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Primate adult brain cell autotransplantation, a new tool for brain repair?

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Abstract

If successful, autologous brain cell transplantation is an attractive approach to repair lesions and restore function of the central nervous system. We demonstrate that monkey adult brain cells obtained from cortical biopsy and kept in culture for 4 weeks exhibit neural progenitor characteristics. After reimplantation into a lesion area of the donor cerebral cortex, these cells can successfully survive and acquire neuronal characteristics over time. These results open new perspectives in the field of brain repair and may lead to future clinical applications.

Keywords: Monkey; Cell autotransplantation; Brain repair

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Introduction

Using neural grafts to restore function after lesions of the central nervous system is a challenging strategy. Most of the transplantation experience acquired the last two decades was focused on fetal neuronal grafts (Isacson, 2003; Lindvall et al., 2004; Richardson et al., 2004). The objective was to replace degenerated neurons in pathologies, such as Parkinson's and Huntington's disease, characterized by degeneration restricted to a limited brain area (Bjorklund and Lindvall, 2000; Brazel and Rao, 2004; Freed et al., 2001; Palfi et al., 1998; Park et al., 2002; Redmond, 2002; Redmond et al., 2001). However, despite the great enthusiasm generated by this approach, ethical controversies, immune rejection, and lack of fetal donors remain a major problem. Therefore, autotransplantation of adult brain cells represents an attractive restoration alternative to bypass the caveats of fetal grafting. Efforts have been made over the past few years to develop appropriate methods to prepare long-term primocultures from primate cortical biopsies (Johansson et al., 1999; Kempermann and Gage, 1999; McKay, 1997; Palm et al., 2000; Uchida et al., 2000). After optimization of the procedure for brain cell culture preparation, using medium with preselected fetal calf serum (pFCS), we succeeded in producing long-term primocultures of adult human brain cells from temporal lobe tissues obtained from epilepsy and trauma neurosurgical patients (Brunet et al., 2002). In a previous study, we have also described the possibility of cryopreserving adult brain tissue to obtain brain cells with characteristics the similar to primoculture (Brunet et al., 2003). Furthermore previous studies suggest that the reconstruction and repair of cortical circuitry responsible for sensory, motor, or cognitive function may be possible by allogeneic stem cell transplantation in the mature mouse neocortex (Hernit-Grant and Macklis, 1996). Here, we propose to demonstrate the feasibility of autotransplantation from brain biopsy to re-implantation of cultured brain cells in a non-human primate model of motor cortex lesion (Fig. 1).

Results and discussion

As for human brain cell culture, using medium with preselected fetal calf serum (pFCS), we succeeded in producing long-term primocultures of brain cells from

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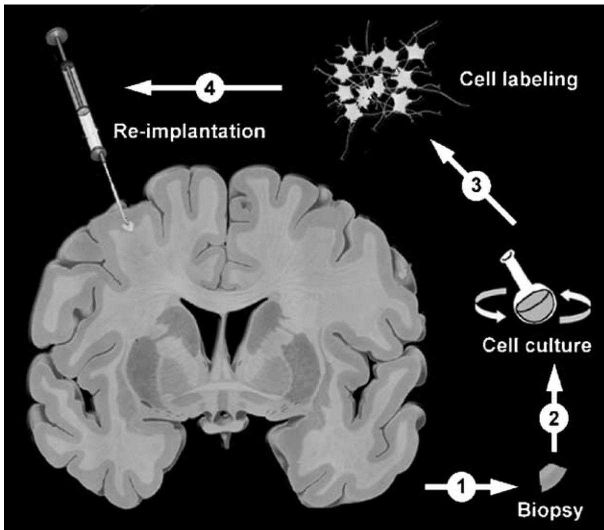

Fig. 1. Scheme of procedure from cortical biopsy to autotransplantation. Schematic representation of the different steps from cortical biopsy to autotransplantation. Pieces of cerebral cortex were obtained from *M. fascicularis* (1). Biopsies were handled as described in supplement to obtain cortical cell aggregates (2). Cell aggregates were pooled and labeled with fluorescent viable dyes PKH26 or PKH67 (Sigma) (3). Labeled cells were stereotaxically re-implanted with a 100-μl Hamilton glass syringe (4).

cortical biopsies of non-human primates (*Macaca fascicularis*) with similar results. In vitro, we could demonstrate that both human and monkey adult brain cells express neuroectodermal and progenitor markers such as GFAP, neurofilament, vimentin, or nestin (data not shown) as we described in a previous studies (Brunet et al., 2002).

In the present study we hypothesized that the monkey cortical grey matter cells kept in vitro for 1 month could survive in vivo, when reimplanted in a normal and a lesioned cortical area of the donor. To obtain the brain cells, a right prefrontal open cortical biopsy was performed in three adult monkeys (6–9 years old; 4–7 kg). The cells from grey matter enriched fraction were then processed in vitro as described for human brain cells previously (Brunet et al., 2002). However, in order to facilitate reimplantation, they were cultured in suspension under slow agitation (50–75 rpm with an ES-W shaker-Kuhner). Cells obtained presented the same morphological characteristics and expressed the same markers (GFAP, vimentin, and nestin), as observed in monolayer cultures. Two weeks after cortical biopsy, as previously described in detail (Liu and Rouiller, 1999), an ibotenic acid excitotoxic lesion was performed in an area of the left primary motor cortex (M1) of the monkeys, restricted to the hand representation. Three weeks later, the cells were stereotaxically re-implanted into the lesioned M1, and into an intact parietal cortical site. Just prior to reimplantation, cells were stained for tracking with fluorescent viable dyes that irreversibly bind to cell membranes (Fig. 1D) (PKH26 for implanted cells in the ibotenic-lesioned site and PKH67 for implanted cells in the intact site) as already described for intracerebrally transplanted cells (Haas et al., 2000).

One month after re-implantation, the first monkey was sacrificed, the brain was removed and 8 series of 50-μm-thick sections were prepared with a cryotome. Sections were observed after Nissl staining or directly under fluorescence microscope after mounting in Vectashield containing DAPI to counterstain nuclei in blue to detect PKH26- or PKH67-stained cells. Analysis confirmed that the autotransplanted cortical grey matter cells could survive equally, at least 1 month, in both the lesioned and the intact sites (Figs. 2A and B). Moreover, migration was observed in distal areas and in the direction of the subventricular zone. Immunohistochemistry revealed that the large majority of the implanted cells were nestin positive (Figs. 2C–E).

Three months after reimplantation, two other monkeys were sacrificed, and brains were processed as above. Surprisingly, no PKH67-labeled cells were detected in the non-lesion reimplantation site and nowhere else in the whole brain (data not shown). This observation confirms that PKH could not be picked up by resident cells. However, PKH26-labeled cells were observed up to 1 cm away from the injection site, surrounding the ibotenic acid lesion (Figs. 3A–D). At the implantation site, PH26-

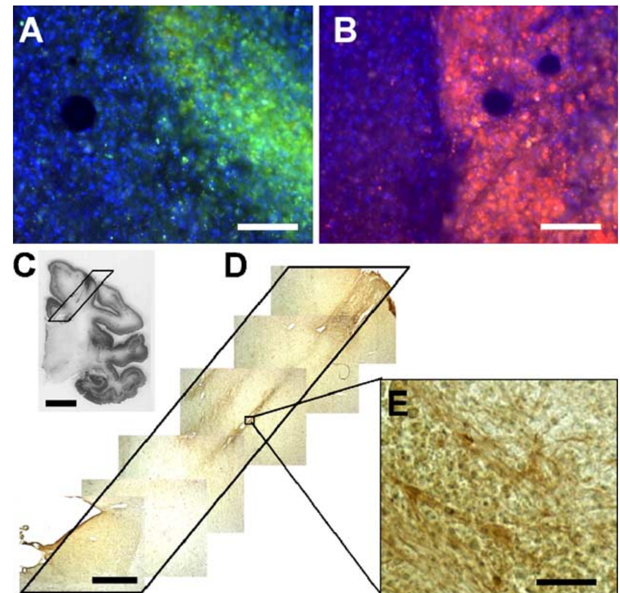

Fig. 2. Brain histology 1 month after adult brain cell re-implantation. (A) PKH67 (green)-labeled monkey autotransplanted brain cells in non-lesioned area. (B) PKH26 (red)-labeled monkey autotransplanted brain cells near the ibotenic acid-induced lesion. (A–C) Nuclei are counter-stained with Dapi (blue). (C) Hematoxylin-stained brain section in the ibotenic acid-induced lesion area, the parallelogram delineates the re-implanted cell invaded area. (D) Reconstruction of magnifications corresponding to the parallelogram in panel C after nestin immunolabeling. (E) Magnification of an area corresponding to the square in panel D, near the ibotenic acid-induced lesion. Note that nestin-positive cells are observed along the ibotenic acid-induced lesion. (A–B: scale bar = 40 μm; C: scale bar = 7 mm; D: scale bar = 1.6 mm; E: scale bar = 60 μm).

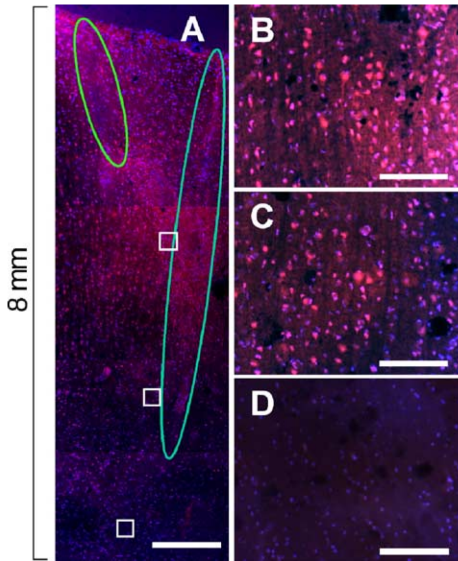

Fig. 3. Brain histology 3 months after adult brain cell re-implantation. All these sections were performed in the ibotenic acid-lesioned motor cortex. (A) Migration of PKH26 (red)-labeled monkey autotransplanted brain cells toward the ibotenic acid-induced lesion. The green ellipse corresponds to the reimplantation site and the blue ellipse represents the ibotenic acid track. (B–D) Magnification of the small squares in panels A, B, and C, respectively, in the middle and at the bottom of ibotenic acid-induced lesion, panel D, in a deeper area without PKH26 reimplanted cells. Nuclei are counterstained with Dapi (blue). (A: scale bar = 1 mm; B–D: scale bar = 75 μm).

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labeled cells still express low level of nestin (Figs. 4A–C). In the lesioned cortical area, many PKH26-labeled cells expressed the neuronal marker MAP2 and presented a neuronal morphology (Figs. 4D–F). No major gliosis was observed.

Taken together with our previous studies (Brunet et al., 2002), these results confirm that adult brain cells can be obtained, cryopreserved, and kept in culture before being reimplanted in the donor, and survive in vivo. At 3 months, reimplanted cells limit their localization around the excitotoxic lesion. Moreover these cells exhibit neuronal characteristics such as MAP2. Nevertheless, the crucial points that remain to be elucidated are their roles in brain repair when applied in different models of neurological disorders. These attractive perspectives and their potential clinical impact lead us to further develop the field of autotransplantation in the central nervous system.

Materials and methods

Preparation of cultured primate brain cells from adult tissue

Pieces of cerebral cortex were obtained from monkey (*M. fascicularis*) in accordance with ethical committee guidelines and authorization of local veterinary authorities. Biopsy was dissected with a razor blade to obtain enriched fractions of grey matter. Primary cultures were generated by mincing and mechanically triturating the tissue with fire-polished glass pipettes of decreasing diameters. The resulting cell suspensions were counted by diluting cell suspension 1:10 in trypan blue stain (T9520 Sigma). Cells were resuspended at 50,000 cells/ml in RPMI 1640 medium (Gibco) supplemented with $NaHCO_3$ 44 mM, 20% fetal bovine serum (FBS), and an antibiotic/antimycotic cocktail (A7292 Sigma) directly into 25 ml-glass Erlenmeyer at 37°C in a water-saturated atmosphere containing 6.5% CO_2/93.5% air under horizontal agitation at 70 rotations/min. A cell suspension was plated on glass coverslips into a 24-well plate in same incubator condition. Fifteen days later, the concentration of serum was reduced to 10%. After 30–45 days when cells became confluent on coverslip, cells were cultured in medium without serum and were maintained in these conditions.

Re-implantation of cultured primate brain cells into donor brain

Cell aggregates from one flask were pooled by centrifugation at 800 rpm, supernatant was recuperated for resuspension. Aggregates were resuspended in 500 μl diluent C with 5 μM fluorescent viable dyes PKH26 or PKH67 (Sigma) for three min. 500 μl FCS was added for 1 min and aggregates washed three time with RPMI

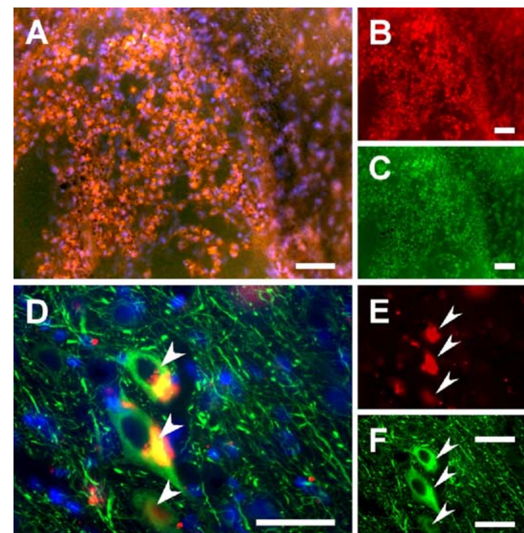

Fig. 4. Immunolabeling of brain section in the ibotenic acid-lesioned motor cortex, 3 months after adult brain cell re-implantation. (A–C) Merged image (A) of PKH26 (red) reimplanted cells (B) that express nestin (green) (C) in the reimplantation site area. (D–F) Merged image (D) of PKH26 (red)-labeled cells (E) that express MAP2 (green) (F) after migration toward the lesioned area. White arrows show three PKH26-positive cells that express MAP2. Nuclei are counterstained with Dapi (blue) (A–F: scale bar = 15 μm).

modified medium. After the last wash, aggregates were resuspended with the recuperated medium previously centrifuged at 4000 rpm to eliminate cellular wastes. Tubes containing fluorescent dye-stained aggregates were completely filled to be transported. For re-implantation aggregates were settled and resuspended in 100 μl. Injection was stereotaxically done with a 100-μl Hamilton glass syringe, cell aggregate suspension was injected by 2 μl/min until 50 μl containing 150,000 cells/site. Into the left motor-lesioned cortex, three sites were injected with PKH26-labeled aggregates. Moreover, three sites were injected with PKH67-labeled aggregates into the normal parietal cortex.

Immunocytochemistry and immunohistochemistry

After sacrifice, monkeys were perfused with paraformaldehyde, brains were then removed, and 8 series of 50-μm-thick sections were prepared with a cryotome. For cell cultures, glass coverslips were fixed with 4% paraformaldehyde in PBS or with acetone. Cell aggregates in suspension were polled by centrifugation, supernatant eliminated, cells were resuspended in 100 μl of medium and drops with resuspended aggregates were placed on a glass slice, dried in a 37°C dry air incubator for 1 h and then fixed with 4% paraformaldehyde in PBS. Brain cryosection, coverslips, or glass slices were incubated in PBS with 0.1% casein and then incubated in PBS 0.3% bovine albumin with antibodies against the following antigens: glial fibrillary acidic protein (GFAP) (monoclonal G3893 Sigma and polyclonal Z0334 Dako, Glostrup, Denmark), nestin (AB 22 polyclonal Chemicon), MAP2 (Sigma). They were then washed and incubated with fluorescent- or horseradish peroxidase-conjugated secondary antibodies. After the last washes, they were revealed for peroxidase activity with diaminobenzidine or mounted in Vectashield® with Dapi to counterstain nuclei before fluorescent microscope or confocal microscope analysis.

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References

Bjorklund, A., Lindvall, O., 2000. Cell replacement therapies for central nervous system disorders. Nat. Neurosci. 3, 537–544.

Brazel, C.Y., Rao, M.S., 2004. Aging and neuronal replacement. Ageing Res. Rev. 3, 465–483.

Brunet, J.F., Pellerin, L., Arsenijevic, Y., Magistretti, P., Villemure, J.G., 2002. A novel method for in vitro production of human glial-like cells from neurosurgical resection tissue. Lab. Invest. 82, 809–812.

Brunet, J.F., Pellerin, L., Magistretti, P., Villemure, J.G., 2003. Cryopreservation of human brain tissue allowing timely production of viable adult human brain cells for autologous transplantation. Cryobiology 47, 179–183.

Freed, C.R., Greene, P.E., Breeze, R.E., Tsai, W.Y., DuMouchel, W., Kao, R., Dillon, S., Winfield, H., Culver, S., Trojanowski, J.Q., et al., 2001. Transplantation of embryonic dopamine neurons for severe Parkinson's disease. N. Engl. J. Med. 344, 710–719.

Haas, S.J., Bauer, P., Rolfs, A., Wree, A., 2000. Immunocytochemical characterization of in vitro PKH26-labelled and intracerebrally transplanted neonatal cells. Acta Histochem. 102, 273–280.

Hernit-Grant, C.S., Macklis, J.D., 1996. Embryonic neurons transplanted to regions of targeted photolytic cell death in adult mouse somatosensory cortex re-form specific callosal projections. Exp. Neurol. 139, 131–142.

Isacson, O., 2003. The production and use of cells as therapeutic agents in neurodegenerative diseases. Lancet Neurol. 2, 417–424.

Johansson, C.B., Momma, S., Clarke, D.L., Risling, M., Lendahl, U., Frisen, J., 1999. Identification of a neural stem cell in the adult mammalian central nervous system. Cell 96, 25–34.

Kempermann, G., Gage, F.H., 1999. New nerve cells for the adult brain. Sci. Am. 280, 48–53.

Lindvall, O., Kokaia, Z., Martinez-Serrano, A., 2004. Stem cell therapy for human neurodegenerative disorders—How to make it work. Nat. Med. 10, S42–S50 (Suppl.)

Liu, Y., Rouiller, E.M., 1999. Mechanisms of recovery of dexterity following unilateral lesion of the sensorimotor cortex in adult monkeys. Exp. Brain Res. 128, 149–159.

McKay, R., 1997. Stem cells in the central nervous system. Science 276, 66–71.

Palfi, S., Conde, F., Riche, D., Brouillet, E., Dautry, C., Mittoux, V., Chibois, A., Peschanski, M., Hantraye, P., 1998. Fetal striatal allografts reverse cognitive deficits in a primate model of Huntington disease. Nat. Med. 4, 963–966.

Palm, K., Salin-Nordstrom, T., Levesque, M.F., Neuman, T., 2000. Fetal and adult human CNS stem cells have similar molecular characteristics and developmental potential. Brain Res. Mol. Brain Res. 78, 192–195.

Park, K., Ourednik, J., Ourednik, V., Taylor, R.M., Aboody, K.S., Auguste, K.I., Lachyankar, M.B., Redmond, D.E., Snyder, E.Y., 2002. Global gene and cell replacement strategies via stem cells. Gene Ther. 9, 613–624 (apy).

Redmond Jr., D.E., 2002. Cellular replacement therapy for Parkinson's disease—Where we are today? Neuroscientist 8, 457–488.

Redmond, D.E.J., Sladek, J.R., Spencer, D.D., 2001. Transplantation of embryonic dopamine neurons for severe Parkinson's disease. N. Engl. J. Med. 345, 146–147.

Richardson, R.M., Fillmore, H.L., Holloway, K.L., Broaddus, W.C., 2004. Progress in cerebral transplantation of expanded neuronal stem cells. J. Neurosurg. 100, 659–671.

Uchida, N., Buck, D.W., He, D., Reitsma, M.J., Masek, M., Phan, T.V., Tsukamoto, A.S., Gage, F.H., Weissman, I.L., 2000. Direct isolation of human central nervous system stem cells. Proc. Natl. Acad. Sci. U. S. A. 97, 14720–14725.

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