

# Neural cell adhesion molecules in rodent brains isolated by monoclonal antibodies with cross-species reactivity

(neural antigens/brain development/phylogenetic relationships)

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**ABSTRACT** Previous studies in this laboratory have led to the identification and purification of a chicken cell surface protein named "neural cell adhesion molecule" (N-CAM) that is involved in neural cell–cell and neurite–neurite interactions. In the present investigation, we have found that a similar molecule exists in the mouse and have confirmed that it is also present in rat neural tissue. A monoclonal antibody to chicken N-CAM that crossreacted with mouse and rat brains and an independently derived monoclonal antibody to mouse N-CAM were used to purify an antigen from perinatal mouse and rat brains. The purified neural antigen resembles chicken N-CAM in its ability to neutralize antibodies that inhibit neural cell aggregation and also in its biochemical properties including molecular weight, sialic acid content, amino acid composition, and autoconversion to a smaller polypeptide. Like chicken N-CAM, the murine molecule is found throughout the nervous system and over the entire neuronal cell surface. These results strongly suggest that the molecule is evolutionarily related to chicken N-CAM and prompt the hypothesis that cell adhesion involving N-CAM is a fundamental mechanism existing in nervous systems of different phylogenetic classes of animals.

Adhesive interactions between the surfaces of embryonic neural cells are presumed to be important in the formation of neural tissue. A cell surface glycoprotein called neural cell adhesion molecule (N-CAM) appears to be centrally involved in such interactions. It has been shown that antibodies against chicken N-CAM disrupt neural cell aggregation (1), fasciculation of fibers growing out of isolated spinal ganglia (2), histogenesis of cultured retina (3), and neuron–muscle interactions *in vitro* (4). The present study was aimed at identifying a similar molecule in perinatal mouse and rat tissue in order to explore the generality of N-CAM-mediated phenomena, to make possible functional studies of the molecule in rodents in which genetic variants are readily available, and to develop adequate reagents for cross-species comparisons.

A murine antigen was identified by means of a monoclonal antibody that recognizes chicken N-CAM and crossreacts with both mouse and rat neural tissue. We report the purification of the antigen by using two distinct and independently derived monoclonal antibodies. This antigen appears to mediate the aggregation of mouse neural cells *in vitro* and is strikingly similar to chicken N-CAM in its biochemical and immunological properties as well as in its distribution in tissues. The results demonstrate that the calcium-independent adhesion mechanism specific for neurons and muscle cells that was originally defined in the chicken also exists in mammals.

## MATERIALS AND METHODS

**Antibodies and Affinity Purification of Antigens.** Hybridoma cells secreting monoclonal antibodies were produced ac-

cording to established procedures (5–7). Mouse P3U-1 myeloma cells and splenocytes from BALB/c mice immunized with chicken tectal membranes or affinity-purified antigen were used for hybridization. Monoclonal antibodies were used for the affinity purification of their antigens by the same method described for chicken N-CAM (7). Briefly, monoclonal antibody (80 mg) was coupled to 40 g of CNBr-activated Sepharose CL-2B (Pharmacia) (8). Brains were dissected from CD1 mice (Charles River Breeding Laboratories) ranging in age from 3 days ante partum to 5 days post partum (perinatal mice). Brains (80 ml) were homogenized with a glass Dounce homogenizer in Ca-Mg-free medium (9) containing Trasylol (200 Klett units/ml; FBA Pharmaceuticals) and DNase I (100  $\mu$ g/ml; Millipore). Plasma membranes were enriched by a one-step sucrose density gradient (0.8 M/2.25 M). The material from the interface was extracted with phosphate-buffered saline ( $P_i$ /NaCl) containing 0.5% Nonidet P-40 (British Drug House, Poole, England) and 1 mM EDTA. The extract was shaken with 40 ml of antibody-conjugated Sepharose beads. These beads were packed into a column, and the bound material was eluted with 50 mM diethylamine in the  $P_i$ /NaCl/Nonidet P-40/EDTA buffer, pH 11.8. The eluate was then neutralized and shaken with Biobeads SM-2 (Bio-Rad) to remove the detergent, dialyzed against water, and lyophilized. All procedures were carried out at 4°C.

Rabbit antibodies were produced by three successive injections of affinity-purified material at 3-week intervals using 400  $\mu$ g in complete Freund's adjuvant, 400  $\mu$ g in incomplete Freund's adjuvant, and finally 100  $\mu$ g in  $P_i$ /NaCl. The rabbits (New Zealand White) were bled 1 week after the third injection.

**Cell Aggregation Assay.** Mouse brain cells were dissociated from day 15–16 embryonic brains by digestion with trypsin (twice crystallized, 20  $\mu$ g/ml, Millipore) at 37°C for 20 min with constant shaking in a medium (SDE medium) consisting of Eagle's minimal essential medium with balanced salts for suspension culture (GIBCO), 50  $\mu$ g of DNase I per ml, and 1 mM EDTA. The tissue was successively triturated with Pasteur pipets of decreasing tip diameters. Calf serum was added to inhibit trypsin activity and the cell suspensions were centrifuged briefly (800  $\times$  g; 15 sec) to remove large clumps. The cells were pelleted (1,700  $\times$  g; 3 min), resuspended, and centrifuged in a step gradient of 3.5% and 35% isotonic albumin (Miles). The cells at the interface were recovered, washed twice in SDE medium, passed through 80- $\mu$ m nylon netting (Nitex, Tetko, Elmsford, NY), and used immediately for the aggregation assay. All procedures except the trypsinization were carried out at 4°C. This procedure yielded a debris-free suspension of single cells whose viability was >95% as measured by trypan blue exclusion.

Aggregation of cells in suspension was measured by determining the rate of decrease of particle number as described (1):

Abbreviations: N-CAM, neural cell adhesion molecule;  $P_i$ /NaCl, phosphate-buffered saline.

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$5 \times 10^6$  cells in a final volume of 2 ml of SDE medium were shaken (100 rpm; 37°C) in scintillation vials. Aliquots were taken at various times, diluted in 1% glutaraldehyde in P<sub>i</sub>/NaCl, and counted in a Cytograf (Ortho Diagnostics, Raritan, NJ).

To test for inhibition of aggregation by antibodies, cells were preincubated with Fab' fragments for 30 min at 4°C. The ability of antigen to neutralize inhibition of aggregation by the antibody was determined after prior incubation (30 min; 4°C) of the Fab' with antigen.

**Analytical Procedures.** Proteins were radiolabeled with <sup>125</sup>I by the lactoperoxidase method (10) for membrane vesicles or the chloramine-T method (11) for purified proteins. Antigens were immunoprecipitated with antibody-coated protein A-Sepharose beads (Pharmacia), released by boiling with NaDodSO<sub>4</sub>, separated by NaDodSO<sub>4</sub>/polyacrylamide gel electrophoresis (12), and visualized by autoradiography.

*Staphylococcus aureus* protease V8 peptide maps were made according to Cleveland *et al.* (13). Protein (30 μg) was resolved by electrophoresis on 7.5% NaDodSO<sub>4</sub>/polyacrylamide gels. Gels were briefly stained, and the broad band in the region of  $M_r$  200,000 was cut out and digested with 5 μg of protease V8 (Miles) in a stacking gel. The resulting peptide fragments were electrophoresed into a 15% NaDodSO<sub>4</sub>/polyacrylamide gel.

Amino acid analysis was performed on a Beckman 121M amino acid analyzer, after hydrolysis in 6M HCl at 110°C for 18 hr. *N*-Acetylneuraminic acid content was determined by the thiobarbituric acid assay (14) after hydrolysis in 0.05 M H<sub>2</sub>SO<sub>4</sub> at 80°C for 4 hr.

Neuraminidase treatment was done by incubating 200 μg of antigen with 0.5 unit of neuraminidase (*Vibrio cholerae*; Calbiochem) at 37°C for 24 hr in 500 μl of 2 mM CaCl<sub>2</sub>/0.2 mM EDTA/50 mM sodium acetate, pH 5.0. Autoconversion of antigen to lower molecular weight form (7) was detected after incubating the intact antigen (1 mg/ml) in 10 mM ammonium bicarbonate/10 mM sodium azide at 37°C for several days.

## RESULTS

**Monoclonal Antibodies to N-CAM-Like Mouse Brain Antigens.** Monoclonal antibodies were initially raised against membrane vesicles from chicken day 9 embryo optic lobes. One of the mouse hybridoma clones, 15G8, produced antibodies that recognized chicken N-CAM as shown by immunoprecipitation of iodinated purified chicken N-CAM. This monoclonal antibody immunoprecipitated material from extracts of lactoperoxidase-iodinated perinatal mouse brain vesicles which migrated as a broad band with an apparent  $M_r$  of ≈200,000 in NaDodSO<sub>4</sub>/polyacrylamide gel electrophoresis (Fig. 1). The similarity of this gel pattern to that of chicken N-CAM prompted us to purify and to characterize further the antigen recognized by 15G8 antibody in mouse brain.

A subcellular fraction enriched in plasma membrane vesicles was prepared from perinatal mouse brains on a one-step sucrose density gradient and extracted with Nonidet P-40. This extract was subjected to affinity chromatography on 15G8 antibody-Sepharose. Characteristics of a preparation from the progeny of 80 pregnant mice are shown in Table 1.

Binding of 15G8 antibody to its antigen was inhibited by high concentrations of sialic acid, and neuraminidase-treated N-CAM was not recognized by the antibody, suggesting that the specificity of this monoclonal antibody involves the sialic acid component of the neural antigen. There are indications that the linkage and amount of the sialic acid component of chicken N-CAM are unusual (7), a fact that may explain the apparent specificity of 15G8 antibody. Nevertheless, we were concerned that undetected crossreactions of the antibody with sialic acid on

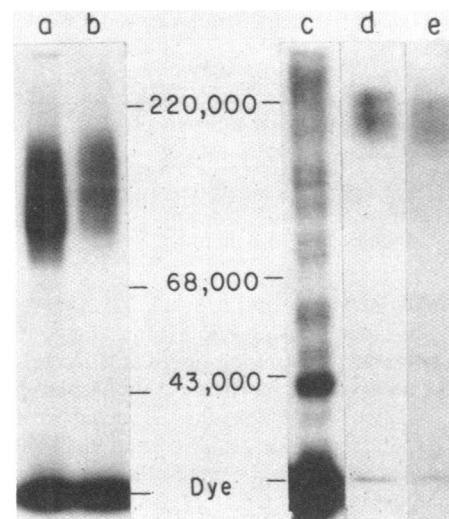


FIG. 1. Autoradiograph of immunoprecipitates resolved by 7.5% NaDodSO<sub>4</sub>/polyacrylamide gel electrophoresis. Lanes: a and b, iodinated chicken N-CAM was immunoprecipitated by a monoclonal antibody specific for chicken N-CAM (7) (lane a) and by monoclonal antibody 15G8 (lane b); c–e, iodinated embryonic mouse brain vesicles—total extract (lane c), extract immunoprecipitated by 15G8 antibody (lane d), and extract immunoprecipitated by 9E11 antibody (lane e).

other glycoproteins or glycolipids might interfere with biochemical and functional analyses. We therefore immunized mice with 15G8 antigen boiled in 2% NaDodSO<sub>4</sub> for 5 min and generated new monoclonal antibodies. One of them, designated 9E11, was an IgG that immunoprecipitated a N-CAM-like molecule from iodinated mouse brain membranes (Fig. 1) as well as from purified 15G8 antigen. The new antibody reacted with antigen that had been treated with neuraminidase or periodate, suggesting that it recognized an antigenic determinant distinct from that recognized by 15G8 antibody. Antigens purified with both antibodies, 15G8 and 9E11, were used for the cell aggregation experiments and for the biochemical studies described in detail below. The N-CAM-like molecules purified from rodent brains by the two different antibodies were similar by all the criteria used in these tests.

**Embryonic Mouse Brain Cell Aggregation.** To assay for molecules involved in neural cell adhesion, we adopted the same immunological approach used to identify chicken N-CAM (1). We first defined an *in vitro* system in which neural cell aggregation is measured by the decrease of single cells during incubation. Mouse brain cells from day 15–16 embryos were dissociated with trypsin at a low concentration (20 μg/ml) in the presence of 1 mM EDTA. Aggregation was 80% after incubation with shaking for 60 min (Fig. 2A)—i.e., the particle number counted at 60 min was only 20% of the original number. Because this aggregation occurred in the presence of EDTA, it is mediated by a calcium-independent cell adhesion mechanism (9, 15).

In this assay system, monovalent Fab' fragments prepared from the IgG of rabbits immunized with mouse brain 15G8 antigen inhibited the aggregation of embryonic mouse brain cells (Fig. 2A). Preincubation of the Fab' with 15G8 antigen decreased the inhibition of aggregation (Fig. 2B). 9E11 antigen had comparable neutralizing activity (Fig. 2C).

Together, these results suggest that the antigen isolated from mouse brain is involved in calcium-independent cell adhesion of embryonic mouse brain cells and that it in fact represents N-CAM.

Table 1. Affinity purification of 15G8 antigen from perinatal mouse brains

| Fraction                          | Protein, mg | Activity, units* | Specific activity, units/mg | Purification, -fold | Yield, % |
|-----------------------------------|-------------|------------------|-----------------------------|---------------------|----------|
| Nonidet P-40 extract of membranes | 630         | 4,851            | 7.7                         | 1                   | 100      |
| Affinity-purified 15G8 antigen    | 4           | 2,220            | 555                         | 72                  | 46       |

\* The activity of a sample was quantitated by its ability to neutralize Fab' fragments that inhibit the aggregation of mouse brain cells. One unit of activity is defined as the amount of material that produces a 25% decrease in the inhibition of adhesion caused by 1 mg of Fab' fragment (1).

**Comparison of Mouse and Chicken N-CAM.** Although the molecules purified by using either monoclonal antibody fulfilled the operational definition of N-CAM (1, 15), it was important to compare their biochemical properties with those of authentic chicken N-CAM. After electrophoresis in 7.5% NaDodSO<sub>4</sub>/polyacrylamide gel, 15G8 and 9E11 antigens from perinatal mouse brain appeared mainly as a broad, continuously stained band extending between  $M_r$  230,000 and 180,000 with some minor bands at lower  $M_r$  (Fig. 3A, lanes 2 and 3). This is similar to the pattern seen with chicken N-CAM (Fig. 3A, lane 5). Incubation of the mouse antigen in low-salt buffer at 37°C for several days in the presence of azide to inhibit bacterial growth caused a gradual conversion of the broad band around  $M_r$  200,000 into a band at  $M_r$  65,000 (Fig. 3A, lane 6). Chicken N-CAM also has this unusual property (7).

When the mouse antigen was treated with neuraminidase, several bands around  $M_r$  140,000 replaced the high molecular weight broad band (Fig. 3A, lane 7), a property shared by neuraminidase-treated chicken N-CAM (7). Boiling of both mouse 15G8 antigen and chicken N-CAM for more than 10 min also resulted in the conversion of the broadly stained band into two discrete bands (data not presented). It had been shown previously that boiling removes sialic acid from chicken N-CAM (7). The sialic acid content of the mouse antigen was high—28–35  $\mu$ g of sialic acid per 100  $\mu$ g of polypeptide—an uncommon characteristic found also in chicken N-CAM (7).

The amino acid compositions of mouse 15G8 antigen, 9E11 antigen, and chicken N-CAM are similar (Table 2). Peptide maps of mouse 15G8 and 9E11 antigens obtained after protease V8 cleavage (11) (Fig. 3B, lanes 12 and 13) also support the conclusion that both monoclonal antibodies recognize the same molecule in the mouse brain. However, as expected, the pep-

tide map of chicken N-CAM was not similar to that of the mouse antigens, implying primary amino acid sequence differences (Fig. 3B, lane 15). Nonetheless, the immunological crossreactivity—shown by the ability of rabbit antibodies against mouse 15G8 antigen to immunoprecipitate iodinated chicken N-CAM and by the ability of rabbit anti-chicken N-CAM antibody to immunoprecipitate iodinated mouse 15G8 antigen—indicates that N-CAMs from the different species share antigenic determinants.

Mouse brain N-CAM was localized in cell cultures and in tissue sections by means of indirect immunofluorescence staining with monoclonal 15G8 and 9E11 antibodies. In cultures of perinatal mouse brain cells, the cells with neuronal morphology were stained but the flat, fibroblast-like cells (which probably included glia) were not. The staining appeared to be uniform over the cell body, neurite, and growth cone (Fig. 4) and resembled the distribution found for chicken N-CAM (16). In frozen sections of perinatal mouse tissue, the antibodies stained both the central and peripheral nervous systems (not shown).

The antigens purified by using 15G8 and 9E11 antibodies were identical by all the criteria detailed above. Because these antigens are involved in cell aggregation and are biochemically and immunologically similar to chicken N-CAM, we conclude that they both are mouse N-CAM, recognized at sialic acid determinants by 15G8 and at protein or other carbohydrate determinants by 9E11.

**Rat N-CAM.** Affinity chromatography also allowed purification of 15G8 antigen from perinatal rat brain. This antigen, after electrophoresis on NaDodSO<sub>4</sub>/polyacrylamide gels, appeared mainly as a broad, continuously stained band between  $M_r$  220,000 and 180,000 (Fig. 3A, lane 4). Rat 15G8 antigen appeared to share some antigenic determinants with mouse N-

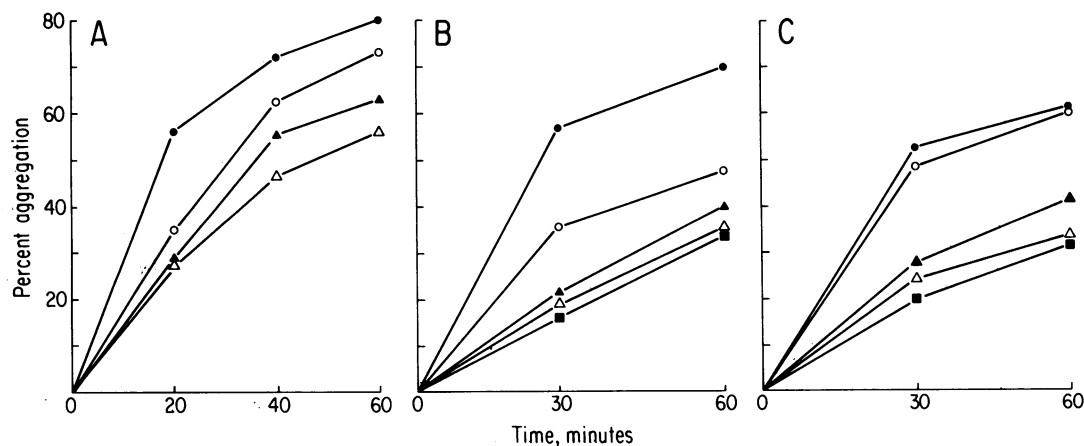


FIG. 2. Aggregation of dissociated day 16 embryonic mouse brain cells. (A) Inhibition of aggregation by rabbit antibody to 15G8 antigen. Percent aggregation is plotted as a function of time in the presence of 1 mg of normal rabbit Fab' (●) or Fab' fragments of the rabbit antibody against 15G8 antigen (○, 0.156 mg; ▲, 0.312 mg; △, 0.625 mg). (B) Neutralization by 15G8 antigen of antibody-induced inhibition of aggregation in the presence of 1 mg of normal rabbit Fab' (●), or 1 mg of Fab' fragments of rabbit antibody against 15G8 antigen preincubated with buffer (■) or 15G8 antigen (△, 1.25  $\mu$ g; ▲, 2.5  $\mu$ g; ○, 5.0  $\mu$ g). (C) Neutralization by 9E11 antigen of antibody-induced inhibition of aggregation in the presence of 1 mg of normal rabbit Fab' (●) or 1 mg of Fab' fragments of rabbit antibody against 15G8 antigen preincubated with buffer (■) or 9E11 antigen (△, 0.7  $\mu$ g; ▲, 2.1  $\mu$ g; ○, 6.3  $\mu$ g).

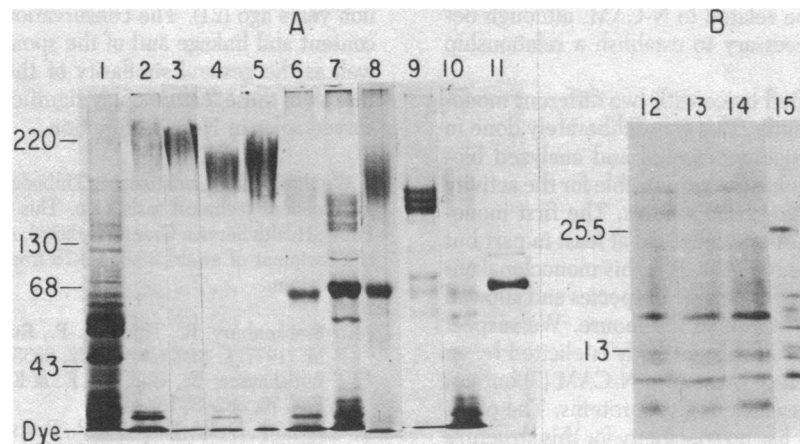


FIG. 3. NaDodSO<sub>4</sub> gel electrophoresis of 15G8 antigens stained with Coomassie blue. (A) In 7.5% gel. Lanes: 1, perinatal mouse brain membrane vesicles; 2, affinity-purified mouse 15G8 antigen; 3, affinity-purified mouse 9E11 antigen; 4, rat 15G8 antigen; 5, chicken N-CAM; 6, autoconversion products of mouse 15G8 antigen; 7, neuraminidase-treated mouse 15G8 antigen (the bands at  $M_r$  90,000 and 68,000 are from the enzyme solution, as shown in lane 11 which contained enzyme only); 8, autoconversion product of rat 15G8 antigen; 9, neuraminidase-treated rat 15G8 antigen; 10, mouse 15G8 antigen incubated in parallel with material in lane 7 in the same buffer without neuraminidase (control). (B) Results of protease V8 digestion of mouse 15G8 antigen (lane 12), mouse 9E11 antigen (lane 13), rat 15G8 antigen (lane 14), and chicken N-CAM (lane 15). The products were electrophoresed on a 15% NaDodSO<sub>4</sub> gel.  $M_r$ s are shown  $\times 10^{-3}$ .

CAM. In the *in vitro* aggregation of embryonic mouse brain cells, the rat antigen neutralized the inhibitory activity of Fab' fragments prepared from rabbit antibodies against mouse 15G8 antigen. Embryonic rat 15G8 antigen also had a high sialic acid content (43  $\mu$ g of sialic acid per 100  $\mu$ g of polypeptide) and amino acid composition (Table 2), protease V8 peptide map (Fig. 3B, lane 14), and other biochemical properties (Fig. 3A, lanes 8 and 9) similar to those of mouse N-CAM. Immunofluorescence staining of cultured perinatal rat brain cells with the monoclonal 15G8 antibody showed that the antigen is on the surface of neuronal cells (not shown). We therefore also conclude that rat brain 15G8 antigen is rat N-CAM.

### DISCUSSION

The experiments described here demonstrate the existence of a large sialoglycoprotein on the surface of mouse brain cells that

Table 2. Amino acid composition of N-CAM molecules

| Amino acid | mol/100 mol of amino acid |                    |                  |                |
|------------|---------------------------|--------------------|------------------|----------------|
|            | Mouse 15G8 antigen        | Mouse 9E11 antigen | Rat 15G8 antigen | Chicken N-CAM* |
| Asx        | 10.2                      | 10.4               | 10.8             | 9.5            |
| Thr        | 7.4                       | 7.2                | 6.5              | 7.2            |
| Ser        | 8.4                       | 8.9                | 8.5              | 9.1            |
| Glx        | 12.1                      | 13.6               | 13.9             | 12.5           |
| Pro        | 6.3                       | 6.9                | 6.0              | 7.1            |
| Gly        | 7.6                       | 7.6                | 8.9              | 5.9            |
| Ala        | 8.5                       | 8.4                | 7.5              | 7.9            |
| Cys        | ND                        | ND                 | ND               | 1.7            |
| Val        | 9.9                       | 7.3                | 7.0              | 8.4            |
| Met        | 1.5                       | 1.5                | 1.5              | 1.1            |
| Ile        | 4.7                       | 5.3                | 5.4              | 6.4            |
| Leu        | 7.4                       | 6.5                | 6.8              | 6.2            |
| Tyr        | 2.3                       | 2.5                | 2.7              | 2.7            |
| Phe        | 3.0                       | 2.9                | 2.9              | 2.6            |
| Lys        | 6.1                       | 7.0                | 6.6              | 6.4            |
| His        | 1.4                       | 1.4                | 1.5              | 1.1            |
| Arg        | 3.1                       | 3.2                | 3.5              | 2.7            |
| Trp        | ND                        | ND                 | ND               | 1.6            |

ND, not determined.

\* From Hoffman *et al.* (7).

is involved in cell-cell adhesion and that is functionally analogous and structurally similar to chicken N-CAM. This conclusion is based on the following data: (i) The molecule neutralizes antibodies that inhibit the aggregation of mouse neural cells. (ii) N-CAMs of mouse and chicken crossreact immunologically. (iii) The amino acid compositions and high sialic acid contents of the molecules are similar. (iv) Mouse N-CAM properties in NaDodSO<sub>4</sub>/polyacrylamide gel electrophoresis are similar to those of chicken N-CAM—migration as a broad band around an apparent  $M_r$  200,000 that is converted to distinct bands of lower molecular weight after treatment with neuraminidase. The precise relationship among the different bands seen in NaDodSO<sub>4</sub>/polyacrylamide gel electrophoresis is not known, although analogous bands in chicken N-CAM have been shown to be structurally related (7). (v) Mouse N-CAM shares with chicken N-CAM the property of being largely converted to a  $M_r$  65,000 polypeptide upon incubation at 37°C. (vi) The antigen is localized on the entire cell membrane of neuronal cells and is distributed in all regions of the nervous system. Other workers (17, 18) have described rodent neuronal cell surface proteins

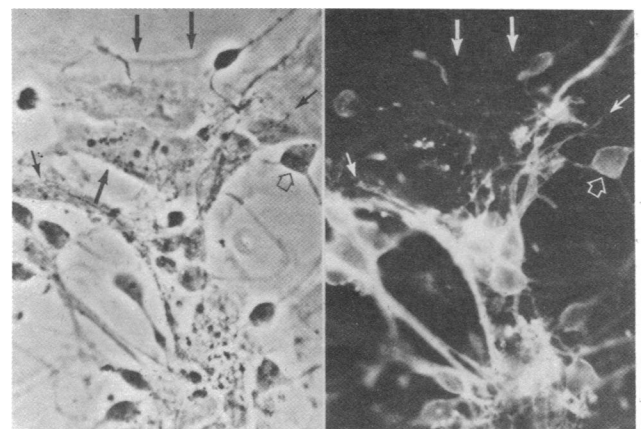


FIG. 4. Phase-contrast (Left) and immunofluorescence (Right) images of mouse brain cell cultures stained by 15G8 antibody. The neuronal cell bodies (open arrow), neurites, and growth cones (thin arrows) are stained; the flat cell (thick arrows) is not stained. ( $\times 410$ .)

which, we surmise, could be related to N-CAM, although detailed functional studies necessary to establish a relationship have not been reported.

Mouse N-CAM was purified twice with two different monoclonal antibodies independently. This was deliberately done in order to verify that the antigens detected and analyzed biochemically were the same molecules responsible for the activity observed in the *in vitro* aggregation studies. The first monoclonal antibody, 15G8, recognizes sialic acid at least in part but apparently is still specific for N-CAM. It is this monoclonal antibody that crossreacts with a wide range of species and allowed the initial identification of N-CAM in the mouse. We suspect that the particular specificity of this antibody is elicited by an unusual polysialic acid structure present in N-CAM (7) but not in most other mammalian or chicken glycoproteins. The other monoclonal antibody, 9E11, has no specificity for this structure and therefore is directed mainly against another antigenic determinant of N-CAM.

We also have confirmed the existence of N-CAM in the rat. The 15G8 antigen purified from perinatal rat brain neutralizes the inhibitory activity of antibodies in a mouse neural cell aggregation assay and behaves like other N-CAMs in NaDodSO<sub>4</sub>/polyacrylamide gel electrophoresis. It has been reported that rat neural tissue contains an antigen called "D2" which has been shown to crossreact immunologically with chicken N-CAM (19). As reported for chicken (2), antibodies against D2 were found to inhibit neurite fasciculation in cultured rat sympathetic ganglia (19). These results suggested that D2 is related to rat N-CAM but the precise relationship has not been confirmed.

Because the neurophysiology and genetics of the mouse have been described more extensively than those of the chicken, the discovery of N-CAM in the mouse provides new and special opportunities for studying N-CAM function. Several defined neurological and developmental mouse mutants have been described (20), and it will be of particular interest to analyze the expression and structure of N-CAM at different developmental stages of these mutants. Moreover, neural cell lines of mouse and rat, analogues of which are not available for the chicken, can be used for the study of N-CAM expression at various states of differentiation.

The identification of N-CAM in the mouse and rat as well as in the chicken implies that this molecule's function has been preserved through evolution since the divergence of avian and mammalian classes in the Carboniferous period about 300 mil-

lion years ago (21). The conservation of the unusual sialic acid content and linkage and of the spontaneous autoconversion as well as the general similarity of these polypeptides indicates that all of these features play significant and evolutionarily conserved roles in N-CAM function.

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1. Brackenbury, R., Thiery, J.-P., Rutishauser, U. & Edelman, G. M. (1977) *J. Biol. Chem.* **252**, 6835-6840.
2. Rutishauser, U., Gall, W. E. & Edelman, G. M. (1978) *J. Cell Biol.* **79**, 382-393.
3. Buskirk, D. R., Thiery, J.-P., Rutishauser, U. & Edelman, G. M. (1980) *Nature (London)* **285**, 488-489.
4. Grumet, M., Rutishauser, U. & Edelman, G. M. (1982) *Nature (London)* **295**, 693-695.
5. Köhler, G. & Milstein, C. (1975) *Nature (London)* **256**, 495-497.
6. Marshak-Rothstein, A., Pink, P., Gridley, T., Ranlet, D. H., Bevan, M. J. & Geffer, M. L. (1979) *J. Immunol.* **122**, 2491-2497.
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.*, in press.
8. Cuatrecasas, P. & Anfinsen, C. E. (1971) *Methods Enzymol.* **22**, 345-378.
9. Takeichi, M. (1977) *J. Cell Biol.* **75**, 464-474.
10. Hubbard, A. L. & Cohn, Z. A. (1972) *J. Cell Biol.* **55**, 390-405.
11. McConahey, P. J. & Dixon, F. J. (1966) *Int. Arch. Allergy Appl. Immunol.* **29**, 185-189.
12. Laemmli, U. K. (1970) *Nature (London)* **227**, 680-685.
13. Cleveland, D. W., Fischer, S. G., Kirshner, M. W. & Laemmli, U. K. (1977) *J. Biol. Chem.* **252**, 1102-1106.
14. Warren, L. (1959) *J. Biol. Chem.* **234**, 1971-1975.
15. Brackenbury, R., Rutishauser, U. & Edelman, G. M. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 387-391.
16. Rutishauser, U., Thiery, J.-P., Brackenbury, R. & Edelman, G. M. (1978) *J. Cell Biol.* **79**, 371-381.
17. Rohrer, H. & Schachner, M. (1980) *J. Neurochem.* **35**, 792-803.
18. Hirn, M., Pierres, M., Deagostini-Bazin, H., Hirsch, M. & Goriidis, C. (1981) *Brain Res.* **214**, 433-439.
19. Jørgensen, O. S., Delouvé, A., Thiery, J.-P. & Edelman, G. M. (1980) *FEBS Lett.* **111**, 39-42.
20. Sidman, R. L., Green, M. C. & Appel, S. H. (1965) *Catalog of the Neurological Mutants of the Mouse* (Harvard Univ. Press, Cambridge, MA).
21. Romer, A. S. (1968) *The Progression of Life* (World, Cleveland, OH).