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TRANSPLANTATION OF NEURAL PROGENITOR CELLS IN CHRONIC SPINAL CORD INJURY

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Abstract—Previous studies demonstrated that neural progenitor cells (NPCs) transplanted into a subacute contusion injury improve motor, sensory, and bladder function. In this study we tested whether transplanted NPCs can also improve functional recovery after chronic spinal cord injury (SCI) alone or in combination with the reduction of glial scar and neurotrophic support. Adult rats received a T10 moderate contusion. Thirteen weeks after the injury they were divided into four groups and received either: 1. Medium (control), 2. NPC transplants, 3. NPC + lentivirus vector expressing chondroitinase, or 4. NPC + lentivirus vectors expressing chondroitinase and neurotrophic factors. During the 8 weeks post-transplantation the animals were tested for functional recovery and eventually analyzed by anatomical and immunohistochemical assays. The behavioral tests for motor and sensory function were performed before and after injury, and weekly after transplantation, with some animals also tested for bladder function at the end of the experiment. Transplant survival in the chronic injury model was variable and showed NPCs at the injury site in 60% of the animals in all transplantation groups. The NPC transplants comprised less than 40% of the injury site, without significant anatomical or histological differences among the groups. All groups also showed similar patterns of functional deficits and recovery in the 12 weeks after injury and in the 8 weeks after transplantation using the Basso, Beattie, and Bresnahan rating score, the grid test, and the Von Frey test for mechanical allodynia. A notable exception was group 4 (NPC together with chondroitinase and neurotrophins), which showed a significant improvement in bladder function. This study underscores the therapeutic challenges facing transplantation strategies in a chronic SCI in which even the inclusion of treatments designed to reduce scarring and increase neurotrophic support produce only modest functional improvements. Further studies will have to identify the combination of acute and chronic interventions that will augment the survival and efficacy of neural cell transplants.
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Key words: neural stem cell transplantation, chronic spinal cord injury, motor and sensory function, bladder function.

INTRODUCTION

It is estimated that the annual incidence of spinal cord injury (SCI) is approximately 12,000 new cases each year (NSCISC, 2013). Injury to the spinal cord results in extensive axonal damage and degeneration, neuronal loss, and severe functional deficits. Three phases occur after SCI: acute (hours to days), sub-acute (days to weeks), and chronic (months to years). Many repair strategies have been proposed and tested in models of acute and sub-acute SCI such as tissue protection using corticosteroids and gangliosides (Hurlbert et al., 2013), anti-inflammatory reagents (Hawthorne and Popovich, 2011; Ren and Young, 2013), factors to promote axonal regeneration such as neurotrophic factors, blockage/removal of inhibitory factors, and tissue/cell transplantation (Cafferty et al., 2007; Hollis and Tuszynski, 2011; Tetzlaff et al., 2011; McCall et al., 2012; Cregg et al., 2014). Indeed, cell transplantation has been shown to be one of the most promising strategies to promote functional recovery in acute and sub-acute SCI. Transplants of Schwann cells, olfactory ensheathing cells, genetically modified fibroblasts, and various neural stem cells have demonstrated some axonal regeneration and partial functional recovery (Murray et al., 2002; Tuszynski et al., 2003; Oudega and Xu, 2006; Barnett and Riddell, 2007; Lopez-Vales et al., 2007; Louro and Pearse, 2008; Deng et al., 2013). Only a few studies, however, have addressed chronic SCI due to the experimental complexities and the daunting challenges associated with axonal growth through a chronic injury (Jin et al., 2002; Barakat et al., 2005; Lu et al., 2007; Tom et al., 2009; Karimi-Abdolrezaee et al., 2010). The glial and extracellular scar, which is formed within days after SCI, becomes well established after several weeks. This “chronic scar,” together with the formation of cystic cavities, creates a greater impediment to axonal regeneration at the chronic stage than immediately after injury. Some studies have shown that it is possible to achieve some axonal growth and even bridge the injury site using combination therapies (Barakat et al., 2005; Lu et al., 2007; Tom et al., 2009). Transplants of modified fibroblasts expressing BDNF in a chronic cervical hemisection lesion induced some supraspinal neurons to regenerate their axons into the transplants, but few bridged the injury site and re-entered the host (Jin et al.,

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Abbreviations: AP, alkaline phosphatase; BBB, Basso, Beattie, and Bresnahan; CGRP, calcitonin gene-related peptide; EUS, external urethral sphincter; MSCs, marrow stromal cells; NPCs, neural progenitor cells; NVC, non-voiding contraction; SCI, spinal cord injury; VFH, Von Frey hair; VR-1, vanilloid receptor type 1.

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2002). Similarly, transplants of modified bone marrow stromal cells (MSCs) expressing NT-3 promoted sensory axon growth through the chronic scar and into the cell graft but did not result in axon growth beyond the lesion (Lu et al., 2007). Combination treatments have shown that modifying the intrinsic growth state of neurons by using conditioning lesions, combined with introduction of genetically modified cells that provide neurotrophins and a gradient of neurotrophins rostral to the lesion site, can induce bridging of sensory axons one year after SCI (Kadoya et al., 2009). All data indicate that axonal regeneration through the lesion area and into host tissue at both the acute and chronic stages of SCI remains a significant challenge.

Our previous studies have demonstrated that transplants of neural progenitor cells (NPCs) into the acute and subacute injured spinal cord survive, generate neurons and glial cells and form a functional relay to connect the lesioned sensory pathway of dorsal column axons (Bonner et al., 2011) and improve function following a contusion injury (Mitsui et al., 2005). In the present study, we applied these approaches to the chronic SCI model and examined whether transplants of NPCs, together with lentivirus vectors expressing chondroitinase (Chase/LV) and lentivirus vectors expressing chondroitinase and growth factor treatments (Chase/LV + BDNF/NT-3/LV) could survive, generate neural cells, and improve functional recovery.

EXPERIMENTAL PROCEDURES

Animals

Adult female rats (225–250 g, Taconic, Germantown, NY, USA) were used in this study. They were housed in an environmentally controlled facility with a 12-h light/dark cycle. Food and water were available *ad libitum*. All procedures were approved by the Institutional Animal Care and Use Committee of Drexel University College of Medicine and were carried out according to the *NIH Guide for the Care and Use of Laboratory Animals.* Fig. 1 shows the outline of experimental design (A) and experimental time line (B).

Surgical procedure

Forty-five rats were used in this study. Thirty-nine rats received a contusion lesion and were divided into four groups (Table 1): Medium ($n = 9$), NPC transplant (NPC, $n = 10$), NPC transplant with Chase/LV (N/C, $n = 10$), NPC transplant with Chase/LV + BDNF/NT-3/LV (N/C/G, $n = 10$). Two rats from N/C/G were sacrificed early due to autophagia.

A laminectomy was performed at T10 after anesthesia with XAK containing Xylazine (6 mg/ml), Acepromazine maleate (0.05 mg/ml), and Ketamine (63 mg/ml) injected intraperitoneally. A contusion lesion was made with the NYU impactor (10 g, 25 mm). Muscle and skin were closed in layers. Thirteen weeks after contusion, animals were re-anesthetized with XAK and placed in a spinal stereotaxic frame and the lesioned region re-exposed without opening the dura. Using a 10-ul Nanofil syringe (World Precision Instruments, Sarasota, FL, USA) with a 33-gauge needle, HBSS (10 μl) or cells (10 μl, 1×10^5 cell/μl) were injected into the lesion center along the midline (4 μl), and rostral and caudal to the lesion along the midline (3 μl/each). HBSS or cells were injected at 20 nl/s using a nanoliter pump controller (World Precision Instruments, Sarasota, FL, USA). One minute after injection, the tip was slowly withdrawn. Rats were placed back in their cages on heating pads and closely observed until awakening. One week after transplantation, rats in the N/C and N/C/G groups received an additional injection of virus with the same procedure as the cell transplants. In the N/C group, rats received Chase/LV injected at lesion/transplant center and 1 mm rostral to caudal to the lesion (1 μl/injection, 2.0×10^6 TU/μl) at midline. In the N/C/G group, rats received Chase/LV injections similar to the N/C group (within the lesion as well as 1 mm rostral and caudal to the lesion), and also

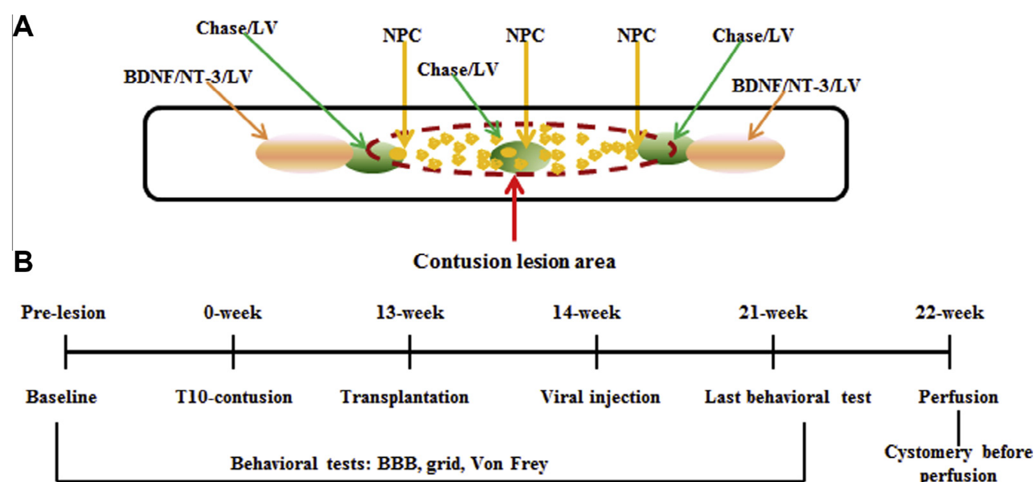

Fig. 1. Outline of experimental design, time line, and groups. Panel A shows the location of transplanted NPCs and viral injection. Panel B shows the time line. Four experimental groups were used in this study: Medium (Control, $n = 9$); NPC ($n = 10$); N/C (NPC + Chase/LV, $n = 10$); N/C/G (NPC + Chase/LV + BDNF/NT-3/LV, $n = 8$).

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