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Trophic and immunoregulatory properties of neural precursor cells: Benefit for intracerebral transplantation

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ABSTRACT

Intracerebral xenotransplantation of porcine fetal neuroblasts (pNB) is considered as an alternative to human neuroblasts for the treatment of neurodegenerative diseases. However, pNB are systematically rejected, even in an immunoprivileged site such as the brain. Within this context, neural stem/precursor cells (NSPC), which were suggested as exhibiting low immunogenicity, appeared as a useful source of xenogeneic cells. To determine the advantage of using porcine NSPC (pNSPC) in xenotransplantation, pNB and pNSPC were grafted into the striatum of rats without immunosuppression. At day 63, all the pNB were rejected while 40% of the rats transplanted with pNSPC exhibited large and healthy grafts with numerous pNF70-positive cells. The absence of inflammation at day 63 and the occasional presence of T cells in pNSPC grafts evoked a weak host immune response which might be partly due to the immunosuppressive properties of the transplanted cells. T cell proliferation assays confirmed such a hypothesis by revealing an inhibitory effect of pNSPC on T cells through a soluble factor. In addition to their immunosuppressive effect, in contrast to pNB, very few pNSPC differentiated into tyrosine hydroxylase-positive neurons but the cells triggered an intense innervation of the striatum by rat dopaminergic fibers coming from the substantia nigra. Further experiments will be required to optimize the use of pNSPC in regenerative medicine but here we show that their immunomodulatory and trophic activities might be of great interest for restorative strategies. This article is part of a Special Issue entitled "Interaction between repair, disease, & inflammation."

Introduction

Neural transplantation is a promising strategy to restore cell function in the human central nervous system (CNS). However, the limited access to human fetal neurons and the ethical concerns regarding their use have fuelled a search for alternative sources of transplantable cells. Among these, cells derived from pig embryos are of great interest (Isacson and Deacon, 1996; Isacson et al., 1995). Fetal pig neurons have the capacity to develop large axons (Deacon et al., 1994) and small-scale clinical trials indicate that neural cells isolated from porcine fetal brains integrate the host tissue after their transplantation into the brain of immunosuppressed patients (Deacon et al., 1997; Fink et al., 2000; Pakzaban and Isacson, 1994). For this reason, fetal porcine neurons have numerous advantages in addition to their wide availability. Their use as donor cells is however highly limited by the host immune response. Indeed, as previously shown for other intracerebral xenografts (Duan et al., 1995; Finsen et al., 1988; Lund et al., 1989; Pollack et al., 1990; Widner and Brundin, 1988), fetal porcine neurons implanted into the brain of immunocompetent rat are systematically rejected. This occurs within 5–7 weeks post-transplantation (Barker et al., 2000; Michel et al., 2006; Remy et al., 2001). The rejection process is accompanied by an infiltration of the graft by microglial and dendritic cells, and a sudden appearance of T lymphocytes (Barker et al., 2000; Michel et al., 2006; Remy et al., 2001). This coincides with a marked accumulation of transcripts encoding monocyte chemoattractants such as MCP-1 and RANTES, as well as proinflammatory lymphokines and Th1 cytokines (Barker et al., 2000; Melchior et al., 2002; Remy et al., 2001). Continuous exposure to high doses of cyclosporine A or treatments with several immunosuppressors prolong the survival of intracerebral xenografts (Cicchetti et al., 2003; Deacon et al., 1997; Fink et al., 2000; Jacoby

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et al., 1999; Larsson et al., 2000, 2001; Pedersen et al., 1997) but few xenogeneic neurons survive and systemic treatments with immunosuppressors produce severe secondary effects (Rezzani, 2006). Progress has therefore to be made in order to control cell rejection even in a relatively immunoprivileged site such as the CNS (Barker and Widner, 2004).

Genetic modifications of porcine neurons are currently performed to evaluate the advantage of a local production of immunosuppressive molecules such as CTLA4-Ig (Martin et al., 2005). Transplantation of multipotent stem cells is another alternative. Xenogeneic mesenchymal stem cells or expanded neural precursors show long-term survival in the brain of immunocompetent animals (Armstrong et al., 2001; Rossignol et al., 2009). Such lengthy survival has been partially attributed to the low immunogenicity of multipotent cells (Armstrong et al., 2001; Odeberg et al., 2005), but recent evidence points to the sizeable immunosuppressive effect of mesenchymal stem cells (for review, see Di Nicola et al., 2002; Krampera et al., 2003; Rasmusson, 2006; Uccelli et al., 2007). Neural precursor cells (NSPC) may also display such immunoregulatory properties. In 2005, Pluchino et al., showed that syngeneic NSPC systemically injected in a mouse model of multiple sclerosis promoted neuroprotection by immunomodulatory mechanisms (Pluchino et al., 2003, 2005). If porcine NSPC (pNSPC) exhibit such immunoregulatory properties, their use would be of great interest for restorative strategies. In fact, NSPC derived from fetal or adult pig brains could be easily expanded upon treatment with bFGF, providing an indefinite source of transplantable cells. Like human NSPC, porcine NSPC are able to generate the three major neural lineages–oligodendrocytes, astrocytes and neurons—both in vitro and following transplantation in vivo (Harrower et al., 2006; Smith and Blakemore, 2000). In addition, experimental work on immunosuppressed rats has shown a good differentiation of pNSPC into neurons with the formation of synapses and the extension of fibers (Harrower et al., 2006).

In the present paper, we show that pNSPC are a valuable source of donor cells, displaying immunosuppressive properties while also producing a trophic effect upon the host dopaminergic system.

Materials and methods

Cell preparation

Porcine embryos were obtained from Large White domestic pigs, 28 days after artificial insemination. Animals were obtained from the Institut National de la Recherche Agronomique (INRA, Nouzilly, France) and killed in the institute's accredited slaughterhouse. After hysterectomy, the embryos were collected and washed in Hank's balanced salt solution (HBSS; Life Technologies Ltd, CergyPontoise, F) before dissection of the brain tissue.

Porcine neuroblasts (pNB) were prepared from G28 ventral mesencephalon as previously described (Remy et al., 2001). After dissection, the pieces of brain tissues were kept in a hibernation medium for up to 3 days. One hour before intracerebral transplantation and freed of meninges, the brain tissues were incubated in 0.1% trypsin (Sigma-Aldrich, Lyon, F) for 20 min at 37 °C. After addition of 10% FCS (Sigma-Aldrich), tissues were washed and incubated for 10 min at 37 °C in HBSS supplemented with 0.01% DNAse (Sigma-Aldrich). Cells were dissociated by gentle trituration, and centrifuged at 750 RPM for 10 min. The pellet was resuspended in HBSS containing 0.01%. DNAse. The viability was assessed by eosin exclusion and the cell suspension was adjusted to a concentration of 2×10^5 cells/µl.

pNSPC were prepared and analyzed from G28 forebrains as previously described (Sergent-Tanguy et al., 2006). Briefly, brain tissues freed of meninges were incubated with 0.25% trypsin for 15 min at 37 °C. Following addition of 10% fetal calf serum (FCS), tissues were exposed to 0.01% of DNase I prior to mechanical trituration. Aggregates were removed by decantation and cells were centrifuged at 750 RPM for 10 min. pNSPC were plated in uncoated dishes in basal culture medium composed of Dulbecco's Modified Eagle Medium (DMEM) and Ham's F12 (1/1, v/v) (Life technologies Ltd.) with 33 mM glucose, 5 mM HEPES (pH 7.2), 2 mM L-glutamine, 5 µg/ml streptomycin and 5 UI/ml penicillin (AS-basal medium), supplemented with 10% FCS (AS-FCS medium). The following day, the floating cells were recovered, washed and resuspended in AS-basal medium supplemented with N2 (Life technologies Ltd.; AS-N2 medium). The cells were then plated in uncoated dishes and expanded as neurospheres for 10 days in the presence of 25 ng/mL basic fibroblast growth factor (bFGF) with a complete change of the N2 medium after 5 days of culture, and addition of bFGF every 3 days. One hour prior to intracerebral transplantation, the cells were mechanically dissociated and adjusted to a concentration of 2×10^5 cells/µl as described for the porcine neuroblasts. To analyze the fate of pNSPC *in vitro*, mechanically dissociated neurospheres were transferred to poly-L-ornithine-coated (PORN, 50 µg/ml, Sigma-Aldrich) glass coverslips and incubated in AS-FCS medium overnight. The following day, the medium was changed and the cells were allowed to differentiate for 7 days in AS-N2 medium (differentiation conditions).

Animals and surgical procedures

Male Lewis 1A rats (250 g) purchased from Janvier CERJ (Rouen, F) were manipulated in compliance with the ethical rules and guidelines of the Institut National de la Santé et de la Recherche Médicale (INSERM). The animals were housed at 22 °C, under 12 h light/12 h dark conditions with ad libitum access to food and water.

Cell transplantation in unlesioned animals

According to a protocol previously described (Michel et al., 2006), 66 unlesioned animals were anesthetized with an intramuscular injection of 2% Rompun (0.3 ml/kg) and 50 mg/ml ketamine (1.3 ml/kg) (PanPharma, Fougères, F) before being held in a stereotaxic frame (Stoelting, Wood Dale, IL, USA). After partial skull removal, pNB or pNSPC were transplanted unilaterally at the following coordinates according to the bregma and the skull surface: anterior, +0.7 mm; lateral, −2.8 mm; ventral, −5.4 mm and −5.8 mm; incisor bar, −3.3 mm. Cells were injected with a 10-µl Hamilton syringe, mounted on an automated microinjector (Phymed, Paris, F). Each site received 1 µl of a suspension at 2×10^5 cells/µl, over a period of 1 min. After 4 min, the syringe was gently withdrawn, the pieces of cranial bone were replaced on the skull, and the skin incisions were sewn. Animals received a total of 400 000 pNSPC or pNB.

At the indicated times (Table 2), the rats were deeply anesthetized to perform transcardiac perfusion with 100 ml of 0.9% NaCl, followed by 250 ml of cold 4% paraformaldehyde (PFA) in phosphate-buffered saline pH 7.4 (PBS). Brains were cryoprotected by a successive immersion in 15% and 30% sucrose. Following this, they were gently frozen in cold isopentane (−35 °C) and stored at −80 °C.

Cell transplantation in 6-OH-dopamine-lesioned animals

To clarify the origin of the TH^+ fibers, nigral dopaminergic neurons were lesioned by injecting the neurotoxin 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle of 2 animals. The rats anesthetized with intraperitoneal injection of Rompun/ketamine (1.6 ml/kg) were placed in a stereotaxic frame (Stoelting, Wood Dale, IL). The neurotoxin dissolved in 0.1% ascorbate/saline (3 µg/µl) was then injected unilaterally (0.5 µl/min) as a single dose of 12 µg at the following coordinates (in mm relative to the bregma and the skull surface): anterior (A) = −4.4; lateral (L) = −1.2; ventral (V) = −7.8; tooth bar at −3.3. Efficiency of the lesion was tested two weeks later by counting the amphetamine-induced rotation (5 mg/kg, IP). Both rats exhibited a number of rotations superior to 200 over a period of 40 min. Two weeks later, the two animals were then transplanted bilaterally