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Lineage-restricted neural precursors survive, migrate, and differentiate following transplantation into the injured adult spinal cord

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Abstract

Fetal spinal cord from embryonic day 14 (E14/FSC) has been used for numerous transplantation studies of injured spinal cord. E14/FSC consists primarily of neuronal (NRP)- and glial (GRP)-restricted precursors. Therefore, we reasoned that comparing the fate of E14/FSC with defined populations of lineage-restricted precursors will test the in vivo properties of these precursors in CNS and allow us to define the sequence of events following their grafting into the injured spinal cord. Using tissue derived from transgenic rats expressing the alkaline phosphatase (AP) marker, we found that E14/FSC exhibited early cell loss at 4 days following acute transplantation into a partial hemisection injury, but the surviving cells expanded to fill the entire injury cavity by 3 weeks. E14/FSC grafts integrated into host tissue, differentiated into neurons, astrocytes, and oligodendrocytes, and demonstrated variability in process extension and migration out of the transplant site. Under similar grafting conditions, defined NRP/GRP cells showed excellent survival, consistent migration out of the injury site and robust differentiation into mature CNS phenotypes, including many neurons. Few immature cells remained at 3 weeks in either grafts. These results suggest that by combining neuronal and glial restricted precursors, it is possible to generate a microenvironmental niche where emerging glial cells, derived from GRPs, support survival and neuronal differentiation of NRPs within the non-neurogenic and non-permissive injured adult spinal cord, even when grafted into acute injury. Furthermore, the NRP/GRP grafts have practical advantages over fetal transplants, making them attractive candidates for neural cell replacement.

Keywords: Fetal graft; Neural progenitor; Neural stem cell; Rat; Spinal cord injury

Introduction

Numerous studies over the last two decades have shown that grafting of embryonic day-14 fetal spinal cord tissue (E14/FSC) into the injured spinal cord rescues axotomized neurons from retrograde cell death and atrophy (Bregman and Reier, 1986; Mori et al., 1997), reduces scar formation (Houle, 1992), and promotes host axonal regeneration (Diener and Bregman, 1998b; Tessler et al., 1988). In the adult, host axons penetrate the graft, but do not transverse transplants to reenter host neuropil. Graft-derived fibers also project into host tissue (Jakeman and Reier, 1991; Reier et al., 1986). Although FSC grafts have been successful in improving functional recovery following complete spinal transection of neonates (Diener and Bregman, 1998a; Howland et al., 1995; Miya et al., 1997), little recovery occurs in adult models (Bregman et al., 1993; Stokes and Reier, 1992). Despite potential benefits, the use of fetal transplants has serious shortcoming including issues of supply, storage, cellular heterogeneity, and quality control.

E14/FSC transplants consist primarily of lineage-restricted neural precursors, both neuronal (NRP)- and glial(GRP)-restricted precursors, as well as small populations of multipotent neural stem cells (NSCs) and differentiated cells (Kalyani and Rao, 1998). Previous studies

Abbreviations: AP, human placental alkaline phosphatase; E14/FSC, embryonic day-14 fetal spinal cord; GRP, glial-restricted precursor; NRP, neuronal-restricted precursor; NSC, multipotent neural stem cell.

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have shown that NSCs grafted into non-neurogenic regions of intact and injured adult CNS survive poorly (Lepore et al., 2004) and differentiate mostly into glial cells (Cao et al., 2001). In contrast, lineage-restricted precursors survive for long periods of time and differentiate into neurons and glia, respectively (Han et al., 2002, 2004; Lepore et al., 2004). However, previous studies have raised concerns about the ability of grafted NSCs and NRPs to survive in the toxic environment of the injury site and to differentiate into mature neurons without the necessary instructive environment (Cao et al., 2002). We therefore reasoned that comparing the fate of E14/FSC transplants and transplants of a defined population of lineage-restricted precursors (NRPs and GRPs) in the injured adult spinal cord, using cells derived from alkaline phosphatase (AP) transgenic rats, will allow for elucidating the sequence of events that follows grafting of fetal cells in the injured adult CNS, including survival, migration, and differentiation.

Using a lateral funiculus injury model, we found an early and extensive cell loss following acute transplants of E14/FSC. Surviving precursors expanded to fill the entire lesion by 3 weeks post-transplantation. E14/FSC grafts integrated and extended long processes into host spinal cord, differentiated into neurons, astrocytes, and oligodendrocytes and showed variability in migration out of the transplant site. Grafts of defined NRP/GRP cells filled the cavity without significant early cell loss, differentiated into mature CNS phenotypes and showed consistent cellular migration out of the injury site, even when grafted into the acute injury. This work suggests that mixed lineage-restricted precursor grafts generate a microenvironment that protects the cells from the detrimental effects of the injured CNS and provides them with a permissive niche for survival, differentiation, and migration. Furthermore, NRP/GRP grafts represent a practical alternative to fetal tissue transplants because of the ability to isolate, expand, store, and genetically manipulate these cells.

Methods

Cell isolation and culture

NRPs and GRPs were isolated from embryonic day-13.5 transgenic Fischer 344 rats that express the marker gene, human placental alkaline phosphatase (AP). This transgenic animal has previously been characterized (Kisseberth et al., 1999; Mujtaba et al., 2002). Briefly, embryos were isolated in DMEM/F12 (Invitrogen; Carlsbad, CA). Trunk segments were incubated in collagenase Type I (10 mg/mL; Worthington Biochemicals; Lakewood, NJ)/dispase II (20 ng/mL; Roche Diagnostics; Indianapolis, IN)/HBSS (Cellgro; Herndon, VA) solution for 8 min at room temperature to remove meninges from the cords. Cords were dissociated using a 0.05% trypsin/EDTA (Invitrogen) solution for 20 min at 37°C. Cells were then plated in NRP complete medium [DMEM-F12, BSA (1 mg/mL; Sigma; St. Louis, MO), B27 (Invitrogen), bFGF (10 μg/mL; Peprotech; Rocky Hill, NJ), Pen-Strep (100 IU/mL; Invitrogen), N2 (10 μL/mL; Invitrogen), NT-3 (10 μg/mL; Peprotech)] on poly-L-lysine (13.3 μg/mL; Sigma)- and laminin (20 μg/mL; Invitrogen)-coated dishes.

Preparation of cells for grafting

Following embryonic dissection, NRPs and GRPs were co-cultured for 3–10 days prior to transplantation. A mixed population of NRPs and GRPs was dissociated from culture flasks using 0.05% trypsin/EDTA, washed and re-suspended at a concentration of 200,000 cells/μL [in Type 1 collagen matrix-Vitrogen (Cohesion; Palo Alto, CA)/basal media mixture] for transplantation. Cells were placed on ice during surgery. After completion of surgery, cell viability was assessed using the trypan blue assay. Viability was always greater than 90%. The composition of the NRP/GRP cultures, with respect to the presence of undifferentiated neural precursors devoid of mature cells, was verified before grafting by staining for the immature neural marker nestin, for markers for NRPs (E-NCAM) and GRPs (A_2B_5), as well as for markers of mature cell types.

Preparation of fetal spinal cord tissue (E14/FSC) for grafting

Whole FSC tissue was isolated from embryonic day-14 rats as previously described (Bregman and McAtee, 1993). To obtain labeled tissue, we used transgenic Fischer 344 rats that express the AP marker gene. Briefly, embryos were isolated in DMEM/F12. Trunk segments were dissected, and the surrounding meninges were removed. Square pieces of tissue (1–3 mm) were kept in DMEM-F12 on ice until placed directly into the spinal cord lesion site (approximately 10 min).

Adult spinal cord injury and transplants

Lateral funiculus injuries were created at the cervical 4 spinal cord level. Adult female Sprague–Dawley rats (approximately 250 g) received intraperitoneal injections of anesthetic cocktail [acepromazine maleate (0.7 mg/kg; Fermenta Animal Health, Kansas City, MO), ketamine (95 mg/kg; Fort Dodge Animal Health; Fort Dodge, IA), and xylazine (10 mg/kg; Bayer, Shawnee Mission, KS)]. The back musculature was excised, and a laminectomy was performed at the cervical 3/4 level. The dura was incised above the dorsal root entry zone. Microscissor cuts were created at the rostral and caudal extents of the injury. Aspiration was used to selectively ablate only the lateral white matter tracts, as well as a minimal portion of the dorsal and ventral gray matter. The dorsal columns and central canal were unaffected. Once hemostasis was