the antibody in histological sections. Direct assessment of this possibility is, however, difficult since quantitative Western blot analysis of the individual components in isolated membrane fractions at early developmental stages is hampered by small quantities and consid-
erable heterogeneity in the available tissue. This heterogeneity does not only pertain to cell type and differentiation state of individual cells, but also to the simultaneous occurrence of adult and embryonic forms of N-CAM (4). It should be noted here that N-CAM$_{180}$ can occur both in the embryonic and adult forms of total N-CAM and that the regulation of N-CAM$_{180}$ expression is not correlated with the degree of sialylation.

In contrast to the observations in histological sections, where N-CAM$_{180}$ expression is restricted to the more differentiated subpopulations of N-CAM-positive neural cells, N-CAM$_{180}$ in vitro is expressed in all N-CAM-positive cerebellar cells. These include the major neural cell types, i.e., astrocytes, oligodendrocytes, and neurons, as has been observed previously (17a, 21, 25, 40). Since cerebellar neurons cease proliferating under monolayer culture conditions (unpublished observations). It is, therefore, conceivable that N-CAM$_{180}$ expression is not correlated with the degree of sialylation.

The fact that N-CAM$_{180}$ expression is first detectable during cytodifferentiation points to a specialized role in cell to cell interactions, as N-CAM$_{180}$ is not expressed during the first migration of granule cell precursors in the primordial external granular layer, but appears at the time of the second migration from the external to the internal granular layer. Interestingly, another neural cell adhesion molecule, the L1 antigen is also absent in the primordial and outer part of the postnatal external granular layer. It is, therefore, conceivable that N-CAM$_{180}$ and L1 antigen are not involved in the first migration, but act in concert before or during the second migration of granule cells (22). In agreement with this hypothesis is the observation that N-CAM and L1 antigen act in synergism in reaggregating cerebellar cells taken at the time of early postnatal granule cell migration (10). This interaction may be transient, since L1 antigen is no longer detectable on cell bodies of granule cells after completion of migration (36). Transient expression of cell surface glycoproteins in the early postnatal cerebellar cortex has been reported (27, 44, 45), but their functional roles and structural relationships to the known cell adhesion molecules remain to be established.

A role of N-CAM$_{180}$ in neurite extension is suggested by the observation that short, filopodia-like processes of neuroblastoma cells do not carry detectable levels of N-CAM$_{180}$, while longer, neurite-like processes do. Also, N-CAM$_{180}$ may be involved in cell contact mechanisms as suggested by the observation that at the sites of close cell apposition N-CAM$_{180}$ is more concentrated than total N-CAM, particularly in DMSO-treated morphologically differentiated neuroblastoma cells. Interestingly, the membrane–cytoskeleton linker protein fodrin is also more concentrated at contact points (unpublished observations). It is noteworthy in this respect that the monoclonal N-CAM$_{180}$ antigen resulted from an immunization of a new cell adhesion molecule that exhibits homotypic interactions (18, 99). It is on embryonic muscle cells and mediates adhesion to nerve cells in vitro. (Nature (Lond.). 259:693–695).

REFERENCES

1. Amano, T., E. Richelson, and M. Nirenberg. 1972. Neurotransmitter synthesis by neuroblastoma clones. Proc. Natl. Acad. Sci. USA. 69:258–263.

2. Keilhauer, G., A. Faissner, and R. Mailhammer. 1985. Differential inhibition of neuron, neuron-astrocyte and astrocyte-astrocyte adhesion by L1, L2 and N-CAM antibodies. Nature (Lond.). 316:725–730.

The authors are grateful to Magdalena Schlott, Sabine Ullrich, Ulrike Bauder, and Hermine Deagostini-Bazin for technical assistance, Drs. L. Eng and W. W. Franke for antibodies, Dr. B. Bizzini for tetanus toxin, Dr. J. A. Holley for a useful suggestion, Dr. C. Lagenaur for help with the membrane preparations, Dr. S. Fushiki for discussions, and Dr. A. Faissner for comments on the manuscript.

This work was supported by Deutsche Forschungsgemeinschaft (Scha 185/9-5) and the Centre National de la Recherche Scientifique (237), and Studienstiftung des deutschen Volkes for a predoctoral fellowship (to E. Pollerberg).

Received for publication 6 June 1985, and in revised form 12 July 1985.
29. Rougon, G., H. Deagostini-Bazin, M. Hirn, and C. Goridis. 1982. Tissue- and developmental stage-specific forms of a neural cell surface antigen linked to differences in glycosylation of a common polypeptide. EMBO (Eur. Mol. Biol. Organ.) J. 1:1239-1244.

30. Rutishauser, U. 1984. Developmental biology of a neural cell adhesion molecule. Nature (Lond.). 310:549-554.

31. Rutishauser, U., and G. M. Edelman. 1980. Effects of fasciculation on the outgrowth of neurites from spinal ganglia in culture. J. Cell Biol. 87:370-378.

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

33. Rutishauser, U., J.-P. Thiery, R. Brackenbury, and G. M. Edelman. 1978. Adhesion among neural cells of the chick embryo. III. Relationship of the surface molecule CAM to cell adhesion and the development of histotypic patterns. J. Cell Biol. 79:371-381.

34. Sadoul, R., M. Hirn, H. Deagostini-Bazin, G. Rougon, and C. Goridis. 1983. Adult and embryonic mouse neural cell adhesion molecules have different binding properties. Nature (Lond.). 304:347-349.

35. Schachner, M., T. Hedley-Whyte, D. Hsu, G. Schoonmaker, and A. Bignami. 1977. Ultrastructural localization of glial fibrillary acidic protein in mouse cerebellum by immunoperoxidase labeling. J. Cell Biol. 75:67-73.

36. Schachner, M., A. Fiscarelli, 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, J.-P. Thiery, and E. Gall, editors. John Wiley & Sons, Inc., New York. In press.

37. Schnitzer, J., and M. Schachner. 1981. Expression of Thy-1, H2 and NS4 cell surface antigens and tetanus toxin receptors in early postnatal and adult mouse cerebellum. J. Neuroimmunol. 1:429-456.

38. Schnitzer, J., and M. Schachner. 1981. Developmental expression of cell specific markers in the mouse cerebellar cortical cells in vitro. J. Neuroimmunol. 1:471-487.

39. 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. 90:435-475.

40. Silver, J., and U. Rutishauser. 1984. Guidance of optic axons in vivo by a preformed adhesive pathway on neuroepithelial epithelium. Dev. Biol. 106:485-499.

41. Sommer, L., and M. Schachner. 1981. Monoclonal antibodies O1 to O4 to oligodendrocyte cell surfaces: an immunocytochemical study in the central nervous system. Dev. Biol. 83:311-327.

42. Thiery, J.-B., R. Brackenbury, U. Rutishauser, and G. M. Edelman. 1977. Adhesion among neural cells of the chick embryo. J. Biol. Chem. 252:4234-4238.

43. Towbin, H., T. Staehelin, and S. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets. Proc. Natl. Acad. Sci. USA. 76:4350-4354.

44. Webb, M., and P. L. Woodhams. 1984. Monoclonal antibodies recognizing cell surface molecules expressed by rat cerebellar interneurons. J. Neuroimmunol. 6:283-300.

45. Zanetta, J. P., G. Roussel, M. S. Ghandour, G. Vincendon, and G. Gombos. 1978. Postnatal development of rat cerebellum: massive and transient accumulation of concanavalin A binding proteins in parallel fiber axolemma. Brain Res. 142:301-319.