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Polypeptide components and binding functions of neuron–glia cell adhesion molecules

(cell–cell adhesion/neuron–glia interaction/neural development/cell surface glycoproteins)

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ABSTRACT **Neuron–glia cell adhesion molecule (Ng-CAM) has previously been shown to be present exclusively on neurons and to mediate adhesion between neuronal membranes and glial cells. In the present study, its chain structure, binding functions, and relation to N-CAM (the other known CAM on neurons) were investigated further. Three polypeptide components of chicken Ng-CAM (M_r 200,000, 135,000, and 80,000) have been isolated. By using specific antisera against each component, the M_r 135,000 and M_r 80,000 components were found to cross-react antigenically with the M_r 200,000 component but not with each other. The conclusion that the M_r 135,000 and 80,000 components are structurally related to different regions of the M_r 200,000 component was further supported by the finding that ^{32}P could be incorporated *in vitro* into the M_r 200,000 and 80,000 components but not into the M_r 135,000 component. Ng-CAM appears to be involved in both neuron–glia adhesion and neuron–neuron adhesion by distinguishable mechanisms that appear to involve different sites or conformations of the molecule. Polyclonal antibodies and a monoclonal antibody against Ng-CAM both inhibited adhesion between glia and neurons derived from brain, cerebellum, and retina. In contrast, antibodies against N-CAM (which inhibit neuron–neuron adhesion) did not inhibit neuron–glia adhesion. These findings confirm the proposed function of Ng-CAM in neuron–glia adhesion. In addition, however, Ng-CAM was found to be involved directly or indirectly in neuron–neuron adhesion. Non-cross-reactive polyclonal anti-Ng-CAM and anti-N-CAM antibodies each inhibited the aggregation of neurons from whole brain and cerebellum and the inhibition was greater when both antibodies were present together. In contrast, monoclonal anti-Ng-CAM antibodies were found that inhibited neuron–glia adhesion but did not inhibit neuronal cell aggregation. The amount of Ng-CAM expressed on neurons was not directly predictive of the effect of anti-Ng-CAM antibodies on their homotypic aggregation. Although Ng-CAM and N-CAM can be expressed simultaneously on individual neurons, the ratio of N-CAM to Ng-CAM ranged from 1.5 for cerebellar cells to 10.0 for retinal cells. While, as expected, retinal cell aggregation was inhibitable only by anti-N-CAM, cerebellar cells, which expressed at least as much Ng-CAM as brain cells, showed significantly less inhibition by anti-Ng-CAM antibodies. These findings raise the possibility that Ng-CAM may actually interact with N-CAM to yield non-linear effects. That Ng-CAM and N-CAM may function differently *in vivo* was suggested by their distribution in sections of brain regions. Within the cerebellum, for example, immunofluorescent anti-N-CAM staining was relatively uniform in all layers; in contrast, anti-Ng-CAM staining was absent on dividing external granule cells and was present in greatest abundance on processes of post-mitotic migratory cells in the molecular layer. These observations are consistent with the hypothesis that Ng-CAM mediates neuron–glia adhesion and is thereby also involved in neuronal migration along radial glial cells.**

Cell–cell adhesion is a major primary process leading to pattern formation within neural tissues. Quantitative assays have been applied to the molecular analysis of adhesion, revealing the existence of separate molecules involved in mechanisms of neuron–neuron and neuron–glia binding in vertebrate species (1–6). Neuron–neuron interaction is mediated by an unusual cell surface glycoprotein containing large amounts of polysialic acid (7); this molecule, called N-CAM (7), forms *trans* homophilic bonds (N-CAM to N-CAM in apposing cells). Neuron–glia interaction is mediated in part by another neuronal cell surface glycoprotein, Ng-CAM, which consists of a number of polypeptide components (4). Ng-CAM binding has been postulated to be *trans* heterophilic because Ng-CAM is either absent or present in very small amounts on glial surfaces (4, 5).

N-CAM and Ng-CAM can occur simultaneously on individual neurons but appear in very different temporal sequences during embryonic development and have different distributions in cells derived from different germ layers. N-CAM, like L-CAM (the liver CAM), appears very early in embryonic development and derives from more than one germ layer (8, 9); for this reason, it has been termed a primary CAM (10). In contrast, Ng-CAM, a secondary CAM, appears first in post-mitotic neurons during periods of fiber tract extension and neuronal migration and is derived only from neuroectoderm (25). In some areas of the central nervous system, a striking difference is seen between N-CAM and Ng-CAM distribution on individual cells; N-CAM appears on both the cell body and processes, whereas Ng-CAM appears mainly on neurites corresponding to axons.

In the present investigation, we define further the relationship among the three polypeptide components of Ng-CAM and provide evidence that one basis for the antigenic cross-reactivity of Ng-CAM with N-CAM (4) rests in determinants present on their N-linked oligosaccharides. Using a cell–cell adhesion assay rather than a vesicle–cell adhesion assay (5), we have confirmed the role of Ng-CAM in neuron–glia binding. Most significantly, we have found that, while Ng-CAM mediates neuron–glia binding, it is also involved along with N-CAM in neuron–neuron interaction by a mechanism distinguishable from that mediating neuron–glia binding. A distinctive functional role for each of the different CAM mechanisms in neural tissue is suggested by immunohistochemical data showing a striking difference in N-CAM and Ng-CAM distribution on cerebellar cells during development.

MATERIALS AND METHODS

Preparation of Antibodies and Ng-CAM. Ng-CAM was immunoaffinity purified from Nonidet P-40 extracts of 14-day

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Abbreviations: CAM, cell adhesion molecule; Ng-CAM, neuron–glia CAM; N-CAM, neural CAM; L-CAM, liver CAM.

chicken embryo brain membranes or from 9-day brain tissue cultured in media containing $^{32}PO_4$ (11) using monoclonal antibody 16F5 coupled to Sepharose CL-2B (4); N-CAM that copurified with Ng-CAM was removed using monoclonal antibody anti-N-CAM no. 1 coupled to Sepharose CL-2B (7). Polyclonal antisera to Ng-CAM were obtained from rabbits immunized with this material. Ng-CAM was also fractionated on NaDodSO$_4$/polyacrylamide gels; segments of the gel containing the M_r 200,000, 135,000, and 80,000 components were individually excised, homogenized, and injected into rabbits. Rabbit antibodies to N-CAM (7) and to brain membranes (5), monoclonal antibodies to N-CAM (7) and to Ng-CAM (4, 5), and IgG fractions and monovalent Fab' fragments (2) were prepared as described. Anti-(brain membrane) antibodies were depleted of anti-N-CAM and anti-Ng-CAM antibodies by incubation with N-CAM or Ng-CAM immobilized onto Sepharose CL-2B (12).

Adhesion Assays. Heterotypic binding of suspensions of fluorescein-labeled neurons to monolayers of glial cells (13) obtained from 9-day chicken embryo forebrains (1×10^7 neurons per 35-mm dish) was performed by using neurons obtained from chicken embryo retina (9-day), whole brain (9-day), and cerebellum (14-day) using 0.002% trypsin/1 mM EDTA (13, 14). Aggregation of cells in suspension was measured as described (2, 4). When cells were co-incubated with both monoclonal and polyclonal antibodies against Ng-CAM, the monoclonal antibodies (1 mg of IgG) were added 15 min prior to addition of the polyclonal antibodies (1 mg of Fab' fragments).

Antibody Binding Assay. Anti-Ng-CAM and anti-N-CAM antibodies were labeled (15) with ^{125}I, and 0.4 mg of IgG was mixed with 5×10^6 cells in 300 μl in Eagle's minimal essential medium with spinner salts/1% bovine serum albumin; after 20 min of incubation at 4°C, the cells were washed by centrifugation through 3.5% bovine serum albumin in the medium and assayed in a γ-ray spectrometer.

Immunofluorescence Studies. Chicken cerebella were fixed with 2.5% paraformaldehyde, impregnated with sucrose, and embedded in Lipshaw compound. Frozen sections (≈2 mm × 3 mm × 10 μm) were labeled (9) for immunofluorescence with antibodies in excess. Quantitative measurements of relative fluorescence were obtained by using a Zeiss PMI photometer (40-μm diaphragm). Measurements from three different fields in each region were averaged and were expressed in arbitrary dimensionless units; the average level of N-CAM staining in the newborn chicken granular layer was defined as 1.0 unit.

RESULTS

Structural Characterization of Ng-CAM. Ng-CAM, fractionated on NaDodSO$_4$/polyacrylamide gels, contained a major component of M_r 135,000, two closely spaced components of M_r 200,000, and a diffuse component of M_r 80,000 (Fig. 1*A*, lane 1). In addition, a degradation product of M_r 60,000 arose during storage. Polyclonal rabbit antibodies to the total Ng-CAM preparation recognized all components of Ng-CAM (lane 2), but several independently derived monoclonal antibodies recognized only components of M_r 135,000 and 200,000 (lane 3). This result and electrophoretic comparisons of the fragments produced by digestion of these two Ng-CAM components with *Staphylococcus aureus* V8 protease (18) (data not shown) suggested that these two polypeptides are structurally related. Antibodies were raised against each of the components of Ng-CAM. The reciprocal recognition of the M_r 135,000 component by antibodies to the M_r 200,000 component (lane 4) and of the M_r 200,000 component by antibodies to the M_r 135,000 component (lane 5) further supported the conclusion that these components are structurally related. However, in addition to recognizing

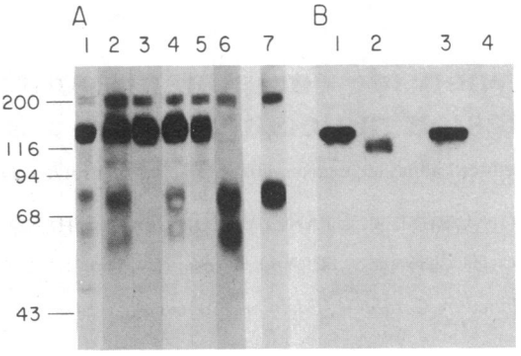

FIG. 1. Antigenic determinants and phosphorylation sites associated with Ng-CAM components. (*A*) Ng-CAM was fractionated on 7.5% polyacrylamide gels (16) and either stained with Coomassie blue (lane 1, 20 μg of protein) or transferred to nitrocellulose (lanes 2–6, 5-μg aliquots) and immunoblotted (4, 17) with polyclonal anti-Ng-CAM antibodies (lane 2), monoclonal antibody 16F5 (lane 3), and antibodies to the individually purified M_r 200,000, 135,000, and 80,000 components of Ng-CAM (lanes 4, 5, and 6, respectively). Lane 7, $^{32}PO_4$-labeled Ng-CAM. (*B*) Ng-CAM (3-μg aliquots) was incubated with no enzyme (lanes 1 and 3) or with endoglycosidase F (11) (lanes 2 and 4) and immunoblotted as above by using monoclonal antibodies 16F5 (lanes 1 and 2) or anti-N-CAM no. 5 (lanes 3 and 4). All lanes except lane 1 in *A* represent autoradiographs of immunoblots performed by using 50 μg of antibody and 1×10^6 cpm of ^{125}I-labeled (protein A). Migrations of standard proteins are indicated by their $M_r \times 10^{-3}$.

the M_r 135,000 component, antibodies to the M_r 200,000 component also recognized the M_r 80,000 component of Ng-CAM (lane 4). Most significantly, specific antibodies to this M_r 80,000 component recognized the component of M_r 200,000 but not the component of M_r 135,000 (lane 6).

Ng-CAM synthesized in brain tissue in the presence of $^{32}PO_4$ incorporated the label into Ng-CAM components of M_r 200,000 and 80,000 but not into the component of M_r 135,000 (Fig. 1*A*, lane 7). Taken together, these results support the interpretation that the M_r 135,000 and 80,000 components of Ng-CAM are both structurally related to the M_r 200,000 component but not to each other. It remains to be determined whether the larger polypeptide is the precursor of the two smaller ones or whether they are all synthesized independently.

Despite the fact that Ng-CAM and N-CAM are different both in structure and function, two independently derived monoclonal antibodies were previously found to react with both CAMs (4). The present studies indicate that one of these antibodies recognizes a determinant associated with the N-linked oligosaccharides of these proteins. Following endoglycosidase F treatment to remove N-linked oligosaccharides, the apparent M_r of the major component of Ng-CAM decreased from 135,000 to 115,000 (Fig. 1*B*, lanes 1 and 2) and recognition of Ng-CAM (Fig. 1*B*, lanes 3 and 4) by the cross-reactive monoclonal antibody anti-(N-CAM) no. 5 (4) was abolished; similar results were obtained for N-CAM. It should be noted that endoglycosidase F did not abrogate other immunoreactivities; different antibodies specific for Ng-CAM and for N-CAM were found to react to the respective CAM even after removal of the carbohydrate.

Different Roles of Ng-CAM and N-CAM in Cell–Cell Adhesion. It has been shown (13) that antibodies to N-CAM did not inhibit neuron–glia adhesion and that antibodies to Ng-CAM inhibited binding of neuronal membrane vesicles to glial cells (5). To evaluate further the role of Ng-CAM and N-CAM in cell–cell interactions, antibodies against Ng-CAM, N-CAM, and chicken brain membranes were analyzed for their capacity to inhibit neuron–glia and neuron–neuron adhesion. The binding to chicken embryo brain glial cells of

neurons prepared from 9-day brain, 9-day retina, and 14-day cerebellum was inhibited by specific monoclonal and polyclonal antibodies to Ng-CAM but not by specific monoclonal and polyclonal antibodies to N-CAM (Table 1). In some experiments, inhibition by polyclonal antibodies to Ng-CAM was as great as 90%. A mixture of antibodies to N-CAM and Ng-CAM had no greater inhibitory effect than did antibodies to Ng-CAM alone. In fact, enhanced binding was observed in the presence of antibodies to N-CAM, probably because the anti-N-CAM promoted the maintenance of the neurons as single cells.

Binding of neurons to glia has been found (13) to be inhibited by polyspecific antisera against chicken embryo brain membranes. To determine whether the antibodies to N-CAM and to Ng-CAM in these antisera were responsible for its inhibitory activity, the antisera were sequentially incubated with immobilized N-CAM and Ng-CAM to remove the respective specific antibodies. The fractions obtained after absorption had the expected specificities as determined by immunoblotting experiments (data not shown). Whereas depletion of antibodies against N-CAM from the polyspecific anti-brain membrane antisera did not deplete their inhibitory activity in the neuron–glia adhesion assay, depletion of antibodies against Ng-CAM did remove the inhibitory activity (Table 1). All of these results confirm that, in these cell preparations, neuron–glia adhesion is mediated by Ng-CAM and not by N-CAM.

The roles of N-CAM and Ng-CAM in neuron–neuron aggregation were compared in a similar fashion (Table 2). As previously described (7), both monoclonal and polyclonal antibodies to N-CAM strongly inhibited neuronal cell aggregation. In contrast, inhibition of cell aggregation by polyclonal antibodies to Ng-CAM depended on the origin of the neuronal cells: the inhibition was strongest for brain cells, weaker for cerebellar cells, and not detectable for retinal cells. A mixture of anti-N-CAM and anti-Ng-CAM antibodies was a somewhat more potent inhibitor of brain and cerebellar cell aggregation than a similar amount of either individual antibody (Table 2). Independent depletion of antibodies to N-CAM and to Ng-CAM from the polyspecific anti-brain membrane antisera confirmed that antibodies to both CAMs were contributing to the inhibition of brain cell aggregation.

We found, in contrast, that certain *monoclonal* antibodies to Ng-CAM inhibited heterotypic neuron–glia adhesion (Table 1) but not homotypic neuronal cell aggregation (Table 2).

Table 1. Heterotypic binding of neurons to glial cells

Antibody	Binding of neurons in suspension to glial cells in monolayers		
	Brain	Retina	Cerebellum
Polyclonal			
Non-immune	468 ± 20	304 ± 17	395 ± 1
Anti-Ng-CAM	191 ± 44	151 ± 4	146 ± 5
Anti-N-CAM	501 ± 21	397 ± 14	374 ± 23
Anti-N-CAM and anti-Ng-CAM	277 ± 19	205 ± 21	211 ± 22
Anti-brain membrane	290 ± 20	ND	186 ± 16
Depleted of anti-N-CAM	304 ± 26	ND	214 ± 24
Depleted of anti-N-CAM and anti-Ng-CAM	473 ± 23	ND	372 ± 10
Monoclonal			
Anti-Ng-CAM (10F6)	198 ± 21	206 ± 9	ND
Anti-N-CAM no. 1	524 ± 38	388 ± 8	ND

Binding to 1 mm^2 of the monolayer was measured (13) in duplicate samples. Note that binding of an aggregate of cells was scored as a single event. For polyclonal antibodies, 1 mg of the Fab′ fraction was used to inhibit binding. Mixtures of antibodies contained 1 mg of each Fab′ fraction. For monoclonal antibodies, 1 mg of the IgG fraction from ion-exchange columns (2) was used. ND, not done.

Table 2. Homotypic binding between neurons

Antibody	Aggregation of neurons, %		
	Brain	Retina	Cerebellum
Polyclonal			
Non-immune	35 ± 2	42 ± 2	45 ± 2
Anti-N-CAM	15 ± 2	20 ± 1	18 ± 2
Anti-Ng-CAM	17 ± 3	40 ± 1	30 ± 2
Anti-N-CAM and anti-Ng-CAM	12 ± 2	17 ± 2	14 ± 2
Anti-brain membrane	14 ± 2	24 ± 2	ND
Depleted of anti-N-CAM	22 ± 3	40 ± 1	ND
Depleted of anti-N-CAM and anti-Ng-CAM	28 ± 2	39 ± 2	ND
Monoclonal			
Anti-Ng-CAM (10F6)	34 ± 3	42 ± 2	ND
Anti-N-CAM no. 1	21 ± 2	25 ± 1	ND

Neuronal cell suspensions were incubated for 15 min at 37°C with rotation at 90 rpm and aggregation was quantitated in terms of percentage decrease in particle number (2, 4). Antibodies were as in Table 1 except that they were used at 0.5 mg per sample; mixtures of antibodies contained 0.25 mg of each antibody per sample. ND, not done.

Polyclonal anti-Ng-CAM antibodies inhibited homotypic neuron–neuron aggregation even in the presence of these monoclonal anti-Ng-CAM antibodies. These findings suggested (*i*) that Ng-CAM acts by different mechanisms in neuron–neuron and neuron–glia adhesion; (*ii*) that different sites on Ng-CAM as well as different ligands for Ng-CAM may be involved in the two cases; and (*iii*) that the presence of a small percentage (≈5%) of glial cells [estimated by their adhesion to collagen and their staining by a monoclonal antibody specific for chicken glial cells (13)] was not the cause of the inhibition of aggregation by polyclonal anti-Ng-CAM antibodies.

In order to evaluate whether the differential effects of anti-N-CAM and anti-Ng-CAM antibodies on retinal, brain, and cerebellar cell aggregation might be due simply to different surface densities of the molecules, the levels of binding of ^{125}I-labeled antibodies against N-CAM and Ng-CAM were determined for cells from each tissue (Table 3). The relatively small amount of Ng-CAM as compared to N-CAM found on retinal neurons is consistent with the fact that aggregation of retinal neurons is not inhibited by polyclonal antibodies to Ng-CAM (Table 2); it may also account for the finding that the control level of retinal cell binding to glia was lower than the level of binding to brain cells or cerebellar cells (Table 1). Nonetheless, the absolute Ng-CAM level cannot be the only factor that determines the influence of Ng-CAM on homotypic neuronal adhesion. The aggregation of cerebellar neurons was significantly less sensitive to polyclonal anti-Ng-CAM antibodies than was the aggregation of total brain neurons, even though the cerebellar cells expressed at least as much Ng-CAM as the brain cells and significantly less N-CAM than cells from the whole brain.

Table 3. Comparison of anti-Ng-CAM and anti-N-CAM binding sites on cells

Cells	Molecules of IgG bound per cell		Binding ratio (anti-N-CAM/anti-Ng-CAM)
	Anti-N-CAM ($\times 10^{-6}$)	Anti-Ng-CAM ($\times 10^{-6}$)	
Retina	2.56 ± 0.27	0.25 ± 0.01	10.0
Brain	2.66 ± 0.59	0.89 ± 0.05	3.0
Cerebellum	1.45 ± 0.28	0.98 ± 0.07	1.5

The binding was calculated from the specific activity of the antibodies and the number of cells counted using a Coulter Counter.

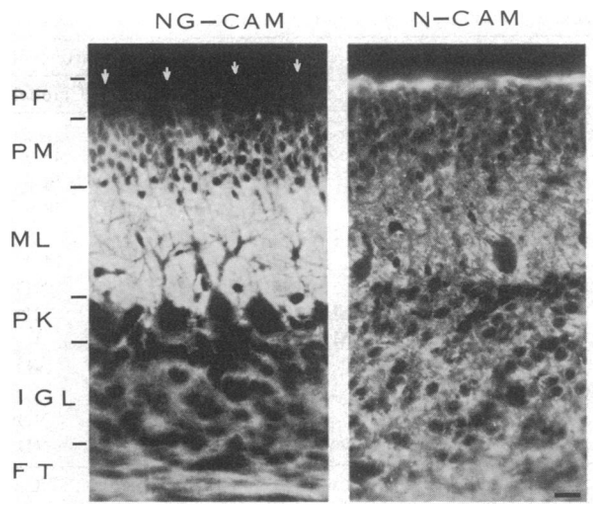

FIG. 2. Localization of Ng-CAM and N-CAM on 17-day embryonic chicken cerebellum. Frozen sections were allowed to react sequentially with rabbit anti-Ng-CAM or anti-N-CAM IgG (20 μg/ml) and fluorescein-conjugated goat anti-rabbit IgG (Miles, 1:50 dilution). Fluorescence micrographs of comparable fields are shown; treatment with preimmune sera gave no staining. Note that staining for N-CAM but not for Ng-CAM (arrows indicate the cerebellar surface) was visualized in the proliferative zone (PF) of the external granular layer. In contrast, in the molecular layer (ML), staining for Ng-CAM is more intense than for N-CAM. PM, premigratory zone; PK, Purkinje cell; IGL, internal granular layer; FT, fiber tract. (Bar = 10 μm.)

Differential Distribution of Ng-CAM and N-CAM in the Cerebellum. In the cerebellum of the chicken embryo, granule cells divide in the proliferative zone of the external granular layer and assume a bipolar shape in the premigratory zone of the external granular layer. The cell bodies then follow the leading process radially across the molecular layer to the internal granular layer (19–21). Immunofluorescence staining of cerebellar sections with anti-N-CAM and anti-Ng-CAM antibodies revealed clear differences in the localization of the two CAMs among these regions (Fig. 2). By using a semiquantitative procedure to analyze these data, Ng-CAM was found to be absent in the proliferative zone (<0.1 unit, see *Materials and Methods*), but present in the premigratory zone (6.0 units), in the internal granular layer (5.5 units), in the fiber tracts (8.6 units), and in greatest abundance (16.0 units) in the molecular layer. In contrast, N-CAM was found to be present in a more uniform manner: proliferative zone (1.6 units), premigratory zone (1.5 units), molecular layer (3.0 units), internal granular layer (2.0 units), and fiber tracts (1.1 units).

In the cerebellum of the newborn chicken, the relative intensity of Ng-CAM staining was dramatically reduced; the intensity of staining dropped sharply in the molecular layer (1.8 units) and in the white matter (1.0 units) but decreased less in the internal granular layer (2.0 units). In contrast, the levels of N-CAM staining were relatively unchanged from those found in the 17-day embryo. These data suggest that the levels of Ng-CAM are high where cell or neurite movements occur (in the molecular layer and in the fiber tracts) and that the levels of Ng-CAM are reduced following the movement period.

DISCUSSION

There are four major conclusions of the present studies: (*i*) Ng-CAM consists of three polypeptides, two of which are antigenically related to the third and largest component but not to each other. Ng-CAM differs greatly from N-CAM but shares at least one saccharide antigenic determinant with it; (*ii*) Ng-CAM unequivocally mediates binding between neuronal and glial cells; (*iii*) Ng-CAM modulates neuronal cell aggregation; whether it acts as a *trans* ligand is not yet clear, but the edvidence opens the possibility that it may interact with N-CAM either *cis* or *trans*; (*iv*) Ng-CAM is expressed on post-mitotic migratory external granule cells and its spatiotemporal distribution is consistent with the hypothesis that it is involved in the migration (21) of these neurons on Bergmann glia. Its distribution and temporal variation in different cerebellar layers both differ dramatically from those of N-CAM, which must therefore serve a different function *in vivo*.

Both the present and previous (4) studies suggest that Ng-CAM contains three separate polypeptide components. The current observation that the M_r 135,000 and 80,000 components are antigenically related to the M_r 200,000 component but not to each other is consistent with the possibility that both are derived from the larger component but by no means proves this point. In addition to the antigenic relationship between the components of M_r 200,000 and 80,000 it is significant that both are phosphorylated, whereas the component of M_r 135,000 is not. Because phosphate is most commonly located in amino acid residues on the cytoplasmic portions of transmembrane proteins (22), these results raise the possibility that the components of M_r 200,000 and 80,000 may span the plasma membrane.

Even at the present stage of knowledge, it is clear that Ng-CAM differs greatly from N-CAM. The previously described antigenic relatedness between Ng-CAM and N-CAM detected by using monoclonal antibody anti-N-CAM no. 5 (4) can be attributed to common N-linked oligosaccharides on both CAMs. The nature of the antigenic determinant recognized by the other cross-reactive monoclonal antibody, anti-N-CAM no. 4 (4), has yet to be identified.

The finding that antibodies against Ng-CAM inhibit neuron–glia adhesion, while antibodies against N-CAM do not inhibit, confirmed that Ng-CAM (but not N-CAM) is involved in the adhesion between neuronal and glial cells. In addition to their ability to perturb neuron–glia adhesion specifically, however, polyclonal anti-Ng-CAM antibodies also inhibited the homotypic aggregation of brain and cerebellar neurons (Tables 1 and 2).

One or more possibilities may account for these results: (*i*) aggregation of the cell population mediated mainly through neuron–glia adhesion with mixed aggregate formation; (*ii*) neuron–neuron adhesion involving interactions between Ng-CAM and N-CAM either at the same cell surface (*cis*) or across cell surfaces (*trans*); and (*iii*) neuron–neuron adhesion involving Ng-CAM on one neuron and a different unidentified ligand on another. Despite the presence of some glial cells in neuron-enriched suspensions, aggregation in these suspensions was not inhibited by monoclonal antibodies to Ng-CAM that do inhibit neuron–glia binding (Table 1). Moreover, the presence of anti-Ng-CAM monoclonal antibodies did not interfere with the inhibition of aggregation by the polyclonal anti-Ng-CAM antibodies. All of these observations suggest that mixed cell aggregation mediated through neuron–glia adhesion by the Ng-CAM mechanism does not occur in the neuron–enriched suspensions and that the polyclonal anti-Ng-CAM is inhibiting adhesion between neurons. The findings are consistent with the idea that different sites on Ng-CAM (or different conformations of Ng-CAM) are involved in neuron–glia adhesion and neuron–neuron interactions.

Though these results rule out the first possibility of mixed neuron–glia aggregation, the other modes of involvement of Ng-CAM in neuron–neuron adhesion have yet to be excluded. The second possibility, that Ng-CAM and N-CAM may interact on neuronal cell surfaces, takes on particular interest in the light of observations that significant amounts of N-

CAM co-purify with Ng-CAM (4) and recent observations indicating that Ng-CAM and N-CAM can bind to each other in solution (unpublished data).

Despite the fact that their mutual binding modes on neurons have not been fully defined, the different binding specificities of N-CAM and Ng-CAM confirmed here suggest that these molecules have different functional roles *in vivo*. In contrast to N-CAM and L-CAM, which appear to be involved in early morphogenesis (8–10), Ng-CAM appears to be expressed on neurons only after they have stopped dividing (Fig. 2); this fact is consistent with the conclusion that it is a secondary CAM involved mainly in histogenesis of neural tissues. The binding properties of Ng-CAM, its absence on precursors of neurons, and its timely appearance on migratory granule cells are consistent with the hypothesis that neuron–glia interaction, which is known to be a vital part of directed neuronal cell migration (19–21), obligatorily involves the Ng-CAM binding mechanism. In fact, recent experiments (unpublished data) have indicated that, when 17-day chicken embryo cerebella were incubated *in vitro*, the migration of external granule cell neurons to the internal granular layer was inhibited in the presence of Fab′ fragments of antibodies to Ng-CAM but not in the presence of various control antibodies, including antibodies to N-CAM.

Several characteristics of chicken Ng-CAM are similar to those of a molecule recently identified in the mouse and called L1 antigen (23): (*i*) each comprises three components of similar molecular weight; (*ii*) immunoblotting experiments using polyclonal antibodies to chicken Ng-CAM have shown the presence of the three Ng-CAM components in extracts of mouse brain (unpublished data); (*iii*) Ng-CAM and L1 antigen show immunolocalization specifically on neurons but not on dividing external granule cells; (*iv*) antibodies to Ng-CAM and L1 antigen inhibit cerebellar cell aggregation; (*v*) for both, different monoclonal antibodies have been found that cross-react with N-CAM: one (4) that recognized both Ng-CAM and N-CAM and the other (23) that recognized both L1 antigen and BSP-2 antigen, which has been shown to be N-CAM (24); and (*vi*) polyclonal antibodies to each molecule inhibit external granule cell migration to the internal granular layer. Although L1 antigen has not been shown to mediate neuron–glia binding, we suggest that L1 antigen is in fact mouse Ng-CAM. Additional biochemical and cell biological characterization of the Ng-CAMs in various species should enable us further to delineate their roles in adhesion and migration of neurons.

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1. Edelman, G. M. (1983) *Science* **219,** 450–457.
2. Brackenbury, R., Thiery, J.-P., Rutishauser, U. & Edelman, G. M. (1977) *J. Biol. Chem.* **252,** 6835–6840.
3. Hoffman, S. & Edelman, G. M. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 5762–5766.
4. Grumet, M., Hoffman, S. & Edelman, G. M. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 267–271.
5. Grumet, M. & Edelman, G. M. (1984) *J. Cell Biol.* **98,** 1746–1756.
6. Hoffman, S., Chuong, C.-M. & Edelman, G. M. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 6881–6885.
7. Hoffman, S., Sorkin, B. C., White, P. C., Brackenbury, R., Mailhammer, R., Rutishauser, U., Cunningham, B. A. & Edelman, G. M. (1982) *J. Biol. Chem.* **257,** 7720–7729.
8. Thiery, J.-P., Duband, J.-L., Rutishauser, U. & Edelman, G. M. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 6737–6741.
9. Edelman, G. M., Gallin, W. J., Delouvée, A., Cunningham, B. A. & Thiery, J.-P. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 4384–4388.
10. Edelman, G. M. (1984) *Annu. Rev. Neurosci.* **7,** 339–377.
11. Sorkin, B. C., Hoffman, S., Edelman, G. M. & Cunningham, B. A. (1984) *Science* **225,** 1476–1478.
12. Cuatrecasas, P. & Anfinsen, C. B. (1971) *Methods Enzymol.* **22,** 345–378.
13. Grumet, M., Rutishauser, U. & Edelman, G. M. (1983) *Science* **222,** 60–62.
14. Brackenbury, R., Rutishauser, U. & Edelman, G. M. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 387–391.
15. McConahey, P. J. & Dixon, F. J. (1966) *Int. Arch. Allergy Appl. Immunol.* **29,** 185–189.
16. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
17. Towbin, H., Staehelin, T. & Gordon, T. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4350–4354.
18. Cleveland, D. W., Fischer, S. C., Kirschner, M. W. & Laemmli, U. K. (1977) *J. Biol. Chem.* **252,** 1102–1106.
19. Mugnaini, E. (1969) in *Neurobiology of Cerebellar Evolution and Development*, ed. Llinas, R. (Am. Med. Assoc. Ed. Res. Found., Chicago), pp. 749–782.
20. Altman, J. (1972) *J. Comp. Neurol.* **145,** 353–398.
21. Rakic, P. (1972) *J. Comp. Neurol.* **141,** 283–312.
22. Wold, F. (1980) *Annu. Rev. Biochem.* **50,** 783–814.
23. Schachner, M., Faissner, A., Knise, J., Lindner, J., Meier, D. H., Rathjen, F. G. & Wernecke, H. (1983) *Cold Spring Harbor Symp. Quant. Biol.* **48,** 557–568.
24. Goridis, C., Deagostini-Bazin, H., Hirn, M., Hirsch, M.-R., Rougon, G., Sadoul, R., Langley, O. K., Gombos, G. & Finne, J. (1984) *Cold Spring Harbor Symp. Quant. Biol.* **48,** 527–537.
25. Thiery, J.-P., Delouvée, A., Grumet, M. & Edelman, G.M., *J. Cell Biol.*, in press.