Cooperation Between PDGF and FGF Converts Slowly Dividing O–2A^{adult} Progenitor Cells to Rapidly Dividing Cells with Characteristics of O–2A^{perinatal} Progenitor Cells

Guus Wolswijk and Mark Noble

Ludwig Institute for Cancer Research, Middlesex Hospital/University College Branch, London W1P 8BT, England

Abstract. We have shown previously that oligodendrocyte-type-2 astrocyte (O–2A) progenitor cells isolated from adult rat optic nerves can be distinguished in vitro from their perinatal counterparts on the basis of their much slower rates of division, differentiation, and migration when grown in the presence of cortical astrocytes or PDGF. This behavior is consistent with in vivo observations that there is only a modest production of oligodendrocytes in the adult CNS. As such a behavior is inconsistent with the likely need for a rapid generation of oligodendrocytes following demyelinating damage to the mature CNS, we have been concerned with identifying in vitro conditions that allow O–2A^{adult} progenitor cells to generate rapidly large numbers of progeny cells. We now provide evidence that many slowly dividing O–2A^{adult} progenitor cells can be converted to rapidly dividing cells by exposing adult optic nerve cultures to both PDGF and bFGF. In addition, these O–2A^{adult} progenitor cells appear to acquire other properties of O–2A^{perinatal} progenitor cells, such as bipolar morphology and high rate of migration. Although many O–2A^{adult} progenitor cells in cultures exposed to bFGF alone also divide rapidly, these cells are multipolar and migrate little in vitro. Oligodendrocytic differentiation of O–2A^{adult} progenitor cells, which express receptors for bFGF in vitro, is almost completely inhibited in cultures exposed to bFGF or bFGF plus PDGF. As bFGF and PDGF appear to be upregulated and/or released after injury to the adult brain, this particular in vitro response of O–2A^{adult} progenitor cells to PDGF and bFGF may be of importance in the generation of large numbers of new oligodendrocytes in vivo following demyelination.

REGENERATION in some adult tissues is associated with rapid division of otherwise slowly dividing or quiescent populations of appropriate precursor cells. For example, in response to damage to skeletal muscle, a pool of quiescent muscle precursor cells (called satellite cells) become mitotically active and generate a population of myoblasts which subsequently fuse to form new muscle fibers (Allen and Rankin, 1990). To date, very little is known about the cellular and molecular mechanisms that promote regenerative events in adult tissues.

The ability of the adult central nervous system (CNS)[1] to recover from damage to oligodendrocytes, the myelin-forming cells of the CNS, has been well documented. Remyelination appears to occur in many experimental models of CNS demyelination (Ludwin, 1981) and there is also evidence of limited myelin repair in patients suffering from the human demyelinating disease, multiple sclerosis (MS) (Prineas and Connell, 1979; Raine et al., 1981; Prineas et al., 1989).

To obtain insights into the mechanisms that may underlie myelin repair, we have been examining the biology of adult oligodendrocyte progenitors, cells that may provide a source for the oligodendrocytes that are needed to repair demyelinated lesion. Two lines of evidence suggest that oligodendrocyte progenitor cells, possibly in addition to mature oligodendrocytes (Aranella and Herndon, 1984; Ludwin, 1984; Ludwin and Bakker, 1988; Wood and Bunge, 1991), may be responsible for the generation of new oligodendrocytes following demyelinating damage. Firstly, Godfraind et al. (1989) found that after virally induced demyelination of mouse spinal cord, a population of cells with the antigenic profile of oligodendrocyte progenitor cells grown in vitro incorporated ^{3}H-thymidine and increased in number during the recovery phase. Secondly, many of the oligodendrocyte lineage cells that proliferate in response to experimentally induced demyelination (Ludwin, 1979, 1984; Arenella and Herndon, 1984) and those that are present at the edges of MS lesions (Raine et al., 1981) have an ultrastructure that resem-

1. *Abbreviations used in this paper*: Astro-CM, cortical astrocyte-conditioned medium; BrdU, 5-bromodeoxyuridine; CNS, central nervous system; Cou, coumarin; DME-BS, defined medium modified from Sato and Bottenstein (1979); Fl, fluorescein; GalC, galactocerebroside; GFAP, glial fibrillary acidic protein; O–2A, oligodendrocyte-type-2 astrocyte; O–2A^{adult} progenitor cells, O–2A progenitor cells isolated from adult optic nerves; O–2A^{perinatal} progenitor cells, O–2A progenitor cells isolated from embryonic or early postnatal optic nerves; PDGF-AA, the AA homodimeric form of PDGF; PF, PDGF-AA plus bFGF; Rd, rhodamine.

bles that of oligodendrocyte progenitor cells isolated from adult CNS tissue and grown in vitro (Wolswijk et al., 1991*a*).

The identification of factors that may play a role in controlling the proliferation and differentiation of oligodendrocyte progenitor cells in regenerative responses in the adult brain is hampered by the relative complexity and inaccessibility of this tissue. To circumvent this, we have been using tissue culture approaches to study the biology of oligodendrocyte progenitor cells isolated from adult rat optic nerves, the simplest myelinated tracts of the CNS. Like their perinatal counterparts (Raff et al., 1983*b*), adult optic nerve-derived oligodendrocyte progenitor cells have the ability to differentiate into type-2 astrocytes in vitro (ffrench-Constant and Raff, 1986; Wolswijk and Noble, 1989). However, oligodendrocyte-type-2 astrocyte (O-2A) progenitor cells derived from the optic nerves of mature and developing rats differ in many properties when such cells are grown in the presence of cortical astrocytes or PDGF (Wolswijk and Noble, 1989; Wren and Noble, 1989; Wolswijk et al., 1991*b*; Wren et al., 1992), the O-2A progenitor mitogen that is produced by cortical astrocytes in vitro (Noble et al., 1988; Raff et al., 1988; Richardson et al., 1988). In these conditions, $O\text{-}2A^{adult}$ progenitor cells can be distinguished from $O\text{-}2A^{perinatal}$ progenitor cells on the basis of their unipolar morphology and $O4^{+}vimentin^{-}$ antigenic phenotype, their much slower rates of proliferation, migration, and differentiation, and also on the basis of the asymmetric manner in which $O\text{-}2A^{adult}$ progenitor cells generate oligodendrocytes. As the $O\text{-}2A^{adult}$ progenitor population appears to be maintained throughout adult life (Wolswijk and Noble, 1989), our observations suggest that $O\text{-}2A^{adult}$ progenitor cells express characteristics of stem cells (Wren et al., 1992). In contrast, $O\text{-}2A^{perinatal}$ progenitor cells appear to be true progenitor cells, because such cells eventually disappear from the optic nerve (Wolswijk and Noble, 1989). The differences between $O\text{-}2A^{adult}$ and $O\text{-}2A^{perinatal}$ progenitor cells appear to be intrinsic to the cells themselves (Wolswijk et al., 1990, 1991*b*) and may be a reflection of the different functions these cells have in adult and developing animals, respectively (see Wolswijk and Noble, 1989; Noble et al., 1991).

The observation that $O\text{-}2A^{adult}$ progenitor cells divide and differentiate slowly and generate oligodendrocytes at a slow rate in the presence of cortical astrocytes or PDGF is consistent with suggestions that there is only a modest production of oligodendrocytes in normal adult brain (McCarthy and Leblond, 1988). However, such a pattern of cellular behavior seems inconsistent with the likely need for rapid oligodendrocyte replacement following demyelinating damage. Therefore, we have been concerned with identifying conditions of growth which would allow $O\text{-}2A^{adult}$ progenitor cells to generate rapidly large numbers of cells and which might be relevant to the understanding of myelin repair in vivo. In our present study, we provide evidence that $O\text{-}2A^{adult}$ progenitor cells are converted to rapidly dividing cells when adult optic nerve cultures are exposed simultaneously to PDGF and basic FGF (bFGF). Many rapidly dividing $O\text{-}2A^{adult}$ progenitor cells present in cultures grown in PDGF plus bFGF (PF) also acquired a bipolar morphology, $O4^{-}vimentin^{+}$ antigenic phenotype, and a high rate of migration, all of which are characteristics of $O\text{-}2A^{perinatal}$ progenitor cells (Raff et al., 1984*b*; Small et al., 1987; Noble et al., 1988; Wolswijk et al., 1990; Sommer, I. and M. Noble, unpublished observations). Furthermore, $O\text{-}2A^{adult}$ progenitor cells in cultures of adult optic nerve exposed to PF were almost completely prevented from oligodendrocytic differentiation. $O\text{-}2A^{adult}$ progenitor cells growing in cultures exposed to bFGF alone expressed some of the characteristics of cells exposed to PF, such as shortened cell cycle lengths and $O4^{-}vimentin^{+}$ antigenic phenotype, but $O\text{-}2A^{adult}$ progenitor cells in these conditions were multipolar and migrate slowly. In addition, bFGF on its own was able to inhibit premature oligodendrocytic differentiation of $O\text{-}2A^{adult}$ progenitor cells. The observation that $O\text{-}2A^{adult}$ progenitor cells bound both iodinated bFGF and anti-FGF receptor antibodies, suggests that bFGF acts directly on these cells. As both PDGF and bFGF may be available to $O\text{-}2A^{adult}$ progenitor cells in areas of damaged adult brain (Logan, 1988, 1990; Lotan and Schwarz, 1992), our data suggest that the pattern of cellular behavior that is displayed by $O\text{-}2A^{adult}$ progenitor cells growing in cultures of adult optic nerve exposed to PF may be of physiological importance in the generation of large numbers of $O\text{-}2A^{adult}$ progenitor cells in vivo following demyelinating damage. This population of cells could then provide a source for the oligodendrocytes that are needed to remyelinate the denuded axons.

Materials and Methods

Primary Cultures of Adult Optic Nerve Cells

Adult optic nerve cells were isolated and cultured as described previously (Wolswijk and Noble, 1989; Wolswijk et al., 1990, 1991*b*). Optic nerves were dissected from adult Wistar or Sprague-Dawley rats (≥2 1/2 mo old), minced finely with a scalpel blade, and incubated in 1.0 ml Leibovitz L-15 medium (Gibco, Paisley, Scotland) containing 333 i.u./ml collagenase (Worthington Biochemical Corporation, Freehold, NJ). After 60-90 min at 37°C, 1.0 ml 30,000 IU/ml bovine pancreas trypsin type III (Sigma, Dorset, England) in Ca^{2+}-, Mg^{2+}-free DME (DME-CMF) was added, followed by a 20-min incubation at 37°C and a centrifugation at 3,000 *g* for 2 min. The supernatant was discarded and the tissue was resuspended and incubated further in 2.0 ml of 15,000 IU/ml trypsin and 0.27 mM EDTA (Sigma) in DME-CMF for 20 min at 37°C. The enzymatic digestion was terminated by addition of 4.0 ml SBTI-DNase (5,200 IU/ml soybean trypsin inhibitor [Sigma], 74 IU/ml bovine pancreas DNAse, and 3.0 mg/ml BSA fraction V [Sigma]). The suspension was centrifuged for 2 min at 3,000 *g* and the tissue was resuspended in 1.0 ml DME + 10% FCS (DME [Gibco] supplemented with 2 mM glutamine [Gibco], 25 μg/ml gentamicin [Gibco] and 10% heat-inactivated FCS [Imperial Laboratories, UK]). A single cell suspension was obtained by trituration of the tissue through a 1-ml blow-out pipette and through 25 and 27 G hypodermic needles attached to a 1-ml syringe. The suspension was diluted further in DME + 10% FCS and plated in 100 μl drops onto glass coverslips (BDH; 13 mm diameter, No. 1) precoated with 20 μg/ml poly-L-lysine (Sigma) or poly-D-lysine (Sigma) at a density of 10-14 coverslips per pair of adult optic nerves. The presence of large amounts of debris and myelin did not allow accurate assessment of the number of cells plated per coverslip. After ~2 h at 37°C, coverslips (which had been kept thus far on raised platforms) were rinsed several times in L-15 medium (to remove debris and myelin) and placed in Falcon 6-well trays (three to four coverslips per well) containing DME-BS (DME with 4.5 g/liter glucose and supplemented with 25 μg/ml gentamicin, 2 mM glutamine, 0.234 IU/ml bovine pancreas insulin [Sigma], 100 μg/ml human transferrin [Sigma], 0.0286% (vol/vol) BSA PATH-O-CYTE* 4 [Miles Laboratories, Inc.], 0.2 μM progesterone [Sigma], 0.1 μM putrescine [Sigma], 0.45 μM L-thyroxine [Sigma], 0.224 μM selenium [Sigma], and 0.5 μM 3,3', 5-triiodo-L-thyronine [Sigma], modified from Bottenstein and Sato [1979] as described previously (Wolswijk and Noble, 1989]) and, where appropriate, supplemented with bFGF or supplemented with both PDGF-AA and bFGF. In most experiments, growth factors were added at a final concentration of 10 ng/ml. Growth factors were added to the cultures daily, while 90% of the culture medium was changed twice a week. Based on immunolabelings of cultures after 1 d in vitro, each coverslip contained

120 ± 18 O-2A^{adult} progenitor cells and 17 ± 2 process-bearing oligodendrocytes (mean ± SEM of five separate isolations). Very few astrocytes survived the isolation procedure: each culture contained only 4 ± 1 flat type-1 astrocyte-like cells and an occasional process-bearing type-2 astrocyte-like cell. (Previous studies have suggested that type-1 astrocytes and type-2 astrocytes are separate and distinct populations of astrocytes in perinatal optic nerve cultures and that type-1 astrocytes resemble in their properties cortical astrocytes [Raff et al., 1983*a*, 1984*a*].) O-2A lineage cells in cultures of adult optic nerve have characteristic process-bearing cells and can be distinguished both morphologically and antigenically from the contaminating non-O-2A lineage cells. O-2A^{adult} progenitor cells labeled with the A2B5 antibody, but not with antibodies against galactocerebroside (GalC, a marker for oligodendrocytes [Raff et al., 1978]) and glial fibrillary acidic protein (GFAP, an astrocyte-specific intermediate filament protein [Bignami et al., 1972]), oligodendrocytes were GalC$^+$ (and initially also A2B5$^+$) and type-2 astrocytes were A2B5$^+$GFAP$^+$. In some conditions described in the present paper, a small number of O-2A^{adult} progenitor cells become A2B5$^-$ (see Table II). However, because such cells had a characteristic process-bearing morphology, they could still be identified unambiguously as O-2A^{adult} progenitor cells. The majority of the non-O-2A lineage cells in the cultures contained vimentin intermediate filaments and had a fibroblast-like morphology. Although a very small proportion of the vimentin$^+$ flat cells bound the A2B5 antibody, they were not included in the population of O-2A lineage cells.

Growth Factors

Purified bovine brain bFGF was purchased from R and D Systems, Inc., while the recombinant human bFGF was obtained from either Boehringer Mannheim UK Ltd. (Lewes, England) or was a kind gift of Larry Coussens (Chiron Corporation, Emeryville, CA). C. George Nascimento (Chiron Corporation) generously supplied us with recombinant human PDGF-AA. Lyophilized growth factors were dissolved in either 0.04 M HCl containing 5% (vol/vol) BSA PATH-O-CYTE* 4 (PDGF-AA) or in L-15 supplemented with 5% BSA PATH-O-CYTE* 4 (bFGF). The solution was then filtered and diluted five times in L-15 to a final concentration of 1 μg/ml, aliquoted, and stored at −20°C. After defrosting, aliquots were kept at 4°C for not more than 2 d. As we obtained similar results with the purified bovine bFGF and the recombinant human bFGF, data from experiments with these two different sources of bFGF were pooled. However, the majority of the experiments were carried out with the recombinant human bFGF obtained from Chiron Corporation. Lyophilized human recombinant ^{125}I-labeled bFGF was obtained from Amersham (starting activity of 900–1,000 Ci/mmol) and was dissolved in L-15 medium containing 5% BSA PATH-O-CYTE* and stored in aliquots at −20°C.

Indirect Immunofluorescence

Before immunolabeling, cultures growing on glass coverslips were fixed in 4.0% paraformaldehyde (TAAB Laboratories Equipment Ltd., Reading, England) in PBS (Gibco) for 15–30 min at room temperature, followed by several rinses in HBSS + 5% NCS (HBSS [Imperial Laboratories] containing 5% heat-inactivated newborn calf serum [NCS] and 0.02 M Hepes [Sigma]). The following antibodies (Abs) were used in the experiments: the mouse IgM mAb A2B5 (hybridoma supernatant, diluted three times; Eisenbarth et al., 1979), the mouse IgM mAb O4 (hybridoma supernatant, diluted three times; Sommer and Schachner, 1981), the mouse IgG_3 anti-GalC mAb (hybridoma supernatant, diluted five times; Ranscht et al., 1982), the mouse IgG_1 antivimentin mAb (Boehringer Mannheim; used at a concentration of 4 μg/ml), the mouse IgG_1 anti-BrdU mAb (Becton Dickinson Immunocytometry Systems, Cowley, England; the Ab solution was diluted 1:100), rabbit anti-cow GFAP polyclonal Abs (Dako Ltd., High Wycombe, England; diluted 1:1000), and rabbit antichicken FGF receptor antiserum (UBI, Inc., Lake Placid, NY; diluted 1:30). To visualize the binding of the mouse mAbs and the polyclonal rabbit Abs, fluorescein (Fl)- or rhodamine (Rd)-conjugated Abs were used (diluted 1:100; Southern Biotechnology Associates Inc., Birmingham, AL). In some experiments, the binding of the anti-GalC mAbs was visualized with biotinylated goat-anti-mouse IgG_3 (Southern Biotechnology Associates Inc.; diluted 1:50), followed by coumarin (Cou)-labeled streptavidin (Molecular Probes Inc., Eugene, OR; diluted 1:50). Abs were diluted in heat-inactivated NCS. Cultures were incubated in 50 μl of the Ab solutions for ∼30 min and were then rinsed several times in HBSS + 5% NCS. In the case of the anti-FGF antibodies, cells were incubated in the antibody solution overnight at 4°C. In addition to the standard double-immunolabeling procedures we have described in detail previously (Wolswijk and Noble, 1989; Wolswijk et al., 1990, 1991*b*), four triple-immunolabelings with three different fluorochromes (fluorescein, rhodamine, and coumarin) were carried out according to the following protocols. (Protocol A) Incubation 1, mAb O4; incubation 2, G-anti-MIgM-Fl plus mAb anti-GalC; incubation 3, mAb A2B5 plus biotinylated G-anti-MIgG$_3$; incubation 4, G-anti-MIgM-Rd plus streptavidin-Cou; incubation 5, methanol (10–20 min, −20°C). This immunolabeling procedure allowed us to calculate the proportion of the O-2A^{adult} progenitor cells (i.e., GalC$^-$ O-2A lineage cells) that were A2B5$^+$O4$^-$ (such cells were Rd$^+$Fl$^-$Cou$^-$) and those that were A2B5$^-$O4$^-$ (such cells were Rd$^-$Fl$^-$Cou$^-$). (Protocol B) Incubation 1, mAb A2B5; incubation 2, G-anti-MIgM-Fl plus mAb anti-GalC; incubation 3, mAb O4 plus biotinylated G-anti-MIgG$_3$; incubation 4, G-anti-MIgM-Rd plus streptavidin-Cou; incubation 5, methanol (10–20 min, −20°C). This immunolabeling procedure allowed us to calculate the proportion of the O-2A^{adult} progenitor cells that were A2B5$^-$O4$^+$ (such cells were Rd$^+$Fl$^-$Cou$^-$) and those that were A2B5$^-$O4$^-$ (such cells were Rd$^-$Fl$^-$Cou$^-$). In addition, this immunolabeling procedure allowed us to determine the proportion of the GalC$^+$ oligodendrocytes that were A2B5$^+$ (such cells were Cou$^+$Fl$^+$Rd$^+$). (Protocol C) Incubation 1, mAbs A2B5, O4, and anti-GalC; incubation 2, G-anti-MIgM-Rd plus biotinylated-G-anti-MIgG$_3$; incubation 3, streptavidin-Cou; incubation 4, methanol (10–20 min, −20°C); incubation 5, antivimentin mAbs; incubation 6, G-anti-MIgG$_1$-Fl. This immunolabeling procedure allowed us to determine the proportion of O-2A^{adult} progenitor cells that contained vimentin intermediate filaments. (Protocol D) Incubations 1–4 were the same as those of Protocol C; incubation 5, anti-GFAP Abs; incubation 6, goat-anti-rabbit Ig-Fl. This procedure allowed us to determine the proportion of the GalC$^-$ O-2A lineage cells that were GFAP$^+$ type-2 astrocytes. After the immunolabeling, coverslips were rinsed several times in HBSS + 5% NCS and distilled water, mounted in a drop of anti-fade (glycerol containing 22 mM 1,4-diazobicycle [2,2,2] octane; Johnson et al., 1982) and sealed with clear nail varnish. Cultures were viewed on a Zeiss Axiophot microscope equipped with phase-contrast and epi-UV illumination and selective filters for rhodamine, fluorescein, and coumarin, using a ×40 Plan NEOFLUAR objective. Immunolabeled cells were photographed using Ilford XP2 400 films.

BrdU Incorporation Assay

To determine whether cells were synthesizing DNA, cultures were incubated in the presence of 10 μM 5-bromodeoxyuridine (BrdU; Sigma) for a total period of 24 h. The incorporation of BrdU into the nuclei of those cells that had synthesized DNA was visualized using anti-BrdU mAbs (Gratzner, 1982), as described before (Wolswijk et al., 1991*b*). Before anti-BrdU mAbs were applied, methanol-fixed (10–20 min, −20°C) cells were exposed first to 0.02% paraformaldehyde in HBBS + 5% NCS for 60 s and then to 0.07 M NaOH for 7–10 min. After each incubation, coverslips were rinsed several times in HBSS + 5% NCS.

^{125}I-bFGF Labeling of Adult Optic Nerve Cultures and Autoradiography

Adult optic nerve cells growing on glass coverslips were washed once in L-15 medium containing 5% FCS (binding buffer) and incubated for 1 h at room temperature in 50 μl binding buffer containing various concentrations of ^{125}I-bFGF with or without a 100-fold excess of unlabeled recombinant human bFGF or unlabeled recombinant human PDGF-AA. After the radiolabeling, cultures were fixed in 4% paraformaldehyde, immunolabeled, washed in distilled water, dehydrated in an ascending series of ethanol, and air-dried. Coverslips were mounted face-up onto glass slides using Gurr fluoromount mountant (BDH Chemicals Ltd.). The following day, slides were dipped twice in Ilford K2 autoradiographic emulsion (diluted in an equal volume of distilled water) and allowed to dry overnight. Slides were exposed at 4°C for varying periods of time (14–32 d) to compensate for differences in the activity of the batch of ^{125}I-bFGF, the activity of which ranged from 550–920 μCi/mmol. Slides were developed in Ilford Contrast-FF (diluted 1:10) and fixed in Ilford Hypam (diluted 1:5). A second coverslip was mounted in anti-fade on top of the cells and was sealed with nail varnish. Cells were examined using a ×100 Plan NEOFLUAR objective and the number of silver grains above the cell body and main process of O-2A^{adult} progenitor cells in the various conditions was determined using a counting grid.

Time-lapse Cinemicroscopy

Time-lapse cinemicroscopy experiments were performed as described before (Wolswijk and Noble, 1989; Wolswijk et al., 1990, 1991*b*). A cell suspension derived from the optic nerves of adult rats was plated in 100-μl drops onto the surface of 3-cm PLL-coated Nunc Petri dishes. After 2 h at 37°C, cultures were rinsed several times in L-15 medium. Cultures were maintained either in DME-BS supplemented with 10 ng/ml bFGF or supplemented with 10 ng/ml PDGF-AA plus 10 ng/ml bFGF. After 1-2 d in vitro, fields (0.77 × 1.08 mm) containing O-2A^{adult} progenitor-like cells were selected for photography and their location was marked by drawing a circle on the bottom of the Petri dish with a diamond needle. O-2A^{adult} progenitors were identified on the basis of their characteristic process-bearing morphology, which was distinct from the fibroblast-like morphology of the majority of the non-O-2A lineage cells in the cultures. Cultures were filmed on Olympus inverted microscopes adapted for time-lapse cinemicroscopy and were maintained at 37°C under 10% CO_2 and 90% air, as described before (Riddle, 1979, 1990), for periods of up to 9 d. Images were recorded every 240 or 360 S on Kodak Infocapture AHU microfilm 1454 films. Before a medium change (which occurred twice a week) or the addition of growth factors (which occurred daily), a Polaroid photograph was taken of the field of photography. This photograph was used later to realign the field of photography. Six films from four separate cultures were analyzed in the case of adult optic nerve cells cultured in bFGF, while seven films from four separate sets of cultures were analyzed in the case of cultures maintained in PDGF-AA and bFGF. Division times, migration rates, and morphologies of individual O-2A^{adult} progenitors were determined. Division times could only be determined of those cells that had remained in the field of photography and had divided at least twice during the filming period. Film frame numbers were used to determine division times. The distance a cell had migrated between divisions was determined with a Hewlett Packard 9874 A digitizer and, to calculate a cell's average rate of migration between divisions, this figure was divided by its division time. Only 1.1% of the O-2A lineage cells in cultures of adult optic nerve that had been exposed to bFGF died during the filming period, while 3.5% of the O-2A lineage cells died in adult optic nerve cultures maintained in PDGF-AA plus bFGF.

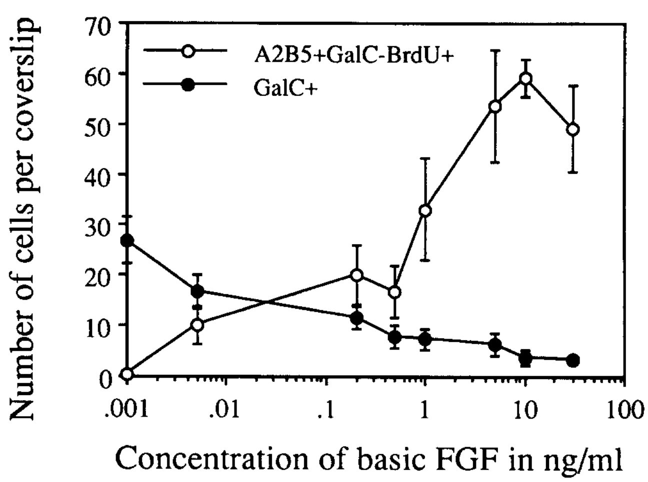

Figure 1. Dose-dependent induction of DNA synthesis in O-2A^{adult} progenitor cells by bFGF. Adult optic nerve cells were cultured in DME-BS supplemented with various concentrations of bFGF for a total of 3 d. For the last 24 h, cells were exposed additionally to 5-bromodeoxyuridine (*BrdU*). The graph shows that the number of O-2A^{adult} progenitor cells (identified as cells that were A2B5$^+$GalC$^-$) that incorporated BrdU into their nucleus increased with increasing doses of bFGF and that at the same time the number of oligodendrocytes (identified on the basis of their expression of GalC) in the cultures decreased. When adult optic nerve cells were grown in the presence of 10 ng/ml bFGF, 46.0 ± 2.5% of the O-2A^{adult} progenitor cells were BrdU$^+$, while 3.0 ± 1.5% of all O-2A lineage cells were oligodendrocytes. Half-maximal effects of bFGF on BrdU uptake by O-2A^{adult} progenitor cells occurred at a dose of ∼1 ng/ml bFGF. Results are expressed as mean ± SEM of five to six cultures of two independent experiments.

Results

bFGF Is a Potent Mitogen for O-2A^{adult} Progenitor Cells

bFGF induced DNA synthesis in O-2A^{adult} progenitor cells in a dose-dependent manner. Cells derived from the optic nerves of adult rats (⩾2 1/2 mo old) were cultured for 3 d in DME-BS supplemented with various concentrations of bFGF. Stimulation of DNA synthesis in O-2A^{adult} progenitor cells (identified as A2B5$^+$GalC$^-$ cells) in response to bFGF was monitored by the incorporation of BrdU during a 24-h terminal pulse. Maximal levels of BrdU incorporation in O-2A^{adult} progenitor cells occurred at a concentration of ∼10 ng/ml bFGF, when 46.0 ± 2.5% O-2A^{adult} progenitor cells were BrdU$^+$ (60 ± 4 cells per culture; Fig. 1). Half-maximal effects occurred with a dose of ∼1 ng/ml bFGF (Fig. 1). Even with a dose as low as 0.05 ng/ml bFGF, 10.1 ± 2.0% of the 103 ± 43 O-2A^{adult} progenitor cells in the cultures had incorporated BrdU. In contrast, no O-2A^{adult} progenitor cells were stimulated to synthesize DNA when adult optic nerve cells were grown in DME-BS lacking growth factors, as shown previously (Wolswijk et al., 1991*b*). This observation suggests that the non-O-2A lineage cells and the few type-1 astrocyte-like cells (4 ± 1 cells/culture) either did not secrete O-2A^{adult} progenitor mitogen(s) (such as PDGF) into the culture medium or did not produce such factor(s) in sufficient quantities to induce division in O-2A^{adult} progenitor cells.

Growth of adult optic nerve cells in the presence of bFGF was associated with a dose-dependent inhibition of oligodendrocytic differentiation of O-2A^{adult} progenitor cells (Fig. 1). bFGF was more effective than PDGF (Wolswijk et al., 1991*b*) or medium conditioned by purified cortical astrocytes (Astro-CM; Wolswijk and Noble, 1989), which contains PDGF (Noble et al., 1988; Raff et al., 1988; Richardson et al., 1988), in preventing the oligodendrocytic differentiation of O-2A^{adult} progenitor cells that is seen in DME-BS lacking growth factors. Only 3.0 ± 1.5% of the O-2A lineage cells in the cultures expressed the oligodendrocyte marker galactocerebroside (GalC; Raff et al., 1978) when adult optic nerve cells were grown for 3 d in the presence of 10 ng/ml bFGF. In contrast, 19.5 ± 2.3% of the O-2A lineage cells were GalC$^+$ in adult optic nerve cultures treated for 3 d with 10 ng/ml PDGF (see also Wolswijk et al., 1991*b*). When adult optic nerve cultures were grown in the absence of growth factors, O-2A^{adult} progenitor cells differentiated prematurely into oligodendrocytes (as described previously [Wolswijk and Noble, 1989]), such that after 3 d of growth in DME-BS, 47.5 ± 3.2% of the O-2A lineage cells expressed GalC.

Very few cells in the cultures expressed the antigenic phenotype of type-2 astrocytes after 5 and 8 d of exposure to bFGF (data not shown), suggesting that bFGF did not induce astrocytic differentiation of O-2A^{adult} progenitor cells.

O-2A^{adult} Progenitor Cells Bind Both ^{125}I-bFGF and FGF Receptor Antibodies

O-2A^{adult} progenitor cells bound ^{125}I-bFGF in a dose-dependent manner and the binding of radiolabeled bFGF was reduced greatly when cells were incubated additionally in an

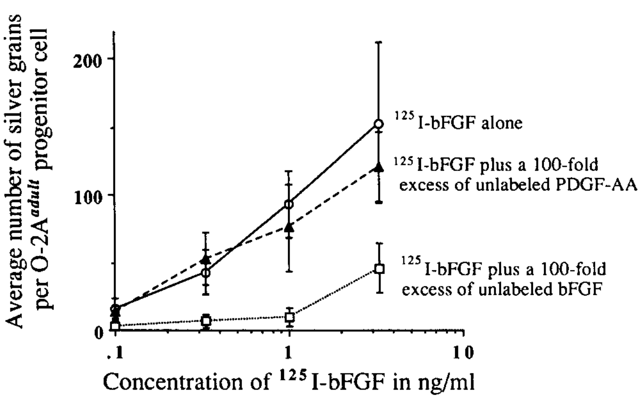

Figure 2. Direct binding of ^{125}I-bFGF to O-2A^{adult} progenitor cells. Adult optic nerve cells were grown for 4 d in 10 ng/ml PDGF-AA and then incubated in 0.1, 0.33, 1.0, or 3.3 ng/ml ^{125}I-bFGF with or without a 100-fold excess of unlabeled bFGF or PDGF-AA, immunolabeled, and processed for autoradiography as described in Materials and Methods. The total number of silver grains above the cell body and main process of ten A2B5$^+$GalC$^-$ O-2A^{adult} progenitor cells in each condition was determined. The graph shows that the number of silver grains above O-2A^{adult} progenitor cells increased with increasing concentrations of ^{125}I-bFGF. Addition of a 100-fold excess of unlabeled bFGF at the indicated concentrations of ^{125}I-bFGF reduced significantly the number of grains/cell (P value < 0.001, t test), while addition of a 100-fold excess unlabeled PDGF-AA at each concentration of ^{125}I-bFGF did not have a significant effect on the binding of radiolabeled bFGF ($0.11 < P < 0.73$). Each symbol represents the mean ± SD of one experiment.

excess of unlabeled bFGF. In these experiments, adult optic nerve cultures that had been maintained for 4 d in 10 ng/ml PDGF-AA were incubated in increasing concentrations of ^{125}I-bFGF, immunolabeled and processed for autoradiography. Adult optic nerve cells were grown in PDGF-AA instead of bFGF to prevent possible down regulation of FGF receptors as a result of exposure to bFGF. The specificity of the binding of radiolabeled bFGF was demonstrated by incubating adult optic nerve cultures in the presence of ^{125}I-bFGF and a 100-fold excess of unlabeled bFGF or PDGF-AA.

The number of silver grains over O-2A^{adult} progenitor cells increased significantly from 16 ± 8 grains/cell at a concentration of 0.1 ng/ml ^{125}I-bFGF to 153 ± 59 grains/cell at a concentration of 3.3 ng/ml ^{125}I-bFGF (Fig. 2). Only 1 ± 1 silver grains were found above O-2A^{adult} progenitor cells that had been incubated with the binding buffer only. The addition of a 100-fold excess unlabeled bFGF at each concentration reduced the binding of ^{125}I-bFGF to the cells by >70% (Fig. 2). For example, the number of silver grains per O-2A^{adult} progenitor cell was reduced by 94.7 ± 2.9% when adult optic nerve cells were exposed to 1.0 ng/ml ^{125}I-bFGF plus a 100-fold excess of unlabeled bFGF (mean ± SEM of three separate experiments). The number of grains per O-2A^{adult} progenitor cell was reduced by only 9.5 ± 7.2% when cells were exposed to 1 ng/ml ^{125}I-bFGF plus a 100-fold excess of unlabeled PDGF-AA; this reduction was not significant. More silver grains were found generally over the non-O-2A lineage cells in the cultures (data not shown).

The possibility that O-2A^{adult} progenitor cells express FGF receptors was substantiated further by the observation that such cells bound antibodies against the chicken FGF receptor. We found that all O-2A^{adult} progenitor cells that had been grown for 3-5 d in 10 ng/ml PDGF-AA or bFGF bound the anti-FGF receptor antibodies (although most cells were only weakly positive; Fig. 3). Oligodendrocytes and the non-O-2A lineage cells in the adult optic nerve cultures also bound the antibody. However, as was the case in the ^{125}I-bFGF binding studies, the non-O-2A lineage cells in the adult optic nerve cultures were generally more strongly labeled with the anti-FGF receptor antibodies than O-2A^{adult} progenitor cells and oligodendrocytes (see Fig. 3). Thus, the ^{125}I-bFGF and immunolabeling studies suggest that O-2A^{adult} progenitor cells express FGF receptors and that bFGF acts directly on these cells.

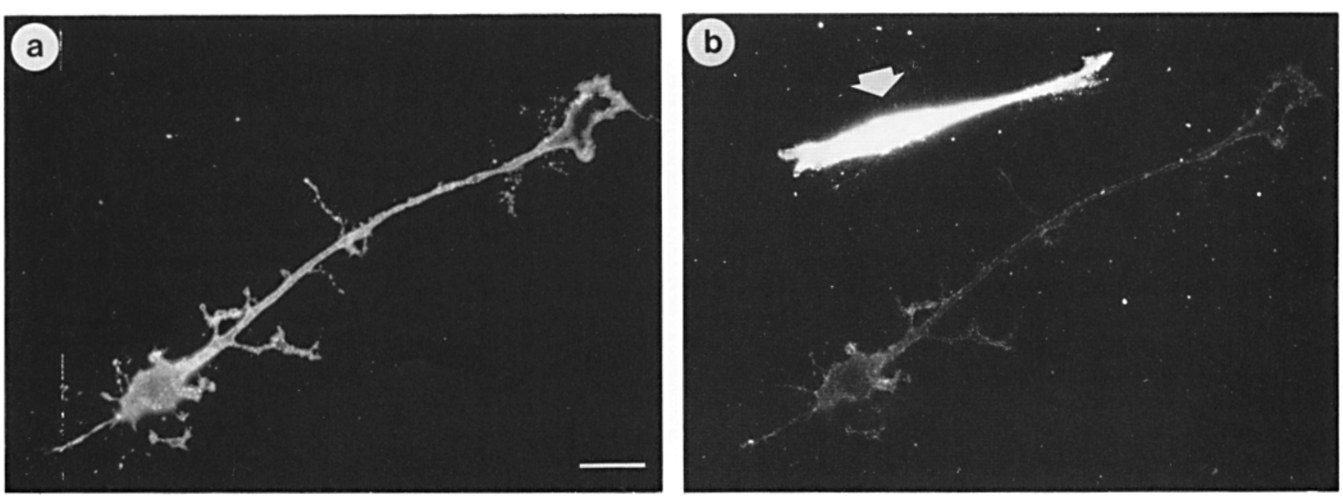

Figure 3. O-2A^{adult} progenitor cells bind anti-FGF receptor antibodies. Adult optic nerve cultures that had been grown for 4 d in 10 ng/ml PDGF-AA were immunolabeled with the A2B5 antibody (*a*) and antichick FGF receptor polyclonal antibodies (*b*) followed by fluorochrome-conjugated second antibodies. Like all other O-2A^{adult} progenitor cells in the culture, the A2B5$^+$ cell shown in the figure was FGF receptor positive. A strongly FGF receptor positive A2B5$^-$ non-O-2A lineage cell is indicated with an arrow. (*a*) Rhodamine optics; (*b*) fluorescein optics. Bar, 10 μm.

Table I. Increases in the Number of O-2A Lineage Cells Following Growth in bFGF or PF

Number of days in vitro	Condition	Total number of O-2A lineage cells	Number of O-2A^{adult} progenitors	Number of oligodendrocytes
1	bFGF	145 ± 38	129 ± 36	17 ± 3
	PF	147 ± 35	128 ± 33	20 ± 3
5	bFGF	547 ± 63	520 ± 64	27 ± 10
	PF	693 ± 114	671 ± 108	22 ± 7
8	bFGF	1,006 ± 18	913 ± 54	93 ± 36
	PF	1,525 ± 111	1,482 ± 107	44 ± 5

Adult optic nerve cells were cultured in DME-BS supplemented with either bFGF or PF. After 1, 5, and 8 d of in vitro growth, cultures were immunolabeled and the total number of O-2A^{adult} progenitor cells and oligodendrocytes present on each coverslip in each condition was determined. A small proportion of the O-2A^{adult} progenitor cells was not labeled with the A2B5 and O4 antibody (see Table II), but such cells were identified as being O-2A^{adult} progenitor cells on the basis of their morphology, which was indistinguishable from O-2A^{adult} progenitor cells that had bound these antibodies. The O-2A lineage population expanded with a doubling time of 60 h between day 1 and day 8 when adult optic nerve cells were cultured in bFGF alone, while the O-2A lineage population in cultures exposed to PF expanded with a 50-h doubling time during the same period. However, the most rapid increase in the number of O-2A lineage cells in both conditions occurred during the first 5 d in culture. The data shown are the mean ± SEM of a minimum of nine cultures from three separate experiments.

The O-2A Lineage Population Expands Rapidly When Cultured in bFGF

Prolonged growth of adult optic nerve cells in the presence of bFGF was associated with more rapid increases in the number of O-2A lineage cells than when such cultures were treated with PDGF-AA or cortical astrocyte-derived PDGF. For example, the average number of O-2A lineage cells per coverslip increased 6.9-fold, from 145 ± 38 cells on day 1 to 1,006 ± 18 cells on day 8, when adult optic nerve cultures were maintained in DME-BS supplemented with 10 ng/ml bFGF (Table I). The increase in the number of O-2A lineage cells over this 7-d period was almost twofold higher than when adult optic nerve cultures were exposed to 10 ng/ml PDGF-AA (3.5-fold increase in the number of O-2A lineage cells during the same period of culture; see also Wolswijk et al., 1991*b*).

The most rapid increase in the number of O-2A lineage cells occurred between day 1 and day 5 (Table I), when the O-2A lineage population increased by 3.8-fold, corresponding to a doubling time for the total O-2A lineage population of 50 h. In contrast, there was only a 1.8-fold increase in the number of O-2A lineage cells between day 5 and day 8 of in vitro growth (Table I), corresponding to a population doubling time of 82 h.

Although the largest expansion occurred in the O-2A^{adult} progenitor population, the number of oligodendrocytes increased 5.5-fold between day 1 and day 8 of in vitro growth (Table I). This suggests that bFGF was not able to inhibit completely oligodendrocytic differentiation of O-2A^{adult} progenitor cells. This was substantiated by the observation that 9.1 ± 6.8% of the oligodendrocytes were labeled with the A2B5 antibody after 8 d in vitro, an antigenic phenotype that appears to characterize newly generated oligodendrocytes (Raff et al., 1983*b*). As following a 24-h pulse with BrdU some oligodendrocytes (<5%) were BrdU$^+$ on day 5 and day 8, the increase in the number of oligodendrocytes could have been due in part to division of these cells. This observation is in agreement with previous suggestions that bFGF is a mitogen for oligodendrocytes (Eccleston and Silberberg, 1985; Saneto and de Vellis, 1985; Bögler et al., 1990).

Many O-2A^{adult} Progenitor Cells Cultured in bFGF Divide Rapidly, Migrate Slowly, and Have a Multipolar Morphology

To determine directly whether O-2A^{adult} progenitors exposed to bFGF had a cell cycle time that was shorter than the 59 ± 5-h cell cycle of O-2A^{adult} progenitor cells treated with PDGF-AA (Wolswijk et al., 1991*b*), adult optic nerve cultures were grown in DME-BS supplemented with 10 ng/ml bFGF and followed between day 1 and day 9 of in vitro growth using time-lapse cinemicroscopy (see Materials and Methods). Cell cycle times of individual O-2A^{adult} progenitor cells (identified on the basis of their characteristic process-bearing morphology) could only be measured if such cells remained in the field of photography and divided at least twice. Thus, O-2A^{adult} progenitor cells that had very long cell cycles (>100 h) were not included in the analysis.

We found that O-2A^{adult} progenitor cells cultured in bFGF had an average cell cycle time of 38.2 ± 3.9 h (n = 45; Fig. 4) and that these cells underwent their first division 58 ± 3 h after the cultures had been prepared. A large variation in the cell cycle lengths of individual O-2A^{adult} progenitor cells was observed (Fig. 4). For example, 23 cells (51%) had a cell cycle time of less than 30 h, while 13 cells (29%) had a cell cycle time of over 50 h. Some O-2A^{adult} progenitor cells did not divide during the filming period (10% of the O-2A^{adult} progenitor cells that were in the field of photography when the filming started), while others only divided once (12.5% of the starting cells).

The time-lapse cinemicroscopy studies also revealed that bFGF was able to induce motility in O-2A^{adult} progenitor cells. Proliferating O-2A^{adult} progenitor cells exposed to bFGF migrated with average speeds of 4.9 ± 0.7 μm/h (Fig. 4), which is very similar to the average rate of migration of O-2A^{adult} progenitors cultured in PDGF-AA (4.1 ± 0.6 μm/h; Wolswijk et al., 1991*b*) or in medium conditioned by purified cortical astrocytes (Astro-CM) (4.3 ± 0.7 μm/h; Wolswijk and Noble, 1989). Although we observed some variation in the migration rates of individual O-2A^{adult} progenitor cells, 84% (38/45 cells) migrated with speeds of <10 μm/h (Fig. 4). The majority of the proliferating and migrating O-2A^{adult} progenitor cells expressed a multipolar morphology (Figs. 4 and 5). However, five cells, all of which were members of one family, had the bipolar morphology characteristic of O-2A^{perinatal} progenitor cells cultured in PDGF or Astro-CM (Temple and Raff, 1986; Fig. 4). Like such O-2A^{perinatal} progenitor cells (Small et al., 1987; Noble et al., 1988), these five cells also divided and migrated rapidly (Fig. 4; average cell cycle time: 15.7 ± 2.4 h; average rate of migration: 14.8 ± 3.8 μm/h).

O-2A^{adult} progenitor cells grown for 5 or 8 d in bFGF were still bipotential and differentiated into oligodendrocytes when the medium of the adult optic nerve cultures was changed to DME-BS and differentiated into type-2 astrocytes when the culture medium was changed to DME + 10% FCS (data not shown).

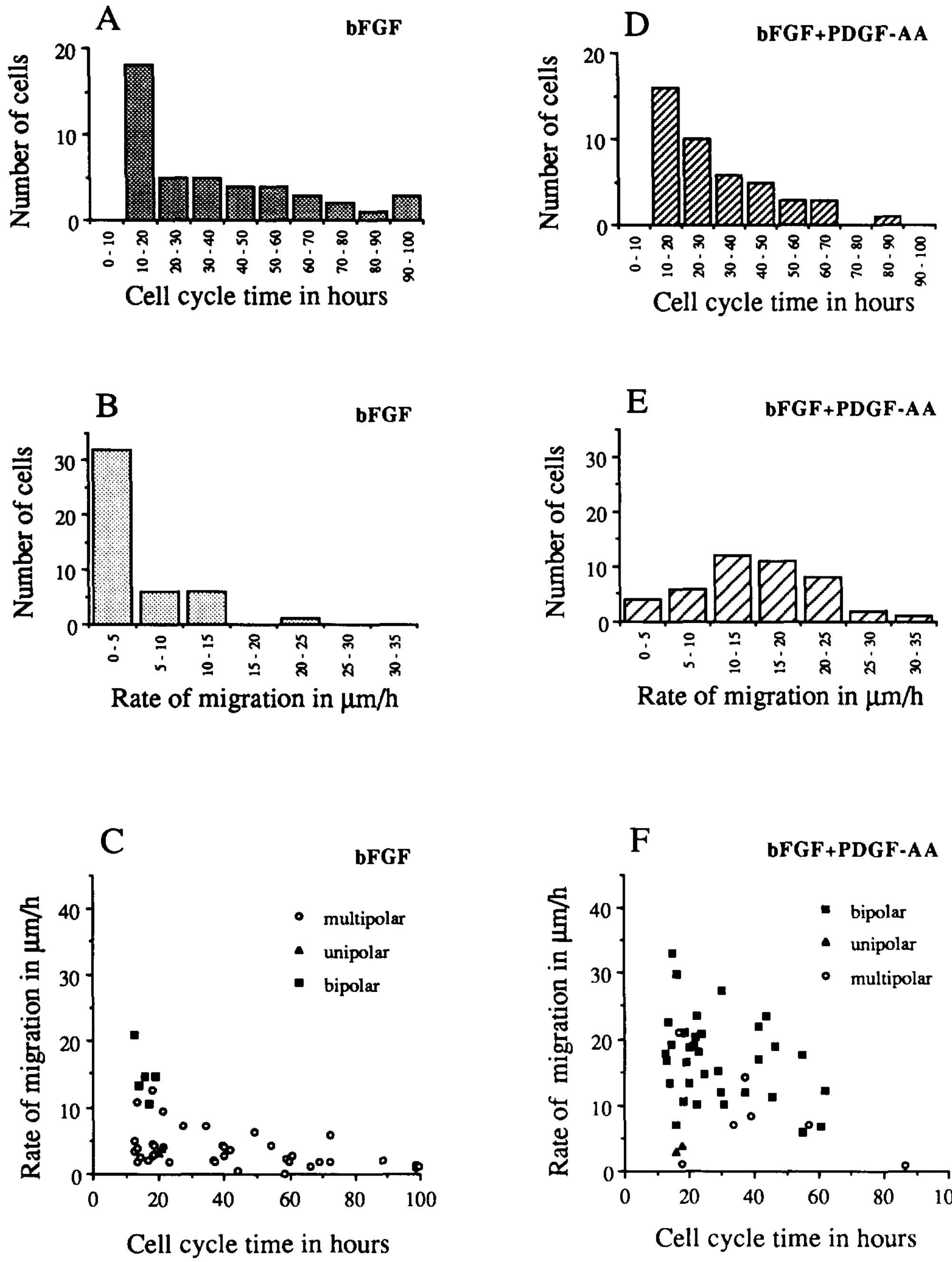

Figure 4. Characteristics of O-2A^{adult} progenitor cells in cultures exposed to bFGF or PF. O-2A^{adult} progenitor cells growing in cultures of adult optic nerve treated with either bFGF or PF were followed using time-lapse cinemicroscopy. The morphology, cell cycle time and rate of migration of individual O-2A^{adult} progenitor cells (identified morphologically) was determined. Filming started on day 1 or day 2 of in vitro growth and lasted for 3-9 d. The 45 O-2A^{adult} progenitor cells that we examined in cultures of adult optic nerve exposed to bFGF had an average cell cycle time of 38.2 ± 3.9 h, an average rate of migration of 4.9 ± 0.7 μm/h and were mostly multipolar. However, there was a large variation in both cell cycle times (*A*) and migration rates (*B*) of individual O-2A^{adult} progenitor cells growing in adult optic nerve cultures exposed to bFGF. Note that five cells, all of which were members of one family, divided and migrated very rapidly and had a bipolar morphology (see *C*). Each symbol in *C* represents one of the 45 O-2A^{adult} progenitor cells that were examined in detail in cultures exposed to bFGF and it gives the cell cycle time, migration rate, and morphology of that cell. The 44 O-2A^{adult} progenitor cells we analyzed in cultures of adult optic nerve exposed to PF predominantly had a bipolar morphology (*F*), had an average cell cycle time of 31.1 ± 2.7 h (*D*), and migrated with average speeds of 14.9 ± 1.1 μm/h (*E*). As with O-2A^{adult} progenitor cells cultured in bFGF, there was a large variation in the cell cycle time and migration rates of individual cells that had been treated with PF. The cell cycle time, migration rate, and morphology of each of the 44 O-2A^{adult} progenitor cells that were examined in cultures exposed to PF is shown in *F*.

The Number of O-2A Lineage Cells Increases More Rapidly When Exposed Simultaneously to bFGF plus PDGF-AA than When These Cells Are Exposed to bFGF Alone

The O-2A lineage population increased more rapidly when adult optic nerve cells were cultured in bFGF plus PDGF-AA(PF) than when such cells were exposed to either PDGF-AA or bFGF alone. For example, the number of O-2A lineage cells on each coverslip increased 10.4-fold between day 1 and day 8 of in vitro growth, from 147 ± 35 cells to 1,525 ± 111 cells in cultures exposed to PF (Table I), corresponding to a population doubling time of ~50 h. As was the case for adult optic nerve cultures grown in bFGF, >90% of the O-2A lineage cells in the cultures were O-2A^{adult} progenitor cells (Table I and II). The most rapid increase in the number of O-2A lineage cells occurred between day 1 and day 5 when the O-2A lineage population expanded with a doubling time of 43 h (Table I).

Two observations suggested that PF was more effective in inhibiting the differentiation of O-2A^{adult} progenitor cells into oligodendrocytes than bFGF. (*a*) The number of oligodendrocytes increased much more slowly when adult optic nerve cells were cultured in PF than when such cells were grown in bFGF alone (Table I). (*b*) The proportion of O-2A lineage cells that were oligodendrocytes was lower in cul-

Table II. Antigenic Phenotype of O-2A^{adult} Progenitor Cells Cultured in bFGF or PF

Characteristic	Number of days in vitro: 1	5	8
A Adult optic nerve cultures exposed to bFGF			
% O-2A lineage cells that were O-2A^{adult} progenitors	87.3 ± 1.1	94.1 ± 1.7	90.3 ± 3.8
% O-2A^{adult} progenitors that were:			
vimentin$^+$	10.5 ± 3.9	96.2 ± 1.3	93.3 ± 1.6
A2B5$^+$04$^-$	9.5 ± 6.5	66.7 ± 1.4	18.0 ± 3.7
A2B5$^+$04$^+$	90.5 ± 6.5	28.7 ± 0.4	75.5 ± 1.1
A2B5$^-$04$^+$	N. O.*	2.6 ± 0.8	4.7 ± 2.0
A2B5$^-$04$^-$	N. O.	2.0 ± 0.8	1.8 ± 1.8
B Adult optic nerve cultures exposed to PF			
% O-2A lineage cells that were O-2A^{adult} progenitors	86.0 ± 1.7	96.8 ± 0.7	97.5 ± 0.5
% O-2A^{adult} progenitors that were:			
vimentin$^+$	12.8 ± 4.9	95.7 ± 3.3	96.3 ± 2.9
A2B5$^+$04$^-$	4.3 ± 3.0	85.2 ± 4.9	69.7 ± 11.0
A2B5$^+$04$^+$	95.7 ± 3.0	9.5 ± 1.4	25.5 ± 3.3
A2B5$^-$04$^+$	N. O.	0.8 ± 0.5	2.1 ± 1.7
A2B5$^-$04$^-$	N. O.	4.5 ± 1.3	3.0 ± 1.8

* N. O., none observed.
Cultures of adult optic nerve incubated in bFGF or PF were immunolabeled after 1, 5, and 8 d in vitro according to a number of different protocols (see Materials and Methods). The proportion of O-2A^{adult} progenitor cells that were A2B5$^+$ and 04$^+$ was deduced from the data that could be determined directly. After 1 d in vitro, the vimentin$^+$ O-2A^{adult} progenitors contained only very small amounts of vimentin filaments and these filaments were located mostly in the tips of their processes. Although most O-2A^{adult} progenitor cells were labeled with the A2B5 and/or 04 antibody, some cells were A2B5$^-$04$^-$ after 5 and 8 d of exposure to bFGF or PF (but not after 3 d in bFGF, such as in the experiment shown in Fig. 1). Such cells were identified on the basis of their characteristic process-bearing morphology, which was indistinguishable from O-2A^{adult} progenitor cells that had bound the A2B5 and/or O4 mAbs and which was considerably different from the non-O-2A lineage cells in the cultures. The immunolabeling experiments suggested that many bFGF-treated and PF-treated O-2A^{adult} progenitors acquired an 04$^-$ vimentin$^+$ antigenic phenotype, although after 8 d of growth in bFGF, many of the O-2A^{adult} progenitors exposed to bFGF had regained 04 positivity. Results are expressed as mean ± SEM of two to four experiments with a minimum of two coverslips per experiment.

tures maintained in PF for 5 or 8 d than in cultures exposed to bFGF alone (Table II). However, exposure of adult optic nerve cultures to PF may not have inhibited completely the differentiation of O-2A^{adult} progenitor cells into oligodendrocytes, as 8.7 ± 3.2% of the oligodendrocytes after 8 d of in vitro growth were A2B5$^+$. As some oligodendrocytes (<10%) were BrdU$^+$ when examined on day 5 and day 8, the small rise in the number of oligodendrocytes between day 1 and day 8 also could have been due partially to division of these cells.

Many O-2A^{adult} Progenitor Cells Exposed Simultaneously to PF Divide and Migrate Rapidly and Possess a Bipolar Morphology

To examine whether O-2A^{adult} progenitor cells exposed to PF divided more rapidly than O-2A^{adult} progenitor cells cultured in bFGF alone, cultures of adult optic nerve were followed between day 1 and day 10 of in vitro growth using time-lapse cinemicroscopy. O-2A^{adult} progenitor cells exposed to PF divided for the first time 60 ± 5 h after the cultures had been prepared. We found that the 44 O-2A^{adult} progenitor cells we examined divided with an average cell cycle time of 31.1 ± 2.7 h (Fig. 4). 26 of these O-2A^{adult} progenitor cells (59%) had a cell cycle length of <30 h (average: 19.1 ± 0.9 h), like O-2A^{perinatal} progenitor cells (Noble et al., 1988). Seven cells (16%) divided very slowly, with cell cycle times of over 50 h (average: 63.5 ± 4.3 h) (Fig. 4). Furthermore, 11.4% of the starting cells did not divide during the filming period, while a further 11.4% divided only once.

Many proliferating O-2A^{adult} progenitor cells growing in cultures exposed to PF migrated rapidly, with average speeds of 14.9 ± 1.1 μm/h (Fig. 4). 25% (11/44 cells) of the O-2A^{adult} progenitor cells, like O-2A^{perinatal} progenitor cells (Small et al., 1987; Noble et al., 1988), were highly motile and migrated with speeds of over 20 μm/h, with some cells achieving average speeds of >30 μm/h (Fig. 4). Only two cells had a rate of migration of <2 μm/h. O-2A^{adult} progenitor cells with a cell cycle time of >50 h tended to migrate at slower rates than those with a cell cycle time of <50 h (8.6 ± 2.0 μm/h [n = 7] versus 16.1 ± 1.2 μm/h [n = 37]).

80% (35/44) of the O-2A^{adult} progenitor cells growing in cultures of adult optic nerve exposed to PF had a bipolar morphology (Figs. 4 and 5) and resembled morphologically O-2A^{perinatal} progenitor cells cultured in PDGF or Astro-CM (Temple and Raff, 1986). In total, 47% (21 cells) of the O-2A^{adult} progenitor cells examined in cultures of adult optic nerve exposed to PF expressed O-2A^{perinatal} progenitor-like characteristics, i.e., were bipolar, divided with a cell cycle of <30 h, and migrated with average speeds of >10 μm/h (Fig. 4).

As was the case for O-2A^{adult} progenitor cells exposed to bFGF alone, cells that had been treated for 5 or 8 d with PF were still able to differentiate into oligodendrocytes or type-2 astrocytes depending on the constituents of the culture medium (data not shown).

Growth of O-2A^{adult} Progenitor Cells in bFGF or PF Is Associated With the Acquisition of an O4$^-$Vimentin$^+$ Antigenic Phenotype

After 1 d of growth in DME-BS supplemented with bFGF or PF, the vast majority of the O-2A^{adult} progenitor cells

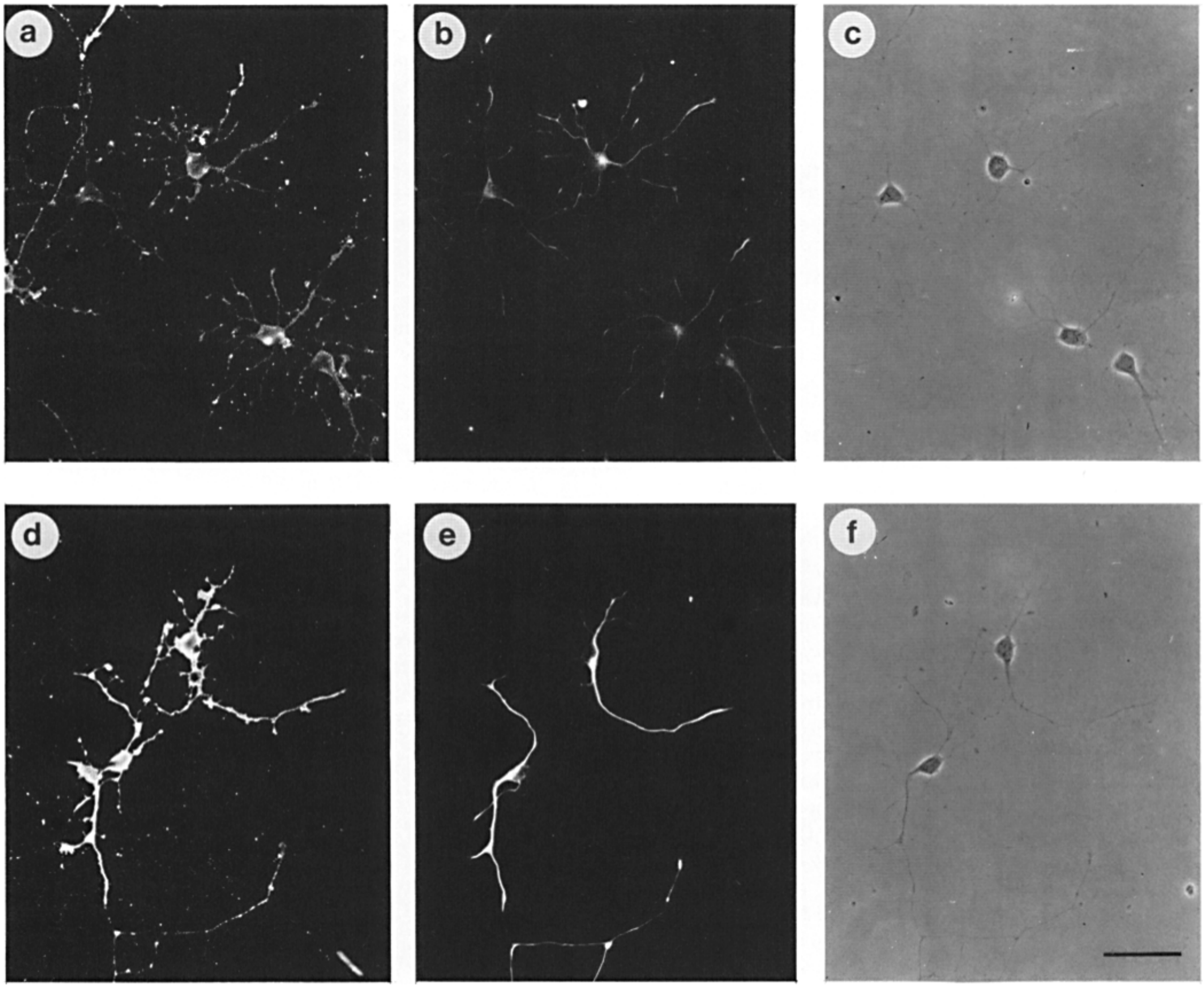

Figure 5. O-2A^{adult} progenitor cells exposed to bFGF are multipolar, vimentin$^+$ cells, while many O-2A^{adult} progenitor cells grown in PF have a bipolar morphology and contain vimentin. Cells derived from the optic nerves of adult rats were maintained for 5 d in either bFGF alone (*a–c*) or PF (*d–f*), immunolabeled with A2B5 (*a* and *d*), anti-GalC (not shown), and antivimentin antibodies (*b* and *e*), followed by fluorochrome-conjugated second antibodies. The photographs illustrate that many O-2A^{adult} progenitor cells cultured in bFGF had a multipolar morphology and acquired vimentin intermediate filaments, and that many O-2A^{adult} progenitor cells maintained in PF were bipolar and vimentin$^+$. Note that because of the limited migratory capacities of O-2A^{adult} progenitor cells exposed to bFGF, colonies generated by O-2A^{adult} progenitor cells were much more compact than those in PF. (*a* and *d*) Rhodamine optics; (*b* and *e*) fluorescein optics; (*c* and *f*) phase-contrast optics. Bar, 50 μm.

were labeled with both A2B5 and O4 mAbs and were devoid of vimentin intermediate filaments (Table II), as shown previously (Wolswijk and Noble, 1989). In contrast, virtually all O-2A^{adult} progenitor cells exposed to either bFGF or PF for several days acquired vimentin filaments in vitro (Table II and Fig. 5). For example, >90% of the O-2A^{adult} progenitor cells contained vimentin in both conditions after 5 and 8 d of culture (Table II). Furthermore, the proportion of O-2A^{adult} progenitor cells that were A2B5$^+$O4$^-$ increased substantially in both conditions from <10% on day 1 to >65% on day 5 (Table II). After 8 d of culture in PF, 69.7 $\pm$ 11.0% O-2A^{adult} progenitor cells were still A2B5$^+$O4$^-$. In contrast, only 18.0 $\pm$ 3.7% of the O-2A^{adult} progenitor cells grown in bFGF alone were A2B5$^+$O4$^-$ after 8 d in vitro, while 75.5 $\pm$ 1.1% of these cells were A2B5$^+$O4$^+$ (Table II). In these conditions, a small proportion (<5%) of the O-2A^{adult} progenitor cells grown in bFGF or PF were A2B5$^-$O4$^-$ when cultures were examined on day 5 and day 8 (Table II). As such cells expressed the morphology characteristic of O-2A^{adult} progenitor cells, they were considered to be O-2A^{adult} progenitor cells. Thus, our immunolabeling studies suggest that many O-2A^{adult} progenitor cells cultured in bFGF or PF acquired the O4$^-$vimentin$^+$ antigenic phenotype typical of O-2A^{perinatal} progenitor cells (Raff et al., 1984*b*; Wolswijk et al., 1990; Sommer, I. and M. Noble, unpublished observations) and that this antigenic phenotype was retained more effectively when O-2A^{adult} progenitor cells were grown in PF than when such cells were cultured in bFGF.

Discussion

We have found that growth of adult optic nerve cells in the presence of bFGF or in the presence of PF was associated with more rapid increases in the number of O-2A lineage cells than when such cells were exposed to PDGF-AA. The O-2A lineage population expanded most rapidly when adult optic nerve cells were cultured in PF. In addition, O-2A^{adult} progenitor cells exposed to either bFGF or PF expressed a distinct pattern of cellular behavior which was unlike that of O-2A^{adult} progenitor cells grown in PDGF-AA. In particular, PF elicited the expression of O-2A^{perinatal} progenitor-like properties in many O-2A^{adult} progenitor cells. In addition, bFGF and PF almost completely prevented the differentiation of O-2A^{adult} progenitor cells into oligodendrocytes.

O-2A^{adult} Progenitor Cells Express Phenotypic Plasticity In Vitro

We have shown previously that O-2A^{adult} progenitor cells exposed to PDGF-AA had a unipolar morphology expressed an O4^{+}vimentin^{-} antigenic phenotype, and divided and migrated slowly (Wolswijk et al., 1991*b*). O-2A^{adult} progenitor cells expressed the same range of properties as cells cultured in the presence of purified cortical astrocytes (Wolswijk and Noble, 1989; Wolswijk et al., 1990), which secrete PDGF in vitro (Noble et al., 1988; Raff et al., 1988; Richardson et al., 1988). However, it appears that the phenotype we have described previously for O-2A^{adult} progenitor cells only applies to cells grown in the presence of PDGF or cortical astrocytes.

In our present study, we have shown that many O-2A^{adult} progenitor cells in cultures of adult optic nerve exposed to bFGF alone were prevented from differentiating into oligodendrocytes, had a multipolar morphology, and divided relatively rapidly, but showed little motility. Many O-2A^{adult} progenitor cells treated with bFGF alone gained vimentin intermediate filaments and became transiently O4^{-}.

Exposure of adult optic nerve cultures to PF elicited the expression in O-2A^{adult} progenitor cells of many of the characteristics we observed previously for O-2A^{perinatal} progenitor cells cultured in PDGF or Astro-CM, such as bipolar morphology, short cell cycle time, high rate of migration, and O4^{-}vimentin^{+} antigenic phenotype. This observation is of particular interest in the light of our recent time-lapse cinemicroscopy studies which have suggested that O-2A^{adult} progenitor cells are derived directly from a subpopulation of O-2A^{perinatal} progenitor cells in cultures of developing optic nerve exposed to Astro-CM (Wren et al., 1992). Thus, our present results suggest that the molecular mechanisms responsible for the expression of the O-2A^{perinatal} progenitor phenotype are not irreversibly inactivated with the generation of O-2A^{adult} progenitor cells.

The observation that O-2A^{adult} progenitor cells bound ^{125}I-bFGF and anti-FGF receptor antibodies suggests that bFGF acts directly on these cells and not indirectly through other non-O-2A lineage cells in the cultures. However, it can not be excluded that growth factors secreted by non-O-2A lineage cells in the cultures cooperated with bFGF or PF to induce the observed change in O-2A^{adult} progenitor phenotype when cultures were exposed to bFGF or PF.

The Population of O-2A^{adult} Progenitor Cells Is Heterogeneous in Its Response to bFGF and PF

It is important to note that O-2A^{adult} progenitor cells were heterogeneous in their response to either bFGF or PF. For example, although the majority of O-2A^{adult} progenitor cells cultured in bFGF were multipolar, a small number of such cells were bipolar. Interestingly, these O-2A^{adult} progenitor cells all belonged to one family and had short cell cycle times and high rates of migration, i.e., had characteristics of O-2A^{perinatal} progenitor cells. Thus, these few O-2A^{adult} progenitor cells were able to express O-2A^{perinatal} progenitor-like properties in cultures exposed to bFGF alone. In addition, some O-2A^{adult} progenitor cells did not divide during the filming period or divided only once when grown in the presence of bFGF or PF. This suggests that some O-2A^{adult} progenitor cells had long cell cycles in such conditions. Similar observations were made in cultures of adult optic nerve cells growing on monolayers of purified cortical astrocytes, where we found that some oligodendrocyte-free O-2A^{adult} progenitor colonies contained $<$16 cells even after 25 d of culture (Wren et al., 1992). Whether such O-2A^{adult} progenitor cells are part of a small population of relatively quiescent cells which only divide occasionally in the presence of mitogen(s) needs to be analyzed further.

The In Vitro Response of O-2A^{adult} Progenitor Cells to PF may be of Importance in the Generation of New Oligodendrocytes in Vivo Following Oligodendrocyte Cell Death

We have suggested previously (Wolswijk and Noble, 1989; Noble et al., 1991) that the pattern of cellular behavior expressed by O-2A^{adult} progenitor cells grown in the presence of cortical astrocytes or PDGF may be appropriate for the generation of the small numbers of new oligodendrocytes that may be needed as part of the slow turnover of glial cells in the adult CNS (McCarthy and Leblond, 1988). However, such a pattern of behavior may not allow the generation of the large numbers of oligodendrocytes that would be needed to repair demyelinated lesions in the adult brain. The simultaneous exposure of O-2A^{adult} progenitor cells to PF would allow rapid increases in the number of O-2A^{adult} progenitor cells (as observed in lesions of mice recovering from virally-induced demyelination [Godfraind et al., 1989]) through an increase in the rate of division of these cells and through active migration of O-2A^{adult} progenitor cells into the lesion site. Subsequent differentiation of these cells into oligodendrocytes, followed by re-ensheathment of the denuded axons, could then restore proper impulse conduction.

Is injury to the mature CNS associated with release of PDGF and FGF into the lesion site? Several studies have suggested that both PDGF and FGF and their mRNAs are upregulated transiently in response to mechanical injury to the adult brain (Finklestein et al., 1988; Logan, 1988, 1990; Nieto-Sampedro et al., 1988; Lotan and Schwartz, 1992). It has been suggested that the increased levels of FGF in lesion sites may be the result of release of FGF from damaged neurones, which express acidic FGF and bFGF and their mRNAs (Pettmann et al., 1986; Janet et al., 1987; Finklestein et al., 1988; Wanaka et al., 1990; Wilcox and Unnerstall, 1991), and due to active synthesis by reactive astrocytes (Finklestein et al., 1988) and invading macrophages (Baird et al., 1986). Furthermore, as recent studies have provided evidence that neurones express PDGF, as well as PDGF transcripts (Yeh et al., 1991; Sasahara et al., 1991), damaged neurones also could be a source of PDGF. A further source of PDGF could be endothelial cells (Hermansson et al., 1988) and the astrocytes that proliferate in response to injury, since studies by Raff et al. (1983*a*) and Miller et al. (1986) have suggested that glial scars may be formed by cortical astrocyte-like cells, the cells that secrete PDGF in vitro. Even though FGF and PDGF may be available to O-2A*adult* progenitor cells in mechanically induced lesions, it remains to be determined whether these factors are also present in lesions induced by viruses, chemicals, or by immunological means, as these are the experimental lesions that have been studied most extensively with respect to myelin repair (Ludwin, 1981). Furthermore, as there is evidence that limited remyelination occurs in the brains of patients suffering from the human demyelinating disease multiple sclerosis (Prineas and Connell, 1979; Raine et al., 1981; Prineas et al., 1989), it will be of interest to investigate whether FGF and PDGF are expressed in regions of demyelinating damage in humans.

The generation of large numbers of O-2A lineage cells as a result of exposure of O-2A*adult* progenitor cells to both PDGF and bFGF in vivo would be clearly inappropriate if continued after sufficient numbers of new cells have been generated. Thus, mechanisms must exist which limit the proliferative response. As the levels of FGF appear to be increased only transiently after CNS damage (Finklestein et al., 1988; Logan, 1988; Nieto-Sampedro et al., 1988), the reduction in local concentrations of FGF and PDGF could be associated with a reduction in the rate of proliferation of O-2A*adult* progenitor cells and their differentiation into oligodendrocytes. In addition, since the increase in the number of O-2A lineage cells in adult optic nerve cultures exposed to PF was less dramatic between day 5 and day 8 of in vitro growth as compared to the increase between day 1 and day 5, it may be that O-2A*adult* progenitor cells are limited intrinsically in their ability to generate large numbers of cells.

In addition to the generation of new oligodendrocytes by O-2A*adult* progenitor cells in response to demyelination, proliferation of mature oligodendrocytes may be important in the recovery process. Uptake of radiolabeled thymidine by mature oligodendrocytes has been observed in vivo after lysolecithin-induced demyelination (Aranella and Herndon, 1984) and after trauma to the adult brain (Ludwin, 1984). In addition, Ludwin and Bakker (1988) found that even differentiated oligodendrocytes attached to myelin incorporated radiolabeled thymidine after wounding of the cortex of adult mice. However, only a comparatively small proportion of mature oligodendrocytes were radiolabeled in these studies. Furthermore, in vitro studies have suggested that oligodendrocytes are able to divide in response to bFGF (Saneto and De Vellis, 1985; Eccleston and Silberberg, 1985; Bogler et al., 1990), when grown on an endothelial cell-derived extracellular matrix (Ovadia et al., 1984) or on dorsal root ganglion neurones (Wood and Bunge, 1986, 1991).

Although PDGF and FGF may play key roles in post-injury responses of the adult brain, other factors may also be important in these processes. The identification of such factors and the ability to identify O-2A*adult* progenitor cells unambiguously in situ would greatly enhance our knowledge of the process of remyelination. This knowledge may prove useful in attempts to enhance myelin repair in diseases in which remyelination is limited, such as in patients with multiple sclerosis.

We wish to thank Peter Riddle and Chris Gilbert for help with the time-lapse cinemicroscopy studies and Larry Coussens and C. George Nascimento (Chiron Corporation) for supplying us generously with recombinant human basic FGF and recombinant human PDGF-AA, respectively. Our colleagues at the Ludwig Institute are thanked for many helpful discussions. Paris Ataliotis, Karen Bevan, Kishore Bakhoo, Marie-José Blouin, Andrew Groves, Damian Wren, and the reviewers are thanked for their criticisms of the manuscript.

This work was supported by a generous grant from the Multiple Sclerosis Society of Great Britain.

Received for publication 11 September 1991 and in revised form 22 April 1992.

References

Allen, R. E., and L. L. Rankin. 1990. Regulation of satellite cells during skeletal muscle growth and development. *Proc. Soc. Exp. Biol. Med.* 194:81–86.

Aranella, L. S., and R. M. Herndon. 1984. Mature oligodendrocyte division following experimental demyelination in adult animals. *Arch. Neurol.* 41: 1162–1165.

Baird, A., F. Esch, P. Mormede, N. Ueno, N. Ling, P. Bohlen, S. Y. Ying, W. D. Wehrenberg, and R. Guillemin. 1986. Molecular characterisation of fibroblast growth factor: distribution and biological activities in various tissues. *Rec. Prog. Horm. Res.* 41:142–205.

Bignami, A., L. F. Eng, D. Dahl, and C. T. Uyeda. 1972. Localization of glial fibrillary acid protein in astrocytes by immunofluorescence. *Brain Res.* 43:429–443.

Bogler, O., D. Wren, S. C. Barnett, H. Land, and M. Noble. 1990. Cooperation between two growth factors promotes extended self-renewal and inhibits differentiation of oligodendrocyte-type-2 astrocyte (O-2A) progenitor cells. *Proc. Natl. Acad. Sci. USA.* 87:6368–6372.

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

Eccleston, P. A., and D. H. Silberberg. 1985. Fibroblast growth factor is a mitogen for oligodendrocytes *in vitro*. *Dev. Brain Res.* 21:315–318.

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

ffrench-Constant, C., and M. C. Raff. 1986. Proliferating bipotential glial progenitor cells in adult rat optic nerve. *Nature (Lond.).* 319:499–502.

Finklestein, S. P., P. J. Apostolides, C. G. Caday, J. Prosser, M. F. Philips, and M. Klagsbrun. 1988. Increased basic fibroblast growth factor (bFGF) immunoreactivity at the site of focal brain wounds. *Brain Res.* 460:253–259.

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

Gratzner, H. G. 1982. Monoclonal antibody 5-bromo and 5-iodeoxyuridine: a new agent for detection of DNA replication. *Science (Wash. DC).* 318: 474–475.

Hermansson, M., M. Nister, C. Betsholz, C.-H. Heldin, B. Westermark, and K. Funa. 1988. Endothelial cell hyperplasia in human glioblastoma: coexpression of mRNA for platelet-derived growth factor (PDGF) B chain and PDGF receptor suggests autocrine growth stimulation. *Proc. Natl. Acad. Sci. USA.* 85:7748–7752.

Janet, T., M. Miehe, B. Pettmann, G. Labourdette, and M. Sensenbrenner. 1987. Ultrastructural localization of fibroblast growth factor in neurons of rat brain. *Neurosci. Lett.* 80:153–157.

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

Logan, A. 1988. Elevation of acidic fibroblast growth factor mRNA in lesioned

rat brain. *Mol. Cell. Endocrinol.* 58:275–278.
Logan, A. 1990. The role of fibroblast growth factors in the central nervous system. *Trends Endocrinol. Metabolism.* 1:149–154.
Lotan, M., and M. Schwarz. 1992. Postinjury changes in platelet derived growth factor-like activity in fish and rat optic nerves: possible implications in regeneration. *J. Neurochem.* 58:1637–1642.
Ludwin, S. K. 1979. An autoradiographic study of cellular proliferation in remyelination of the central nervous system. *Am. J. Pathol.* 95:683–696.
Ludwin, S. K. 1981. Pathology of demyelination and remyelination. *In* Demyelinating Disease: Basic and Clinical Electrophysiology. S. G. Waxman and J. M. Ritchie, editors. Raven Press, New York. 123–168.
Ludwin, S. K. 1984. Proliferation of mature oligodendrocytes after trauma to the central nervous system. *Nature (Lond.).* 308:274–275.
Ludwin, S. K., and D. A. Bakker. 1988. Can oligodendrocytes attached to myelin proliferate? *J. Neurosci.* 8:1239–1244.
McCarthy, G. F., and C. P. Leblond. 1988. Radiographic evidence for slow astrocyte turnover and modest oligodendrocyte production in the corpus callosum of adult mice infused with ^{3}H-thymidine. *J. Comp. Neurol.* 271: 589–603.
McKinnon, R. D., T. Matsui, M. Dubois-Dalcq, and S. A. Aaronson. 1990. FGF modulates the PDGF-driven pathway of oligodendrocyte development. *Neuron.* 5:603–614.
Miller, R. H., E. R. Abney, S. David, C. ffrench-Constant, R. Lindsay, R. Patel, J. Stone, and M. C. Raff. 1986. Is reactive gliosis a property of a distinct subpopulation of astrocytes? *J. Neurosci.* 6:22–29.
Nieto-Sampedro, M., R. Lim, D. J. Hicklin, and C. W. Cotman. 1988. Early release of glia maturation factor and acidic fibroblast growth factor after rat brain injury. *Neurosci. Lett.* 86:361–365.
Noble, M., K. Murray, P. Stroobant, M. D. Waterfield, and P. Riddle. 1988. Platelet-derived growth factor promotes division and motility and inhibits premature differentiation of the oligodendrocyte/type-2 astrocyte progenitor cell. *Nature (Lond.).* 333:560–562.
Noble, M., P. Ataliotis, S. C. Barnett, K. Bevan, O. Bögler, A. Groves, P. Jat, G. Wolswijk, and D. Wren. 1991. Development, regeneration and neoplasia of glial cells in the central nervous system. *Ann. NY Acad. Sci.* 633: 35–47.
Ovadia, H., I. Lubetzki-Korn, T. Brenner, O. Abramsky, R. Fridman, and I. Vlodavsky. 1984. Adult rat oligodendrocytes grown *in vitro* on an extracellular matrix have the ability to proliferate. *Brain Res.* 322:93–100.
Pettmann, B., G. Labourdette, M. Weibel, and M. Sensenbrenner. 1986. The brain fibroblast growth factor (FGF) is localized in neurons. *Neurosci. Lett.* 68:175–180.
Prineas, J. W., and F. Connell. 1979. Remyelination in multiple sclerosis. *Ann. Neurol.* 5:22–31.
Prineas, J. W., E. E. Kwon, P. Z. Goldenberg, A. A. Ilyas, R. H. Quarles, J. A. Benjamins, and T. J. Sprinkle. 1989. Multiple sclerosis: oligodendrocyte proliferation and differentiation in fresh lesions. *Lab. Invest.* 61:489–503.
Raff, M. C., R. Mirsky, K. L. Fields, R. P. Lisak, S. H. Dorfman, D. H. Silberberg, N. A. Gregson, S. Leibowitz, and M.C. Kennedy. 1978. Galactocerebroside is a specific cellsurface antigenic marker for oligodendrocytes in culture. *Nature (Lond.).* 274:813–816.
Raff, M. C., E. R. Abney, J. Cohen, R. Lindsay, and M. Noble. 1983*a*. Two types of astrocytes in cultures of developing rat white matter: differences in morphology, surface gangliosides, and growth characteristics. *J. Neurosci.* 3:1289–1300.
Raff, M. C., R. H. Miller, and M. Noble. 1983*b*. A glial progenitor cell that develops *in vitro* into an astrocyte or an oligodendrocyte depending on the culture medium. *Nature (Lond.).* 303:390–396.
Raff, M. C., E. R. Abney, and R. H. Miller. 1984*a*. Two glial lineages diverge prenatally in rat optic nerve. *Dev. Biol.* 106:53–60.
Raff, M. C., B. P. Williams, and R. H. Miller. 1984*b*. The *in vitro* differentiation of a bipotential glial progenitor cell. *EMBO (Eur. Mol. Biol. Organ.) J.* 3:1857–1864.
Raff, M. C., L. E. Lillien, W. D. Richardson, J. F. Burne, and M. D. Noble. 1988. Platelet-derived growth factor from astrocytes drives the clock that times oligodendrocyte development in culture. *Nature (Lond.).* 333:562–565.
Raine, C. S., L. Scheinberg, and J. M. Waltz. 1981. Multiple sclerosis: oligodendrocyte survival and proliferation in an active established lesion. *Lab. Invest.* 45:534–546.
Ranscht, B., P. A. Clapshaw, J. Price, M. Noble, and W. Seifert. 1982. Development of oligodendrocytes and Schwann cells studied with a monoclonal antibody against galactocerebroside. *Proc. Natl. Acad. Sci. USA.* 79:2709–2713.
Richardson, W. D., N. Pringle, M. J. Mosley, B. Westermark, and M. Dubois-Dalcq. 1988. A role for platelet-derived growth factor in normal gliogenesis in the central nervous system. *Cell.* 53:309–319.
Riddle, P. N. 1979. Time-Lapse Cinemicroscopy. Biological Techniques Series. J. E. Treherne and P. H. Rubery, editors. Academic Press, London.
Riddle, P. N. 1990. Time-lapse cinemicroscopy. *In* Methods in Molecular Biology. Vol. 5. Animal Cell Culture. J. W. Pollard and J. M. Walker, editors. The Humana Press, Inc., Clifton, NJ. 415–446.
Saneto, R. P., and J. de Vellis. 1985. Characterization of cultured rat oligodendrocytes proliferating in a serum-free, chemically defined medium. *Proc. Natl. Acad. Sci. USA.* 82:3509–3513.
Sasahara, M., J. W. U. Fries, E. W. Raines, A. M. Gown, L. E. Westrum, M. P. Frosch, D. T. Bonthron, R. Ross, and T. Collins. 1991. PDGF B-chain in neurons of the central nervous system, posterior pituitary, and in a transgenic model. *Cell.* 64:217–227.
Small, R. K., P. Riddle, and M. Noble. 1987. Evidence for migration of oligodendrocyte-type 2 astrocyte progenitor cells into the developing rat optic nerve. *Nature (Lond.).* 328:155–157.
Sommer, I., and M. Schachner. 1981. Monoclonal antibodies (O1 to O4) to oligodendrocyte cell surfaces: an immunocytological study in the central nervous system. *Dev. Biol.* 83:311–327.
Temple, S., and M. C. Raff. 1986. Clonal analysis of oligodendrocyte development in culture: evidence for a developmental clock that counts cell divisions. *Cell.* 44:773–779.
Wanaka, A., E. M. Johnson, Jr., and J. Milbrandt. 1990. Localization of FGF receptor mRNA in the adult rat central nervous system by in situ hybridization. *Neuron.* 5:267–281.
Wilcox, B. J., and J.R. Unnerstall. 1991. Expression of acidic fibroblast growth factor mRNA in the developing and adult rat brain. *Neuron.* 6:397–409.
Wolswijk, G., and M. Noble. 1989. Identification of an adult-specific glial progenitor cell. *Development (Camb.).* 105:387–400.
Wolswijk, G., P. N. Riddle, and M. Noble. 1990. Coexistence of perinatal and adult forms of a glial progenitor cell during development of the rat optic nerve. *Development (Camb.).* 109:691–698.
Wolswijk, G., P. M. G. Munro, P. N. Riddle, and M. Noble. 1991*a*. Origin, growth factor responses and ultrastructural characteristics of an adult-specific glial progenitor cell. *Ann. NY Acad. Sci.* 633:502–504.
Wolswijk, G., P. N. Riddle, and M. Noble. 1991*b*. Platelet-derived growth factor is mitogenic for O-2A^{adult} progenitor cells. *Glia.* 4:495–503.
Wood, P. M., and R. P. Bunge. 1986. Evidence that axons are mitogenic for oligodendrocytes isolated from adult animals. *Nature.* 320:756–758.
Wood, P. M., and R. P. Bunge. 1991. The origin of the remyelinating cells in the adult central nervous system: the role of the mature oligodendrocyte. *Glia.* 4:225–232.
Wren, D., and M. Noble. 1989. Oligodendrocytes and oligodendrocyte-type-2 astrocyte progenitor cells of adult rats are specifically susceptible to the lytic effects of complement in the absence of antibody. *Proc. Natl. Acad. Sci. USA.* 86:9025–9029.
Wren, D., G. Wolswijk, and M. Noble. 1992. In vitro analysis of the origin and the maintenance of O-2A^{adult} progenitor cells. *J. Cell Biol.* 116:167–176.
Yeh, H.-J., K. G. Ruit, Y.-X. Wang, W. C. Parks, W. D. Snider, and T. F. Deuel. 1991. PDGF A-chain gene is expressed by mammalian neurons during development and in maturity. *Cell.* 64:209–216.