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Brief Communication

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

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 reimplantation 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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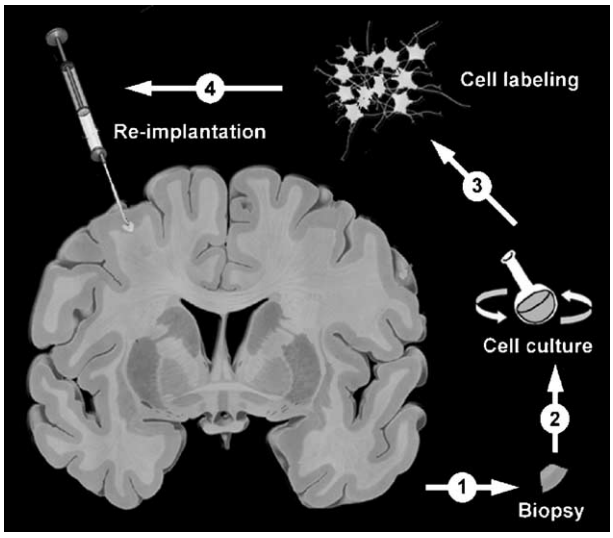

Fig. 1. Scheme of procedure from cortical biopsy to autotransplantation. Schematic representation of the different steps from cortical biopsy to autotransplantation. Pieces of cerebral cortex were obtained from *M. fascicularis* (1). Biopsies were handled as described in supplement to obtain cortical 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-

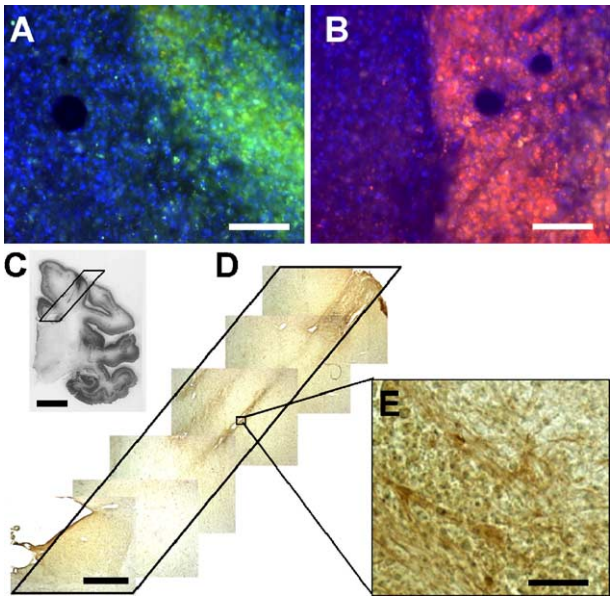

Fig. 2. Brain histology 1 month after adult brain cell re-implantation. (A) PKH67 (green)-labeled monkey autotransplanted brain cells in non-lesioned area. (B) PKH26 (red)-labeled monkey autotransplanted brain cells near the ibotenic acid-induced lesion. (A–C) Nuclei are counter-stained with Dapi (blue). (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).