Individual Neural Cell Types Express Immunologically Distinct N-CAM Forms

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ABSTRACT The neural cell adhesion molecules, or N-CAMs, are a group of structurally and immunologically related glycoproteins found in vertebrate neural tissues. Adult brain N-CAMs have apparent molecular weights of 180,000, 140,000, and 120,000. In this article we identify, using monoclonal antibody (Mab) 3G6.41, an immunologically distinct adult rat N-CAM form and show that this form is selectively expressed by some clonal neural cell lines. Consecutive immunoprecipitation experiments indicate that rabbit anti-N-CAM can remove from solubilized cerebellar neuron primary cultures all 180,000- and 140,000-mol-wt N-CAM molecules that react with Mab 3G6.41. However Mab 3G6.41 cannot remove all N-CAM molecules that react with rabbit anti-N-CAM. Rabbit anti-N-CAM binds to and immunoprecipitates N-CAM forms from the rat neuronal cell lines B35, B65, and B104, the glial lines B12 and C6, and L6 myoblasts. Mab 3G6.41 does not bind to or immunoprecipitate N-CAM from the B12 and B65 lines but does react with the other four lines by both criteria. Many cells in primary cultures of postnatal rat that express glial fibrillary acidic protein also bind Mab 3G6.41. Thus a unique form of rat N-CAM recognized by Mab 3G6.41 is found on some but not all neuronal, glial, and muscle cells.

Cell–cell interaction is of central importance to the establishment and maintenance of functional connections in the developing nervous system. Specific adhesion between cells has been proposed as the basis for precise neural connectivity (32). The BSP-2 glycoproteins originally identified using a monoclonal antibody against neonatal mouse brain glycoprotein extracts (18) have been implicated in the cell–cell adhesion of neurons (29). BSP-2 is composed of a group of related glycoproteins with apparent molecular weights (M_r's) of 180,000, 140,000, and 120,000, in the adult mouse cerebellum (17) and is immunologically similar or identical to the previously identified chick neural cell adhesion molecules or N-CAMs[1] (6, 7, 12) and the mammalian D2 protein (17). Hence, for uniformity of terminology these mammalian proteins will also hereafter be called N-CAM. It has been suggested that control of the specificity of cell–cell interactions involving these molecules may be due either to temporal expression of N-CAM or to alterations in the structure of the expressed molecules (9).

In embryonic brain N-CAM is found as a unique polysialylated form of the adult polypeptide (10, 11, 26). Liposomes containing sialylated N-CAM show less binding to N-CAM on cell surfaces than do liposomes with nonsialylated N-CAM (19, 29). This structural and potential functional difference between embryonic and adult N-CAM forms can be assayed immunologically with a monoclonal antibody (Mab) that binds to the polysialyl chains (6). The adult 180,000-, 140,000-, and 120,000-mol-wt forms have been shown to vary primarily in the size of their cytoplasmic domain (13). However it is not clear whether each of these molecular weight classes represents a single polypeptide or a group of polypeptides of similar M_r. Likewise, the immunologic relationships among the molecular weight forms of adult N-CAM are less clear. Recently differences in the reactivity of Mab's with adult N-CAM from brain and muscle or various brain regions have been reported (6).

Two of us (Drs. Williams and Akeson) have reported that the monoclonal antibody 3G6.41 immunoprecipitated 185,000- and 140,000-M_r polypeptides from iodinated cere-

[1] *Abbreviations used in this paper:* DME, Dulbecco's modified Eagle's medium; Mab, monoclonal antibody; N-CAM, neural cell adhesion molecule; Ng-CAM, polypeptide that may mediate the adhesion between neurons and glia.

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bellar neuronal cultures (34). We report here that the 185,000- and 140,000 molecules recognized by Mab 3G6.41 are a subset of those precipitated by rabbit anti-N-CAM from rat primary neuronal cultures. Some individual neural clonal cell lines do not express the form(s) of N-CAM that reacts with Mab 3G6.41 whereas other lines express N-CAM forms that react with both rabbit anti-N-CAM and Mab 3G6.41.

MATERIALS AND METHODS

Cell Cultures: Clonal cell lines B12, B35, B65, B104, and L6 (generously donated by Dr. David Schubert, Salk Institute) and the C6 cell line (from American Type Culture Collection, Rockville, MD) were maintained in Dulbecco's modified Eagle's medium (DME) with 10% fetal calf serum. Postnatal cerebellar cultures dissociated as previously described (34) were plated in a 1:1 mix of Ham's F12 media and DME with 10% horse serum and 24 mm K^+ at a density of 10^7 cells in poly-l-lysine-coated (3) 60-mm plastic petri dishes (Falcon Labware Div., Becton-Dickinson & Co., Oxnard, CA).

Antibodies and Assay of Antibody Binding to Cells: The monoclonal antibody 3G6.41, isotype $\gamma 2b, \kappa$ was produced from the fusion of X63-Ag8.653 myeloma cells (653 myeloma; from Dr. Daryl Doyle, State University of New York at Buffalo) with spleen cells from BALB/c × C57BL6 F_1 (Jackson Laboratory, Bar Harbor, ME) mice immunized with adult rat forebrain synaptic plasma membranes as described (34). Polyclonal antiserum against N-CAM polypeptides was generated in rabbits using as an immunogen rat N-CAM that had been affinity purified with a monoclonal anti-N-CAM column (12, 13). This serum blocks cell-cell aggregation. As compared with a rabbit anti-mouse N-CAM serum (7; the gift of Dr. G. M. Edelman, The Rockefeller University) both sera totally remove all iodinated (12) or [^{3}H]-methionine-labeled (Goridis, C., unpublished data) molecules that react with the other sera. Some earlier articles used the acronym BSP-2 for the N-CAM molecules, but BSP-2 and mammalian N-CAM are immunologically identical (12). Hence we will use the term N-CAM here.

Cultured cells were removed with EDTA in pH 7.4 phosphate-buffered saline and washed in 0.01 M Tris HCl pH 7.4, 0.15 M NaCl with 0.5% bovine serum albumin. We quantitated binding of Mab 3G6.41 and anti-BSP-2 sera using ^{125}I-labeled protein A in the previously described antibody-excess assay (25). Double-label immunofluorescence analysis of antibody binding using Mab 3G6.41 and anti-glial fibrillary acidic protein sera (Dako Corp., Santa Barbara, CA) was performed as previously described (1).

Polypeptide Labeling and Immunoprecipitation: Cell-surface radioiodination of clonal cell lines and primary cultures, detergent solubilization of membranes, and immunoprecipitations were done as described (34). Postnatal day 7 cerebellar cultures (prepared as above) were biosynthetically labeled by replacement of the serum-containing medium with DME containing only 1 mg/ml glucose and the supplements described for serum-free culture of neurons (5). After 3 d in culture, 20 μCi of [^{3}H]fucose (ICN Pharmaceuticals Inc., Irvine, CA) were added to the cultures in 2 ml of this serum-free medium. After 43 h of incubation, the cell membranes were solubilized and polypeptides were immunoprecipitated as above.

Gel Electrophoresis and Immunoblotting: Immunoprecipitates were boiled for 5 min in sample buffer (containing 5% 2-mercapto-ethanol) and electrophoresed on 6% running gels by the method of Laemmli (20). Autoradiography (34) and fluorography (21) of gels were done as described.

For immunoblotting proteins from adult rat whole brain membranes were solubilized in 1% Nonidet P-40, immunoprecipitated with monoclonal antibody 3G6.41 as described (34), resolved on SDS gels, and electrophoretically transferred to nitrocellulose strips (33). Immunoblotting of the strips with Mab P61 was done as described (12) except that bound Mab P61 was detected using rabbit anti-rat 1/300 dilution followed by ^{125}I protein A 1.6×10^6 cpm/ml (specific activity, 7.7×10^6 cpm/μg protein).

RESULTS

Relationship of Polypeptides Immunoprecipitated by Mab 3G6.41 and Anti-N-CAM

We identified the polypeptides that bind Mab 3G6.41 by lactoperoxidase cell-surface labeling of viable neuronal postnatal rat cerebellum cultures and subsequent solubilization of membrane proteins in nonionic detergent and immunoprecipitation. Mab 3G6.41, but not control ascites containing Mab of the same isotype, precipitated a pair of polypeptides with M_r's of 185,000 and 140,000 (Fig. 1, lanes *A* and *B*). Both polypeptides are glycoproteins since they can also be immunoprecipitated from [^{3}H]fucose biosynthetically labeled cerebellar primary culture (Fig. 1, lanes *C* and *D*). The M_r's and glycoprotein nature of the polypeptides immunoprecipitated by 3G6.41 suggest these molecules may be similar or identical to the previously described N-CAM (10, 17) class of neuronal cell surface macromolecules.

To compare directly the relationship between these 3G6.41 140,000- and 180,000-mol-wt proteins with the previously described 140,000- and 180,000-mol-wt N-CAM polypeptides, we performed sequential precipitations. In this approach

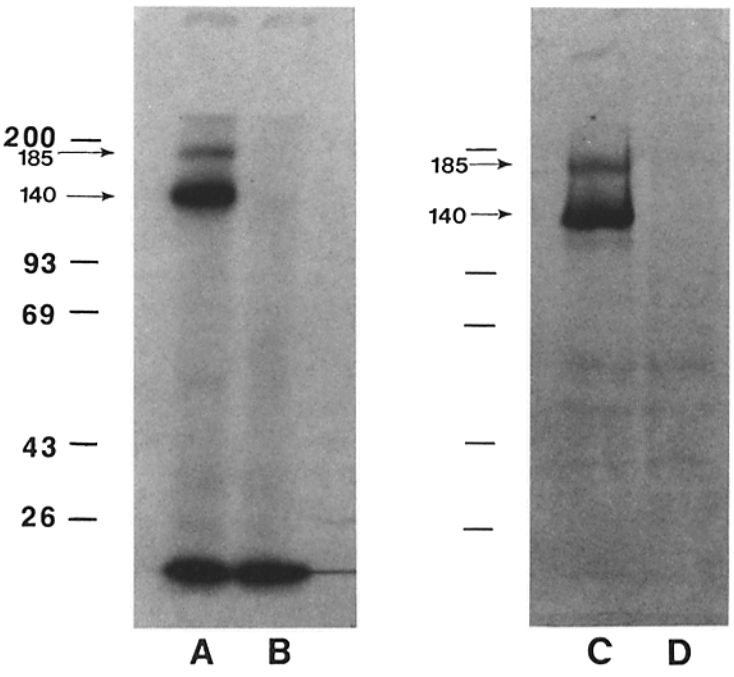

FIGURE 1 Autoradiographs of polypeptides from cerebellar primary cultures lactoperoxidase cell-surface labeled with ^{125}I (*left*) or biosynthetically labeled using [^{3}H]fucose (*right*) then solubilized and immunoprecipitated with Mab 3G6.41 (lanes *A* and *C*) or control ascites containing an Mab of the same isotype as Mab 3G6.41 (lanes *B* and *D*). Larger numbers to left of figure indicate position of molecular weight markers in thousands. 200,000 mol wt, myosin heavy chain; 93,000 mol wt, phosphorylase B; 69,000 mol wt, bovine serum albumin; 43,000 mol wt, ovalbumin; and 26,000 mol wt, α-chymotrypsinogen.

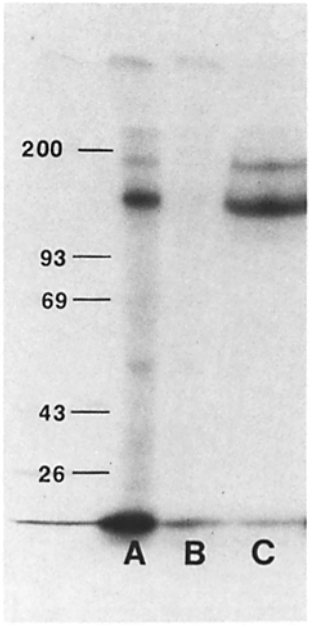

FIGURE 2 Sequential immunoprecipitation of 3G6.41 and then N-CAM antigens. ^{125}I-labeled cerebellar culture lysates were immunoprecipitated with 5 μl Mab 3G6.41 (lane *A*). Supernatant from the first precipitation was immunoprecipitated with two more rounds of 5 μl Mab 3G6.41. The third immunoprecipitate had no bands at M_r 140,000 and 180,000 (lane *B*). Rabbit anti N-CAM (5 μl) was able to precipitate additional M_r 140,000 and 180,000 bands from the supernatant remaining after these three rounds of immunoprecipitation with 3G6.41 (lane *C*).

three rounds of precipitation with Mab 3G6.41 were followed by precipitation of material remaining in the final 3G6.41 supernatant with rabbit anti-N-CAM. Mab 3G6.41 reactive molecules were completely removed by two rounds of precipitation with Mab 3G6.41 (Fig. 2, lane *A*) since the third round of 3G6.41 showed no precipitate (Fig. 2, lane *B*). A fourth round of precipitation using *Staphylococcus aureus* suspension alone to remove possible residual 3G6.41 antibody molecules also had no immunoprecipitated bands (not shown). When this final supernatant was immunoprecipitated with anti-N-CAM a significant amount of N-CAM polypeptides was found even after maximal precipitation with 3G6.41 (Fig. 2, lane *C*). The polypeptides precipitated by Mab 3G6.41 and anti-N-CAM have indistinguishable M_r's in this and other experiments (below), which suggests their identity. However Mab 3G6.41 appears to recognize only a portion of the total population of 140,000- and 180,000-mol-wt polypeptides that are recognized by the rabbit anti-N-CAM.

In a reciprocal experiment rabbit anti-N-CAM or nonimmune rabbit serum was used for three consecutive immunoprecipitations of iodinated rat cerebral cultures (Fig. *3*, panels *1–3*). After clearance with *S. aureus*, a final immunoprecipitation with Mab 3G6.41 gave no bands from anti-N-CAM-pretreated supernatants (broken line, panel *5* of Fig. 3). Large amounts of the 3G6.41 reactive bands were left if the first immunoprecipitations were done with nonimmune rabbit serum (solid line, panel *5* of Fig. 3). Therefore all the 3G6.41 reactive molecules from these primary cultures were immunoprecipitated by rabbit anti-N-CAM.

For an additional test of the relationship of the polypeptides precipitated by Mab 3G6.41 and previously defined N-CAMs, we used Mab P61, which has been shown to react exclusively with N-CAMs (12). Immunoprecipitates of detergent-solubilized unlabeled rat brain proteins were made with Mab 3G6.41 or normal mouse sera, electrophoresed, and transferred to nitrocellulose for immunoblotting. Mab P61 reacts strongly with the 140,000- and 180,000-mol-wt bands precipitated by Mab 3G6.41 (Fig. 4), indicating these molecules express this previously defined N-CAM antigenic site.

Comparison of Mab 3G6.41 and Anti-N-CAM Binding and Immunoprecipitated Polypeptides with Clonal Neural Cell Lines

Since it appeared that Mab 3G6.41 may recognize a subset of the anti-N-CAM-reactive molecules we undertook the screening of nervous system cell lines of various phenotypes to determine if 3G6.41 reactivity was differentially distributed among anti-N-CAM positive cell lines. We also examined a

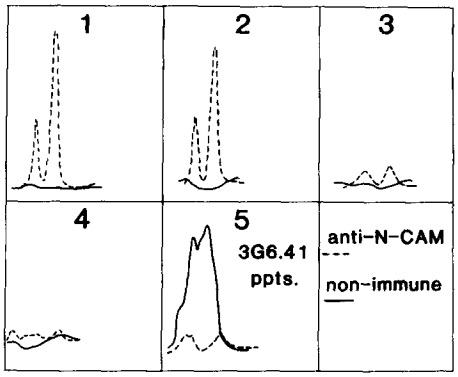

FIGURE 3 Sequential immunoprecipitation of N-CAM and then 3G6.41 antigens. ^{125}I-labeled embryonic rat whole brain cultures were immunoprecipitated with three consecutive aliquots of anti-N-CAM (dashed lines, panels *1–3*) to remove all N-CAM molecules or nonimmune rabbit serum (solid lines) and then with *S. aureus* alone (panel *4*) as in Fig. 2. Each final supernatant was then precipitated with Mab 3G6.41. Panel *5* shows that N-CAM-like components are precipitated by 3G6.41 after initial precipitations with nonimmune serum (solid line) but no 3G6.41 reactive molecules are left after initial precipitations with anti N-CAM (dashed line). Density scans are in arbitrary units and were performed on films after 24 (panel *1*) or 112 h of exposure (panels *2–5*). Panel *1* was also scanned at twofold lesser sensitivity so the peak heights in panel *1* are actually ~8 times those in panels *2–5*. Immunoprecipitates were made by mixing preformed *S. aureus*-antibody complexes with the detergent soluble material for 2 h for each consecutive precipitate.

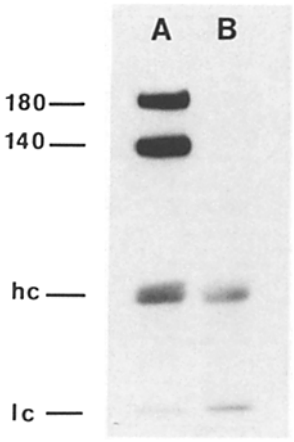

FIGURE 4 Monoclonal anti-N-CAM P61 immunoblotting of 180,000 and 140,000-mol-wt polypeptides immunoprecipitated by Mab 3G6.41. Immunoprecipitates of unlabeled adult whole-brain detergent extracts using Mab 3G6.41 (lane *A*) or parent myeloma conditioned media plus 1% normal mouse serum (lane *B*) were immunoblotted with Mab P61 after electrophoresis on SDS gels and transfer to nitrocellulose. Numbers to left of figure indicate apparent molecular weights in thousands. Bands marked *hc* and *lc* represent heavy chain and light chain of IgG respectively, present in the immunoprecipitates. These react with rabbit anti-rat IgG used as second antibody in the immunoblots. Exposure time, 2 h.

TABLE I. *Binding of Rabbit Anti-N-CAM and Mab 3G6.41 to Neuronal, Glial, and Muscle Cell Lines*

Cell line	^{125}I-Protein A bound	
	Anti-N-CAM	Mab 3G6.41
	ng/2 × 10^5 cells	
B12	7.2 ± 0.33 (6)	0.0 ± 0.02 (6)
B35	6.8 ± 0.14 (6)	0.6 ± 0.17 (6)
B65	3.6 ± 0.25 (6)	0.0 ± 0.03 (6)
B104	10.2 ± 1.2 (2)	0.6 ± 0.01 (2)
C6	9.0 ± 0.55 (3)	0.6 ± 0.01 (2)
L6	21.8 ± 0.12 (6)	2.8 ± 0.20 (6)

For anti-N-CAM determinations, nanograms of ^{125}I-protein A were calculated from the counts per minute bound with a predetermined excess anti-N-CAM serum minus the counts per minute bound with normal rabbit serum. The resulting counts per minute bound were then divided by the specific activity (counts per minute per nanogram) of the ^{125}I-protein A (typically 5–10 × 10^3). For 3G6.41, Mab containing media was used and background control media was 1% normal mouse serum in parent myeloma (653) conditioned media. Data shown are averages with standard deviations of two or more determinations as noted in parenthesis. In a typical experiment 25,000–150,000 cpm of ^{125}I bound to anti-N-CAM-treated cells and 4,000–20,000 cpm bound to Mab 3G6.41-treated cells.

muscle cell line because N-CAM or N-CAM-like molecules have been reported to be expressed on chicken muscle (16, 22). Six rat cell lines were found to be anti-N-CAM positive: B35, B65, and B104 are neuronal cell lines, whereas B12 and C6 are glial in phenotype and L6K is a skeletal myoblast. Four of these lines, B35, B104, C6, and L6K, were also found to react with Mab 3G6.41 (Table I). Thus, the 3G6.41 and N-CAM antigens are found on cell lines with neuronal, glial, and myoblast phenotypes. Two cell lines, B12 and B65, that bind anti-N-CAM showed no significant binding of Mab 3G6.41. The finding of two cell lines that express N-CAM molecules but not the 3G6.41 antigen further indicates that 3G6.41 recognizes a subset of the anti-N-CAM-reactive molecules. For the cell lines that bound both antibodies the ratio of molecules of anti-N-CAM IgG bound to molecules of Mab 3G6.41 IgG bound varied from 8 to 1 (L6) to 17 to 1 (B104). These results suggest that 3G6.41 reactive molecules are a variable proportion of the total anti-N-CAM-binding population. The percentage of the N-CAM molecules that bind Mab 3G6.41 is difficult to evaluate rigorously with this method because the multiplicity of binding of rabbit anti-N-CAM IgG molecules to individual antigen molecules is unknown.

To test if Mab 3G6.41 bound to unique molecular forms on these cell lines, they were surface iodinated and immunoprecipitated with Mab 3G6.41 or rabbit anti-N-CAM in parallel. Anti-N-CAM immunoprecipitates from three cell lines, B35, C6, and L6 that bound both Mab 3G6.41 and anti-N-CAM all showed the presence of the 140,000 and 120,000 M_r polypeptides and to a lesser extent of a polypeptide band at ~180,000 M_r (Fig. 5, lanes *B*, *D*, and *F*). In the Mab 3G6.41 immunoprecipitates we consistently saw the 140,000-mol-wt component in each of these three cell lines (Fig. 5, lanes *A*, *C*, and *E*). As with the ^{125}I-labeled cerebellar cultures, we did not observe the 120,000 M_r component in any of the Mab 3G6.41 immunoprecipitates. From the C6 glial cell line Mab 3G6.41 immunoprecipitated a band with M_r 180,000–190,000-mol-wt that comigrated with the highest M_r band precipitated by anti-N-CAM. This upper component was not seen in the L6 myoblast or B35 neuronal cell lines.

From the B12 glial cell line, which bound rabbit anti-N-CAM but not Mab 3G6.41, anti-N-CAM immunoprecipitated broad bands of almost identical intensity centered at ~145,000 and 180,000 M_r (Fig. 6, lane *B*) and a less intense 120,000 M_r component. Mab 3G6.41 did not immunoprecipitate these polypeptides from B12 cells. Rabbit anti-N-CAM also precipitated 140,000- and 120,000-mol-wt components from the B65 neuronal line (Fig. 6, lane *F*). However, we did not see a 180,000-mol-wt component in this precipitate. Again, as expected from the lack of detectable antibody binding to whole cells, Mab 3G6.41 did not precipitate any of these N-CAM components from the B65 cell line (Fig. 6, lane *G*).

As summarized in Table II, Mab 3G6.41 can immunoprecipitate polypeptides in the 140,000-mol-wt range (all cells tested) and also the 180,000-mol-wt range (C6 cells and primary neuron cultures). However, the 120,000-mol-wt form of N-CAM does not seem to contain the 3G6.41 antigenic site. The apparent molecular weights of the 140,000- and 180,000-mol-wt region polypeptides precipitated by Mab 3G6.41 and rabbit anti-N-CAM overlap entirely. Thus these experiments do not support the hypothesis that Mab 3G6.41 reacts with a unique size class of N-CAM molecules.

N-CAM Expression by Primary Astrocytes

The reactivity of Mab 3G6.41 with C6 astrocytoma cells as well as our earlier observation that 3G6.41 reacts with cells of non-neuronal morphology in cerebellar primary cultures suggest that rat astrocytes may express N-CAM like surface components. This point has not been entirely clear, as rat cerebellar Bergmann glia (17), mouse glial cell lines (2), and neuroepithelial cells adjacent to the chick embryo visual pathway (31) have been reported to stain with anti-N-CAM. In contrast no staining with anti-N-CAM of non-neuronal cells in chick or mouse brain primary cultures was detected

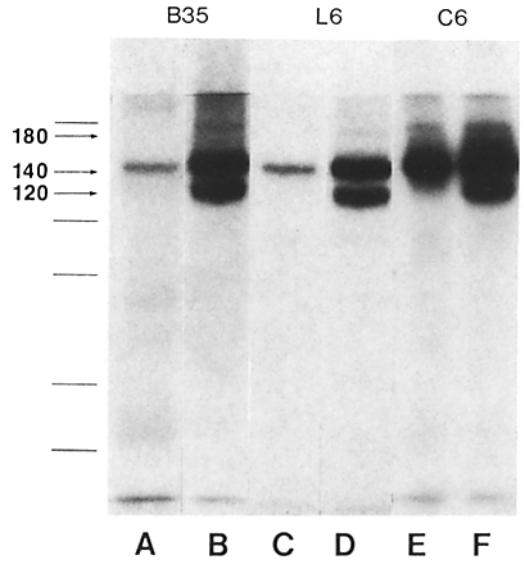

FIGURE 5 Autoradiograph of ^{125}I-polypeptides from surface iodinated B35 neuronal, L6 myoblast, and C6 glial cell lines immunoprecipitated with Mab 3G6.41 (lanes *A*, *C*, and *E*) or anti-N-CAM sera (lanes *B*, *D*, and *F*). Calibration to left of figure is as in Fig. 1. Input trichloroacetic acid-precipitable counts per minute used in immunoprecipitates were for B35, 3 × 10^5; for L6, 1 × 10^5; and for C6, 1 × 10^5. Autoradiograph exposure, 10 d.

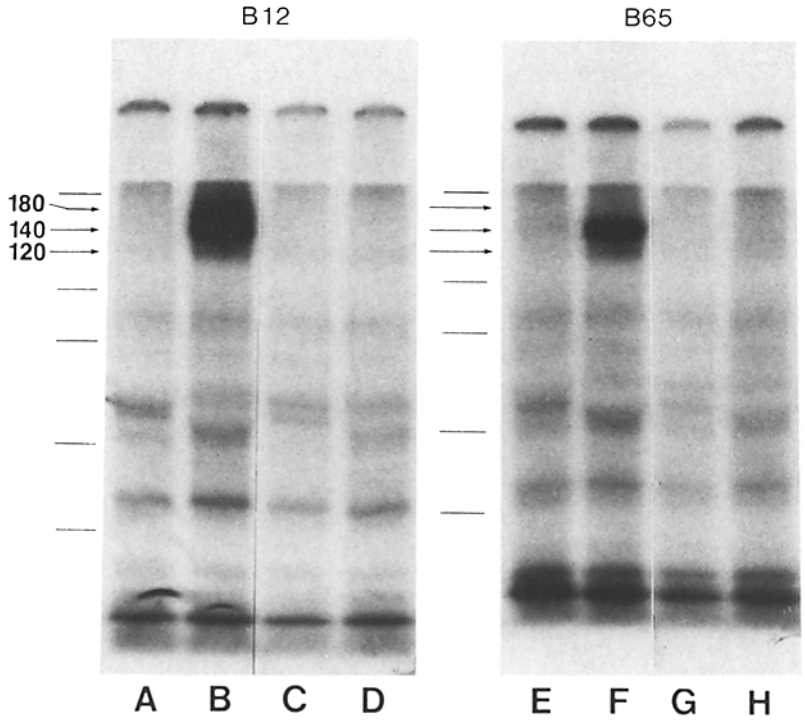

FIGURE 6 Autoradiographs of ^{125}I polypeptides immunoprecipitated from cell lines that bind rabbit anti-N-CAM but not Mab 3G6.41. Labeled extract from B12 glial cell line (*left*) or B65 neuronal line (*right*) immunoprecipitated with Mab 3G6.41 (lanes *A* and *E*), anti-N-CAM sera (lanes *B* and *F*), normal rabbit sera (lanes *C* and *G*), or mouse control ascites with Mab of same isotype as Mab 3G6.41 (lanes *D* and *H*). Numbers and bars to left of figure are the same as for Fig. 2. Input trichloroacetic acid-precipitable counts per minute used in immunoprecipitates: B12, 3×10^5; B65, 3×10^5. Autoradiograph, exposures, 7 d.

TABLE II. *Molecular Weights of ^{125}I-Polypeptides Precipitated from Cloned Cell Lines by Mab 3G6.41 and Anti-N-CAM Sera*

Cell line	Antibody	180,000 mol wt range	140,000 mol wt range	120,000 mol wt range
B35	N-CAM	+ (183–193)	+ (135–157)	+ (117–130)
	3G6.41	–	+ (135–153)	–
B65	N-CAM	–	+ (133–152)	+ (110–120)
	3G6.41	–	–	–
C6	N-CAM	+ (180–190)	+ (127–160)	+ (110–120)
	3G6.41	+ (180–190)	+ (130–155)	–
B12	N-CAM	+ (155–185)	+ (135–155)	+ (110–120)
	3G6.41	–	–	–
L6	N-CAM	+ (183–193)	+ (137–157)	+ (113–127)
	3G6.41	–	+ (137–150)	–

Numbers in parentheses are the observed ranges of M_r (in thousands) for each band in autoradiograms from two or three experiments. A minus indicates that no band could be detected in that region after 7–10 d of exposure.

(7, 15, 16). To test this possibility newborn rat brain primary cultures were double labeled with Mab 3G6.41 and rabbit anti-glial fibrillary acidic protein, an intermediate filament type unique to astrocytes (4). Third passage whole brain astrocyte cultures that contained no neurons had many cells that bound both antibodies (Fig. 7). Most of the anti-glial fibrillary acidic protein–labeled cells also bound Mab 3G6.41. Fluorescence was often particularly intense at regions of cell–cell contact (Fig. 7, arrowheads). A cell unlabeled by either antibody is outlined in the figure. Thus rat astrocytes clearly express components immunologically similar to N-CAM.

DISCUSSION

Distinct neural cell adhesion molecules appear to be expressed in the same brain region and in some cases the same cell type. Mouse cerebellar granule cells express the L1 cell adhesion molecules consisting of 200,000- and 140,000-mol-wt glycoproteins (24) and also N-CAM molecules (18). Individual neurons cultured from chick embryo brains have been shown to express both N-CAM and also Ng-CAM, a 135,000-mol-wt polypeptide that has been suggested to mediate the adhesion between neurons and glia (14, 15). Through the use of specific antibody reagents (23, 30), N-CAM and L1 have been shown to be immunologically distinct; however N-CAM and Ng-CAM share at least one antigenic site (15). Further diversity among the 140,000-M_r glycoproteins has been observed using the monoclonal antibody NSP-4 (27), which recognizes a family of molecules in mouse brain distinct from N-CAMs, Ng-CAM, and L1. Sets of nervous system cells that are perhaps functionally or developmentally distinct, may express combinations of these cell adhesion molecules.

Diversity in the molecular forms of the N-CAM molecules themselves during neural development is characterized by a shift from highly sialylated N-CAM glycoproteins in embryonic and early postnatal mammals to much less sialylated polypeptides with distinct M_r's of 180,000, 140,000, and 120,000 in adults (17, 26). Differences among the lengths of these adult type chains appears to be due to the size of their cytoplasmic extensions. N-CAM of all M_r's reconstituted into liposomes and then trypsinized yields different sized fragments which remain liposome bound and hence contain the cytoplasmic domain (12, 13). Variations that may occur within each of the components of the adult forms have been less well characterized. One Mab directed against a nonsialic acid portion of N-CAM has been reported to react with adult cerebral but not cerebellar N-CAM (6, 7).

Mab 3G6.41 appears to recognize a subclass of the adult form of N-CAM cell adhesion molecules. This conclusion is based on the following evidence: (*a*) Mab 3G6.41 and monoclonal anti-N-CAM (18) recognize neuronal cell surface glycoproteins of 140,000 and ~180,000 M_r in cerebellar cultures. The immunoprecipitated bands using Mab 3G6.41 were reproducibly less intense on the autoradiographs than the bands immunoprecipitated by anti-N-CAM sera, implying that Mab

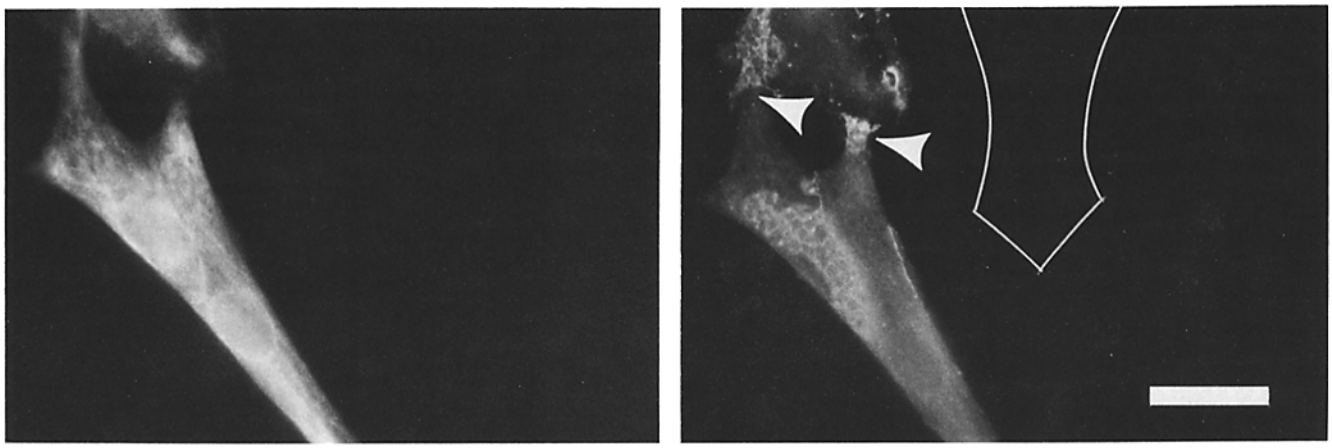

FIGURE 7 Simultaneous detection of rabbit anti-glial fibrillary acidic protein and Mab 3G6.41 binding. Glial cultures were double stained and the same field was photographed with fluorescein filters for glial filaments (*left*) and rhodamine filters for Mab 3G6.41 (*right*). Note intense 3G6.41 fluorescence at the region of the cell–cell contact (arrowheads) and the large, presumably fibroblastic, cell that lacks both labels (outlined to show location). Bar, 20 μm. × 800.

3G6.41 can immunoprecipitate only a fraction of the total molecules in each band immunoprecipitated by anti–N-CAM. (*b*) Precipitation with rabbit anti–N-CAM removed all Mab 3G6.41–reactive bands. However exhaustive precipitation with Mab 3G6.41 could not remove all of the 140,000- and 180,000-mol-wt molecules that react with rabbit anti–N-CAM. (*c*) Polypeptides of 180,000- and 140,000-mol-wt immunoprecipitated by Mab 3G6.41 express the antigenic site recognized by Mab P61 on N-CAMs. (*d*) An alternative explanation that Mab 3G6.41 recognizes a possible N-CAM binding protein present in the cell extracts that could then secondarily precipitate a fraction of the N-CAM molecules is unlikely because no other bands were specifically immunoprecipitated in either the ^{125}I- or [^{3}H]fucose-labeled cell extracts and because all cell lines and primary cultures where Mab 3G6.41 binding has been examined at the molecular level have an immunoprecipitable 140,000-mol-wt band. (*e*) Two independently derived cell lines were shown to express some N-CAM polypeptides but not the polypeptides recognized by Mab 3G6.41. The expression of the N-CAM polypeptide subtypes thus appears to be separable into two classes of cells. The first (C6, L6, B35, B104, and cerebellar primary neurons) express both Mab 3G6.41 binding and other N-CAM forms. The second (B12 and B65) express only other N-CAM forms and lack the Mab 3G6.41–binding forms.

The identification of the molecules precipitated by Mab 3G6.41 as N-CAMs relies heavily on comparisons with molecules that react with rabbit and monoclonal anti–N-CAMs. The specificity of these antibodies was documented in Materials and Methods and prior publications. In addition we have begun to analyze one-dimensional maps of proteolytic cleavage products (8) of 3G6.41 and rabbit anti–N-CAM-precipitated molecules. The maps are very similar for the two populations, which further indicates that the major polypeptide precipitated by each antibody is very similar in structure (unpublished data). However, the antigenic site recognized by Mab 3G6.41 might consist of a limited primary amino acid sequence that is present on only some of the N-CAM molecules. Alternatively Mab 3G6.41 may recognize a specific conformation or posttranslational modification that is present on only some of the N-CAM molecules. Since the antigenic site for Mab 3G6.41 has been shown to be insensitive to neuraminadase treatment (34) it is unlikely that Mab 3G6.41 recognizes the sialic acid components of the N-CAM molecules. Other posttranslational modifications that may contribute to the 3G6.41 antigenic site include differences in glycosylation other than sialic acid, phosphorylation, sulfation, and covalent linkage of lipids.

Other anti–N-CAM antibodies have been reported not to react with cultures of mouse glia (15, 18). However, permanent glial cell lines established from mouse cerebella do express N-CAM (2). We observed expression of N-CAM on rat glia that did not appear to depend on culture conditions since Mab 3G6.41 reacted with primary cultures, three times passaged, and clonal rat glial cells.

The function of the unique form of N-CAM recognized by the monoclonal antibody 3G6.41 is not known. N-CAM interaction has been characterized as homophilic, i.e., N-CAM on one cell directly interacts with the N-CAM of another cell (28). Thus, it is possible that expression of N-CAM recognized by Mab 3G6.41 may promote the mutual adhesion of 3G6.41 positive cells. Since the cell types that express the form of N-CAM recognized by Mab 3G6.41 include rat neurons, astrocytes, and muscle, it is possible that the 3G6.41 form of N-CAM influences several cell–cell interactions including neuron–glial interaction. Alternatively, the 3G6.41 site on the N-CAM molecules may be involved in other cell–cell or cell–matrix interactions.

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