DEVELOPMENTAL BIOLOGY 167, 596–608 (1995)

Oligodendroblasts Distinguished from O-2A Glial Progenitors by Surface Phenotype ($O4^+GalC^-$) and Response to Cytokines Using Signal Transducer LIFRβ

A. L. GARD, W. C. WILLIAMS II, AND M. R. BURRELL

Department of Structural and Cellular Biology, College of Medicine, University of South Alabama, Mobile, Alabama 36688

Accepted October 27, 1994

The developmental potential of progenitors at two final stages of the macroglial lineage giving rise to oligodendrocytes in postnatal rat brain was studied in response to defined and serum inducers of astrocyte gene expression. Cell immunoselection [with G_{D3} ganglioside, O4 and galactocerebroside (GalC) antibodies] was used to isolate $G^+_{D3}O4^-$ and $O4^+GalC^-$ phenotypes directly from premyelinating cerebrum. In a basal defined culture medium, $G^+_{D3}O4^-$ progenitors differentiated infrequently into oligodendrocytes on a growth substratum comprised of meningeal cell-derived extracellular matrix. Their conversion into astrocytes, as determined by immunofluorescence analysis of glial fibrillary acidic protein expression, was induced by oncostatin-M as well as leukemia inhibitory factor (LIF) and ciliary neurotrophic factor, but not interleukin-6, and required extracellular matrix. By comparison, $O4^+GalC^-$ progenitors were refractory to astrocyte induction under these conditions, as in short-term cultures of optic nerve, and differentiated into myelinogenic oligodendrocytes instead. Only in response to an overriding stimulus in fetal bovine serum did $O4^+GalC^-$ progenitors, like their immediate precursors, become astrocytic. These data functionally distinguish two classes of astrocyte-inducing agents to provide clear evidence of an oligodendroblast, a progenitor defined by surface phenotype ($O4^+GalC^-$) and an altered response of the oligodendrocyte lineage to cytokines using signal transducer LIFRβ. © 1995 Academic Press, Inc.

INTRODUCTION

Over the past decade, studies using optic nerve glia have suggested that environmental cues divert oligodendrocyte precursor cells (O-2A) to an astrocytic phenotype designated type-2 (Raff *et al.*, 1983a; Raff, 1989, and references therein). An extraneural source of proteinaceous type-2 astrocyte-inducing activity was found in fetal bovine serum (Raff *et al.*, 1983b) and identified as a 50-kDa protein entity also evident in human serum and plasma (Levison and McCarthy, 1991). The detection of an inducer protein expressed in brain cultures (Lillien *et al.*, 1988) and postnatal rat optic nerve (Hughes and Raff, 1987; Hughes *et al.*, 1988) led to the proposal that within the CNS, ciliary neurotrophic factor (CNTF), a polyfunctional neurotrophin localized predominantly to other astrocytes (Stockli *et al.*, 1991; Dobrea *et al.*, 1992; Rudge *et al.*, 1992), drives type-2 astrocyte development (Hughes *et al.*, 1988; Lillien *et al.*, 1988, 1990; Lillien and Raff, 1990). Differing from induction by FBS, however, the CNTF-mediated response required meningeal cell-derived extracellular matrix components for stabilization (Lillien *et al.*, 1990) and may have affected fewer O-2A cells as they matured (Hughes and Raff, 1987).

In vivo findings indicate that bipotential progenitors contribute to gliogenesis, although to a lesser extent than previously conceptualized (Raff, 1989). Despite *in vitro* evidence to the contrary, few, if any, detectable type-2 astrocytes have been identified in postnatal rat optic nerve (Skoff, 1990; Fulton *et al.*, 1992), where CNTF is particularly abundant (Stockli *et al.*, 1991). Fate studies of progenitors infected with retroviruses encoding heritable markers (Grove *et al.*, 1993; Luskin *et al.*, 1993; Price and Thurlow, 1988) concur that the bulk of oligodendrocytes and astrocytes in forebrain descend prenatally along separate progenitor lines, as suggested by other light and electron microscopic observations (Levine and Goldman, 1988; Skoff and Knapp, 1991). However, this approach has recently demonstrated in the rat forebrain subventricular zone a population of bipotential, postnatally generated progenitors whose decision to form oligodendrocytes or astrocytes is made upon completing migration (Levison and Goldman, 1993a) and depends on their destination within the hemispheres (Levison *et al.*, 1993).

Here we have reexamined the differentiation potential of oligodendrocyte progenitors as a function of their progression through two successive stages defined by antibodies to cell surface components (Gard and Pfeiffer, 1990; Warrington and Pfeiffer, 1992). In the rat, migratory progenitors that express G_{D3} ganglioside

0012-1606/95 $6.00

upon departure from germinal subventricular zones (Levine and Goldman, 1988; Reynolds and Wilkin, 1988; Hardy and Reynolds, 1991) correspond to motile, generally bipolar cells bearing monoclonal antibody (mab) A2B5-reactive gangliosides *in vitro* (Raff *et al.*, 1983b; Levi *et al.*, 1986, 1987). The immediate progenitors of oligodendrocytes label with mab O4 (Sommer and Schachner, 1981) and, distinct from their A2B5/$G_{D3}^+O4^-$ precursors, represent a multipolar, postmigratory stage (Dubois-Dalcq, 1987; Warrington and Pfeiffer, 1992; Warrington *et al.*, 1993) that is also mitogenically active (Gard and Pfeiffer, 1990, 1993; McKinnon *et al.*, 1990; Warrington and Pfeiffer, 1992; Hardy and Reynolds, 1993; Fok-Seang and Miller, 1994). Some evidence that $O4^+$ progenitors have already entered an oligodendrocyte differentiation program *in vivo* (Gard and Pfeiffer, 1989) is discordant with studies suggesting that they, like their A2B5/$G_{D3}^+O4^-$ precursors, are bipotential in response to FBS (Levi *et al.*, 1987; Aloisi *et al.*, 1988; Trotter and Schachner, 1989) and express GFAP in demyelinating disease (Godfraind *et al.*, 1989).

In this study an immunopanning technique (Gard and Pfeiffer, 1989) was used to compare, in cultures challenged with different type-2 astrocyte-inducing agents, the differentiation potential of $G_{D3}^+O4^-$ and $O4^+GalC^-$ progenitors isolated directly from early postnatal rat forebrain. The results show that in response to CNTF only the former are bipotential and clearly identify two additional members of the same cytokine subfamily, leukemia inhibitory factor (LIF) and oncostatin-M (OSM), as "type-2" astrocyte inducers with identical target-cell specificity. In contrast, $O4^+GalC^-$ progenitors are committed to oligodendrocyte differentiation in the presence of these cues yet retain the plasticity to develop into astrocytes if exposed to an overriding stimulus in FBS.

EXPERIMENTAL PROCEDURES

Growth Factors and Other Type-2 Astrocyte Inducers

Recombinant rat CNTF (Masiakowski *et al.*, 1991; gift of Regeneron Pharmaceuticals), recombinant human OSM and recombinant murine IL-6 and LIF (R & D Systems) were stored (−70°C) as stock dilutions (≥100 μg/ml) containing 0.1% (v/v) crystalline bovine serum albumin (BSA; A-4161, Sigma). Fetal bovine serum (FBS; Hyclone "defined") was heat-inactivated prior to storage (−20°C) in small aliquots. A high-salt extract of rat optic nerves was prepared (Hughes and Raff, 1987) as modified by Lillien *et al.* (1990).

Immunoreagents

Mouse monoclonal antibodies (mabs) A2B5 (Eisenbarth *et al.*, 1979), R24 (anti-G_{D3}; Pukel *et al.*, 1982), and O-series markers (Sommer and Schachner, 1981) O4 (Bansal *et al.*, 1992) and O1 (anti-GalC; Bansal *et al.*, 1989) were concentrated by membrane ultrafiltration and used to label the surface of live oligodendrocyte lineage cells. Rat monoclonal anti-GFAP and rabbit polyclonal anti-MBP were the gifts of V. Lee (Lee *et al.*, 1984) and S. E. Pfeiffer (Barbarese and Pfeiffer, 1981). Secondary antibodies (Jackson Immunoresearch Labs) consisted of $F(ab)'_2$ fragments of donkey anti-rat IgG conjugated to rhodamine (TRITC); goat anti-mouse IgG (γ chain-specific) conjugated to fluorescein (GAM_G-FITC), 7-amino-4-methylcoumarin-3-acetic acid (GAM_G-AMCA) or nothing; and goat anti-mouse IgM (μ chain-specific) in conjugated (GAM_M-FITC) or unconjugated form.

Immunopanning

From tissue. Cerebra from rat littermates at Postnatal Day (P) 4–5 (CD; Charles River) were used as a source of $G_{D3}^+O4^-$ and $O4^+GalC^-$ progenitors purified by immunopanning, as detailed elsewhere (Gard and Pfeiffer, 1989) with modifications (Gard *et al.*, 1993; Gard and Pfeiffer, 1993). Briefly, pooled tissue from three littermates was dissociated into a single-cell suspension from which $O4^+GalC^+$ oligodendrocytes, accounting for ~15% of the total $O4^+$ cell population, were removed by mab O1-mediated lysis at 25°C using nontoxic guinea pig complement. Following the removal of macrophages by differential adherence, the remaining $O4^+GalC^-$ progenitor population was labeled with mab O4 and then incubated in polystyrene petri dishes precoated with affinity-purified GAM_M. In parallel, cells dissociated from three additional littermates were depleted of G_{D3}^+ $O4^+$ progenitors (by mab O4-mediated complement lysis) and macrophages, treated with mab R_{24} to label remaining $G_{D3}^+O4^-$ progenitors and incubated in dishes precoated with affinity-purified GAM_G (Warrington *et al.*, 1993). Yields for each stage averaged $1\text{–}1.5 \times 10^6$ total cells with >92% purity. Minor contaminants in $G_{D3}^+O4^-$ isolates were $GFAP^+$ cells (~1%), $O4^+GalC^-$ progenitors (<2%) and unidentified cells, whereas $O4^+GalC^-$ isolates contained a few $G_{D3}^+O4^-$ progenitors (<2%), $O4^+GalC^+$ oligodendrocytes (<2%) and unidentified cells but were essentially devoid of astrocytes.

From germinal cultures. Mixed cell-type cultures of dissociated rat cerebra were initiated in poly-DL-ornithine-coated (50 μg ml^{-1}, 30 min, 37°C) T-75 flasks at a seeding density of 2×10^7 cells per flask (Gard *et al.*, 1988) and grown in BDM containing 10% FBS. On Day 4 the cultures were rinsed twice with 10 ml of DMEM to remove residual serum and dissociated with 8 ml of 0.025% (w/v) trypsin (15 min, 37°C) in a Hepes-buffered (20 m*M*, pH 7.4) Earle's balanced salt solution (EBSS) supplemented with 0.3% (w/v) BSA and 14 m*M* D-glu-

cose (Hepes-EBSS). After centrifugation (100*g*, 5 min) in 5% (v/v) FBS, cells recovered from two flasks were resuspended with ice-cold Hepes-EBSS in a single 50-ml glass conical centrifuge tube to which unfractionated mab O1 hybridoma culture supernatant was added to 10% (v/v) final concentration. After 15 min the cells were centrifuged (as above) and subjected to the same selection sequence (complement lysis, differential adherence, and immunopanning) used for $O4^+GalC^-$ progenitor purification from tissue (above).

Cell Culture

Isolated cerebral progenitors were seeded (12,000 per 30-μl droplet) onto coverslips (Chance Propper; 9 mm) precoated with various substrata (below) and transferred into 48-well trays (Costar) to which 250 μl of medium was added per well. Basally defined culture medium (BDM), a derivation of the N2 formulation (Bottenstein and Sato, 1979), consisted of DMEM (Gibco 430-2100) containing sodium bicarbonate (1.85 g/liter), D-glucose (4.5 g/liter), L-glutamine (2 m*M*), human transferrin (50 μg/ml), bovine insulin (5 μg/ml), 3,3'5-triiodo-L-thyronine (30 n*M*), sodium selenite (30 n*M*), sodium pyruvate (10 m*M*), d-biotin (10 n*M*), hydrocortisone (10 n*M*), 0.01% (w/v) crystalline BSA, penicillin (100 IU/ml), streptomycin sulfate (100 μg/ml) and tested growth factors. The medium was changed (50%) every 2 days.

Optic nerve cells were dissociated from 4-day-old rat optic nerves as previously described (Gard and Pfeiffer, 1990) and seeded (10,000) on polyornithine-coated (above) 6-mm coverslips in 48-well trays. Growth media consisted of BDM containing 0.5% FBS (added to promote modest cell survival).

Meningeal cell-derived extracellular matrix (Lillien *et al.*, 1990) was prepared from primary meningeal cell cultures (Gard and Pfeiffer, 1990) that were passaged onto poly-DL-ornithine-coated (50 μg/ml, 1 hr, 37°C) coverslips (9 mm) in a 100-mm dish. After reaching confluency in DMEM containing 10% FBS, the cultures were rinsed twice with 10 ml PBS and lysed on a rotary shaker with two changes (2 min each) of 20 m*M* ammonium hydroxide solution prepared daily and rinsed with PBS. Polyornithine-coated coverslips were alternatively treated with 0.01% (w/v) BSA or PBS (1–2 hr). All prepared substrata were immersed in PBS (37°C) until used (within 2 hr).

Immunofluorescence Microscopy

Cultures were labeled for 20–30 min at 4°C with antibodies, each diluted in EBSS-Hepes containing 5% (v/v) normal goat serum or normal donkey serum. Cells to be doubly labeled for surface antigens and GFAP were treated live with an IgM [A2B5 (1:40), O4 (1:40), or O1 (1:40)] or IgG [R24 (1:20)] marker, followed by FITC-GAM_G or -GAM_M (1:40), and fixed (3 min, −20°C) in ethanol:acetic acid (95:5). These cultures were then labeled with rat anti-GFAP (1:5) followed by TRITC-donkey anti-rabbit IgG (1:40). Other cultures were doubly labeled with O1 and MBP as described previously (Gard and Pfeiffer, 1989). For triple labeling, cultures were treated with a mixture of R_{24} (1:20) and O4 (1:40), followed by AMCA-GAM_G (1:40) and FITC-GAM_M (1:40) and then fixed and labeled for GFAP as described above. Rinses with two 5-min changes of EBSS-Hepes followed each step. Coverslips were inverted in a droplet of buffered (pH 8.6) glycerol containing 2.5% (w/v) diazobicyclo-(2,2,2) octane to retard image fading (Johnson *et al.*, 1982).

RESULTS

Response to Cytokines: CNTF, LIF, OSM, and IL-6

To determine whether $G^+_{D3}O4^-$ and $O4^+GalC^-$ progenitors are bipotential in premyelinating rat cerebrum, populations representing both phenotypes were isolated separately from tissue by immunopanning, initiated as developmental stage-specific cultures, and challenged with CNTF in parallel. Also tested for activity were LIF, OSM, and IL-6. Receptor activation by IL-6 entails homodimerization of the "β" signaling subunit transducer protein gp130 (Murakami *et al.*, 1993), whereas CNTF, LIF, and OSM heterodimerize gp130 and the related protein, LIFRβ (Davis *et al.*, 1993). The cells were seeded on meningeal culture-derived extracellular matrix to promote stable type-2 astrocyte development under serumless conditions (Lillien *et al.*, 1990) and examined 1 and 2 days later by immunofluorescence labeling for surface G_{D3} ganglioside or O4 antigens in combination with GFAP.

GFAP expression was induced only in cultures initiated with $G^+_{D3}O4^-$ progenitors (Fig. 1). Fewer than 2% of seeded $G^+_{D3}O4^-$ cells were $GFAP^+$ upon isolation. Undetectable 18 hr after seeding, induction was distinct by Day 2, when the proportion of G^+_{D3} cells expressing GFAP increased with CNTF doses >0.001 ng/ml to a plateau of ~50% at 0.1–10 ng/ml (ED_{50} = 10 pg/ml), 6-fold over background with CNTF at doses <0.001 ng/ml (Fig. 1A). Also showing activity, LIF (ED_{50} = 40 pg/ml) and OSM (ED_{50} = 110 pg/ml) were as efficacious as CNTF, varying only in potency (Figs. 1B and 1C), whereas IL-6 was ineffective (Fig. 1D). This experiment was repeated three times with nearly identical results. In all cases, growth on meningeal cell-derived extracellular matrix was required for induction (Table 1) and increased the percentage of $G^+_{D3}O4^-$ progenitors express-

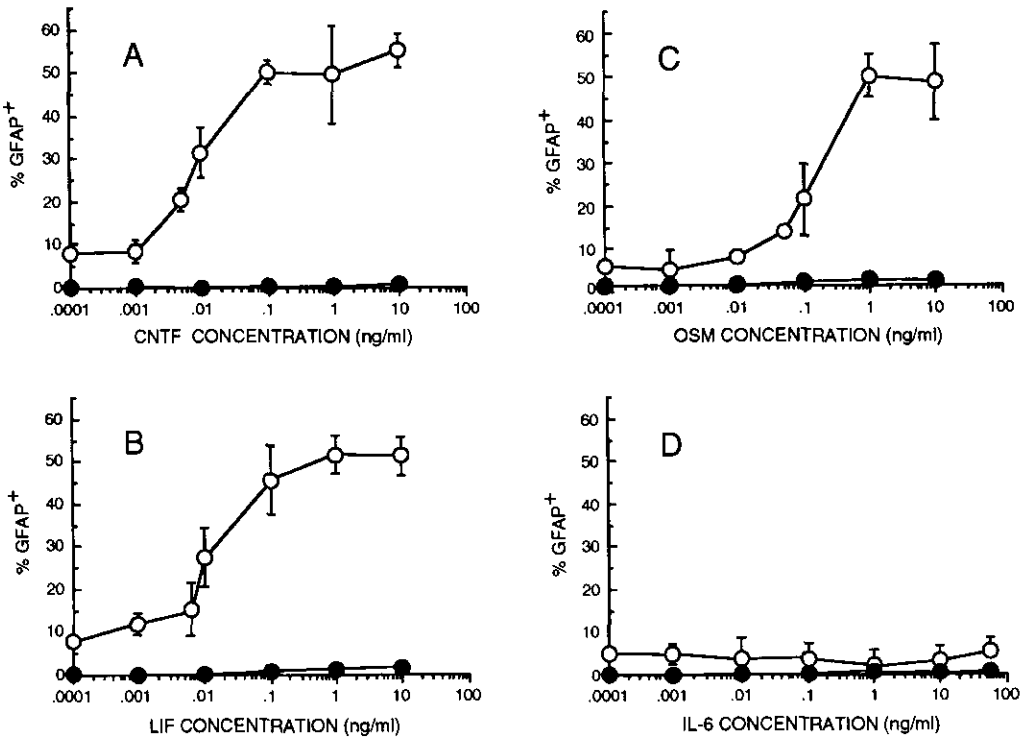

FIG. 1. Growth factors inducing GFAP expression in cultured cerebral oligodendrocyte progenitors according to phenotypic stage. Progenitors at successive $G_{D3}^+O4^-$ (○) and $O4^+GalC^-$ (●) stages were isolated in parallel by immunopanning, seeded on substratum of meningeal cell-derived ECM and cultured in BDM containing CNTF (A), LIF (B), OSM (C), or IL-6 (D) at the indicated concentrations. After 48 hr, the cultures labeled by indirect immunofluorescence for G_{D3} (○) or O4 antigens (●) in combination with GFAP. Data represent the mean (±SD) proportion of oligodendrocyte lineage cells doubly labeled in triplicate cultures.

ing GFAP 9- to 10-fold over background on a polyornithine substratum alone.

Figure 2 shows the striking difference of effect that CNTF had on cultures initiated with the two progenitor phenotypes. $G_{D3}^+O4^-$ progenitors relabeled with mab R_{24} 2 days after seeding had regenerated 2-4 unbranched processes, and many were clearly $G_{D3}^+GFAP^+$ (Figs. 2A-2C), indicative of a type-2 astrocytic phenotype (Lillien and Raff, 1990). A small proportion (~10%) having evolved a more complex morphology by this time were $O4^+GFAP^+$ (Figs. 2D and 2E). This dual phenotype probably arose from seeded $G_{D3}^+O4^-$ cells, since contamination of the starting population with $O4^+$ progenitors was negligible (<1%). In contrast to their precursors, $O4^+GalC^-$ progenitors were uniformly $GFAP^-$ upon isolation (Gard and Pfeiffer, 1989) and remained so during the 2-day CNTF challenge as the majority evolved their morphology to resemble more complex, newly differentiated oligodendrocytes (Figs. 2F-2H). Culturing in LIF or OSM produced essentially the same effect as CNTF (data not shown).

TABLE 1
EFFECT OF MENINGEAL EXTRACELLULAR MATRIX ON TYPE-2 ASTROCYTE INDUCTION BY CNTF, LIF, AND OSM

Substratum	% of G_{D3}^+ Cells expressing GFAP in: CNTF	LIF	OSM
Polyornithine + BSA	5.6 ± 0.3	5.4 ± 1.3	4.7 ± 0.6
Polyornithine + extracellular matrix	55.1 ± 3.9	51.2 ± 4.6	41.1 ± 8.8

Note. Cerebral $G_{D3}^+O4^-$ cells recovered from P4 littermates were seeded (12,000) on coverslips (9 mm) coated with the indicated substrata (see Experimental Procedures) and grown in BDM supplemented with LIF, CNTF, OSM (each at 10 ng/ml), or nothing. After 2 days in culture the cells were doubly labeled for G_{D3} and GFAP by immunofluorescence. Data are the means (±SD) of three cultures from a single representative experiment carried out in duplicate.

Survival of the $O4^+GalC^-$ cell population 2 days after culture was high on extracellular matrix, exceeding 90% of the seed, regardless of whether inducing factors were included in the medium. These cells were mitogenically inactive, with a labeling index of bromodeoxyuridine incorporation (6 hr) of <2%, and invariably differentiated into $GalC^+$ oligodendrocytes at a rate (~15-18% by Day 1, increasing to ~50-55% by Day 2) similar to that oc-

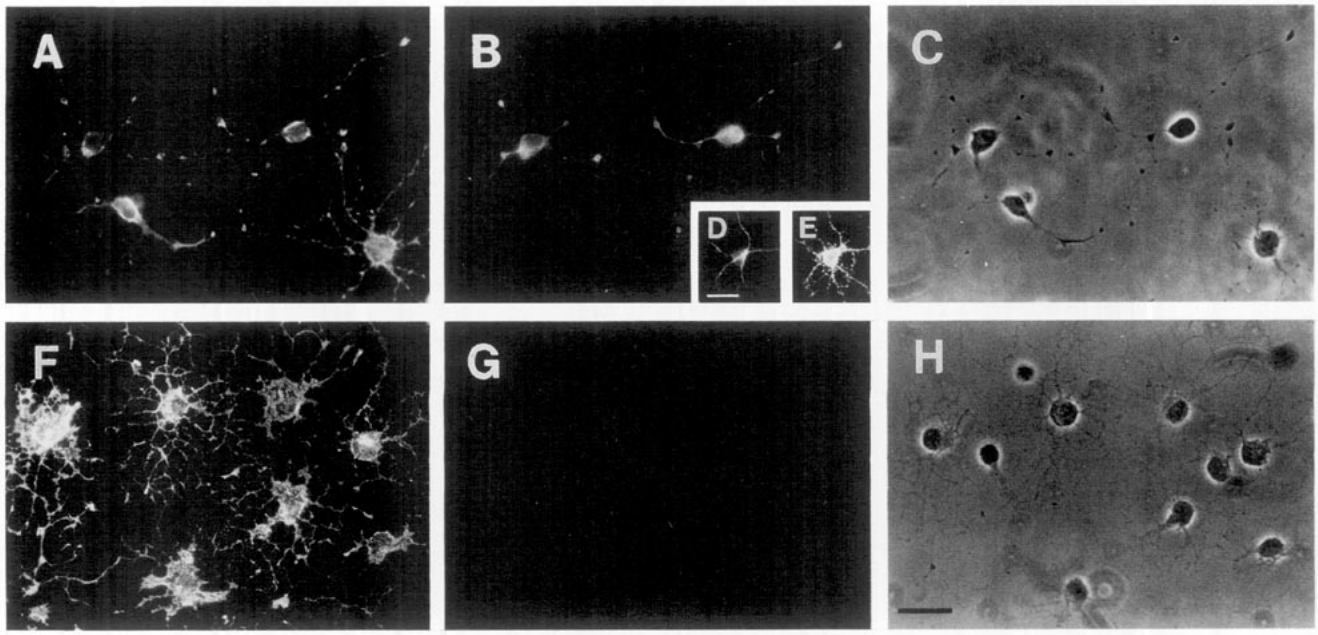

FIG. 2. Immunostaining of cerebral oligodendrocyte progenitors responsive and refractory to the type-2 astrocyte-inducing effect of CNTF in culture. Progenitor populations isolated at $G^+_{D3}O4^-$ (A–E) and $O4^+GalC^-$ (F–H) stages were cultured as described in the Fig. 1 legend in 1 ng/ml CNTF and analyzed 48 hr later [(A–B, D–E, F–G) double label indirect immunofluorescence; (C and H) phase contrast]. Two of four G^+_{D3} cells reacting with R_{24} antibody [FITC, (A)] in cultures initiated with $G^+_{D3}O4^-$ progenitors label with GFAP antibody [TRITC, (B)]. A minor population of these cells acquire both O4 [FITC, (D)] and GFAP [TRITC, (E)]. Progenitors captured at the more advanced $O4^+GalC^-$ stage, labeled with O4 antibody [FITC, (F)], are $GFAP^-$ [TRITC, (G)]. Bars, 20 μm (A–H).

curring in BDM on a polyornithine substratum alone (Gard and Pfeiffer, 1993). Thus, neither accelerated death nor rapid terminal differentiation accounted for the lack of bipotentiality in $O4^+$ progenitors sampled from cerebrum.

In another experiment, we compared the short-term response of progenitors arising in postnatal rat optic nerve to CNTF and endogenous inducing activity extracted from this region (Hughes and Raff, 1987; Lillien *et al.*, 1990). Tricolor immunofluorescence enabled the detection GFAP among $G^+_{D3}O4^-$ and $O4^+GalC^-$ progenitors copopulating the same cultures (Fig. 3) 1 day after dissociation from P4 tissue (Gard and Pfeiffer, 1990). Both CNTF (10 ng/ml) and optic nerve tissue extract (5 μg protein/ml) significantly stimulated the percentage of $G^+_{D3}O4^-$ progenitors coexpressing GFAP, whereas $O4^+GalC^-$ progenitors were unaffected (Figs. 3 and 4). Thus, aside from responding earlier (within 24 hr) and without prepared extracellular matrix as compared to purified cultures (Figs. 1 and 2), optic nerve progenitors behaved no differently than their cerebral counterparts.

Fate of Cultured $G^+_{D3}O4^-$ and $O4^+GalC^-$ Progenitors

Having determined that $G^+_{D3}O4^-$, but not $O4^+GalC^-$, progenitors can switch to an astrocytic phenotype in a serumless medium, the developmental fate of both stages was compared after 5 days of culture under inducing (10 ng/ml CNTF or LIF) and noninducing conditions. At this time the extent of differentiation into oligodendrocytes or astrocytes was assessed by double-label immunofluorescence (Fig. 3) using antibodies to GFAP and the myelin markers, GalC, and myelin basic protein (MBP).

Cultures initiated with $G^+_{D3}O4^-$ progenitors in the presence of CNTF or LIF were heterogenous by this time and developed few oligodendrocytes. The majority of cells were astrocytic (Table 2) with a stellate morphology (Figs. 5A–5C) and appeared to have lost G_{D3} ganglioside from their surface as reported by others (Norton and Farooq, 1989; 1993). Of the <5% of $GFAP^+$ cells that remained G^+_{D3}, most had an expansive, polygonal shape (data not shown) reminescent of the "type-3" variety (Vaysse and Goldman *et al.*, 1992). Approximately 25% of total cells remained bipolar with an undifferentiated oligodendrocyte precursor phenotype ($G^+_{D3}O4^-GFAP^-$). In the absence of CNTF/LIF, astrocytes comprised a significantly smaller proportion of total cells (Table 2), the majority of which retained the starting G^+_{D3} phenotype (data not shown).

By comparison, $O4^+GalC^-$ progenitors differentiated predominantly into $GalC^+$ oligodendrocytes (Table 2; Figs. 5D–5F) regardless of whether CNTF or LIF was

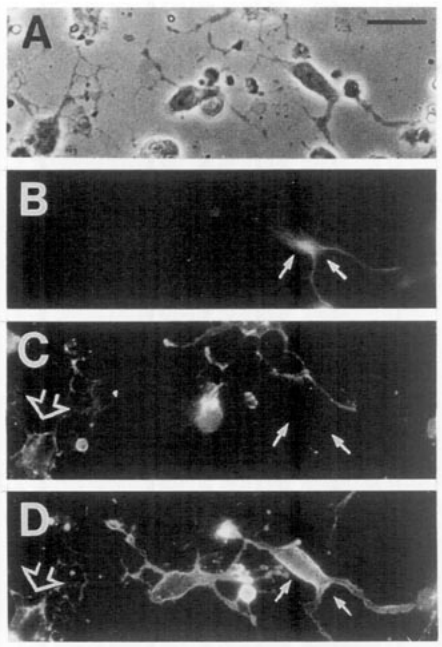

FIG. 3. Immunofluorescence analysis of CNTF-induced GFAP expression in oligodendrocyte progenitors cultured from postnatal optic nerve. Mixed cell-type cultures of dissociated P4 rat optic nerves were plated on polyornithine-coated coverslips in BDM containing CNTF (1 ng/ml) and analyzed 24 hr later [(A) phase contrast; (B–D) triple-label indirect immunofluorescence]. $GFAP^+$ filaments [TRITC, (B)] are shown in one (small arrows) of two G^+_{D3} oligodendrocyte precursors reacting with R_{24} [AMCA, (D)], but not O4 [FITC, (C)], which labels a more complex progenitor in this field (hollow arrow). Bar, 22 μm (A–D).

added to the medium, and >90% of these had matured to evolve their processes into flattened myelin-like membranes that reacted intensely with MBP antibodies

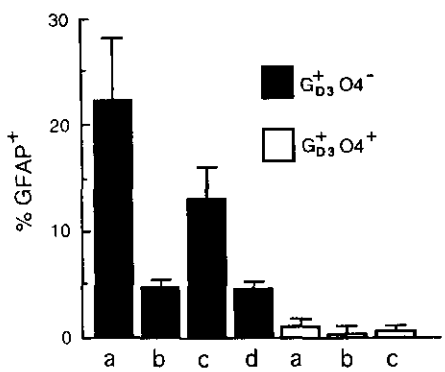

FIG. 4. Frequency of induced GFAP expression among cultured optic nerve progenitors distinguished by surface phenotype. Mixed cell-type cultures prepared and analyzed as described in the Fig. 3 legend were grown in BDM supplemented with (a) CNTF (1 ng/ml), (b) FCS (0.5%), (c) optic nerve extract protein (5 μg/ml), or (d) nothing. Data represent the mean (±SD) proportion of $G^+_{D3}O4^-$ and $G^+_{D3}O4^+$ cells that coexpressed GFAP in triplicate cultures from which ≥300 cells for each surface phenotype were scored.

TABLE 2

IN VITRO DIFFERENTIATION POTENTIAL OF OLIGODENDROCYTE PROGENITORS COMPARED AT TWO SUCCESSIVE STAGES OF THE LINEAGE IN PREMYELINATING RAT CEREBRUM

	$G^+_{D3}O4^-$		$O4^+GalC^-$	
Inducer	%$GalC^+$	%$GFAP^+$	%$GalC^+$	%$GFAP^+$
None	8.4 ± 3.5	29.9 ± 8.3	87.8 ± 6.2	9.0 ± 1.1
CNTF	13.3 ± 2.5	65.2 ± 15.7	88.9 ± 4.1	6.6 ± 0.8
LIF	6.4 ± 4.1	60.0 ± 4.4	87.2 ± 4.7	9.0 ± 3.8

Note. Cultures initiated with progenitor populations enriched for the indicated surface phenotypes were grown on meningeal cell-derived extracellular matrix in BDM supplemented with 10 ng/ml CNTF or LIF or nothing and doubly labeled for GFAP and GalC by immunofluorescence 5 days later. Data are expressed as the mean percentage (±SD) of total cells positive for the indicated marker in triplicate cultures.

(Figs. 5G–5I). Before differentiating under these conditions, $O4^+$ progenitors did not transiently revert to the less mature $A2B5/G^+_{D3}O4^-$ stage as occurs when seeded on a substratum of astrocyte-derived extracellular matrix (Agresti *et al.*, 1991) or in the presence of exogenous PDGF (Gard and Pfeiffer, 1993). Nonoligodendrocytes, comprising ~10% of total cells, consisted of undifferentiated progenitors ($O4^+GFAP^-$), those with a dual phenotype ($O4^+GFAP^+$), and unidentified cells at a ratio of ~8:1:1. Nevertheless, a large majority of $O4^+GalC^-$ progenitors were able to execute a myelinogenic program under identical conditions that converted their immediate precursors to an astrocytic phenotype.

Response to FBS

We next determined whether cerebral $O4^+GalC^-$ progenitors are as refractory to the type-2 astrocyte-inducing effect of FBS as they are to CNTF, LIF and OSM (Figs. 1 and 2). To control for differences in germinal environment, the response of $O4^+GalC^-$ progenitors arising in serum-fed (10% FBS), primary cultures (4-day-old) of newborns was compared to their counterparts emerging in P4–5 cerebrum. Progenitors purified from both sources in parallel were plated on a polyornithine substratum in BDM containing 10% FBS and examined for GFAP expression by immunofluorescence as function of increasing age.

Despite their refractoriness to defined inducers, $O4^+$ progenitors were converted by FBS into astrocytes (Fig. 6), as were $G^+_{D3}O4^-$ progenitors examined in parallel (data not shown). Differing upon isolation, $O4^+$ progenitors raised in mixed cell-type cultures frequently contained GFAP, unlike their counterparts born *in vivo* (Table 3; Figs. 6A and 6B and 6F and 6G).

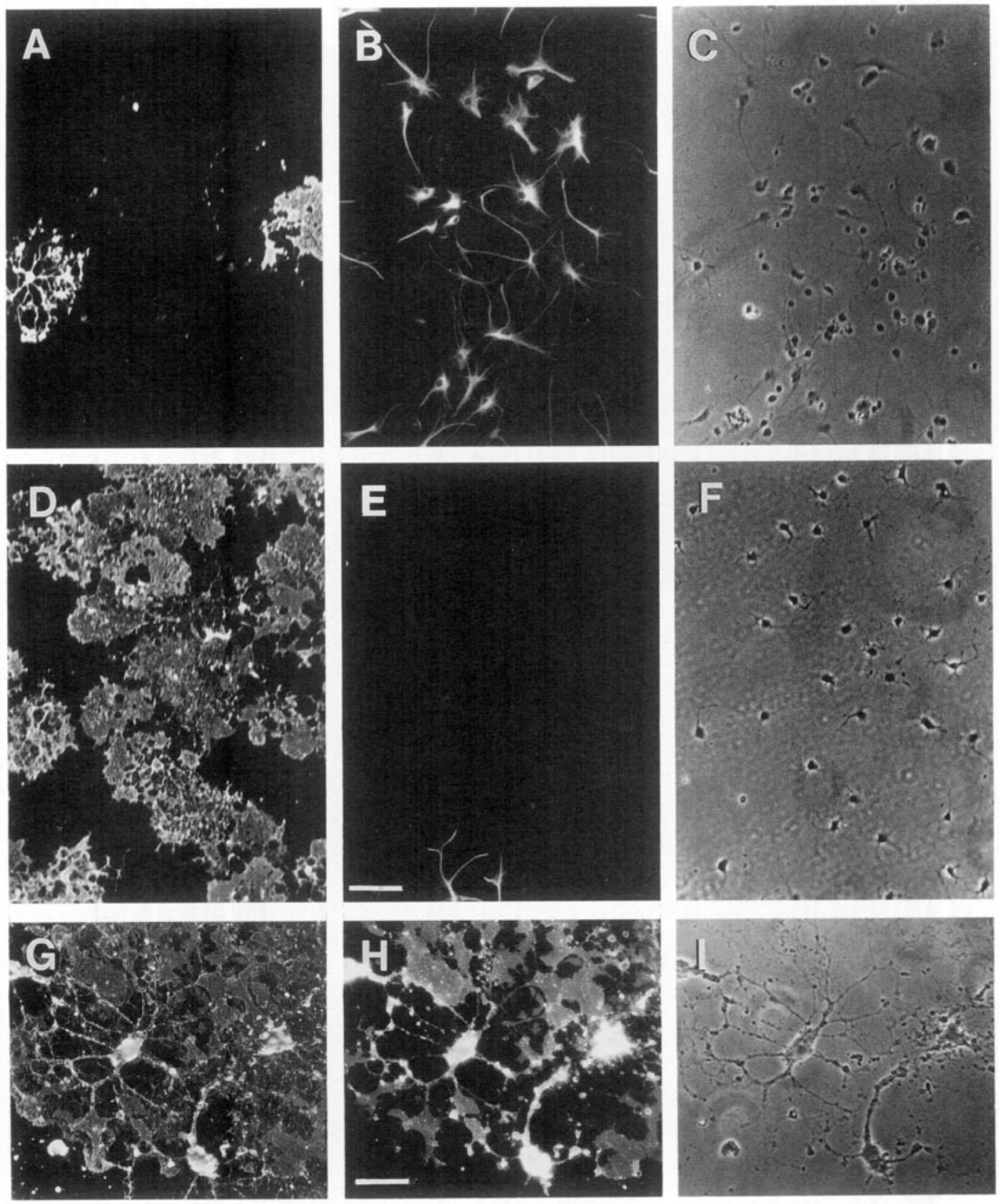

FIG. 5. Fate of oligodendrocyte progenitors cultured in CNTF as a function of developmental stage. Cells isolated from P4–5 cerebra with the phenotypes $G^{+}_{D3}O4^{-}$ (A–C) and $O4^{+}GalC^{+}$ (D–I) were cultured on extracellular matrix in BDM containing 1 ng/ml CNTF and analyzed 5 days later [(A, B, D, E, G, H) double label immunofluorescence; (C, F, I) phase contrast] for GalC [FITC (A, D, G)] in combination with GFAP [TRITC (B, E)] or MBP [TRITC (H)]. Scale bars, 50 μm (A–D); 30 μm (G–I).

Once separated from other cell types and recultured in 10% FBS, the proportion of culture-derived progenitors that coexpressed GFAP remained constant for 24 hr, while $O4^{+}$ cells that were sampled directly from tissue remained $GFAP^{-}$. Thereafter, GFAP appeared in both populations and with increasing frequency, occurring in as many as 80–90% of total $O4^{+}$ cells by Day 5 with variability among experiments (Table 3; Figs. 6C–6E and 6H–6J). At this time diminished $O4^{-}$ immunostaining and relatively enhanced stellation distinguished the culture-derived cells. We conclude that progenitors born *in vivo* were as plastic as those arising *in vitro* if exposed to the appropriate stimulus (FBS, but not CNTF).

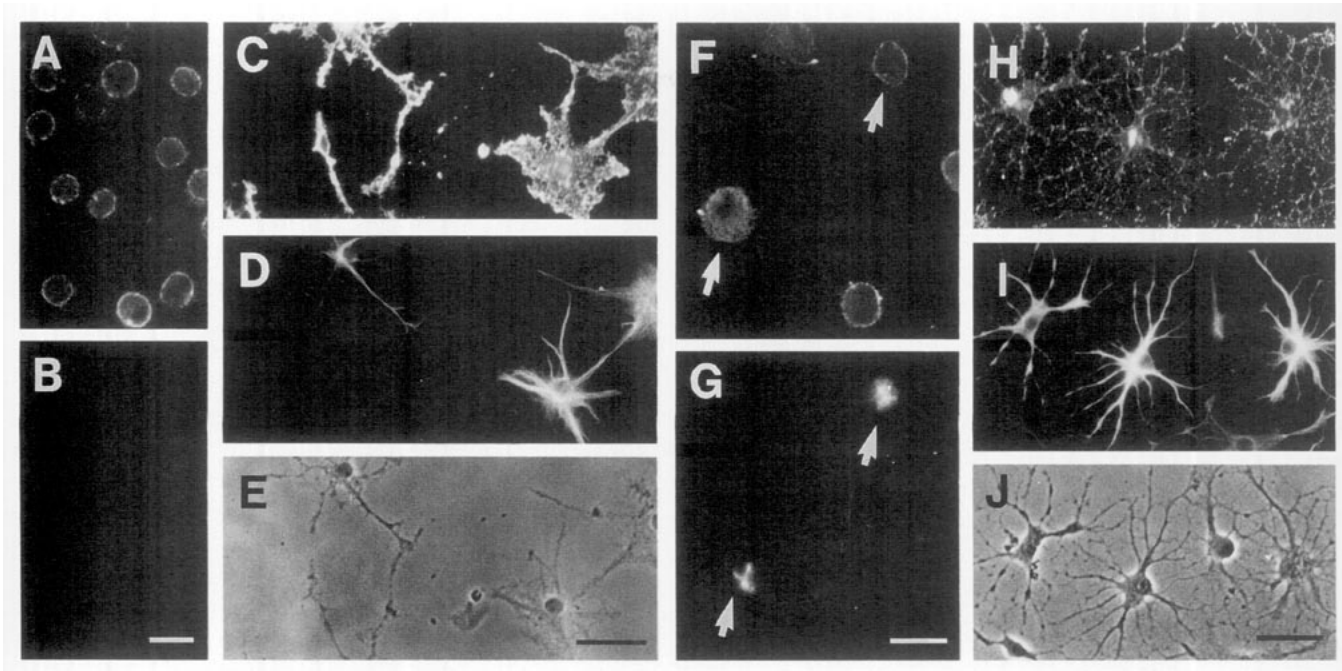

FIG. 6. FBS-induced GFAP expression in oligodendrocyte progenitors ($O4^+GalC^-$) as a function of origin in cerebrum and cerebral-derived germinal culture. $O4^+GalC^-$ progenitors purified from cerebrum (A–E) and germinal cerebral cultures (F–J) were plated on polyornithine-coated coverslips and analyzed [(A and B, C and D, F and G, H and I) double label immunofluorescence; (E and J) phase contrast] immediately after attachment (A, B, F, G) or following 5 days of culture in BDM containing 10% FBS (C–E, H–J). Progenitors expressing surface O4 [FITC, (A, C, F, H)] were labeled internally for GFAP [TRITC, (B, D, G, I)]. Examples of culture-derived $O4^+$ cells that contain GFAP upon isolation are indicated [arrows, (F and G)]. Bars, 15 μm (A, B, F, G); 30 μm (C–E, H–J).

Astrocytes in Postnatal Cerebrum and Optic Nerve Are $A2B5^+$ but $O4^-$

To determine if astrocytes with a similar dual $O4^+GFAP^+$ phenotype reside in rat brain at the equivalent age, cells dissociated from P10 cerebra and optic nerves were examined by immunofluorescence labeling. Live cells singly marked with A2B5, O4, and GalC antibodies accounted for ~65 (60)%, 10 (49)%, and 3 (13)% of the total dissociated from cerebrum (optic nerve), respectively, indicating that both progenitor stages ($A2B5^+O4^-$ and $O4^+GalC^-$) and oligodendrocytes ($O4^+GalC^+$) were represented (Gard and Pfeiffer, 1990). Both regions yielded a significant subpopulation of astrocytes with surface A2B5 immunoreactivity (Table 4) consistent with the finding of Miller *et al.* (1985). However, convincing examples of astrocytes immunostained for O4 on their surface were never found.

TABLE 3

TIMECOURSE OF FBS-INDUCED GFAP EXPRESSION IN $O4^+GalC^-$ PROGENITORS ISOLATED FROM CEREBRUM AND CEREBRUM-DERIVED GERMINAL CULTURES

Source	Progenitor yield per littermate ($\times 10^5$)	% $GFAP^+$ on Day: 0	1	3	5
Tissue	3.8 ± 0.5	0, 0	0, 0	38, 45	49, 88
Culture	1.9 ± 0.6	19, 20	20, 19	50, 90	86, 89

Note. $O4^+GalC^-$ progenitors were isolated in parallel from P4–5 cerebra and age-matched germinal cultures of P0–1 cerebra (initiated from the same litter) by immunopanning (see Experimental Procedures). The purified cells were seeded on polyornithine-coated coverslips in DMEM containing 10% FBS and doubly labeled by indirect immunofluorescence for O4 and GFAP at the indicated times. Yield data represent the means (±SEM) of three independent purifications, each representing 3–6 littermates. Immunofluorescence data are the mean percentages of $O4^+$ cells coexpressing GFAP in duplicate experiments (1, 2), each representing ≥300 scored cells.

DISCUSSION

The data provide three principal findings concerning the developmental potential of oligodendrocyte progenitors in postnatal rat brain. First, cell immunoselection and primary culture were coupled to delineate bipotential progenitors from those committed to an oligodendrocyte differentiation program. Arising in sequence, these cells are identified by the antigenic phenotypes $A2B5/G^+_{D3}O4^-$ and $O4^+GalC^-$ and their response to CNTF as a measure of type-2 astrocyte inducibility. Second, the results show that related lymphokines, OSM

TABLE 4
FREQUENCY OF ASTROCYTES EXPRESSING SURFACE ANTIGENS FOR THE OLIGODENDROCYTE LINEAGE IN DISSOCIATED RAT CEREBRUM AND OPTIC NERVE AT P10

Region	%GFAP$^+$ Cells	% of GFAP$^+$ cells expressing: A2B5	O4	GalC
Cerebrum	4.5 ± 0.9	21.0 ± 2.4	0	0
Optic nerve	7.4 ± 3.3	11.2 ± 4.7	0	0

Note. Dissociates yielding averages of 1.2, and 0.04 × 10^6 cells per cerebrum and optic nerve, respectively, at P10 were attached to a polyornithine substratum at 10^5 (cerebrum) or 10^4 (optic nerve) cells per coverslip and doubly labeled by immunofluorescence for the indicated surface markers and GFAP (see Experimental Procedures). Data are expressed as the mean (±SEM) of three independent experiments and ≥300 scored GFAP$^+$ cells per labeling permutation.

and LIF, but not IL-6, can fully mimic CNTF as effectors of astrocyte gene expression for the earlier bipotential stage. Third, mature progenitors (O4$^+$GalC$^-$) that are refractory to these cues remain sensitive to an overriding stimulus in FBS, thereby distinguishing two classes of astrocyte-inducing agents.

Our culture paradigm differs from many previous studies. Given the possibility that one or more non-CNTF inducer proteins in serum (Levison and McCarthy, 1991) are normally excluded from parenchyma by the blood–brain barrier, bipotentiality was measured by responsiveness to CNTF (Hughes *et al.*, 1988; Lillien and Raff, 1990), which is expressed in the developing CNS (Stockli *et al.*, 1991; Dobrea *et al.*, 1992). Building on evidence that broadly defined O-2A cells (A2B5$^+$GalC$^-$) become less sensitive to endogenous CNTF-like inducing activity with increasing perinatal age (Hughes and Raff, 1987), two successive maturational stages within this compartment (A2B5/$G^+_{D3}O4^-$ and O4$^+$GalC$^-$) were compared. Because oligodendrocyte lineage cells are plastic when generated in conventional FBS-containing germinal cultures (Ingraham and McCarthy, 1989), we purified them directly from tissue (Gard and Pfeiffer, 1989).

Distinction of an Immunocytochemically Defined Oligodendroblast

Under serumless culture conditions, O-2A progenitors are converted by CNTF to type-2 astrocytes on a substratum of meningeal cell-derived extracellular matrix (Lillien *et al.*, 1990). Our results show that $G^+_{D3}O4^-$ progenitors isolated from rat cerebrum respond in this way, whereas O4$^+$GalC$^-$ progenitors do not (Figs. 1, 2, and 4) and differentiate into oligodendrocytes instead (Fig. 5). These data provide compelling evidence that oligodendrocytes derive from a committed progenitor, i.e., an oligodendroblast (Fig. 7) proposed originally from ultrastructural features (Skoff *et al.*, 1976; Imamoto *et al.*, 1978) and identified here with a mab (O4; Sommer and Schachner, 1981) commonly used for glial lineage analyses (Pfeiffer *et al.*, 1993, and references therein).

This striking difference in progenitor responsiveness probably does not reflect the loss of CNTF receptors. When grown on a simple polycationic substatum, postmitotic GalC$^+$ oligodendrocytes react trophically to CNTF (Louis *et al.*, 1993; Barres *et al.*, 1993), as do immunopurified oligodendroblasts (A. L. Gard, unpublished observations), although this effect was presently obscured by good survival occurring on extracellular matrix alone. The $G^+_{D3}O4^-$ to O4$^+$GalC$^-$ transition more likely heralds a change in response to the unidentified extracellular matrix component facilitating astrocyte induction or modulation of CNTF receptor-mediated signaling to the nucleus (Bonni *et al.*, 1993; Stahl *et al.*, 1994). Other comparative studies of these stages in the perinatal rat lineage predict that progenitor commitment correlates with postmigratory development (Warrington *et al.*, 1993), multipolar process outgrowth (Dubois-Dalcq, 1987), and a switch in mitogens utilized from PDGF to FGF-2 (Gard and Pfeiffer, 1993; Hardy and Reynolds, 1993; Fok-Seang and Miller, 1994).

Neither CNTF nor LIF significantly enhanced the differentiation of oligodendrocyte progenitors in this study. Recall that even in the absence of added factors, nearly all oligodendroblasts maintained in BDM differentiated into myelinogenic (MBP$^+$) oligodendrocytes on extracellular matrix (Fig. 5; Table 2), just as the majority do that survive on a polyornithine substratum alone (Gard and Pfeiffer, 1989). Under the same conditions most precursors captured at the preceding $G^+_{D3}O4^-$ stage failed to develop into oligodendrocytes (Table 2), con-

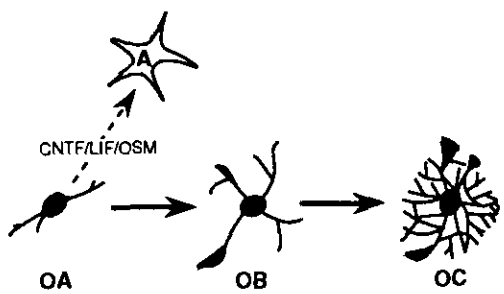

FIG. 7. Bipotential progenitor (OA) and oligodendroblast (OB) stages of the oligodendrocyte (OC) lineage in cerebrum as defined by phenotype (OA, A2B5/$G^+_{D3}O4^-$; OB, O4$^+$GalC$^+$; OC, O4$^+$GalC$^+$) and their *in vitro* responsiveness to astrocyte-inducing cytokines using signal transducer LIFRβ. Committment to either macroglial cell type is modeled as a postmigratory decision (Levison and Goldman, 1993; Warrington *et al.*, 1993) of bipotential progenitors retermed "OA" to reflect evidence for the non-type-2 phenotype of astrocytes (A) generated this way in forebrain cultures (Levison *et al.*, 1993).

ceivably because their progression was inhibited by extracellular matrix-associated molecules (Lillien *et al.*, 1990) or required additional cues (Dutly and Schwab, 1991; Agresti *et al.*, 1991) missing from our culture system. Thus our results do not support a role for CNTF in stimulating oligodendrocyte differentiation as shown in another defined culture medium (N2; Mayer *et al.*, 1994).

Progenitors cultured from postnatal optic nerve responded to CNTF and endogenous CNTF-like activity in the same developmental stage-specific manner as their cerebral counterparts (Figs. 3 and 4). Nevertheless, astrocytes with pharmacological properties of an *in vitro*-derived type-2 phenotype have not been found *in vivo* (Fulton *et al.*, 1992), nor have morphological (Butt and Ransom, 1993; Butt *et al.*, 1994) and thymidine autoradiographic studies (Skoff, 1990) of this tract substantiated the hypothesis of a second, functionally distinct astrocyte lineage (Raff, 1989), despite its high CNTF content (Stockli *et al.*, 1991). Considering the progenitor pool in nerve is predominantly $O4^+GalC^-$ by P7 (Gard and Pfeiffer, 1990; Wolswijk *et al.*, 1990), our data imply the lineage is already largely committed to myelinogenesis by the time when endogenous astrocyte-inducing activity (CNTF) first accumulates (Lillien *et al.*, 1988; Stockli *et al.*, 1991).

Multiple Effectors of Astrocyte Gene Expression in Oligodendrocyte Progenitors: Developmental and Pathological Implications

Unlike in heterogeneous optic nerve cultures, each astrocyte-inducing cytokine applied to immunopurified $G^+_{D3}O4^-$ progenitors required extracellular matrix for activity (Table 1). Concurring with Mayer *et al.* (1994), these results confirm suspicion that CNTF and extracellular matrix-associated molecules by themselves lack intrinsic astrocyte-inducing activity (Lillien *et al.*, 1990). But is their collaboration physiologically relevant for bipotential progenitors not found at the pial surface? The capacity of cultured endothelial cell-derived extracellular matrix to specifically mimic the meningeal effect (Lillien *et al.*, 1990; Mayer *et al.*, 1994) has directed others to think the natural, nondiffusible component of the astrocyte-inducing stimulus is vascular contact instead (Levison and Goldman, 1993b).

Belonging to a structurally related cytokine family (Bazan, 1991), OSM, LIF and CNTF can evoke overlapping hematopoietic and neuronal effects (Bruce *et al.*, 1993; Rao *et al.*, 1992) attributed to heterodimerization of shared "β" signaling subunits gp130 and LIFRβ (Gearing *et al.*, 1992; Baumann *et al.*, 1993; Stahl *et al.*, 1993). By showing these factors act interchangeably as astrocyte-inducers *in vitro*, our data support their modeled action through a common receptor (Ip and Yancopoulos, 1992) and intracellular signaling pathway (Stahl *et al.*, 1994) in glia while cautioning that CNTF may not be the ligand driving astrocyte development from bipotential progenitors migrating into rat striatum and neocortex (Levison and Goldman, 1993a; Levison *et al.*, 1993). Unlike LIF and OSM, CNTF lacks a signal peptide (Stockli *et al.*, 1989), suggesting that only through nonconventional release or cell damage (Lillien *et al.*, 1988; Rudge *et al.*, 1992) is it accessible to oligodendrocyte lineage cells. Whereas the expression of CNTF and LIF in non-injured forebrain is reportedly negligible (Stockli *et al.*, 1991; Ip *et al.*, 1993; Yamamori, 1991; Patterson and Fann, 1992), OSM and other gp130-associated cytokines, e.g., interleukin-11 (Yin and Yang, 1994) await study. The ineffectiveness of IL-6 in our hands suggests that homodimerization of transducer gp130 is insufficient to induce astrocyte conversion. However, we cannot exclude the possibility that absence of the obligatory binding protein, IL6Rα (Taga *et al.*, 1989), rendered IL-6 inactive.

The apparent loss of G_{D3} by type-2 astrocytes presently formed in serumless medium is consistent with other descriptions of an unstable phenotype in FBS (Goldman *et al.*, 1986; Norton and Farooq, 1989, 1993; Levison *et al.*, 1993). This observation strengthens doubt that authentic, bipotentially derived astrocytes normally sustain a traceable type-2 phenotype (Levison *et al.*, 1993) or relate to $G^+_{D3}GFAP^+$ and $A2B5^+GFAP^+$ populations arising in tissue dissociates (Miller *et al.*, 1985; Curtis *et al.*, 1988; this study). For reactive astrocytes, however, there is good immunohistochemical evidence of inducible G_{D3} expression as shown in specific murine cerebellar mutations (Levine *et al.*, 1986; but see Miller *et al.*, 1986; Levine and Goldman, 1988; Reynolds and Wilkin, 1988). Whether this reflects a pathological reaction by astrocytes or bipotential progenitors that become astrocytes remains to be determined.

Although unaffected by CNTF/LIF/OSM, cerebral $O4^+GalC^-$ progenitors clearly expressed GFAP when exposed to FBS, regardless of their origin in tissue or germinal culture (Fig. 6). This finding coupled with the absence of detectable $O4^+$ astrocytes *in vivo* (Table 4) leads us to conclude that GFAP expression occurring this late in the oligodendrocyte lineage (e.g., Aloisi *et al.*, 1988; Trotter and Schachner, 1989; Agresti *et al.*, 1991) in brain is ectopic and reflects a different pathway taken than development. It may signify the capacity of adult oligodendrocyte progenitors, which are normally $O4^+GFAP^-$ (Armstrong *et al.*, 1992; Wolswijk and Noble, 1989), to form reactive astrocytes, as evidenced by the marked accumulation of $O4^+GFAP^+$ cells following experimental viral-induced demyelination in mice (Godfraind *et al.*, 1989).

The astroglia-inducing molecule (AIM) in FBS is be-

lieved to circulate in blood as an active 12- to 18-kDa acidic protein complexed to a larger binding protein (Levison and McCarthy, 1991) and is thus smaller in size than known LIFRβ ligands. Further, the effect of AIM on oligodendroblasts suggests it works through a different, non-CNTF receptor-mediated astrocyte-inducing mechanism and raises the possibility that astrocytes generated by AIM and CNTF-like factors differ pharmacologically. In light of the reduced role ascribed to interleukin-1β in reactive gliosis (Sievers *et al.*, 1993), a humoral astrocyte-inducing cue may have special clinical relevance to this process in lesions, e.g., multiple sclerosis placques, where blood brain barrier damage exposes oligodendroblasts otherwise destined for remyelination to humoral factors.

As the CNTF cytokine subfamily grows, the likelihood of its contribution to astrocyte pathology increases. Apart from exposure to humoral factors, neovascularization (Lawrence *et al.*, 1984), gliomeningeal scarring (Rudge and Silver, 1990), and increased neural expression of CNTF (Ip *et al.*, 1993) and LIF (Aloisi *et al.*, 1994) associated with traumatic injury or neuroinflammatory disease may present oligodendrocyte progenitors regardless of stage with a signaling environment more conducive to astrocyte committment. Finally, knowledge that OSM functions pleiotropically as an astrocyte inducer (Fig. 1) and is secreted by activated T cells and macrophages (Brown *et al.*, 1987; Zarling *et al.*, 1986) poses another signaling dimension for glial progenitors confronted by immune infiltrates in lesion foci. Culturing adult human oligodendrocyte progenitors (Armstrong *et al.*, 1992) now makes many of these possibilities testable.

The authors gratefully acknowledge Regeneron Pharmaceuticals for the gift of rat recombinant CNTF. We especially thank Drs. Sean Murphy and John Rudge for critically reading the manuscript. This work was supported by Shannon Award and Grant NS29648 (A.L.G.) from NINDS.

REFERENCES

Agresti, C., Aloisi, F., and Levi, G. (1991). Heterotypic and homotypic cellular interactions influencing the growth and differentiation of bipotential oligodendrocyte-type-2 astrocyte progenitors in culture. *Dev. Biol.* **144,** 16–29.

Aloisi, F., Agresti, C., D'Urso, D., and Levi, G. (1988). Differentiation of bipotential glial precursors into oligodendrocytes is promoted by interaction with type-1 astrocytes in cerebellar cultures. *Proc. Natl. Acad. Sci. USA* **85,** 6167–6171.

Aloisi, F., Rosa, S., Testa, U., Bonsi, P., Russo, G., Peschle, C., and Levi, G. (1994). Regulation of leukemia inhibitory factor synthesis in cultured human astrocytes. *J. Immunol.* **152,** 5022–5031.

Armstrong, R. C., Dorn, H. H., Kufta, C. V., Friedman, E., and Dubois-Dalcq, M. E. (1992). Pre-oligodendrocytes from adult human CNS. *J. Neurosci.* **12,** 1538–1547.

Bansal, R., Stefansson, K., and Pfeiffer, S. E. (1992). Proligodendroblast antigen (POA), a developmental antigen expressed by A007/O4-positive oligodendrocyte progenitors prior to the appearance of sulfatide and galactocerebroside. *J. Neurochem.* **58,** 2221–2229.

Bansal, R., Warrington, A., Gard, A. L., Ranscht, B., and Pfeiffer, S. E. (1989). Multiple and novel specificities of monoclonal antibodies O1, O4 and R-mAb used in the analysis of oligodendrocyte development. *J. Neurosci. Res.* **24,** 548–557.

Barbarese, E., and Pfeiffer, S. E. (1981). Developmental regulation of myelin basic protein in dispersed cultures. *Proc. Natl. Acad. Sci. USA* **78,** 1953–1957.

Barres, B. A., Schmid, R., Sendtner, M., and Raff, M. C. (1993). Multiple extracellular signals are required for long-term oligodendrocyte survival. *Development* **118,** 283–295.

Baumann, H., Ziegler, S. F., Mosley, B., Morella, K. K., Pajovic, S., and Gearing, D. P. (1993). Reconstitution of the response to leukemia inhibitory factor, oncostatin M, and ciliary neurotrophic factor in hepatoma cells. *J. Biol. Chem.* **268,** 8414–8417.

Bazan, J. F. (1991). Neuropoietic cytokines in the hematopoietic field. *Neuron* **7,** 197–208.

Bonni, A., Frank, D. A., Schindler, C., and Greenberg, M. E. (1993). Characterization of a pathway for ciliary neurotrophic factor signaling to the nucleus. *Science* **262,** 1575–1579.

Bottenstein, J. E., and Sato, G. (1979). Growth of a rat neuroblastoma line in serum-free supplemented medium. *Proc. Natl. Acad. Sci. USA* **76,** 514–517.

Brown, T. J., Lioubin, M. N., and Marquardt, H. (1987). Purification and characterization of cytostatic lymphokines produced by activated T lymphocytes: Synergistic antiproliferative activity of transforming growth factor beta-1, interferon-gamma, and oncostatin M for human melanoma cells. *J. Immunol.* **139,** 2977–2983.

Bruce, A. G., Linsley, P. S., and Rose, T. M. (1993). *Oncostatin M. Prog. Growth Factor Res.* **4,** 157–170.

Butt, A. M., and Ransom, B. R. (1993). Morphology of astrocytes and oligodendrocytes during development in the intact rat optic nerve. *J. Comp. Neurol.* **338,** 141–158.

Butt, A. M., Duncan, A., and Berry, M. (1994). Astrocyte associations with nodes of Ranvier: Ultrstructural analysis of HRP-filled astrocytes in the mouse optic nerve. *J. Neurocytol.* **23,** 486–499.

Curtis, R., Cohen, J., Fok-Seang, J., Hanley, M. R., Gregson, N. A., Reynolds, R., and Wilkin, G. P. (1988). Development of macroglial cells in rat cerebellum. I. Use of antibodies to follow early *in vivo* development and migration of oligodendrocytes. *J. Neurocytol.* **17,** 43–54.

Davis, S., Aldrich, T. H., Stahl, N., Pan, L., Taga, T., Kishimoto, T., Ip, N. Y., and Yancopoulos, G. D. (1993). LIFRβ and gp130 as heterodimerizing signal transducers of the tripartite CNTF receptor. *Science* **260,** 1805–1808.

Dobrea, G. M., Unnerstall, J. R., and Rao, M. S. (1992). The expression of CNTF message and immunoreactivity in the central and peripheral nervous system of the rat. *Dev. Brain Res.* **66,** 209–219.

Dubois-Dalcq, M. (1987). Characterization of a slowly proliferative cell along the oligodendrocyte differentiation pathway. *EMBO J.* **6,** 2587–2595.

Dutly, F., and Schwab, M. E. (1991). Neurons and astrocytes influence the development of purified O-2A progenitor cells. *Glia* **4,** 559–571.

Eisenbarth, G. S., Walsh, F. S., and Nirenberg, M. (1979). Monoclonal antibody to a plasma membrane antigen of neurons. *Proc. Natl. Acad. Sci. USA* **76,** 4913–4917.

Fok-Seang, J., and Miller, R. H. (1994). Distribution and differentiation of A2B5$^+$ glial precursors in the developing rat spinal cord. *J. Neurosci. Res.* **37,** 219–235.

Fulton, B. P., Burne, J. F., and Raff, M. C. (1992). Visualization of O-2A progenitor cells in developing and adult rat optic nerve by quisqualate-stimulated cobalt uptake. *J. Neurosci.* **12,** 4816–4833.

Gard, A. L., and Pfeiffer, S. E. (1989). Oligodendrocyte progenitors isolated directly from developing telencephalon at a specific phenotypic stage: Myelinogenic potential in a defined environment. *Development* **106,** 119–132.

Gard, A. L., and Pfeiffer, S. E. (1990). Two proliferative stages of the oligodendrocyte lineage ($A2B5^+O4^-$ and $O4^+GalC^-$) under different mitogenic control. *Neuron.* **5,** 615–625.

Gard, A. L., and Pfeiffer, S. E. (1993). Glial cell mitogens bFGF and PDGF differentially regulate development of $O4^+GalC^-$ oligodendrocyte progenitors. *Dev. Biol.* **159,** 618–630.

Gard, A. L., Pfeiffer, S. E., and Williams, W. C., II, (1993). Immunopanning and developmental stage-specific culture of oligodendrocyte progenitors ($O4^+GalC^-$) directly from postnatal rodent cerebrum. *Neuroprotocols* **2,** 209–220.

Gard, A. L., Warrington, A. E., and Pfeiffer, S. E. (1988). Direct microculture enyme-linked immunosorbent assay for studying neural cells: Oligodendrocytes. *J. Neurosci. Res.* **20,** 46–53.

Gearing, D. P., Comeau, M. R., Friend, D. J., Gimpel, S. D., Thut, C. J., McGourty, J. M., Brasher, K. K., King, J. A., Gillis, S., Mosley, B., Ziegler, S. F., and Cosman, D. (1992). The IL-6 signal transducer, gp130: An oncostatin receptor and affinity converter for the LIF receptor. *Science* **255,** 1434–1437.

Godfraind, C., Friedrich, V. L., Holmes, K. V., and Dubois-Dalcq, M. (1989). In vivo analysis of glial cell phenotypes during a viral demyelinating disease in mice. *J. Cell Biol.* **109,** 2405–2416.

Goldman, J. E., Geier, S. S., and Hirano, M. (1986). Differentiation of astrocytes and oligodendrocytes from germinal matrix cells in primary culture. *J. Neurosci.* **6,** 52–60.

Grove, E. A., Williams, B. P., Da-Qing, L., Hajihosseini, M., Friedrich, A., and Price, J. (1993). Multiple restricted lineages in the embryonic rat cerebral cortex. *Development* **117,** 553–561.

Hardy, R., and Reynolds, R. (1991). Proliferation and differentiation potential of rat forebrain oligodendroglial progenitors both *in vitro* and *in vivo*. *Development* **111,** 1061–1080.

Hardy, R., and Reynolds, R. (1993). Rat cerebral cortical neurons in primary culture release a mitogen specific for early ($G^+_{D3}O4^-$) oligodendroglial progenitors. *J. Neurosci. Res.* **34,** 589–600.

Hughes, S. M., Lillien, L. E., Raff, M. C., Rohrer, H., and Sendtner, M. (1988). Ciliary neurotrophic factor induces type-2 astrocyte differentiation in culture. *Nature* **335,** 70–73.

Hughes, S. M., and Raff, M. C. (1987). An inducer protein may control the timing of fate switching in a bipotential glial progenitor cell in rat optic nerve. *Development* **101,** 157–167.

Imamoto, K., Paterson, J. A., and Leblond, C. (1978). Radioautographic investigation of gliogenesis in the corpus callosum of young rats. I. Sequential changes in oligodendrocytes. *J. Comp. Neurol.* **180,** 115–138.

Ingraham, C. A., and McCarthy, K. D. (1989). Plasticity of process-bearing glial cell cultures from neonatal rat cerebral cortical tissue. *J. Neurosci.* **9,** 63–69.

Ip, N. Y., and Yancopoulos, G. D. (1992). Ciliary neurotrophic factor and its receptor complex. *Prog. Growth Factor Res.* **4,** 139–155.

Ip, N. Y., Wiegard, S. J., Morse, J., and Rudge, J. S. (1993). Injury-induced regulation of ciliary neurotrophic factor mRNA in the adult rat brain. *Eur. J. Neurosci.* **5,** 25–33.

Johnson, G. D., Davidson, R. S., McNamee, K. C., Russell, G., Goodwin, D., and Holborow, E. J. (1982). Fading of immunofluorescence during microscopy: A study of the phenomenon and its remedy. *J. Immunol. Methods* **55,** 231–242.

Lawrence, J. M., Huang, S. K., and Raisman, G. (1984). Vascular and astrocytic reactions during establishment of hippocampal transplants in adult host brain. *Neuroscience* **12,** 745–760.

Lee, V., Page, C. D., Wu, H. L., and Schlaepfer, W. W. (1984). Monoclonal antibodies to gel-excised glial filament protein and their reactivity with other intermediate filament proteins. *J. Neurochem.* **42,** 25–32.

Levi, G., Aliosi, F., and Wilkin, G. P. (1987). Differentiation of cerebellar bipotential glial precursors into oligodendrocytes in primary culture: Developmental profile of surface antigensand mitotic activity. *J. Neurosci. Res.* **18,** 407–417.

Levi, G., Gallo, V., and Ciotti, M. T. (1986). Bipotential precursors of fibrous astrocytes and oligodendrocytes in rat cerebellar cultures express distinct surface features and "neuron-like" γ-aminobutyric acid transport. *Proc. Natl. Acad. Sci. USA* **83,** 1504–1508.

Levine, S. M., and Goldman, J. E. (1988). Embryonic divergence of oligodendrocyte and astrocyte lineages in developing rat cerebrum. *J. Neurosci.* **8,** 3992–4006.

Levine, S. M., Seyfried, T. N., Yu, R. K., and Goldman, J. E. (1986). Immunocytochemical localization of G_{D3} ganglioside to astrocytes in murine cerebellar mutants. *Brain Res.* **374,** 260–269.

Levison, S. W., Chuang, C., Bernetta, J., and Goldman, J. E. (1993). The migrational patterns and developmental fates of glial precursors in the rat subventricular zone are temporally regulated. *Development* **119,** 611–622.

Levison, S. W., and Goldman, J. E. (1993a). Both oligodendrocytes and astrocytes develop from progenitors in the subventricular zone of postnatal rat forebrain. *Neuron* **10,** 201–212.

Levison, S. W., and Goldman, J. E. (1993b). Astrocyte origins. *In* "Astrocytes: Pharmacology and Function" (S. Murphy, Ed.), pp. 1–22. Academic Press, San Diego.

Levison, S. W., and McCarthy, K. D. (1991). Characterization and partial purification of AIM: A plasma protein that induces rat cerebral type 2 astroglia from bipotential glial progenitors. *J. Neurochem.* **57,** 782–794.

Lillien, L. E., Sendtner, M., Rohrer, H., Hughes, S. M., and Raff, M. C. (1988). Type-2 astrocyte development in rat brain cultures is initiated by a CNTF-like protein produced by type-1 astrocytes. *Neuron* **1,** 485–494.

Lillien, L. E., and Raff, M. C. (1990). Analysis of the cell-cell interactions that control type-2 astrocyte development in vitro. *Neuron* **4,** 525–534.

Lillien, L. E., Sendtner, M., and Raff, M. C. (1990). Extracellular matrix-associated molecules collaborate with ciliary neurotrophic factor to induce type-2 astrocyte development. *J. Cell Biol.* **111,** 635–644.

Louis, J.-C., Magal, E., Takayama, S., and Varon, S. (1993). CNTF protection of oligodendrocytes against natural and tumor necrosis factor-induced death. *Science* **259,** 689–692.

Luskin, M. B., Parnavelas, J. G., and Barfield, J. A. (1993). Neurons, astrocytes and oligodendrocytes of the rat cerebral cortex originate from separate progenitor cells: An ultrastructural analysis of clonally related cells. *J. Neurosci.* **13,** 1730–1750.

Masiakowski, P., Liu, H., Radziejewski, C., Lottspeich, F., Oberthuer, W., Wong, V., Lindsay, R. M., Furth, M. E., and Panayotatos, N. (1991). Recombinant human and rat ciliary neurotrophic factors. *J. Neurochem.* **57,** 1003–1012.

Mayer, M., Bhakoo, K., and Noble, M. (1994). Ciliary neurotrophic factor and leukemia inhibitory factor promote the generation, maturation and survival of oligodendrocytes in vitro. *Development* **120,** 143–153.

McKinnon, R. D., Toshimitsu, M., Dubois-Dalcq, M., and Aaronson, S. (1990). FGF modulates the PDGF-driven pathway of oligodendrocyte development. *Neuron* **5,** 603–614.

Miller, R. H., Abney, E. R., David, S., ffrench-Constant, C., Lindsay, R., Patel, R., Stone, J., and Raff, M. C. (1986). Is reactive gliosis a property of a distinct subpopulation of astrocytes? *J. Neurosci.* **6,** 22–29.

Miller, R. H., David, S., Patel, R., Abney, E. R., and Raff, M. C. (1985). A quantitative immunohistochemical study of macroglial cell devel-

opment in the rat optic nerve: In vivo evidence for two distinct astrocyte lineages. *Dev. Biol.* **111,** 35–41.

Murakami, M., Hibi, M., Nakagawa, T., Yasukawa, K., Yamanishi, K., Taga, T., and Kishimoto, T. (1993). IL-6-induced homodimerization of gp130 and associated activation of a tyrosine kinase. *Science* **260,** 1808–1810.

Norton, W. T., and Farooq, M. (1989). Astrocytes cultured from mature brain derive from glial progenitors. *J. Neurosci.* **9,** 769–775.

Norton, W. T., and Farooq, M. (1993). Differentiation of glial precursors from developing rat brain in vitro. *Dev. Brain Res.* **72,** 193–202.

Patterson, P. H., and Fann, M.-J. (1992). Further studies of the distribution of CDF/LIF mRNA. *Ciba Found. Symp.* **167,** 125–140.

Pfeiffer, S. E., Warrington, A. E., and Bansal, R. (1993). The oligodendrocyte and its many cellular processes. *Trends Cell Biol.* **3,** 191–197.

Price, J., and Thurlow, L. (1988). Cell lineage in the rat cerebral cortex: A study using retroviral-mediated gene transfer. *Development* **104,** 473–482.

Pukel, C. S., Lloyd, K. O., Travassos, L. R., Dippold, W. G., Oettgen, H. F., and Old, L. J. (1982). GD_3, a prominent ganglioside of human melanoma. Detection and characterization by mouse monoclonal antibody. *J. Exp. Med.* **155,** 1133–1147.

Raff, M. C. (1989). Glial cell diversification in the rat optic nerve. *Science* **243,** 1509–1524.

Raff, M. C., Abney, E. R., Cohen, J., Lindsay, R., and Noble, M. (1983a). Two types of astrocytes in cultures of developing white matter: Differences in morphology, surface gangliosides and growth characteristics. *J. Neurosci.* **3,** 1289–1300.

Raff, M. C., Miller, R. H., and Noble, M. (1983b). A glial progenitor that develops in vitro into an astrocyte or an oligodendrocyte depending on the culture medium. *Nature* **303,** 389–396.

Rao, M. S., Symes, A., Malik, N., Shoyab, M., Fink, J. S., and Landis, S. C. (1992). Oncostatin M regulates VIP expression in a human neuroblastoma cell line. *Neuroreport* **3,** 865–868.

Reynolds, R., and Wilkin, G. P. (1988). Oligodendroglial progenitor cells but not oligodendroglia divide during normal development of the rat cerebellum. *J. Neurocytol.* **20,** 216–224.

Rudge, J. S., Alderson, R. F., Pasnikowski, E., McClain, J., Ip, N. Y., and Lindsay, R. M. (1992). Expression of ciliary neurotrophic factor and the neurotrophins—Nerve growth factor, brain-derived neurotrophic factor and neurotrophin-3 in cultured rat hippocampal astrocytes. *Eur. J. Neurosci.* **4,** 459–471.

Rudge, J., and Silver, J. (1990). Inhibition of neurite outgrowth on astroglial scars *in vitro*. *J. Neurosci.* **10,** 3594–3603.

Sievers, J., Struckhoff, G., and Puchner, M. (1993). Interleukin-1β does not induce reactive vascularization or scar formation in the immature rat brain. *Int. J. Dev. Neurosci.* **11,** 281–293.

Skoff, R. P. (1990). Gliogenesis in rat optic nerve: Astrocytes are generated in a single wave before oligodendrocytes. *Dev. Biol.* **139,** 149–168.

Skoff, R. P., and Knapp, P. E. (1991). Division of astroblasts and oligodendroblasts in postnatal rodent brain: Evidence for separate astrocyte and oligodendrocyte lineages. *Glia* **4,** 165–174.

Skoff, R. P., Price, D. L., and Stocks, A. (1976). Electron microscopic autoradiographic studies of gliogenesis in the rat optic nerve. I. Cell proliferation. *J. Comp. Neurol.* **169,** 291–312.

Sommer, I., and Schachner, M. (1981). Monoclonal antibodies (O1 and O4) to oligodendrocyte cell surfaces: An immunocytochemical study in the central nervous system. *Dev. Biol.* **83,** 11–327.

Stahl, N., Davis, S., Wong, V., Taga, T., Kishimoto, T., Ip, N. Y., and Yancopoulos, G. D. (1993). Cross-linking identifies leukemia inhibitory factor-binding protein as a ciliary neurotrophic factor receptor component. *J. Biol. Chem.* **268,** 7628–7631.

Stahl, N., Boulton, T. G., Farruggella, T., Ip, N. Y., Davis, S., Witthuhn, B. A., Quelle, F. W., Silvennoinen, O., Barbieri, G., Pellegrini, S., Ihle, J. N., and Yancopoulos, G. D. (1994). Association and activation of Jak-Tyk kinases by CNTF-LIF-OSM-IL-6β receptor components. *Science* **263,** 92–95.

Stockli, K. A., Lottspeich, F., Sendtner, M., Masiakowski, P., Carroll, P., Gotz, R., Lindholm, D., and Thoenen, H. (1989). Molecular cloning, expression and regional distribution of rat ciliary neurotrophic factor. *Nature* **342,** 920–923.

Stockli, K. A., Lillien, L. E., Naher-Noe, M., Breitfeld, G., Hughes, R. A., Raff, M. C., Thoenen, H., and Sendtner, M. (1991). Regional distribution, developmental changes and cellular localization of CNTF mRNA and protein in rat brain. *J. Cell Biol.* **115,** 447–459.

Taga, T., Higi, M., Hirata, Y., Yanasaki, K., Yasukawa, K., Matsuda, T., Hirona, T., and Kishimoto, T. (1989). Interleukin-6 triggers the association of its receptor with a possible signal transducer, gp130. *Cell* **58,** 573–581.

Trotter, J., and Schachner, M. (1989). Cells positive for the O4 surface antigen isolated by cell sorting are able to differentiate into astrocytes or oligodendrocytes. *Dev. Brain Res.* **46,** 115–122.

Vaysse, P. J.-J., and Goldman, J. E. (1992). A distinct type of G^+_{D3} flat astrocyte in rat CNS cultures. *J. Neurosci.* **12,** 330–337.

Warrington, A. E., Barbarese, E., and Pfeiffer, S. E. (1993). Differential myelinogenic capacity of specific developmental stages of the oligodendrocyte lineage upon transplantiation into hypomyelinating hosts. *J. Neurosci. Res.* **34,** 1–13.

Warrington, A. E., and Pfeiffer, S. E. (1992). Morphology, proliferation and differentiation of oligodendrocytes in unfixed postnatal rat cerebellum. *J. Neurosci. Res.* **33,** 338–353.

Wolswijk, G., and Noble, M. (1989). Identification of an adult-specific glial progenitor cell. *Development* **105,** 387–400.

Wolswijk, G., Riddle, P. N., and Noble, M. (1990). Coexistence of perinatal and adult forms of a glial progenitor cell during development of the rat optic nerve. *Development* **109,** 691–698.

Yamamori, T. (1991). Localization of cholinergic differentiation factor/leukemia inhibitory factor mRNA in the rat brain and peripheral tissues. *Proc. Natl. Acad. Sci. USA* **88,** 7298–7302.

Yin, T., and Yang, Y.-C. (1994). Mitogen-activated protein kinases and ribosomal S6 protein kinases are involved in signaling pathways shared by interleukin-11, interleukin-6, leukemia inhibitory factor, and oncostatin M in mouse 3T3-L1 cells. *J. Biol. Chem.* **269,** 3731–3738.

Zarling, J. M., Shoyab, M., Marquardt, H., Hanson, M. B., Lioubin, M. N., and Todaro, G. J. (1986). Oncostatin M: a growth regulator produced by differentiated histiocytic lymphoma cells. *Proc. Natl. Acad. Sci. USA* **83,** 9739–9743.