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Glial Cells of the O-2A Lineage Bind Preferentially to *N*-Cadherin and Develop Distinct Morphologies

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Environmental cues guiding glial cell behavior during development or regeneration of the nervous system are provided by both soluble and nondiffusible factors. We examined the influences of purified extracellular matrix molecules and cell adhesion molecules on the development and proliferation of glial cells from neonatal rat optic nerves. Dissociated optic nerve glia were plated on fibronectin, laminin, collagen type IV, L1, *N*-cadherin, and N-CAM. Cultures were grown in chemically defined medium to promote formation of oligodendrocytes. Other cultures were grown in 10% serum to support type-1 astrocytes and the differentiation of O-2A progenitor cells to type-2 astrocytes. In short-term adhesion assays to measure cell affinity for the different substrates, cells in the O-2A lineage bound preferentially to *N*-cadherin while type-1 astrocytes preferentially bound to extracellular matrix components. The cells in the O-2A lineage also developed distinctive morphologies on different substrates after incubation for 4 days. Type-2 astrocytes and oligodendrocytes produced very large membranous expansions on *N*-cadherin. Measurements of BrdU incorporation indicated that the substrates did not significantly influence cell proliferation rates. Our results showed that O-2A progenitor cells, oligodendrocytes, type-1 astrocytes, and type-2 astrocytes possess different complements of receptors for the adhesion molecules in their environment and that their morphological differentiation can be dramatically altered by these extracellular signals. © 1993 Academic Press, Inc.

INTRODUCTION

Many important events during neural development involve interactions between a cell and components of its environment. Such interactions can involve contacts with other cells by cell adhesion molecules (CAM).[1] Other interactions may involve very large molecules secreted into the extracellular space, constituting the extracellular matrix (ECM). The interaction of a cell with the ECM involves substrate adhesion molecules (SAM), which selectively bind to components of the extracellular matrix. Neuronal migration, differentiation, and axonal growth clearly depend on specific adhesive interactions. Previous studies in other systems, for example in neural crest (Perris *et al.*, 1988; Stemple and Anderson, 1992) and in retinal pigmented epithelium (Reh *et al.*, 1987), have shown that ECM can determine the phenotypic fate of a cell. It is possible that instructive adhesive influences play important roles in the functional specialization and spatial distribution of glial cells that produce specific associations of astrocytes with blood vessels and oligodendrocytes with axons. The developing rat optic nerve provides a model system used in many recent investigations of glial functions. The major constituents in cell cultures derived from dissociated optic nerves are type-1 astrocytes, type-2 astrocytes, oligodendrocytes, and their progenitors (Raff *et al.*, 1990). The specific glial cell types that subsequently develop are determined by manipulation of the culture conditions. We studied the regulation of glial cell behavior by CAMs and SAMs in this system to evaluate the potential roles of adhesion molecules in glial development and response to nervous system damage.

Many CAMs have been identified that are potentially important in glial biology. While some of these molecules are expressed on the surfaces on the glia, others are found only on surfaces of the cells that glia contact. We selected three well-characterized CAMs for these studies. Neural cell adhesion molecule (N-CAM) is important for neurite growth on glia in several systems (Bixby *et al.*, 1988; Drazba and Lemmon, 1990). N-CAM is expressed in three different forms in the CNS, with molecular weights of 180, 140, and 120 kDa. The 180-kDa form is exclusively neuronal, while 140- and 120-kDa forms are also found on glia (Noble *et al.*, 1985; Trotter *et al.*, 1989; Bartsch *et al.*, 1990; Bhat and Silberberg, 1990; Hekmat *et al.*, 1990). Both neurons and glia express *N*-

[1] Abbreviations used: CAM, cell adhesion molecule; SAM, substrate adhesion molecule; ECM, extracellular matrix; N-CAM, neural cell adhesion molecule; PBS, phosphate-buffered saline; FCS, fetal calf serum; GC, galactocerebroside; BrdU, bromodeoxyuridine; BSA, bovine serum albumin; Coll IV, collagen type IV; Ln, laminin; Fn, fibronectin; N-cad, N-cadherin.

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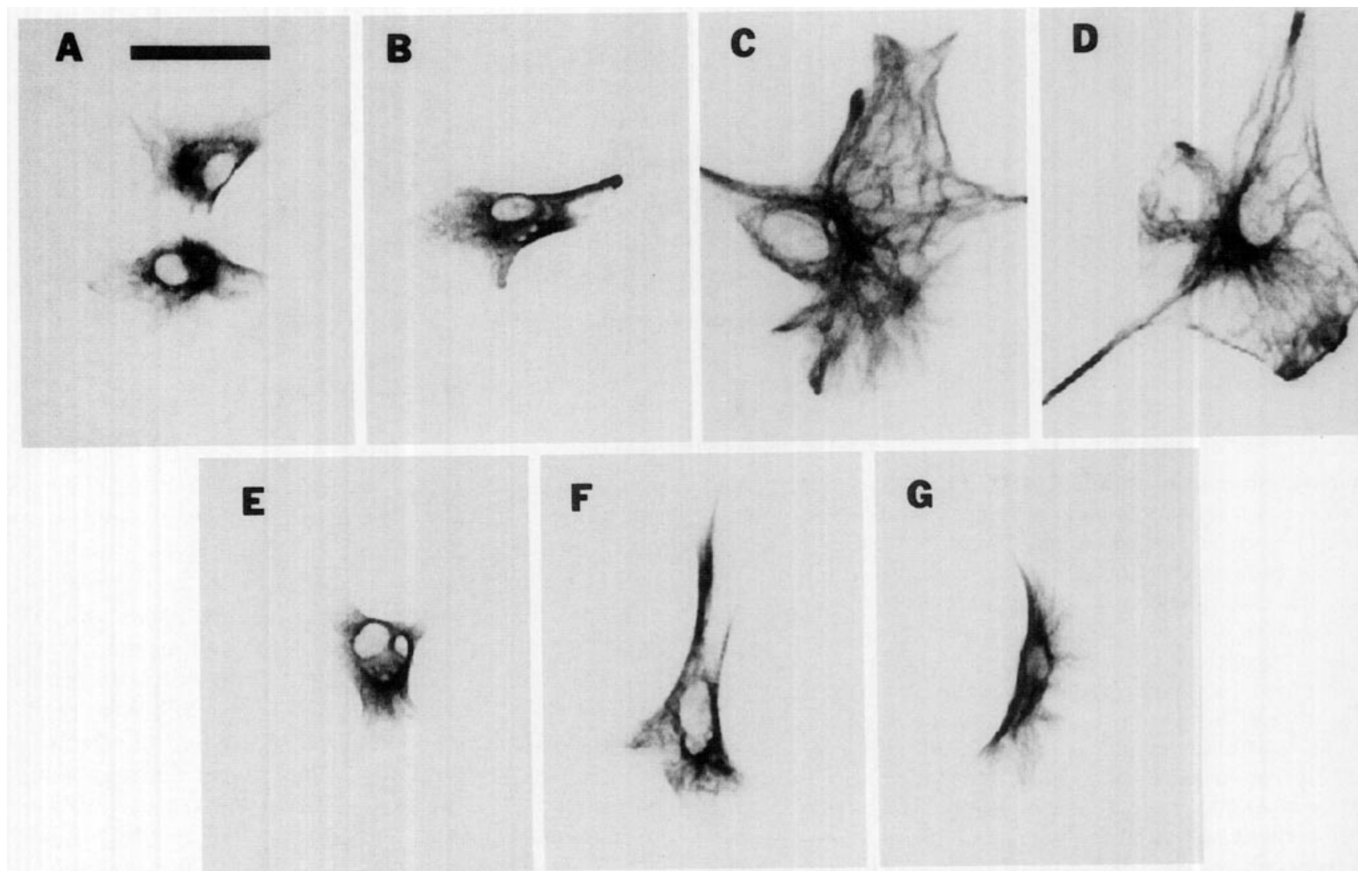


FIG. 1. Examples of type-1 astrocyte (A2B5$^-$, GFAP$^+$) morphology on the different substrates after 4 days *in vitro*. No consistent differences in cell shape were detected, although type-1 astrocytes were larger on ECM components (see Fig. 5). These cultures were grown in FCS medium on BSA (A), collagen IV (B), fibronectin (C), laminin (D), L1 (E), *N*-cadherin (F), and N-CAM (G). Fluorescent images of GFAP-stained type-1 astrocytes were captured with a video camera, digitized, and printed with reversed gray scale to create the black on white images shown here. Calibration bar, 50 μm.

cadherin, a Ca^{2+}-dependent CAM that is important in neurite growth on glia in the CNS (Bixby *et al.*, 1988; Drazba and Lemmon, 1990) and PNS (Letourneau *et al.*, 1990). Although not well studied in glial-glial adhesion, *N*-cadherin acts in a homophilic binding mechanism, and thus it probably is a factor in glial-glial interactions. The third CAM, L1 (also called NILE, and the related molecules Ng-CAM, 8D9, and G4), is expressed on neurons, and it appears to mediate adhesion to glia by binding to a heterophilic binding partner on glia (Grumet and Edelman, 1988; Drazba and Lemmon, 1990). In the PNS, L1 is essential for the initiation of axon myelination by Schwann cells (Seilheimer *et al.*, 1989; Wood and Bunge, 1991). L1 is expressed on most CNS projection axons during development and it is present in much higher concentrations on axons than on dendrites or neuronal somas. These characteristics suggest that L1 may signal the presence of axons to oligodendrocytes and it could be involved in mediating their interactions.

Glia interact with some of the major ECM components in the developing and mature brain. Radial glia in the developing cortex (Stewart and Pearlman, 1987) and in cerebellum (Hatten *et al.*, 1982) are associated with fibronectin. Laminin or a related molecule is expressed by CNS glia *in vitro* (Liesi *et al.*, 1983) and *in vivo* (Liesi *et al.*, 1984). Collagen, a major ECM protein, is an essential factor in the stimulation of Schwann cells to ensheath axons (Bunge *et al.*, 1986). Type IV collagen is found in the basal lamina investing vascular structures and thus is opposed to the perivascular processes of astrocytes. Since astrocytes can express integrins that bind to collagen (Tawil *et al.*, 1990), collagen type IV in the basal lamina may interact with astrocytes.

In the present investigation, we attached purified adhesion molecules to a nitrocellulose substrate to study their influence on glial behavior *in vitro*. We found that subpopulations of astrocytes have different preferences for adhesion to defined substrates bound to nitrocellulose. Type-1 astrocytes showed a strong preference for

ECM components while type-2 astrocytes and O-2A progenitors bound preferentially to fibronectin and *N*-cadherin. Moreover, association with a specific class of adhesion molecule profoundly affected glial morphogenesis. Type-2 astrocytes and oligodendrocytes exhibited strikingly different morphologies on *N*-cadherin than on other substrates such as fibronectin. These results are consistent with the concept that adhesion molecules play a crucial role in glial development *in vivo.*

MATERIALS AND METHODS

Preparation of Substrates

Fibronectin (bovine, plasma; GIBCO), laminin (mouse; Upstate Biotechnology, Inc.), and type IV collagen (murine; Collaborative Research) were obtained from commercial sources. L1, N-CAM, and *N*-cadherin were prepared with monoclonal antibody affinity columns as described previously (Lemmon and McLoon, 1986; Bixby and Zhang, 1990). L1 was purified from rat brain with Mab 74-5H7 which binds L1 from a variety of mammalian species (Lemmon *et al.*, 1989). N-CAM was purified from E14 chick brain with Mab 12F8 which provided a preparation of polysialated N-CAM capable of supporting cell attachment (Chung *et al.*, 1991; Abosch and Lagenaur, 1993). Chicken *N*-cadherin was collected with Mab NCD-2 from the hybridoma cell line obtained from M. Takeichi and G. Grunwald. This Mab produced purified *N*-cadherin that supports cell attachment on nitrocellulose (Bixby and Jhabvala, 1990). Monoclonal antibody affinity columns were prepared using CNBr-activated Sepharose (Pharmacia), according to supplier instructions. Plasma membranes from chick or rat brains were prepared with sucrose density gradients. These membranes were solubilized in sodium deoxycholate and centrifuged at 100,000*g* for 60 min, and the supernatants were passed through the affinity columns. The columns were washed and eluted with a procedure that optimized preservation of activity of the particular CAM. Typically, 50 m*M* diethylamine, pH 11.5, was used. The eluate was neutralized immediately and dialyzed against phosphate-buffered saline (PBS) to remove the elution buffer. Fractionated samples were tested for purity by polyacrylamide gel electrophoresis. Substrate molecules were attached to nitrocellulose-coated plastic tissue culture dishes (Lagenaur and Lemmon, 1987).

Preparation of Glia

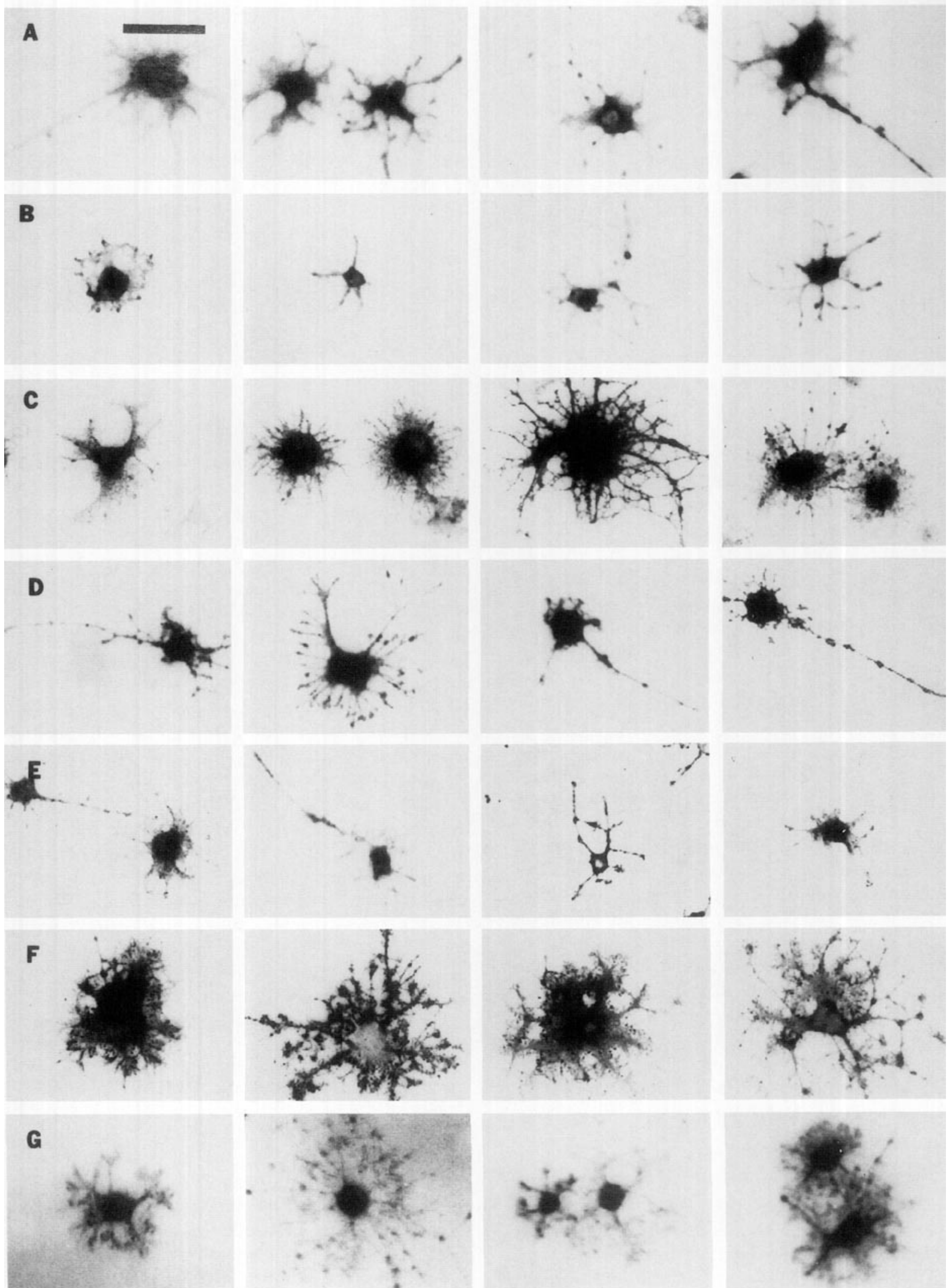
Rat optic nerve glia were prepared using procedures developed by Raff and associates (Raff *et al.*, 1983). Briefly, optic nerves were removed from rat pups anesthetized with methoxyfurane and sacrificed by decapitation at Postnatal Day 7. The nerves were minced and then incubated in 0.2% collagenase followed by 0.1% trypsin. The tissue was triturated in 0.04 mg/ml DNase, and single cells were harvested by centrifugation. The cells were plated in DME/Hams F12 (1/1) supplemented with 10% fetal calf serum (FCS medium) at a density of 40,000 cells/cm^2, incubated at 37°C for 2 hr to allow cell attachment, and then cultured in media that select for the development of alternative glial cell types *in vitro.* In experiments to study astrocytes, the attached cells were fed with FCS medium which produced cultures consisting predominantly of astrocytes. Alternatively, cultures to be used for studies of oligodendrocytes were fed with a serum-free, chemically defined medium (N2; Bottenstein and Sato, 1979; Raff *et al.*, 1983; Lillien and Raff, 1990) which does not support development or proliferation of astrocytes.

Immunostaining of Cell Cultures

The glial cells present under various conditions were characterized by indirect double immunofluorescence. Cultures were rinsed briefly in fresh culture medium and then fixed for 1 hr in 4% paraformaldehyde, 0.01% glutaraldehyde at 4°C. For antibody staining of cell surface components, cultures were incubated for 1 hr at 4°C with anti-galactocerebroside (GC) (Ranscht *et al.*, 1982) or A2B5 (Eisenbarth *et al.*, 1979) diluted in PBS with 10% horse serum, followed by fluorochrome-conjugated goat anti-mouse IgG antibodies for 1 hr. For staining of GFAP, cultures labeled with A2B5 were incubated for 1 hr with anti-GFAP diluted in PBS with 10% horse serum and 0.1% saponin. These cells were incubated in biotinylated goat anti-mouse IgG followed by avidin-Texas Red and goat anti-rabbit IgG. The specificity of the immunostaining was controlled by: (1) omission of the primary antibody and (2) incubation of cells labeled by each primary antibody with the noncorresponding secondary antibody system. For this investigation, type-2 astrocytes were defined as cells that coexpressed GFAP and A2B5, whereas type-1 astrocytes expressed GFAP but not A2B5.

Cell Counting, Morphometry, and Statistical Analysis

The cells were counted on a Leitz Ortholux II fluorescence microscope, imaged with a silicon-intensified video camera (DAGE 65 or Hamamatsu C2400-08) and then recorded on an optical memory disk recorder (Panasonic). The cell areas of type-1 astrocytes, type-2 astrocytes, and oligodendrocytes were measured with an Image 1 image analysis system from digitized images of GFAP, A2B5, and GC fluorescence patterns, respectively. The area of a cell was calculated by computer as the calibrated area enclosed by a manual trace of the outer boundary of the cell processes. All results were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analyzed by one-way ANOVA using the Scheffe F test to determine significance levels. All bar charts present the mean values ± SEM.

Short-Term Adhesion Assays

Short-term adhesion assays were performed to determine the efficiency of cell binding to the substrates. Astrocytes and progenitor cells for these experiments were obtained by culturing dissociated optic nerve cells on poly-L-lysine/nitrocellulose-coated dishes for 2 days. Oligodendrocytes were produced by seeding dissociated cells on *N*-cadherin/nitrocellulose-coated dishes and cultivation for 3 days in N2 medium. The cells were detached with Ca^{2+}- and Mg^{2+}-free buffer, washed by centrifugation, and resuspended in FCS medium. A 5-μl aliquot containing 3000 suspended cells was dispensed to each 0.3-cm^2 well on substrate/nitrocellulose-coated dishes. All wells receiving astrocytes and progenitors were seeded with the same number of cells and all wells receiving oligodendrocytes were seeded with the same number of cells. After a 60-min incubation at 37°C in FCS medium with 10% fetal calf serum, the plates were washed gently to remove unattached cells. The cells were fixed and immunolabeled as described above and counted by fluorescence microscopy.

Determination of Cell Proliferation

Cell proliferation was evaluated by immunofluorescence of incorporated bromodeoxyuridine (BrdU; Gratzner, 1982) in 38-hr cultures that were established in substrate/nitrocellulose-coated dishes. The cultures were exposed to 50 μ*M* BrdU during the last 10 hr of culture and then fixed for immunostaining. Fixed cells were incubated with A2B5 and then permeabilized with 5% acetic acid/95% ethanol at −20°C for 90 sec. The cells were treated with 2 *N* HCl for 10 min to denature the DNA, followed by 0.1 *M* sodium borate pH 8.5 for 10 min. The primary anti-BrdU antibody (diluted 1:5) and anti-GFAP were added for 45 min, followed by biotinylated goat anti-mouse IgG for 45 min. Fluorochrome-conjugated avidin and goat anti-rabbit IgG were added for 45 min. The specificity of the immunostaining was controlled by: (1) omission of the primary antibody and (2) incubation of cells labelled by each primary antibody with the noncorresponding secondary antibody system.

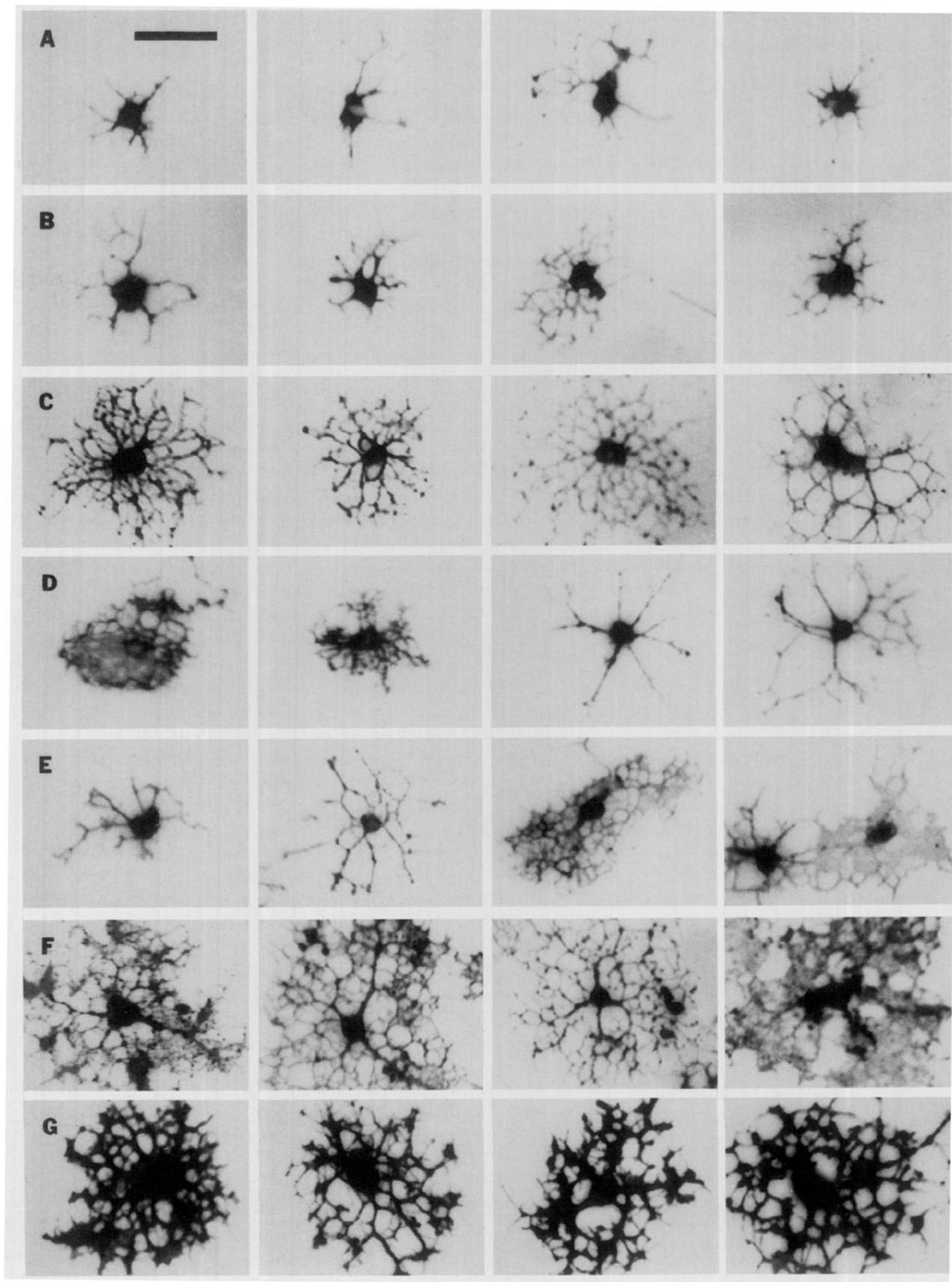
RESULTS

Substrate Effects on Glial Morphology

Incubation of glial cultures for 4 days on purified adhesion molecules produced substrate-specific cell shapes in some classes of glia. Although type-1 astrocytes (Fig. 1) did not appear to develop characteristic morphologies on these substrates, type-2 astrocytes (Fig. 2) and oligodendrocytes (Fig. 3) both developed substrate-specific cell shapes. Type-2 astrocytes on BSA typically had broad processes which produced cell borders with smooth scalloped edges (Fig. 2A). Some cells also possessed slender radiating processes generally 30 to 50 μm in length with one or two unbranched processes as long as 160 μm in length. On collagen type IV, only a few short processes generally extended from the cell bodies of type-2 astrocytes. The processes of type-2 astrocytes on fibronectin were more numerous and longer (compare Figs. 2B and 2C). The type-2 astrocytes on laminin often developed elongated cell shapes with processes that ended in small swellings. On L1, the type-2 astrocytes that developed were smaller cells whose processes were generally smooth with few membranous enlargements. Type-2 astrocytes on *N*-cadherin had 8–10 compact cellular extensions from the cell body and usually lacked highly elongated cellular processes. The major cytoplasmic extensions of these cells formed a profusion of smaller branches terminating in flattened swellings that produced a discontinuous membrane sheet. Many of these small branching processes lacked evidence of GFAP (Fig. 4). On N-CAM, type-2 astrocytes with a profusion of terminal enlargements were found as well as cells bearing a network of fine branching processes.

Oligodendrocytes (Fig. 3) on BSA typically had five to eight short primary processes with few interconnecting branches but occasional cells bore one or two longer processes. Oligodendrocytes on collagen IV were quite similar to those on BSA but there was more branching of the short processes and small lamellar regions were observed more frequently. Oligodendrocytes on fibronectin had smaller cell bodies than those on BSA and they had an elaborate process network that is characteristic of mature oligodendrocytes. On this substrate, the interconnecting elements of the network usually consisted of slender processes. On laminin, oligodendrocytes had a network of thicker processes. Some cells had regions

FIG. 2. Comparison of type-2 astrocyte ($A2B5^+$, $GFAP^+$) morphology on the different substrates in 4-day cultures grown in FCS medium. Rows show A2B5 staining of representative cells in cultures plated on BSA (A), collagen IV (B), fibronectin (C), laminin (D), L1 (E), *N*-cadherin (F), and N-CAM (G). Note the relative abundance of terminal expansions of cytoplasmic processes on *N*-cadherin (F). Fluorescent images of A2B5-stained type-2 astrocytes were captured with a video camera, digitized, and printed with reversed gray scale to create the black on white images shown here. Calibration bar, 50 μm.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with sheet-like membrane. Stellate cells with radiating processes having few branches were also observed on this substrate. Oligodendrocytes on L1 had a limited, often asymmetric, process network. Oligodendrocyte morphology on N-CAM was characterized by a process network with thick interconnecting elements. On *N*-cadherin, oligodendrocytes had their most elaborate process network. The network developed from five to six major processes that emanated from the cell body and then branched many times to fine processes. Frequently, elements distal to the cell body expanded to form lamellae that appeared to enlarge cellular contact with the substrate. By 6 days *in vitro*, this lamellar-type morphology characterized 77% of oligodendrocytes on *N*-cadherin but only 8% of oligodendrocytes on fibronectin (Fig. 5). The lamellar development of oligodendrocytes on the other substrates resembled the more filamentous morphology of cells on fibronectin.

Measurements of Cell Size after 4 Days in Culture

Glial cells displayed substrate-associated size differences after 4 days in culture (Fig. 6). Type-1 astrocytes on ECM components (collagen IV and fibronectin) were significantly ($P < 0.05$) larger than those on cell adhesion molecules (L1, *N*-cadherin, and N-CAM). In contrast, the largest type-2 astrocytes and oligodendrocytes were found on *N*-cadherin and fibronectin. All glial cell types were smaller growing on laminin than on fibronectin.

Glial Cell Density

Quantitation of cell numbers after 4 days in culture revealed glial cell preferences for certain substrates over others (Fig. 7). Type-1 astrocytes attained significantly ($P < 0.05$) higher cell densities on the ECM substrates (collagen IV, fibronectin, and laminin). Far fewer type-1 astrocytes were found in cultures grown on *N*-cadherin and this cell type was seen rarely on L1, N-CAM, or BSA. Conversely, type-2 astrocytes and oligodendrocytes were most numerous in cultures grown on *N*-cadherin. Although many type-2 astrocytes were found on fibronectin, these cells were relatively sparse on the other ECMs. The increased numbers of type-2 astrocytes and oligodendrocytes found on *N*-cadherin and fibronectin relative to those on other substrates could result from several factors including: (a) increased initial binding rates to these substrates; (b) increased proliferation rates on *N*-cadherin and fibronectin; and (c) enhanced affinity for *N*-cadherin and fibronectin by the O-2A progenitor cell from which they develop. Differences in the initial binding rates were tested with short-term binding assays. Optic nerve cells cultured for 2 days were detached in Hank's Ca^{2+}- and Mg^{2+}-free PBS, seeded on different substrates and allowed to attach for 60 min, and washed to remove nonadherent cells. We found that type-1 astrocytes attached preferentially to ECM components and that O-2A progenitor cells and type-2 astrocytes bound preferentially to *N*-cadherin and fibronectin while oligodendrocytes showed a striking preference for *N*-cadherin (Fig. 8). Glial cell proliferation rates were tested by pulse-labeling cells with BrdU for the last 10 hr of a 38-hr culture period and then immunostaining with anti-BrdU. Oligodendrocytes divided at very low rates on all substrates in our cultures (data not shown). We found no statistically significant differences in the proliferation rates of glial cells on BSA, collagen IV, fibronectin, laminin, *N*-cadherin, and N-CAM (Table 1). Insufficient numbers of type-1 astrocytes appeared on BSA, L1, and N-CAM for a valid statistical comparison. Type-2 astrocytes and O-2A progenitors on L1 were excluded from the analysis for the same reason.

DISCUSSION

During studies on axon growth, we noted that relatively few astrocytes from P0 rat cerebellum bound to L1 when compared to laminin, yet those astrocytes that did attach to L1 expressed the process-bearing "type-2" shape while most of the astrocytes on laminin had the fibroblast-like type-1 morphology. On the basis of this intriguing observation we undertook a series of experiments to examine systematically how different adhesion molecules influence the development of glia in cell cultures derived from dissociated rat optic nerves. The major constituents in these cultures are astrocytes, oligodendrocytes, and their progenitors (Raff *et al.*, 1990). There is excellent agreement about the existence of an "O-2A progenitor cell" that can produce either type-2 astrocytes or oligodendrocytes *in vitro*. Whether the O-2A progenitor also gives rise to a "type-2 like" astrocyte *in vivo* is controversial (Noble, 1991; Skoff and Knapp, 1991; Fulton *et al.*, 1992; Noble and Wolswijk, 1992). In our experiments, the identities of astrocytes were defined by their antigenic phenotypes *in vitro*:

FIG. 3. Comparison of oligodendrocyte (GC^+) morphology on various substrates in 4-day cultures grown in N2 medium. Each row shows GC staining of representative cells in cultures plated on BSA (A), collagen IV (B), fibronectin (C), laminin (D), L1 (E), *N*-cadherin (F), and N-CAM (G). The complexity of the oligodendrocyte process network varies widely among the different substrates. Note the lamellar regions that were characteristic of oligodendrocytes on *N*-cadherin (F). Fluorescent images of GC-stained type-2 astrocytes were captured with a video camera, digitized, and printed with reversed gray scale to create the black on white images shown here. Calibration bar, 50 μm.

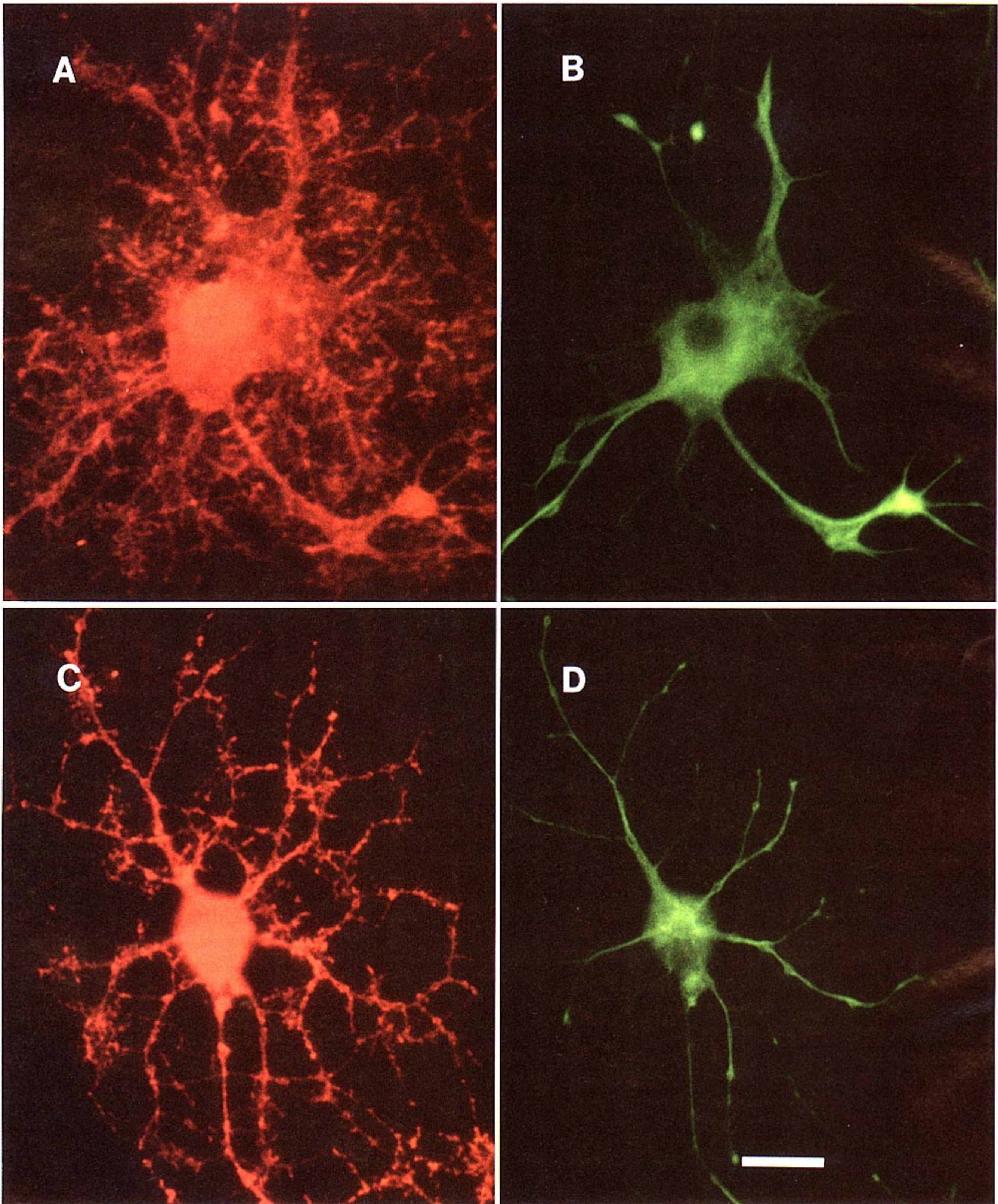

FIG. 4. Double immunolabeling of type-2 astrocytes grown on *N*-cadherin (A, B) or laminin (C, D) for 4 days in FCS medium. (A, C) A2B5 staining (Texas Red). (B, D) GFAP staining (fluorescein). Membranous expansions of cytoplasmic processes on *N*-cadherin were stained by A2B5 but they generally lack GFAP. Cells with numerous flattened vesicles were found infrequently on laminin or the other substrates tested. Calibration bar, 20 μm.

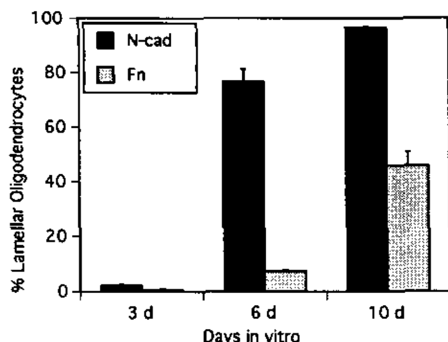

FIG. 5. Expansion of oligodendrocyte lamellar membranes *in vitro*. Glial cultures were plated on *N*-cadherin and fibronectin, fed with N2 medium, incubated for 3, 6, and 10 days, and then fixed and immunostained with anti-GC. An oligodendrocyte was counted as "lamellar-type" if more than 40% of the region used for the cell area measurement was covered by lamellar expansions. The number of lamellar-type oligodendrocytes in the cultures was expressed as a percentage of total oligodendrocytes in each well. At time intervals beyond 3 days *in vitro*, oligodendrocytes with lamellar regions were much more numerous on *N*-cadherin than on fibronectin ($P < 0.01$).

$GFAP^+$, $A2B5^-$ cells were considered type-1 astrocytes and $GFAP^+$, $A2B5^-$ cells were considered type-2 astrocytes. Although our studies are unable to resolve the current controversy about the optic nerve glia system, our results confirmed the idea that different adhesion molecules can exert potent influences on glial morphology and size. After 4 days *in vitro*, type-1 astrocytes grew to much larger sizes on extracellular matrix components than on N-CAM, *N*-cadherin, and L1, with fibronectin producing cells that were more than four times larger than those on the CAMs. Type-2 astrocytes and oligodendrocytes grew to the largest sizes on *N*-cadherin and fibronectin, being more than 50% larger than those growing on the other substrates.

The short-term binding assays further demonstrated that type-1 astrocytes express a different repertoire of functional receptors than cells in the O-2A cell lineage. The type-1 astrocytes bound preferentially to ECM components while the cells in the O-2A lineage bound preferentially to *N*-cadherin and, in the case of type-2 astrocytes and O-2A progenitor cells, to fibronectin. These results suggest that oligodendrocytes but not astrocytes lose their ability to bind to fibronectin during differentiation. However, to produce sufficient numbers of cells for these experiments, oligodendrocytes were grown on *N*-cadherin although type-1 astrocytes, type-2 astrocytes, and O-2A progenitors were raised on poly-L-lysine/nitrocellulose. This cultivation protocol might have preselected for oligodendrocytes with suppressed expression of integrins. This possibility has not yet been tested. The substrates did not influence the proliferation rates of cells of the O-2A lineage. The proliferation rates of type-1 astrocytes were typically elevated on *N*-cadherin compared to those on ECM components, although this increase failed our statistical tests for significance.

A limited number of studies have been done on the effects of adhesion molecules on astrocytes (Goetschy *et al.*, 1987; Pixley *et al.*, 1987; Tawil *et al.*, 1990). Most of these have been studies using brain or cortical astrocytes and no distinction was made between type-1 and type-2 astrocytes. In our experiments we found that these two classes of cells respond very differently to different adhesion molecules. This may make comparisons

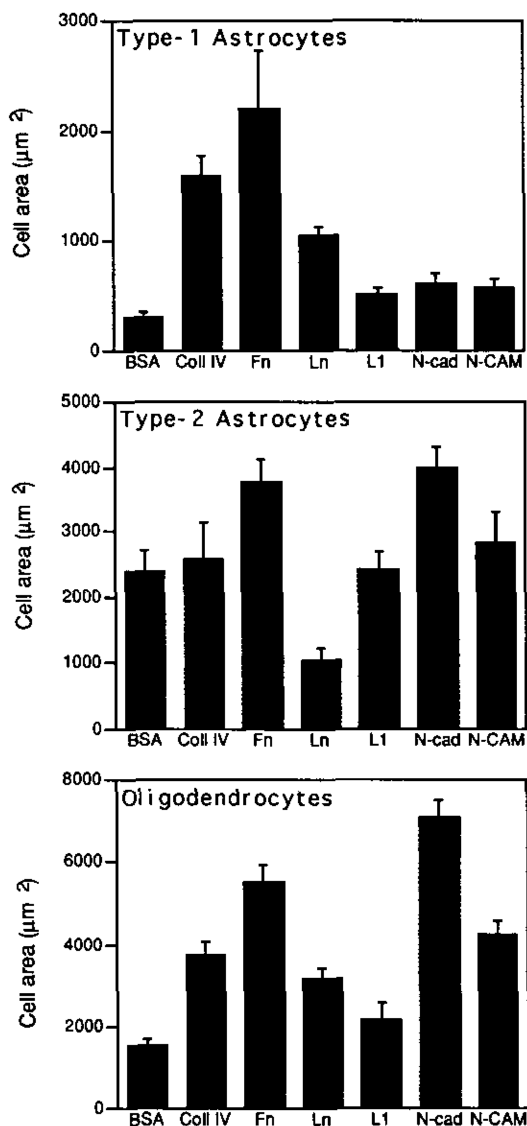

FIG. 6. Substrate-associated size differences within each glial cell type at 4 days *in vitro*. Type-1 astrocytes on ECM components (collagen IV and fibronectin) were significantly ($P < 0.05$) larger than those on cell adhesion molecules (L1, *N*-cadherin, and N-CAM) or the substrate control (BSA). In contrast, the largest type-2 astrocytes and oligodendrocytes were found on *N*-cadherin and fibronectin.

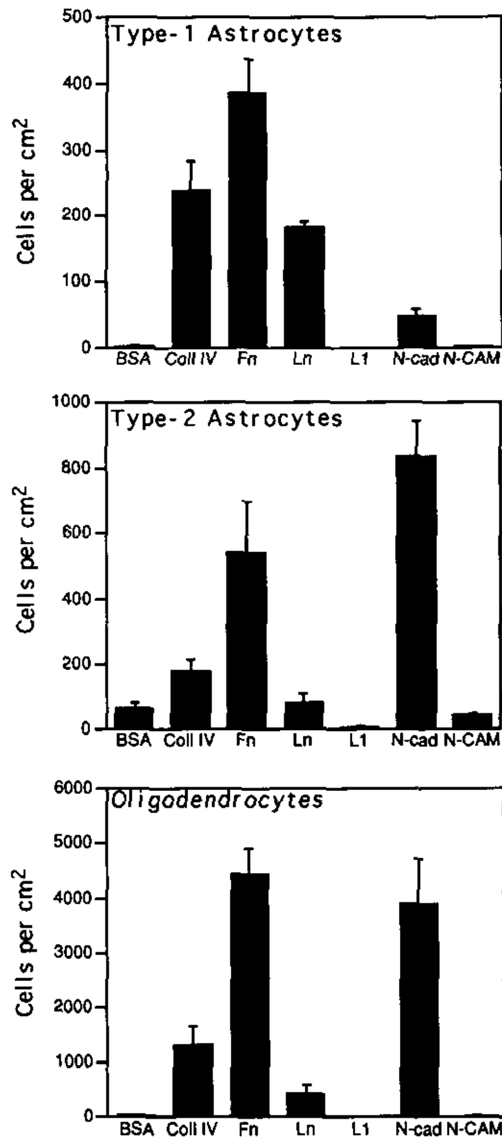

FIG. 7. Substrate-associated differences in the culture density of each glial cell type at 4 days *in vitro*. Type-1 astrocytes attain significantly ($P < 0.05$) higher cell densities on the ECM substrates (collagen IV, fibronectin, and laminin). Conversely O-2A lineage cells are far more numerous in cultures plated on *N*-cadherin and fibronectin than on collagen IV and laminin. Each bar represents the mean of five replicate wells.

of our results with the earlier work difficult. For example, it is possible that earlier results were due to selection of different types of astrocytes based on differential adhesion or to alteration of astrocyte behavior following attachment. It is also likely that glia from different parts of the CNS may respond differently to different adhesive interactions (Rousselet *et al.*, 1988). The proliferation rate of cortical astrocytes was reported to be enhanced by fibronectin and decreased by collagen when compared to untreated glass coverslips (Goetschy *et al.*, 1987). We, however, observed no significant differences in proliferation rates of either optic nerve type-1 or type-2 astrocytes on fibronectin compared to collagen IV. Possibly, this discrepancy is due to the low sensitivity of our methods which would be unable to detect less than a twofold difference in rates.

Our results suggest a potential modification of the widely used method of McCarthy and de Vellis (1980) for the purification of astrocytes. In this procedure, dissociated brain cells are plated on tissue culture plastic to which astrocytes can bind but neurons do not. After enriched astrocyte cultures have been produced, it may be possible to use dishes coated with either collagen IV or laminin to selectively purify type-1 astrocytes. Alternatively, *N*-cadherin-coated plates or dishes coated with anti-*N*-cadherin antibodies may be used for selectively purifying type-2 astrocytes. This approach may allow biochemical or cell biological studies on purified populations of astrocytes.

There have been numerous studies on how cell contact-mediated interactions influence the differentiation of Schwann cells, the myelin-forming cells of the peripheral nervous system (Bunge *et al.*, 1986, 1989). L1 has been implicated in the interactions between neurons and Schwann cells that lead to the production of compact myelin (Seilheimer *et al.*, 1989; Wood *et al.*, 1990), while *N*-cadherin appears to be very important in the initial interactions between nerve growth cones and Schwann cells (Letourneau *et al.*, 1991). Collagen has also been found to play a crucial role in this process (Bunge *et al.*, 1989). Much less is known about how CAMs and SAMs produce signals that mediate oligodendrocyte differentiation and myelin production. Oligodendrocytes growing on poly-L-lysine plates produce significantly more myelin-specific proteins than oligodendrocytes grown in suspension culture (Yim *et al.*, 1986). This was interpreted as indicating that adhesion is important in the switch to myelinogenic metabolism. Similarly, peptides that inhibit integrin function also inhibit oligodendrocyte production of myelin-specific membrane proteins when the oligodendrocytes are grown on an astroglial matrix (Cardwell and Rome, 1988). These results indicate that adhesive interactions can regulate oligodendrocyte differentiation. Our results showing that *N*-cadherin induces large increases in the surface area and in the amount of membrane produced by oligodendrocytes and suggests that *N*-cadherin may play an especially important part in oligodendrocyte differentiation. It remains to be tested whether *N*-cadherin specifically affects myelin formation.

Glial cells of the different lineages appear to serve separate functions in the optic nerve. Type-1 astrocytes form the glial limiting membrane, provide a structural framework for the nerve, and play a part in the forma-

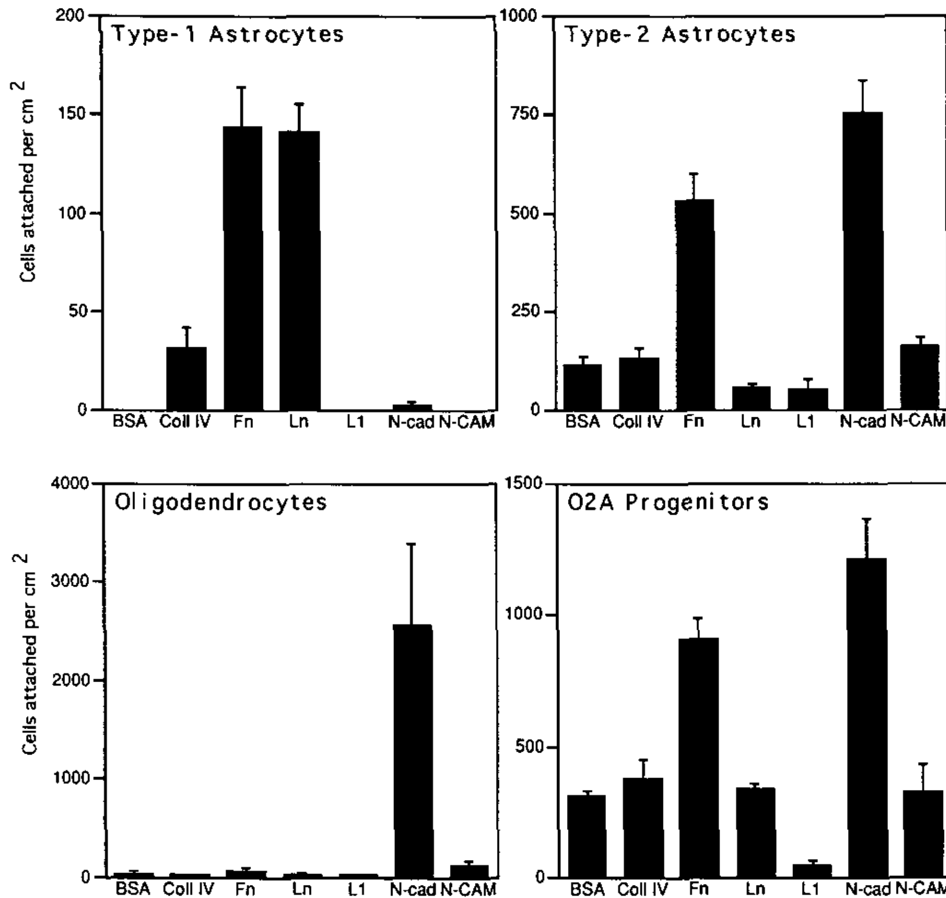

FIG. 8. Short-term adhesion of glial cells to substrates. O-2A lineage cells selectively bind to *N*-cadherin whereas type-1 astrocytes preferentially bind to the ECM components collagen IV ($P > 0.05$), fibronectin, and laminin ($P > 0.01$). Although type-2 astrocytes and O-2A progenitors also adhered to fibronectin, oligodendrocytes failed to bind in significant numbers to this substrate. Each bar represents mean cell attachment in four replicate wells.

tion of the blood-brain barrier. On the other hand, differentiated O-2A lineage cells are specialized for myelinating axons and helping to organize the nodes of Ranvier. The two cell lineages are likely to have structural differences that account for this specialization. We found that type-1 astrocytes exhibited preferences for adhesion molecules that differed markedly from the preferences shown by cells of the O-2A lineage. This result indicates that the glial cell types express different sets of CAMs and SAMs. Such differences would be a crucial factor in glial development for they may provide mechanisms whereby cells from different lineages can choose different migration pathways through developing, regenerating, or damaged regions of the nervous system. For example, type-1 astrocytes migrate from transplants into the surrounding CNS and associate closely with host blood vessels (Lindsey and Raisman, 1984; Smith and Miller, 1991). On the other hand, migration of O-2A progenitors from the optic chiasm into the optic nerve during development is probably guided by axons. This migration serves to distribute these cells to regions that will become myelinated (Small *et al.*, 1987). At the lamina cribosa, specialized type-1 astrocytes appear to form a barrier that prevents the migration of O-2A cells into the retina (ffrench-Constant *et al.*, 1988). Glial cells of the two lineages also respond differently to localized cues in other microenvironments such as blood vessel basal laminae, synaptic membranes, or axons. The use of CAMs and SAMs to provide localized differentiation signals would allow glia to produce appropriate structural and functional specializations in regions where abrupt transitions occur, such as between gray and white matter and at junctions between the PNS and CNS. There is now ample evidence that CAMs and SAMs can generate signals that can influence cellular second messenger systems (Kornberg *et al.*, 1991; Atashi *et al.*, 1992). Thus adhesion molecules could serve as differentiation signals or they could act to concentrate and confine diffusible differentiation factors to discrete targets (Singer, 1992). The influences of these

TABLE 1
EFFECT OF SUBSTRATES ON BRDU INCORPORATION[a]

Substrate	% BrdU positive cells		
	Type-1 astrocytes	Type-2 astrocytes	Progenitors
BSA	[b]	26.3 ± 3.0	13.2 ± 2.7
Collagen IV	13.8 ± 3.0	30.5 ± 8.0	16.0 ± 2.9
Fibronectin	15.2 ± 4.7	27.5 ± 3.0	13.5 ± 2.5
Laminin	22.0 ± 6.6	33.3 ± 3.0	15.8 ± 1.3
N-Cadherin	32.8 ± 7.8	19.4 ± 3.5	11.8 ± 3.6
NCAM	[b]	31.7 ± 6.4	20.8 ± 8.7

[a] Values are the mean of four replicate wells ± SEM. Differences between substrates were not significant ($P < 0.05$) when results were analyzed by one-way ANOVA.

[b] Cell numbers were too low on this substrate for statistical comparison.

adhesion molecules on glial migration rates and differentiation will require further studies *in vivo* and *in vitro*.

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