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Alternative Sources of Neurons and Glia From Somatic Stem Cells

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Stem cell populations have been shown to be extremely versatile: they can generate differentiated cells specific to the tissue in which they reside and descendents that are of different germ layer origin. This raises the possibility of obtaining neuronal cells from new biological source of the same adult human subjects. In this study, we found that epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) cooperated to induce the proliferation, self-renewal, and expansion of neural stem cell-like population isolated from several newborn and adult mouse tissues: muscle and hematopoietic tissues. This population, in both primary culture and secondary expanded clones, formed spheres of undifferentiated cells that were induced to differentiate into neurons, astrocytes, and oligodendrocytes. Brain engraftment of the somatic-derived neural stem cells generated neuronal phenotypes, demonstrating the great plasticity of these cells with potential clinical application.

Key words: Neural stem cells; Transdifferentiation; Multipotent precursor; Gene therapy; Neuronal differentiation

INTRODUCTION

Considerable interest currently surrounds a population of multipotential stem cells that could be transplanted to provide a means for the systemic rather than local repair of damaged tissues. Recently, neural progenitors (NPs) with the capacity to give rise to all major cell types of the mature central nervous system (CNS) have been isolated from the developing or adult CNS (17,21,34). They become more restricted in number during development and remain as a small, relatively quiescent population of dividing cells in the subventricular regions of the adult CNS. These neural progenitors can be grown in vitro in the presence of either epidermal growth factor (EGF) or basic fibroblast growth factor (bFGF), as a population of continuously dividing progenitors capable of differentiating into both neurons and glia (19,21,23,25,33). The benefices of the neural stem cell transplantation as a means of restoring CNS diseases or spinal cord injury are well recognized (1,7,27, 32). The use of human embryonic NSCs raises ethical issue as well as practical problems: the behavior of an embryonic cell in an adult environment and the need for immunosuppression. One apparent advantage of using adult stem cells, therefore, is that they could be derived from and transplanted to the same patient, thereby avoiding potential tissue rejection. The identification of a source of readily accessible neural progenitors (NPs) that can be obtained without permanent damage from the individual requiring transplantation therapy could provide a great benefit. An important biological finding from several studies is that stem cells have a certain degree of flexibility in their differentiating capacity, resulting in lineage interconversion between precursor cells originating from the same and different germ layer (1,10.13,14). Such plasticity is supported by experiments in which bone marrow-derived stem cells can yield not only all cells of the blood but also muscle cells (mesodermal derivatives), and cells bearing neuronal markers (neuroectoderm) (3,18,30). However, there is also evidence that NPs can give rise to hemopoietic and muscle cells (2,35). The study of this surprising lineage transition that appears to be dictated by the local environment as well as the multipotency of stem cells may prove useful for

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autologous stem cell therapy. We investigated whether NPs are restricted to the central nervous system or can be obtained from nonneuronal tissues.

MATERIALS AND METHODS

Generation of Floating Spheres

Cells were isolated from 2-day-old newborn and 2-month adult muscle, bone marrow, and blood tissues of C57BL/10J mice (Charles River Laboratory). Muscle tissues were minced into smaller pieces using razor blades. The bone marrow was obtained flushing femurs with EBSS and whole blood was collected in 20 mM EDTA during intracardiac saline perfusion. Briefly, the saline solution was infused by a peristaltic pump in the left ventricular cavity and blood collected from the excised right atrium. Cells were enzymatically dissociated by adding 0.2% collagenase type XI and 0.1% trypsin for 1 h at 37°C. Somatic tissue cell extract was preplated on the culture noncoated flasks (12,31). This method offers the advantage to isolate different populations of tissue-derived cells based on the number of preplates performed on the culture noncoated flasks. Preplate 1 (pp1) represented a population of tissue-derived cells that adhered in the first 24 h after isolation. It is a highly enriched culture of fibroblasts from muscle at pp1. Preplate 2 (pp2), which represented a population of tissue-derived cells that did not adhere, was used as target population. The growth media used was composed of DMEM/F-12 (1:1), 20% FBS, including HEPES buffer (5 mM), glucose (0.6%), sodium bicarbonate (3 mM), and glutamine (2 mM). A defined hormone and salt mixture composed of insulin (25 μg/ml), transferrin (100 μg/ml), progesterone (20 nM), putrescine (60 μM), and sodium selenite (30 nM) was also used. To the above medium, epidermal growth factor (EGF) (20 ng/ml) and fibroblast growth factor (bFGF) (10 ng/ml) (murine recombinant; Chiron Corporation, Emeryville, CA) were added as mitogens. This medium support the proliferation of EGF + bFGF-responsive neuronal and glial progenitors characterized by the formation of spheres of undifferentiated cells (24).

Self-Maintenance and Differentiation of Somatic-Derived Spheres

After mechanical and enzymatic treatment of tissues, cells (10^3 cells/cm^2) were grown in the presence of EGF and bFGF and analyzed for the presence of spheres. To test whether the somatic-derived progenitors exhibit self-maintenance, single cells generated from primary EGF + bFGF-responsive somatic-derived spheres were plated into 96-well plates. For plating single cells, a single primary somatic-generated sphere was collected in vitro with a pipette, mechanically dissociated, and serially diluted to yield approximately one to two cells per 10 μl aliquot. A 10-μl aliquot was added to each well of a 96-well plate containing 200 μl of EGF + bFGF-containing medium. All wells that contained one viable cell 24 h later were scored 8 days later for the presence of spheres. Under these conditions, secondary single spheres were transferred to 500-μl Eppendorf tubes containing 200 μl of medium, triturated 20–40 times, and replated as single cells into a new 96-well plate. The plates were scored 8 days later for the number of tertiary spheres derived from a single secondary sphere (Table 1). In experiments of differentiation, primary or secondary single isolated spheres were plated on glass coverslips in individual wells of 24-well (1.0 ml/well) (Nunc, USA) culture dishes in DMEM/F-12 medium with the hormone and salt mixture and EGF + bFGF. Medium was not changed for the rest of the experiment and coverslips were processed 21–25 days later for indirect immunocytochemistry. In a second experiment, the muscle and bone marrow floating secondary spheres were cocultured with the cells of the same tissue of origin (5×10^3 cells/cm^2) in DMEM medium (Gibco, Rockville, MD), supplemented with 5% fetal calf serum (FCS). In this experiment, when the primary cultures of the somatic-derived cells obtained at pp1 reached 30% of confluence, free-floating secondary spheres were added to the culture. The behavior of the cells within the adhered spheres in coculture was observed by time-lapse videomicroscopy and characterized by immunocytochemistry. All scenes were recorded on videotape using a silicon-intensified-target videocamera (VE 1000-SIT; Dage MTI, Michigan City, IN), a time and frame counter (ELCA, Treviglio, Italy) and a high picture quality SVHS Panasonic VCR (Panasonic, Boston, MA). The recordings

Table 1. Isolation and Self-Renewal Capacity of Murine Somatic-Derived NPs

	Number of Secondary and Tertiary Spheres Obtained From	
Tissue Origin of Spheres	Newborn Donor Mice	Adult Donor Mice
Muscle	35 ± 4 (12 ± 3)	13 ± 1 (11 ± 2)
Bone marrow	12 ± 1 (7 ± 3)	2 ± 1 (3 ± 2)
Blood*	2 ± 1 (3 ± 1)	—

After mechanical and enzymatic treatment of tissues, cells (10^3 cells/cm^2) were grown in the presence of EGF and bFGF and analyzed for the presence of spheres. Dissociation of single primary spheres into single cells, all of which are plated into one well, results in more than one secondary sphere. Secondary sphere average could be differentiate in neurons and glia or could generate tertiary clones (indicated in parentheses). All data are given from 10 experiments as mean ± SEM.
*Blood-derived spheres were obtained after 4 weeks from the blood of 10 newborn mice after intracardiac saline perfusion. However, no spheres were isolated from the total blood obtained from 10 adult mice.

were digitized on Casablanca digital system (MS Macrosystem Computer GmbH, Witten, Germany).

Western Blot and PCR Analysis of Somatic-Derived Spheres

Western blot conditions are specified in Torrente et al. (31). Briefly, the concentration of proteins was determined using the Lowry technique. One hundred micrograms of extracted proteins was separated on 6% polyacrylamide gels and electrotransferred onto nitrocellulose membranes (Biorad, Toronto, Canada). To ensure that equivalent amounts of proteins were loaded for each sample, the membrane was stained with Ponceau S. (Sigma). Membranes were subsequently incubated with monoclonal antibodies directed against nestin, MAP-2, β-tubulin III (Novocastra, Newcastle, UK) and revealed using a commercially available chemiluminescence kit (Ultra ECL, Pierce, Rockford, IL). Membranes were then exposed to BioMax autoradiographic films (Kodak, Rochester, NY), which were developed and scanned with a densitometer. Nested RT-PCR method (15) was used to amplify genomic DNA. Primers for GAPDH, c-met, m-cadherin, myf-5, nestin, GFAP, c-kit, and CD34 were obtained from previous reports (6,20,29). Thirty-five cycles at 60°C annealing were used to amplify the following MAP-2 and β-tubulin III outside primers used in the first PCR: 5′-CAA GGA CCA GCC TGC AGC TCT G-3′ and 3′-G ACG TCC TGG GGT CAC GAG CTA-5′ (MAP-2); 5′-GTC CTA GAT GTC GTG CGG AA-3′ and 3′-GAC TGC CAC GGA CTC GAG TGC-5′ (β-tubulin III). After the first round of PCR, the reaction was diluted 1:100 into separate secondary PCR reactions (40 cycles of 94°C for 1 min and 75°C annealing) using the following primers: 5′-GAT GGA ATT CCC TGA GCA GGA G-3′ and 3′-C AAA TCG ACG GGG TTA TAG AGG ACC-5′ (MAP-2); 5′-GAC TGC CTG CAG GGC TTC CAG-3′ and 3′-GTG AAG AAG TAC GGG CCG AAG-5′ (β-tubulin III).

In Vitro Immunohistochemistry Analysis

Neuronal differentiation was tested using the following antibodies. Mouse monoclonal antibodies against the 200 kDa neurofilament (Sigma 1:500) and neuronal specific enolase (NSE) (1:20); glial fibrillary acidic protein (GFAP) (1:50) was from Boehringer Mannheim (Indianapolis, IN); mouse monoclonal antibodies to β-tubulin (type III; 1:100) and mouse monoclonal antibody to microtubule-associated protein (MAP-2) (1:200) were from Sigma; goat antibody to nestin (1:500) and galactocerebroside (Gal-C) were from Promega (South San Francisco, CA). Indirect immunostaining of cells within the spheres and their progeny was also done using monoclonal antibodies against the CD31 (1:100; Santa Cruz), Flk-1 (1:20), CD34 (1:50; Becton Dickinson, Mountain View, CA), Sca-1 (1:250; Becton Dickinson), CD45 (1:100; Becton Dickinson), CD14 (1:50; Dako), CD11b (1:200; Dako), desmin (1:20; Sigma), myosin heavy chain (MyHC) (1:200; Ylem), cytokeratin (1:150; Dako), S100 and vimentin (1:200; Santa Cruz), CD4 and CD8 (1:100; Santa Cruz). Indirect immunocytochemistry was carried out with spheres attached to glass coverslips, either immediately after plating (for nestin) or after 21–25 days in vitro (for coculture labeling and for neuronal phenotypes). Coverslips tested for neuronal markers were fixed in 4% paraformaldehyde (in PBS, pH 7.2) for 30 min, followed by three (10 min each) washes in PBS, pH 7.2. Nonneuronal antibodies were tested into coverslips fixed in ethanol 70% in PBS for 1 min and permeabilized for 5 min with 0.5% Triton X-100 in PBS. All antibodies were incubated with the coverslips for 1 h at room temperature. After washing with PBS, cells were incubated with appropriate secondary FITC/TRITC-conjugated IgG antibodies (1:100) for 1 h at room temperature and examined by epifluorescence microscopy.

Somatic-Derived Neural Progenitor Transplantation

NPs clonally derived from muscle or bone marrow secondary spheres were labeled with PKH26 (31) and injected (10^6 cells in 5 μl of PBS) into the right striatum ($n = 40$; 10 mice for each group) with the aid of a stereotaxic apparatus (coordinates: A/P, +0.5; M/L −3.1; D/V, −4.8) of C57BL/10J mice. Fibroblasts were also injected into the brain as control. Five days later, brain and muscle tissues were removed, frozen in liquid nitrogen-cooled isopentane, and cut on a cryostat into 7-μm serial sections. For neuronal immunostaining, sections were fixed in 4% paraformaldehyde and incubated with nestin, vimentin, MAP-2, β-tubulin III, GFAP, and neurofilament antibodies. For myogenic immunostaining the sections were fixed in ethanol 70% in PBS for 5 min and incubated with desmin and MyHC antibodies.

Grafted cells were detected by immunofluorescence assays for PKH-26-positive cells and the number of cells that had differentiated was counted in 100 serial sections, adjacent to the area of injection. Cell nuclei were stained for 15 min at 37°C with 4′,6-diamidino-2-phenylindole (DAPI). The nuclear staining revealed the number of grafted cells within the PKH-positive areas.

RESULTS

Clonal NPs Expansion, Self-Renewal, and Differentiation From Nonneuronal Tissues

A tissue culture system to facilitate enrichment of somatic-derived progenitor cells was used (31). Muscle, bone marrow, and blood tissues were removed from neonatal and adult C57BL/10J mice. Individual cells isolated at pp2 were plated in growth medium containing

EGF and bFGF (5). After 5 days in vitro, floating clusters of cells with morphological resemblance to neurosphere were seen in muscle cultures. Spherical clusters were also present in bone marrow cultures, 2 weeks after plating. Surprisingly, within the blood-derived cells we also found few spherical clusters after 4 weeks in culture though floating spheres were rare, especially from hematopoietic tissues of adult animals (Table 1). Self-maintenance was demonstrated by serial subcloning experiments where single primary spheres were dissociated and replated in 96-well plates as 1 cell/well. Single cell-derived primary spheres generate clonally derived secondary spheres capable of producing, after their dissociation, tertiary spheres that could be differentiated into neurons and glia. A subset of clones proliferated and gave rise to secondary spheres in a different proportion depending on the tissue of origin, but there was a reduction in neurosphere yield with increasing passages (Table 1). These data indicate that somatic-derived primary spheres exhibit self-renewal, generating secondary spheres after dissociation, just as CNS stem cells do under similar culture conditions.

Most cells within somatic-derived spheres were positive for nestin, the major cytoskeletal protein expressed in neuronal progenitors (16) (Fig. 1), whereas these cells were negative for Sca-1, Flk-1 (both markers of hematopoietic stem cells), and CD45 (a marker of all mature nucleated hematopoietic cells). However, the RT-PCR analysis of the floating spheres showed the expression of nestin, c-met (tyrosine kinase receptor, ligand HGF/scatter factor), c-kit (coding for the SCF receptor), and CD34.

When individual spheres from somatic-derived cultures were dissociated and replated as single cells under clonal condition in growth medium, some cells proliferated and differentiated 21–25 days later to acquire the typical morphology of neural cells (Fig. 1). Specific immunolabeling of these cell populations showed that $20.1 \pm 1.2\%$ of the total cell number ($n = 10$) expressed the neuron-specific markers, β-tubulin III, MAP2, neurofilament, and NSE (Fig. 1); $64.7 \pm 2.1\%$ ($n = 10$) of total cell number (TCN) were positive for the astroglial marker, GFAP, whereas there was $1.9 \pm 2.0\%$ TCN ($n = 6$) expressing the oligodendroglial antigen, galactocerebroside (GalC) (Fig. 1). The expression of neuronal and glial markers was similar in all sphere-derived progeny from somatic tissues as muscle, bone marrow, and blood (data not shown). Some immunocytochemical data were confirmed by both RT-PCR and Western blot (Fig. 2A, B). However, the primary culture (i.e., pp1) of somatic-derived cells did not stain with neuronal antibodies.

Table 2. Summary of Immunoreactivity of Secondary Free-Floating Spheres Before and After Their Adhesion in Coculture Experiments

Antigen Expression	Floating Spheres	Spheres Cocultured in: Muscle	Spheres Cocultured in: Bone Marrow
Nestin	+	+	+
Sca-1	–	–	+
CD34	+/–	+	+
Flk-1	–	–	–
CD31	–	+	–
β-tubIII	–	+	+
MAP-2	–	–	–
NSE	–	–	–
GalC	–	–	–
Desmin	–	+	–
MyHC	–	+	–
Cytokeratin	–	–	–
Vimentin	–	–	–

In this experiment, when the primary cultures of the somatic-derived cells obtained at pp1 reached 30% of confluence, free-floating secondary spheres of the same origin were added to the culture. The behavior of the cells within the adhered spheres in coculture was observed by time-lapse video-microscopy and characterized by immunocytochemistry.

Multipotentiality of Somatic-Derived NP Cells

To verify the effect of local environment on NPs differentiation, floating secondary spheres obtained from muscle and bone marrow were cocultured with cells of the same tissue of origin and tested for the expression of neural, myogenic, hemopoietic, and endothelial markers by immunocytochemistry. The behavior of clonal somatic-derived spheres in coculture was observed by time-lapse video-microscopy. When spheres were adhered, the replication within the spheres resumed in two stages. First, in a period of about 20 min, rounded cells detached from the spheres, forming roughly spherical, phase-bright cells. Second, over a 60-min period, the surrounding spherical cell of the spheres formed new cells with extended processes that migrated to the substratum as if searching for the appropriate interaction with cocultured cells. After 10 days of coculture with muscle-derived cells, we found adherent secondary spheres, which contained rare desmin- and MyHC-positive cells (myogenic markers) at the periphery and few CD34, CD31, uncommitted/committed hematopoietic progenitor, and vascular endothelial cells in the center of the neurospheres. Few contracting myotubes were also present near several secondary spheres (Fig. 3E). Coexpression of both myogenic and neuronal phenotypes (MyHC and β-tubulin III) was observed in <1% of total cell number (TCN) after 7 days in culture (Fig. 3F). This phenomenon was transient as cells expressing β-tubulin III switched to a myogenic phenotype. Few S100-positive cells (Schwann cell marker) were also found near

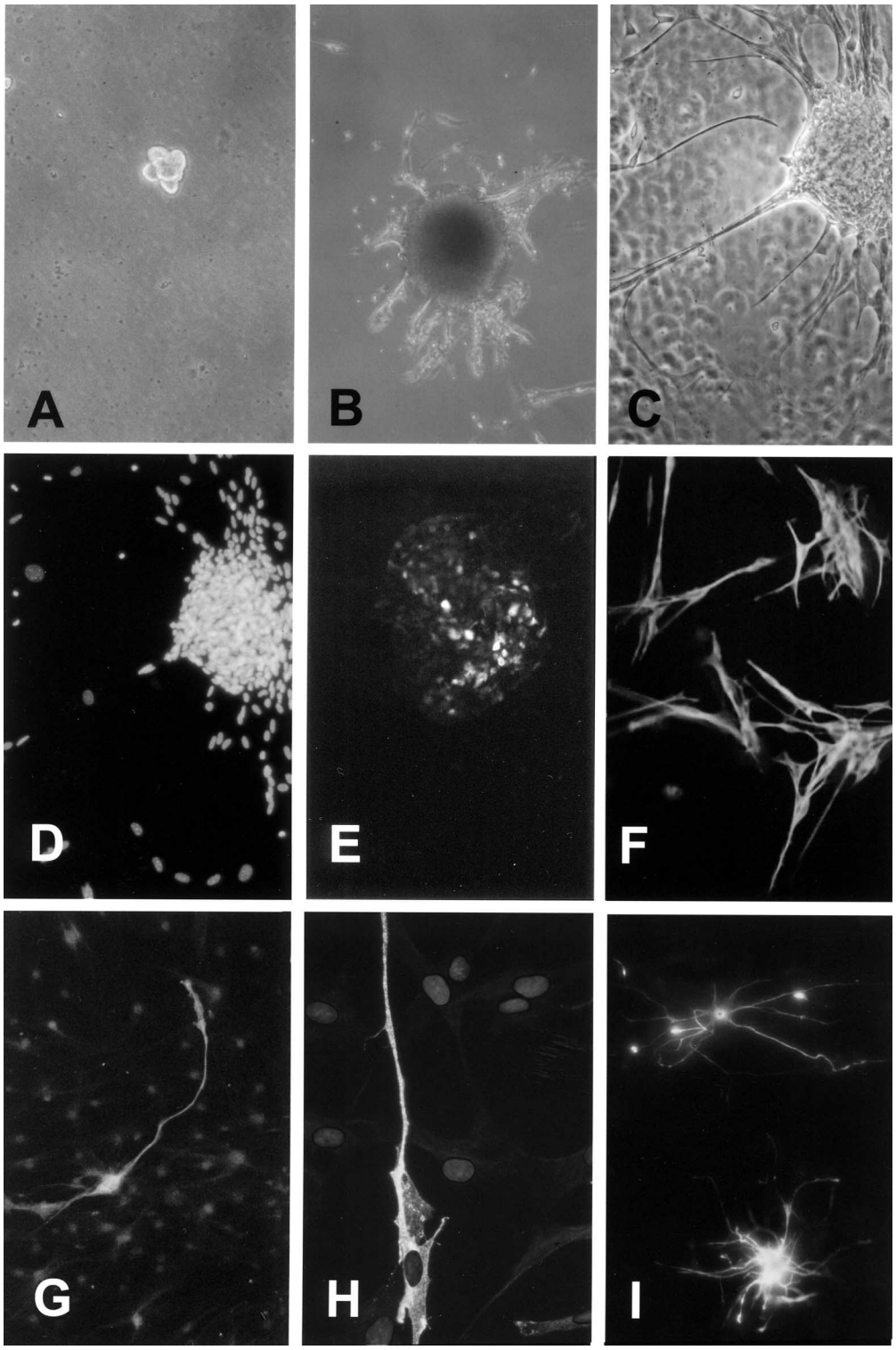

Figure 1. Cloning of newborn and adult C57BL/10J single cells obtained from floating spheres derived from the muscle, bone marrow, and blood tissues. Single cells proliferated, giving rise to floating clusters after 3 days (A), which differentiate by plating these cells in 1% fetal bovine serum, in the absence of growth factors (B). Staining of cell nuclei with DAPI showed that cells within the sphere colony continue to proliferate, forming a large colony with attached processes after 9 days (C, D). Many cells within the colony expressed the NSC antigen, nestin (E). The progeny of adhered muscle-derived spheres expressed the neuron-specific markers, β-tubulin III (F), neurofilament (G), neuronal specific enolase (NSE) (H), and oligodendroglial antigen, galactocerebroside (GalC) (I).

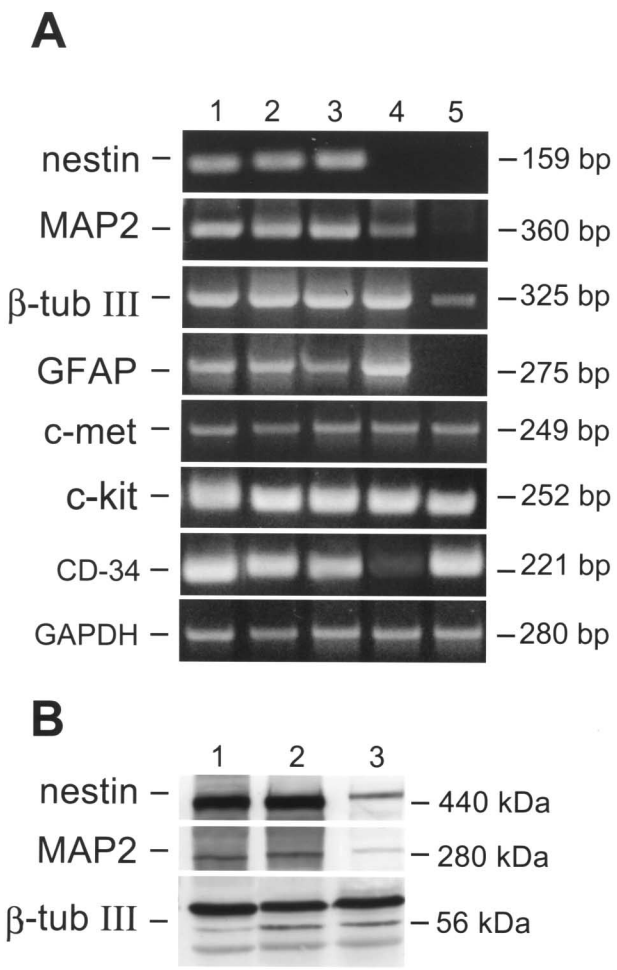

Figure 2. Expression of neuronal genes in 11-day differentiated cloned sphere colonies. (A) RT-PCR analysis of total RNA of cloned spheres isolated from bone marrow (lane 1), muscle (lane 2), and blood (lane 3) tissues of newborn mouse. Mature murine brain (lane 4) and muscle (lane 5) tissues were used as control. Nested nestin RT-PCR resulted in a 159-base pair product present in all cloned spheres, confirming the neuronal progenitor characteristics. Nestin expression was not detected in control tissues (lanes 4 and 5). Expression of c-met, c-kit, and CD34 was also found. Neuronal differentiation could be identified in the same culture well by the expression of MAP-2, β-tubulin III, and GFAP products. GAPDH (glyceraldehyde-3phosphate dehydrogenase) was amplified in the same reaction tube as an internal control. (B) Immunoblotting analysis of cloned spheres examined by RT-PCR in lane 1 through lane 3 confirmed the expression of neuronal antigens.

the muscle-derived secondary spheres in one of six experiments (data not shown). Moreover, after 10–21 days in culture, cells expressing markers of neuronal and glial differentiation were found (Table 2). Conversely, secondary spheres cocultured with bone marrow-derived cells showed few Sca-1, CD34-positive cells, and different types of sprouting progeny still attached at the periphery of the secondary spheres such as CD4, CD8 (T lymphocytes), CD14, and CD11b (myeloid cells)-positive cells (2). By contrast, adhered secondary spheres from the blood tissue showed low expression of nestin and CD45 (Fig. 4).

Engrafted Somatic-Derived NP Cells Can Differentiate Into Neuronal Cells

In a second series of experiments, we examined the question of whether progenitors isolated from the nonneuronal tissues can exhibit in vivo neurogenic properties after implantation into the brain of adult recipients. PKH26-labeled neural progenitors derived from muscle and bone marrow were injected in the ipsilateral right striatum region of the brain of C57BL/10J mice ($n = 12$). Five days after transplantation, PKH26-positive cells were still detected in the striatum. Injected cells expressed neuronal β-tubulin III and MAP-2 markers (Fig. 5 and Table 3), but no desmin, MyHC, GFAP, and nestin-positive cells were seen (data not shown). Control injections into the striatum with muscle fibroblasts did not result in neuronal antigen expression. All these data indicate that signaling from the environment can induce differentiation of somatic-derived NPs.

DISCUSSION

The limited capacity for structural repair in the mammalian brain is in part explained by the inability of the mature CNS to generate new cellular elements in response to damage.

Stem cells have great promise as a source for introducing new neurons and glial cells to the damaged CNS. These cells are cryo-preservable and retain full proliferation and differentiation capacity even in long-term cultures (11). Once transplanted into the brain, neural stem cells can adapt to the region of engraftment by differentiating into the appropriate neuronal subpopulations (22, 26,28). We isolated a somatic-derived NPs population from several newborn and adult tissues. This population of stem cells responds in vitro to mitotic medium of NSCs and have in vivo a broad developmental capacity after their transplantation in neurogenic (brain) regions of adult mouse. The expression of phenotypic markers of site-specific differentiation within the grafted regions is probably the effect of the local microenvironment on the behavior of the somatic-derived NP cells.

We noted no signs of tumor growth or nonneural tissue in the transplant brain recipients. Although long-term survival studies will be necessary to comprehensively address the safety of somatic-derived NPs transplantation, our observations suggest that the prolonged propagation in growth factors promoting NSC proliferation may eliminate undesired cells in the donor cell population and provide an alternative source for CNS trans-

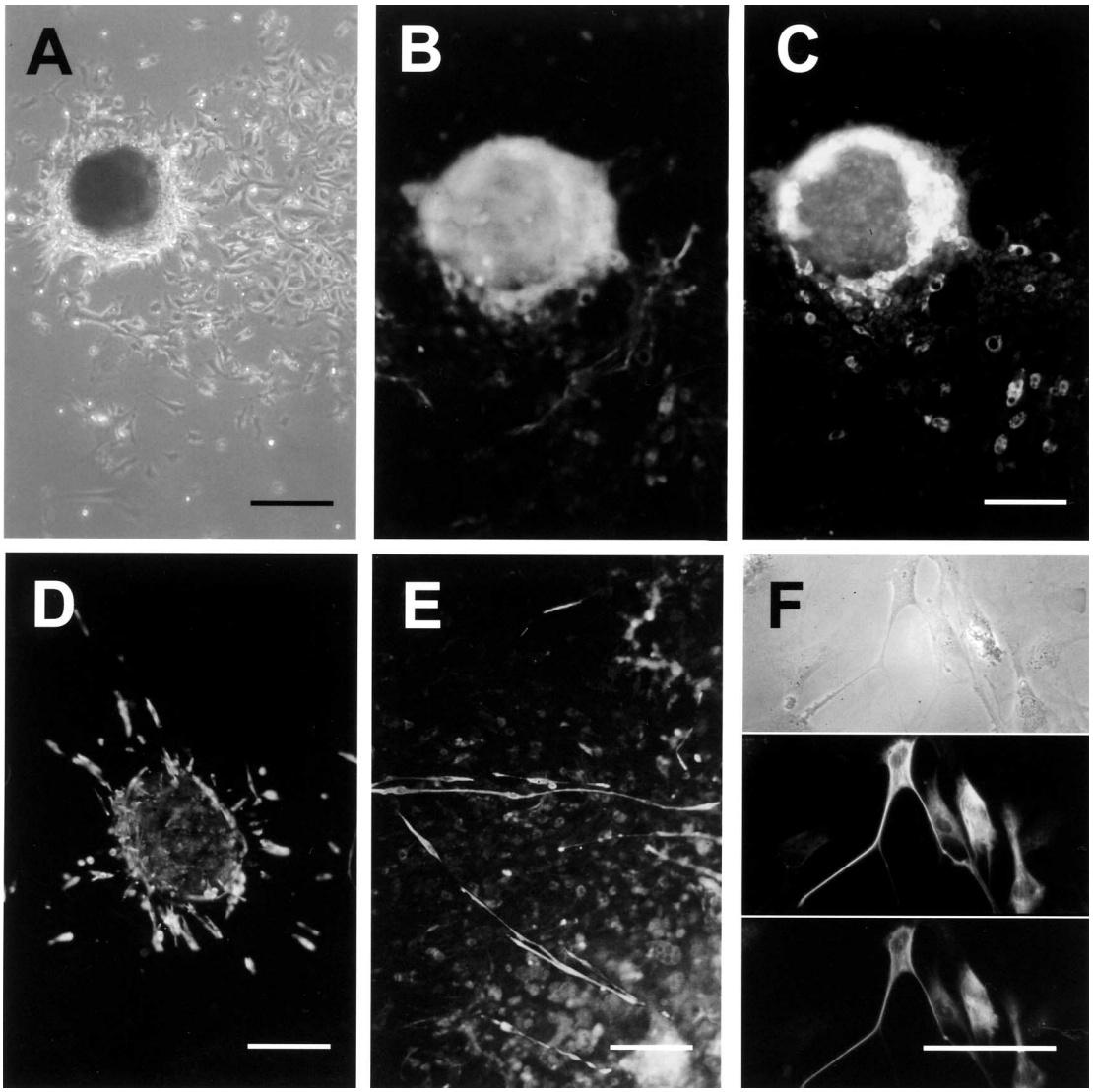

Figure 3. Somatic-derived secondary spheres were cocultured with cells (pp1) originating from the same tissue. In the muscle-derived cell cultures (A), expression of CD34 (B) and CD31 (C) within the adherent secondary spheres was seen after 3 days in vitro (DIV). The progeny of these colonies were desmin positive (D, after 10 DIV), providing few MyHC-positive myotubes near several secondary spheres after 18 DIV (E). Coexpression of both myogenic (MyHC in the bottom panel in F) and neuronal (β-tubulin III in the middle panel in F) markers was observed in few cells after 7 DIV.

plantation therapy. If multipotent somatic-derived stem cells share neural stem cell potential, then why don't these cells display NPs characteristics in vivo? Several hypotheses come readily to mind. In the first, somatic-derived progenitors maintain their tissue-specific identities due to the surrounding environmental factors. Indeed, several articles support the new view that somatic progenitors can change fate when grafted to heterologous adult mice tissues. It is remarkable that in this case, the NSCs of neuroectodermal origin changed germ layers and contributed to the mesodermally derived hematopoietic cells (2). Even more surprising are results which show that bone marrow can differentiate into neurons and glia in the brain of adult recipient mice (3,18). It may be that the neurosphere-generating cells from our tissue culture represent a phenomenon of in vitro transdetermination between somatic progenitors originating from different germ layers. This event led to a somatic-derived neural progenitor that is functionally related to the multipotent subependymal cells described by Alvarez-Buylla and colleagues (8,9). On the other hand, it is possible that common pluripotent cells, resembling those of mouse pluripotent stem cells termed embryonic stem (ES) cells capable of commitment into many progenitors and cell types, reside in all body tissues. This hypothesis may be supported by recent reports showing

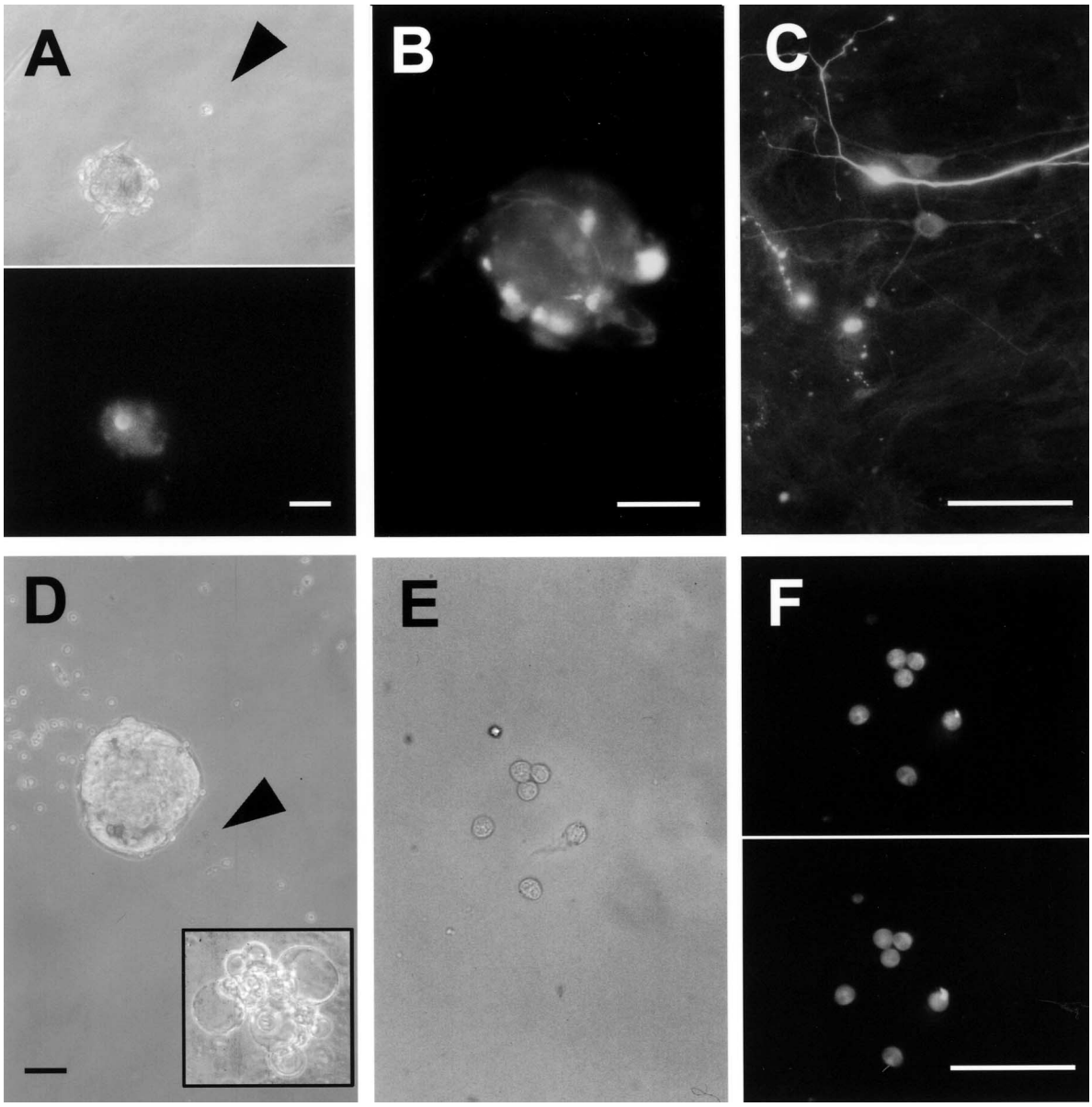

Figure 4. Immunostaining of floating clonal neurospheres cocultured with cells of the same tissue of origin. Blood-derived spheres expressed CD45 protein (A) and produced rounded cells that detached from the spheres (arrowhead in A). Many (but not all) of the cells within the spheres exhibit nestin antigen (B). Eight weeks after inducing differentiation the progeny of blood-derived spheres showed bipolar neuron-like morphology whose body and processes expressed neurofilament protein (C). Numerous cells of bone marrow-derived spheres detached from the spheres and reaggregates forming new spheroids (inset in D) that were positive for nestin (not shown) or adhered as single rounded cells (E) that expressed both Sca-1 (top panel of F) and CD34 (bottom panel of F).

the finding that applying bFGF and EGF to ES cells permits efficient in vitro generation of precursors for oligodendrocytes and astrocytes (4). Moreover, adult NSCs were present in all germ layers in chimeric mice following injection into the blastocyst (5).

During experiments of cocultures, cells in each somatic-derived sphere undergo proliferation and differentiation through a variety of tissue markers. In fact, progeny of spheres exhibit immunoreactivity for muscle, neuronal, skin, and hematopoietic antigens. Thus, somatic-derived spheres showed high differentiation plasticity when subjected to different microenvironments. Although most cells within the floating spheres were positive for nestin, the expression of Sca-1 and CD34 in the center of cocultured adhered spheres may be considered as a consequence of lineage interconversion of so-

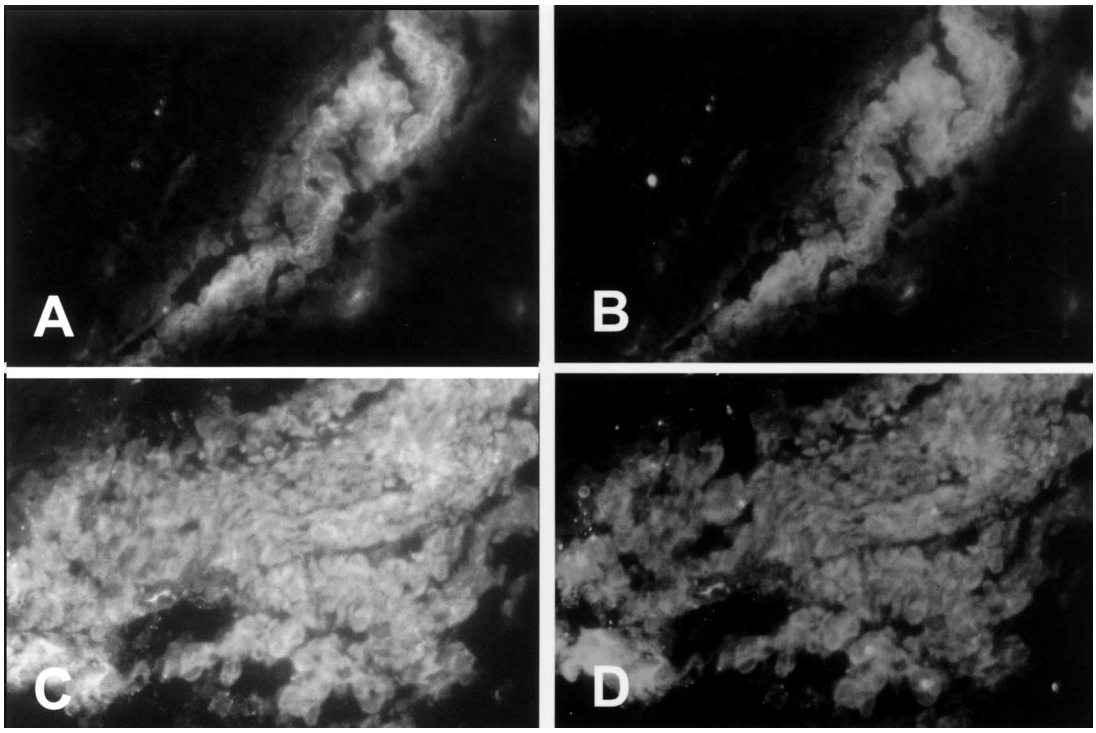

Figure 5. Transplantation of single cells obtained from secondary floating spheres derived from the muscle into the adult murine striatum. Clonal floating neurospheres were dissociated and prelabeled with PKH-26 dye (10^6 cells/graft). Five microliters of 10^6 labeled cells in phosphate-buffered saline was injected into the right striatum ($n = 40$; 10 mice for each group) with the aid of a stereotaxic apparatus (coordinates: A/P, +0.5; M/L −3.1; D/V, −4.8) of C57BL/10J mice. Five days after transplantation into the ipsilateral right striatum few PKH-26 (A, C left panel)-positive cells coexpressed β-tubulin III (B) and MAP-2 neuronal antigen (D).

matic stem cells. Additionally, the RT-PCR analysis revealed that somatic-derived NPs expressed the c-kit tyrosine kinase growth factor receptor, which is also expressed in hematopoietic stem cells but not in brain-derived NSCs.

Each somatic-derived sphere could thus be considered as a developing microsystem that is presumably the result of different stem/progenitor cell types, whose variation may be due to their in vitro isolation from different somatic tissues or at different stages of their maturation. These data would indicate that somatic stem cells have multipotential activities such as neurohematopoietic and neuromuscular activities.

The availability of human NPs from nonneuronal tissues and the possibility of autologous transplantation provide exciting perspectives for the treatment of CNS human diseases. Further tests are necessary to determine whether human NPs isolation from several somatic tissues may be applied similarly to their murine counterparts.

Table 3. Neuronal Marker Expression 5 Days After Somatic-Derived NSC Transplantation

Origin of Injected NSCs	Brain Injection	
	β-Tubulin III	MAP-2
Muscle	78 ± 2.3% ($n = 3$)	55.6 ± 0.7% ($n = 3$)
Bone marrow	70 ± 2.5% ($n = 3$)	66 ± 1.7% ($n = 3$)

Results are presented as mean ± SEM of the injected cells.

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