

Survival and differentiation of adult neuronal progenitor cells transplanted to the adult brain

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ABSTRACT The dentate gyrus of the hippocampus is one of the few areas of the adult brain that undergoes neurogenesis. In the present study, cells capable of proliferation and neurogenesis were isolated and cultured from the adult rat hippocampus. In defined medium containing basic fibroblast growth factor (FGF-2), cells can survive, proliferate, and express neuronal and glial markers. Cells have been maintained in culture for 1 year through multiple passages. These cultured adult cells were labeled *in vitro* with bromodeoxyuridine and adenovirus expressing β -galactosidase and were transplanted to the adult rat hippocampus. Surviving cells were evident through 3 months postimplantation with no evidence of tumor formation. Within 2 months postgrafting, labeled cells were found in the dentate gyrus, where they differentiated into neurons only in the intact region of the granule cell layer. Our results indicate that FGF-2 responsive progenitors can be isolated from the adult hippocampus and that these cells retain the capacity to generate mature neurons when grafted into the adult rat brain.

Most neurons in the adult central nervous system are terminally differentiated, exist through the life of the organism, and are not replaced when they die. However, evidence exists that small populations of neurons continue to be born in the adult ventricular zone (1–3), olfactory system (4–6), and hippocampus (7–10). In the adult hippocampus, newly born neurons originate from putative stem cells that exist in the subgranular zone of the dentate gyrus. Progeny of these putative stem cells differentiate into neurons in the granule cell layer within a month of the cells' birth, and this late neurogenesis continues throughout the adult life of the rodent (11–15). Paralleling these *in vivo* findings, *in vitro* studies have shown that the precursor cells isolated from adult mouse ventricular zone and forebrain have the capacity for *in vitro* neurogenesis when stimulated with epidermal growth factor (16) or basic fibroblast growth factor (FGF-2) (17), respectively. FGF-2 is a potent mitogen for fetal cells isolated from different areas of the brain (18–22). This proliferative property of FGF-2 has allowed the isolation and culturing of fetal hippocampal cells for over a year through multiple passages (21), some of which expressed neuronal phenotypes *in vitro*. These studies have raised the question whether the FGF-2 responsive cells can be isolated and cultured from the adult brain, particularly in the hippocampus, where neurogenesis occurs even in adulthood. In the present study we report that a population of FGF-2 responsive progenitor cells can be isolated and cultured from the adult rat hippocampus. Cells in culture express precursor, glial, and neuronal cell markers. Upon implantation into the adult rat hippocampus, cells migrate and differentiate into mature neurons or glia, depending on the terminal site of migration. Such a progenitor population will be valuable for

exploring cues that influence the proliferation and differentiation of neuronal progenitor cells and for neuronal replacement strategies for the damaged brain.

MATERIALS AND METHODS

Cell Culture Conditions. Adult (>3 months) female Fischer 344 rats were anesthetized with a mixture of ketamine (44 mg/kg), acepromazine (0.75 mg/kg), and xylozine (4 mg/kg) in 0.9% NaCl and sacrificed in accordance with procedures approved by the University of California at San Diego and by the National Institutes of Health. Hippocampi (from one rat) were dissected bilaterally and the ependymal lining, subventricular zone, and choroid plexus were removed. Tissue was washed in cold sterile Dulbecco's phosphate-buffered saline (PBS-D) and cells were dissociated as described (21). Briefly, after enzyme treatment (0.01% papain, 0.1% neutral protease, and 0.01% DNase I), cells were centrifuged and resuspended in Dulbecco's modified Eagle medium (DMEM)/F12 (1:1) high-glucose medium (Irvine Scientific) containing 2.5 mM L-glutamine and 10% fetal bovine serum (Sigma), plated at 1×10^6 viable cells onto uncoated plastic tissue culture flasks (75 cm², Costar), and maintained at 37°C with 5% CO₂. The next day, the medium was replaced with serum-free medium: DMEM/F12 containing N2 supplement (GIBCO) and 20 ng of FGF-2 per ml (recombinant human FGF-2; gift from A. Baird, The Whittier Institute). Cultures were incubated for 2–4 weeks with half of the medium changed every 3–4 days with fresh medium containing FGF-2 until they were confluent enough to be passaged. Complete medium change was done for actively growing cultures every 3–4 days. The cells were maintained and passaged as described before (21, 22). Cells have been cultured for >1.5 years through 33 passages.

Grafting Procedures. Cultured cells were labeled with BrdUrd or an adenoviral vector prior to transplantation. For BrdUrd labeling, cells were incubated for 4 days with fresh N2 medium containing 20 ng of FGF-2 per ml and 5 μ M BrdUrd (Boehringer Mannheim). For viral labeling, E1a-deleted type 5 adenoviral vectors carrying a cytomegalovirus immediate early promoter-driven *LacZ* gene (kindly provided by I. Verma, The Salk Institute) were prepared by infecting naive 293 cells with viral stocks at a multiplicity of infection of ≈ 10 . Two days later, adenovirus-containing supernatant was harvested and cellular debris was removed by centrifugation at $5000 \times g$ for 5 min, and the cell-free supernatants were used to infect the adult-derived progenitor cultures. Viral titers

Abbreviations: FGF-2, basic fibroblast growth factor; β -Gal, β -galactosidase; NSE, neuron-specific enolase; GFAP, glial fibrillary acidic protein; MAP, microtubule-associated protein; GAD, glutamic acid decarboxylase; FITC, fluorescein isothiocyanate; GalC, galactocerebroside C; NF, neurofilament; DAPI, 4',6-diamidino-2-phenylindole; BrdUrd, bromodeoxyuridine.

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ranged between 5×10^7 and 10^8 . Cells were transduced by replacing the medium with fresh medium containing 10% virus-containing supernatant from 293 cells. The next day, the cells were harvested with trypsin, extensively washed with PBS-D, and suspended at a density of 50,000 per μl in Dulbecco's PBS and 20 ng of FGF-2 per ml. A 5- μl Hamilton syringe with a 26-gauge beveled needle was used to slowly inject 1 μl of cells into the dorsal hippocampus (AP, -3.6 ; ML, ± 2.0 ; DV, -4.0) of anesthetized adult female Fischer 344 rats. The syringe was raised slightly and an additional 0.5 μl of cells was injected. The syringe was raised 1 mm and left in place for 2 min to minimize cell diffusion up the needle track. At 1, 4, 8, and 12 weeks postimplantation, three rats for each time point were sacrificed and their brains were prepared for light or electron microscopy. Detection of *LacZ* transgene expression was accomplished using an anti- β -galactosidase (β -Gal) antibody.

Immunodetection. Cells were cultured in polyornithine/laminin-coated slide chambers (Lab-Tek, Nunc) and were fixed for 15 min in phosphate-buffered 4% paraformaldehyde. Grafted animals were deeply anesthetized and perfused with phosphate-buffered 4% paraformaldehyde. Brains were removed and prepared as free-floating coronal freezing microtome sections (40 μm).

BrdUrd pretreatment. Sections processed for BrdUrd immunodetection were pretreated for 2 hr in 50% formamide/2 \times SSC at 65°C followed by a 30-min incubation in 2 M HCl at 37°C.

Immunofluorescence. Cells or sections were incubated with antibody diluted in PBS containing 0.3% Triton X-100 and 5% goat or donkey normal serum (PBS/TS). Triton X-100 was omitted for the A2B5 and O4 antibodies. After rinses in PBS/TS, the primary antibody was detected with fluorescein isothiocyanate (FITC)-, Texas red-, Rhodamine-, or Cy5-labeled secondary antibodies (Jackson Immunochemicals, West Grove, PA, 1:250 in PBS/TS) or biotinylated secondary antibodies (1:80, Jackson Immunochemicals) and streptavidin/Texas red (1:500, Jackson Immunochemicals). A mouse anti-BrdUrd antibody directly coupled to FITC (Boehringer Mannheim) was used when combined with another monoclonal antibody for double labeling. For nuclear counterstain, cells were treated with 10 ng of 4',6-diamidino-2-phenylindole (DAPI) per ml. Labeled cells and sections were visualized using confocal scanning laser microscopy (Bio-Rad MRC600) and color images were generated using Adobe Photoshop (Adobe Systems, Mountain View, CA).

Immunoperoxidase. Cells were processed according to published procedures (22) and sections were stained using the avidin/biotin-peroxidase system (Vectastain Elite, Vector Laboratories) and diaminobenzidine.

Antibodies. Mouse (mo), rabbit (rb), or guinea pig (gp) primary antibodies and final dilutions were as follows: mo α -A2B5, 1:1000 (Boehringer Mannheim); mo α -BrdUrd, 1:400 (Boehringer Mannheim); mo α -BrdUrd-FITC, 1:20 (Boehringer Mannheim); mo α -calbindin, 1:1000 (Sigma); rb α -calretinin, 1:2000 (Chemicon); mo α -ED1, 1:1000 (Chemicon); rb α -fibronectin, 1:100 (Telios Pharmaceuticals, San Diego); rb α -glutamic acid decarboxylase (GAD), 1:50 (Chemicon); mo α - β -Gal, 1:5000 (Promega); rb α -galactocerebroside C (GalC), 1:250 (Advanced Immunochemical, Long Beach, CA); rabbit α -glial fibrillary acidic protein (GFAP), 1:500 (Chemicon); gp α -GFAP, 1:500 (Advanced Immunochemical); mo α -microtubule-associated protein 2 (MAP2), 1:5000 (Sigma); mo α -MAP5, 1:5000 (Chemicon); mo α -myelin basic protein (MBP), 1:250 (Boehringer Mannheim); rb α -nestin, 1:2000 (kindly provided by R. G. D. McKay); rb α -neurofilament 68 (NF68), 1:250 (Chemicon); rb α -NF150, 1:2000 (Chemicon); rb α -NF200, 1:1000 (Chemicon); mo α -NeuN (A60), 1:10 (kindly provided by R. J. Mullen); rb α -neuron-specific eno-

lase (NSE), 1:5000 (Chemicon); mo α -O4, 1:2 (kindly provided by O. Boegler); mo α -Tau, 1:250 (Boehringer Mannheim).

Electron Microscopy. Cultures were fixed in 2% glutaraldehyde in 100 mM phosphate buffer and incubated in 1% aqueous osmium tetroxide. Grafted animals were transcardially perfused with 4% paraformaldehyde/0.1% glutaraldehyde. The brains were removed and 60- μm -thick coronal vibratome sections were produced. Sections were pretreated and processed for BrdUrd immunoperoxidase as described above. Sufficient ultrastructural detail was preserved despite the harshness of the BrdUrd pretreatment procedure. Cultured cells and vibratome sections were processed for electron microscopy according to published procedures (21). Thin sections (70 nm) were examined in a Phillips CM-10 transmission electron microscope.

Quantitation. *In vitro*. Logarithmic-phase hippocampal cultures were fixed and processed for immunofluorescence as described above. Cell nuclei were counterstained with DAPI to identify individual cells. For each marker, 500–1000 cells sampled systematically from standardized fields at $\times 40$ magnification were examined and scored for immunoreactivity. For comparison between cultures, sister cultures were stained for different markers at the same passage number. The experiments were repeated four times at passages 11, 14, 15, and 21. In all cases, the different cultures yielded roughly the same percentage of cells in each category with a variance of about 5–10%.

In vivo. Every sixth section through the dorsal hippocampus was stained immunohistochemically for BrdUrd and the number of BrdUrd-positive cells was quantified using modified stereological procedures. Animals evaluated at 8–12 weeks postgrafting were pooled for these data. The hippocampal profile in each section was sampled in a systematic, uniform random manner applying the optical disector procedure (23, 24). The results of this procedure provided a numerical density (cells per unit volume). The volume of the hippocampus sampled extended 1.2 mm in the rostral–caudal axis with the midpoint of the sample centered at the graft injection site and was calculated using the Cavalieri procedure (24). The data from the optical disector and the Cavalieri procedure were combined to yield an estimate of the number of BrdUrd-positive cells in this series of sections. Although these data are not a true stereological estimate of cell number within the entire hippocampus, they provide a standardized estimate for the purposes of assessing cell survival following engraftment. To determine the frequency at which BrdUrd-positive cells demonstrated a neuronal or glial phenotype, sections were triple labeled with immunofluorescence and collected as confocal images. Sections were triple labeled with anti-NeuN, rabbit anti-GFAP, and anti-BrdUrd coupled with FITC as described above. Grafted cells that were immunopositive for BrdUrd were examined at 8 and 12 weeks postgrafting for colocalization with either NeuN to indicate a neuronal phenotype or GFAP to indicate an astrocytic phenotype. Triple labeling of sections for GFAP, NeuN, and BrdUrd showed that NeuN and GFAP were not expressed in the same BrdUrd-positive cell. The number of cells for each phenotype was pooled among the grafts and the results were expressed as a percentage of BrdUrd-positive cells examined.

RESULTS

During the first 2 months following plating into defined medium supplemented with FGF-2, a mixed population of cells was present. Many of the cells proliferate slowly, as indexed by BrdUrd labeling and increasing numbers *in vitro* (data not shown). With successive passages, a more restricted population of cells emerged with a doubling time of about 2 days and a more homogeneous morphology. These cells are predominantly phase bright and rounded with thin processes,

with a minority of the population displaying a flat appearance. Withdrawal of FGF-2 from the cultures results in complete cell death within 1 week. This result indicates that the adult-derived cells are dependent upon FGF-2 for survival.

Immunocytochemical analyses of cells performed in early and late passaged cultures showed identical patterns of staining. The majority of the hippocampal-derived cells express proteins found in undifferentiated progenitor cells, such as nestin, A2B5, and O4 (Fig. 1A; Table 1). Although glial progenitors appear to be present in the adult cultures, as indicated by labeling for A2B5 and O4, <2% of these cells progress to a more differentiated glial phenotype (Fig. 1B; Table 1: GalC, MBP, GFAP). Rather, a majority of the cells (85–92%) express GAD, NSE, MAP2, and MAP5 (Fig. 1D; Table 1), markers typically considered to be indicative of a neuronal phenotype. From these results, we inferred that the overlap of glial (O4) and neuronal markers (NSE, MAP2, GAD) in the same cells may indicate that the adult-derived cells are a multipotent progenitor population that has not yet committed to a specific neural fate. However, it is also possible that coexpression of glial and neuronal markers may reflect an unusual property of the cultured population. Single cell cloning of the population will help to distinguish between these possibilities by clarifying if multipotent progenitors are present within the cultures.

The cultured cells do not express NeuN, a nuclear marker reported to be present in terminally differentiated neurons (25), or tau. However, there is evidence to suggest that some of the cells progress toward a mature neuronal phenotype since a small percentage expresses calretinin or calbindin (Fig. 1C; Table 1), proteins that are present within hippocampal neurons. In ultrastructural analyses, some cells within the cultures display large round euchromatic nuclei with a distinct nucleolus (Fig. 2A) and somatic extensions that branch and contain well-organized arrays of microtubules (Fig. 2B). Further, some processes with microtubules have been found to be ensheathed

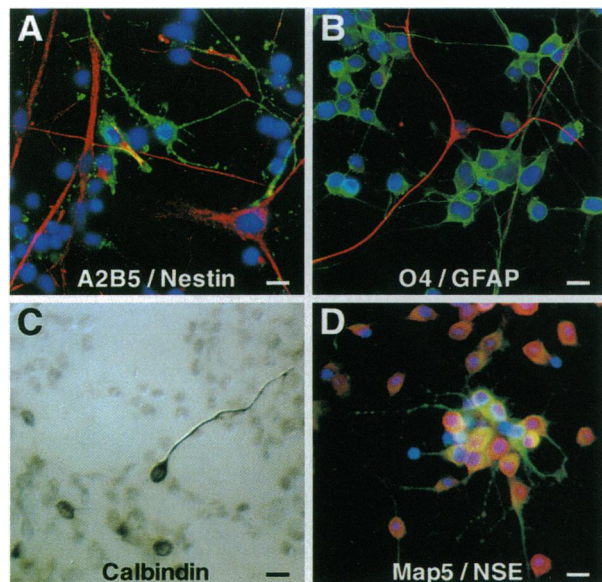


FIG. 1. Epifluorescent confocal micrographs of hippocampal cells maintained in culture for 14 passages. A variety of process-bearing cell morphologies was observed and many of these expressed markers for glial and neuronal lineages. (A) The A2B5 antigen (green) and nestin (red) are present in separate populations; however, some cells express both markers (yellow). (B) The O4 antigen (green) and GFAP (red) are found in mutually exclusive populations of cells. (C) Immunoperoxidase detection of calbindin. (D) MAP5 (green) is found in a subpopulation of NSE-positive (red) cells. Cells are yellow where both markers overlap. Nuclei are stained with DAPI (blue). (Bars = 10 μ m.)

Table 1. Expression of different antigenic markers by adult-derived hippocampal cells after 7 months in culture

Lineage	Marker	% positive
Immature	Nestin*	80
	A2B5*	31
Glial	O4	87
	GalC	0.3
	MBP	<0.01
	GFAP	2
Neuronal	NSE*	92
	MAP2*	85
	MAP5	6
	NF68	ND
	NF150*	41
	NF200†	<1
	NeuN	ND
	Tau	ND
	GAD	88
	Calbindin	5
	Calretinin	13
Other	ED1	ND
	Fibronectin	ND

ND, not detectable.

*Markers expressed in glial and neuronal cells *in vitro* as described in the literature.

†Although all other markers were equivalent between immunofluorescence and immunoperoxidase staining, NF200 showed a greater percentage of positive cells with immunoperoxidase staining.

by several layers of myelin, suggesting the maturing nature of some oligodendroglia and neurons in the culture (Fig. 2C). Although present, cells with the mature, differentiated ultrastructural neuronal morphology described above are rare.

The adult-derived population was transplanted into the hippocampus of adult Fischer 344 rats to assess whether the cultured cells differentiate in response to endogenous local cues. Cells were marked with BrdUrd and/or an adenoviral vector carrying *LacZ* prior to transplantation to facilitate *in vivo* identification. Control experiments in which *LacZ*- or BrdUrd-labeled cells were either sonicated or freeze-thawed prior to grafting indicated that dead or dying cells do not transfer the markers to endogenous host cells. Thus, labeling observed *in vivo* reflects the culture-derived population. Rats implanted with 75,000 cultured cells were sacrificed 1, 4, 8, and 12 weeks postimplantation and assessed for graft cell survival and differentiation. Quantitative estimates of BrdUrd-labeled cells revealed 26,000–31,000 grafted cells present in the hippocampus 8–12 weeks postimplantation. Immunoreactive labeling for β -Gal showed decreasing numbers of *LacZ*-expressing cells at longer times postgrafting, which may reflect loss of cells or transgene expression following transplantation. Conversely, loss of *LacZ* expression may occur if the cultured cells proliferated *in vivo*, since the adenoviral particle remains episomal and is subject to degradation or random loss following division. The quantitative analyses of BrdUrd-labeled cells may not detect such proliferation due to dilution of the BrdUrd below detection levels, although the lack of any obvious solid mass formation or tissue distortion strongly suggests that extensive proliferation of the cultured cells does not occur postimplantation.

Grafted cells were distributed up to 3 mm from the site of implantation, although most cells remained localized near the injection track. Injections into the hippocampus can result in tissue damage to the dorsal leaf of the dentate gyrus (26), and there was a clear localization of grafted cells in this area of damage (Fig. 3A and B). β -Gal-labeled cells with astroglial and oligodendroglial morphologies were detected as early as 1 week postgrafting. These cells were distributed in the corpus callosum (Fig. 3C), stratum radiatum, stratum moleculare, and

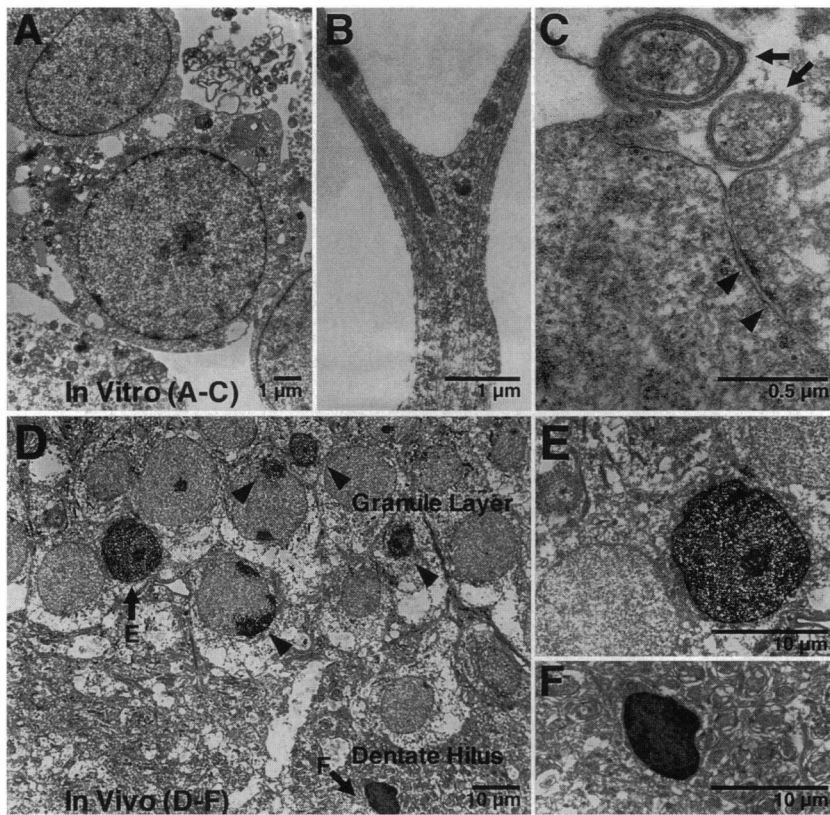


FIG. 2. Ultrastructure of adult progenitor cells *in vitro* (A–C) and following transplantation (D–F). (A) Cells *in vitro* showing progression to differentiation have oval or round euchromatic nuclei. (B) Surrounding these cells are numerous processes, some of which appear dendritic. (C) In addition, processes are occasionally observed wrapped in thin myelin sheaths (arrows), and membrane thickenings (arrowheads) are also observed, suggesting synaptogenesis may be occurring. (D) Grafted cells labeled with BrdUrd (arrowheads) in the subgranular zone and integrated within the dentate granule cell layer. A number of cells with neuronal (E) or glial (F) morphology were found to be immunopositive for BrdUrd (arrowheads), with the cells indicated (arrows) shown at higher magnification (E and F). Typical BrdUrd staining was evident from complete or discrete nuclear labeling.

the dorsal leaf of the dentate gyrus, where damage was evident. Confirmation of the glial phenotype was provided by double

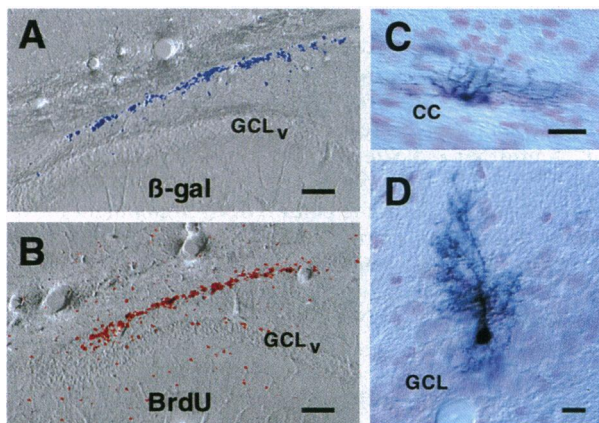


FIG. 3. Localization and morphology of adult hippocampal-derived cells implanted into the hippocampus of adult rats. Cultured cells were marked with the *LacZ* transgene and/or BrdUrd prior to implantation. (A and B) Immunofluorescent signals were collected as confocal laser images, digitally color enhanced, and superimposed upon Nomarski interference contrast images to visualize the anatomical structures. (A) β -Gal immunoreactive cells (blue) aligned within the granule cell layer (GCL) of the hippocampus 12 weeks postimplantation. (B) BrdUrd-labeled nuclei (red) of an adjacent section showed a broad distribution of grafted cells throughout the hippocampus. The majority of labeled cells were in the damaged dorsal leaf of the GCL. Cells with the large, round nuclei characteristic of neurons were primarily observed within the ventral leaf of the GCL (GCLv). (C) β -Gal immunoreactive cell within the corpus callosum (CC) dorsal to the hippocampal injection site 4 weeks postgrafting. This cell displayed a morphology suggestive of an oligodendrocyte. (D) β -Gal immunoreactive cell within an intact region of the GCL 4 weeks postgrafting that showed the elaborate dendritic branching into the molecular layer that is characteristic of endogenous granule neurons. (Bars in A and B = 100 μ m; bars in C and D = 15 μ m.)

labeling for BrdUrd and GFAP (Table 2; Fig. 4 C and D) and ultrastructural analysis of the BrdUrd-positive cells (Fig. 2F). Cells displaying neuronal characteristics were observed only in the granule cell layer of the dentate gyrus (Table 2; Fig. 4 A and B). β -Gal-labeled cells within this region occasionally displayed polarized dendritic processes arborizing into the molecular layer (Fig. 3D). At the ultrastructural level, BrdUrd-positive cells within the intact neuronal regions displayed large round nuclei with distinct nucleoli (Fig. 2 D and E) consistent with a neuronal phenotype and similar to the host granule cells of this region. The finding that much greater numbers of adult-derived hippocampal cells differentiated into mature neurons or glia *in vivo* (Table 2) compared to *in vitro* (Table 1) indicates that there are signals within the adult brain that are crucial for the maturation of the progenitors.

Table 2. Distribution of *in vivo* grafted BrdUrd-positive cell phenotype after 8 weeks

Neuronal lamina	BrdUrd ⁺ cells	Phenotype frequency*		
		NeuN ⁺	GFAP ⁺	Unlabeled
DG damaged	100	3 (3)	47 (47)	50 (50)
DG intact	64	40 (63)	0 (0)	24 (37)
Area CA1	19	0 (0)	10 (53)	9 (47)
Area CA3	36	0 (0)	15 (42)	21 (58)
Corpus callosum	100	0 (0)	5 (5)	95 (95)

Distribution of neuronal (NeuN) and astrocytic (GFAP) phenotypes of BrdUrd-positive cells within neuronal cell layers of the hippocampus [dentate gyrus (DG), area CA1, area CA3] and corpus callosum as determined by triple labeling. Unlabeled cells are BrdUrd-positive cells that are negative for NeuN and GFAP. Regions of the dentate gyrus that were damaged by the grafting procedure (DG damaged) were sampled separately. Because of the high abundance of BrdUrd-positive cells within these regions and in the corpus callosum, an upper limit of 100 sampled cells was imposed.

*Percentage in parentheses.

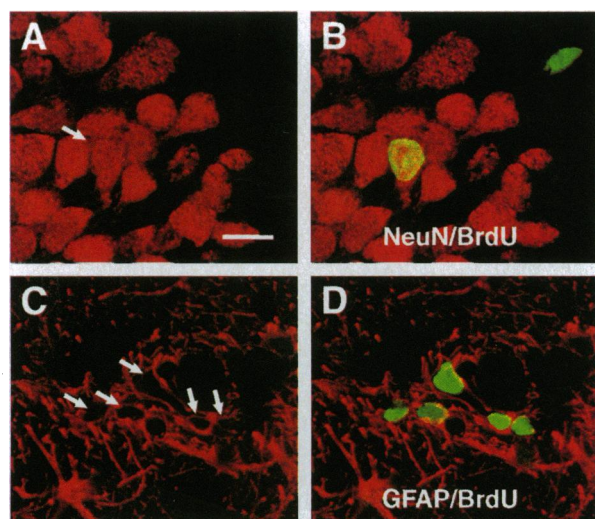


FIG. 4. Neuronal and glial phenotype of grafted cells 12 weeks postimplantation in the adult rat hippocampus. Immunofluorescence labeling for BrdUrd was combined with labeling for NeuN or GFAP and analyzed with a confocal scanning laser microscope. (A and B) NeuN immunoreactive neurons (red) forming the granular layer of the dentate gyrus. Yellow color indicates a grafted cell within the granular layer double-labeled for NeuN (red) and BrdUrd (green and arrow in A). (C and D) BrdUrd immunoreactive grafted cells (green in D and arrows in C) colocalize with rabbit α -GFAP immunoreactivity (red) primarily at the injection site and in the dorsal leaf of the dentate gyrus. (Bar = 10 μ m.)

DISCUSSION

Our results demonstrate that progenitor cells can be isolated from the adult rat hippocampus and serially passaged *in vitro* in defined serum-free medium supplemented with 20 ng of FGF-2 per ml. Further, these adult-derived cells retain the potential for survival and terminal differentiation into mature glia and neurons *in vivo*, as evidenced by the ultrastructural morphology, *LacZ* labeled morphology, and the differentiated markers GFAP and NeuN. The *in vivo* fate of these cells is clearly influenced by exogenous factors. Cells surviving within the granule cell layer of the hippocampus often differentiated into neurons, whereas those cells residing outside of this region often exhibited glial markers and morphology. These findings are consistent with observations that immortalized clonal progenitors derived from the developing brain also exhibit site-specific differentiation *in vivo* (27–29). Since we have not used clonal cultures, whether different cell types are derived from a common precursor *in vivo* will be determined when clonal lines are available for grafting. Since the granule cell layer of the dentate gyrus is one of the sites of neurogenesis in the adult brain (11–15), the present results suggest that the hippocampal adult progenitors and host stem cells respond similarly to endogenous cues that regulate neurogenesis. Whether the cells we have isolated *in vitro* and transplanted back into the brain are derived from the same population of neural stem cells that resides within the adult subgranular zone of the dentate gyrus *in situ* remains to be determined. The markers expressed by cultured progenitor cells (e.g., O4, MAP2, NSE) may be useful for identifying progenitors *in situ*; however, isolation of the cells in a culture environment may dramatically alter the cell phenotype. Since most cells in culture expressed proteins described in the literature as typical precursor (A2B5, nestin), glial (O4), and neuronal (NSE, MAP2, GAD) markers (Table 1), it is inferred that they are coexpressed in the same cell. This may reflect the multipotentiality of the cells and surprisingly immature nature of these presumptive phenotype-specific markers or a unique response

to the culture environment. Unique markers for host stem cells are needed to determine how closely the cultured cells resemble the host progenitor cells and their responses to local cues *in vivo*. If the endogenous stem cell and cultured progenitor cell are the same cells, the culture and grafting of these cells provide a unique model system to determine the signals regulating proliferation and differentiation of neuronal progenitors *in vivo*. These proliferative cells can be genetically manipulated and used in *ex vivo* gene transfer as a source of neurons for transplantation. Further characterization of adult precursor cells should prove useful for developing strategies to directly manipulate endogenous stem cells within the central nervous system.

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