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COMPRESSION INJURY IN THE MOUSE SPINAL CORD ELICITS A SPECIFIC PROLIFERATIVE RESPONSE AND DISTINCT CELL FATE ACQUISITION ALONG ROSTRO-CAUDAL AND DORSO-VENTRAL AXES

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14 **Abstract—Harnessing the regenerative capabilities of endogenous precursor cells in the spinal cord may be a useful tool for clinical treatments aimed at replacing cells lost as a consequence of disease or trauma. To better understand the proliferative properties and differentiation potential of the adult spinal cord after injury, we used a mouse model of compression spinal cord injury (SCI). After injury, adult mice were administered BrdU to label mitotic cells and sacrificed at different time-points for immunohistochemical analysis. Our data show that the rate of proliferation increased in all regions of the spinal cord 1 day after injury, peaked after 3 days, and remained elevated for at least 14 days after injury. Proliferation was greater at the injury epicenter than in rostral and caudal adjacent spinal segments. The number of proliferative cells and rate of proliferation varied between dorsal and ventral regions of the spinal cord and between the gray and white matter. Newly generated cells expressed markers for progenitor cells (Nestin and Olig2), oligodendrocytes (Sox10), astrocytes (S100b and glial fibrillary acidic protein), and microglia (Iba1), but not neuronal markers (Map2 and NeuN). Marker expression varied with regard to the dorso-ventral region, rostro-caudal proximity to the injury epicenter, and time after injury. At early time-points after injury, BrdU^{+} cells mainly expressed microglial/macrophage and astrocytic markers, while at these same time-points in the control spinal cord the mitotic cells predominately expressed oligodendrocyte and oligodendrocyte progenitor cell markers. The profile of proliferation and cell fate marker expression indicates that after moderate compression, the spinal cord has the capacity to generate a variety of glial cells but not neurons, and that this pattern is space and time specific. Future studies should focus on ways to control proliferation and cell fate after injury to aid the development of cell replacement treatments for SCI.** **© 2013 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: spinal cord injury, precursor cells, proliferation, cell fate.

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Q2 *Abbreviations:* BMS, Basso Mouse Scale; CC, central canal; DGM, dorsal gray matter; DWM, dorsal white matter; GFAP, glial fibrillary acidic protein; PBS, phosphate buffered saline; PFA, paraformaldehyde; SCI, spinal cord injury; VGM, ventral gray matter; VWM, ventral white matter.

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INTRODUCTION

After spinal cord injury (SCI), a process of neuronal and glial cell death begins immediately (Grossman et al., 2001). This process also induces the release of cytokines, growth factors, and cytotoxic amino acids (Zai et al., 2005; Eftekharpour et al., 2008; Ruff et al., 2008), resulting in further cell death and damage. The consequences of SCI include impairments in sensation and motor function and a host of clinical complications (Hawryluk et al., 2008). Despite the initial degenerative responses, the mammalian spinal cord is capable of some repair through endogenous mechanisms such as axonal sprouting (Weidner et al., 2001; Menet et al., 2003; Fouad et al., 2004; Liebscher et al., 2005; Gensel et al., 2006; Okano et al., 2007) and proliferation of precursor cells (Vaquero et al., 1981, 1987; Johansson et al., 1999; Namiki and Tator, 1999; Horner et al., 2000; McTigue et al., 2001; Takahashi et al., 2003; Mothe and Tator, 2005; Zai et al., 2005; Zai and Wrathall, 2005; Horky et al., 2006; Lytle and Wrathall, 2007; Meletis et al., 2008; Sellers et al., 2009; Barnabe-Heider et al., 2010). (Q3; lines 17–37)

Previous studies reported the existence of ependymal cell proliferation after SCI (Vaquero et al., 1981, 1987; Beattie et al., 1997; Mothe and Tator, 2005; Meletis et al., 2008; Barnabe-Heider et al., 2010) More recent studies have focused on proliferation within other regions of the spinal cord, particularly the subpial white matter (Horner et al., 2000; Petit et al., 2011). As there may be two, or more, populations of cells responding to injury, the precise identity and niche of precursor cells remain unclear. We know that glial progenitor cells, which proliferate in both intact and injured spinal cord tissue, are scattered throughout the spinal cord and contribute to the recovery of oligodendrocyte and astrocyte numbers after injury (Horner et al., 2000; Barnabe-Heider et al., 2010). The potential to modulate the proliferation and differentiation of endogenous precursor cells represents an attractive therapeutic (lines 38–54)

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target for SCI. Although recent human clinical trials have demonstrated safe transplantation of stem cells into the human spinal cord (Karussis et al., 2010; Mazzini et al., 2010; Glass et al., 2012), the introduction of allogeneic stem cells still raises safety and ethical concerns. The capacity to modify endogenous precursor cell properties is thus a good alternative to stem cell transplantation.

Previous studies on progenitor cell properties after SCI have quantified levels of cell proliferation within specific areas of the spinal cord or the overall proliferation of specific progenitor cell types. In the current study, we investigated the level of proliferation and cell fate of newly generated cells within specific anatomical regions – dorsal and ventral portions of the white and gray matter – and specific segments – rostral and caudal to the injury site. Distinct cell populations, located within different regions of the spinal cord, fulfill specific functions. For example, the dorsal gray matter (DGM) is populated by sensory interneurons whereas the ventral gray matter (VGM) contains the motor neurons, and both regions contain glial cells (Watson et al., 2009). The white matter of the spinal cord consists primarily of oligodendrocytes and bundles of axons organized into distinct tracts with specific functions (Watson et al., 2009). Specific cell populations may be the result of distinct pools of precursor cells with distinct differentiation potentials, thus generating differential responses across anatomical regions of the spinal cord. Successful regenerative therapies will need to account for the anatomical organization of the spinal cord and the unique needs of each region for repair.

Prior work in this field has focused on transection or contusion models of SCI, whereas the proliferative response to the lateral compression injury model of SCI has not been previously characterized to the same extent. The compression model of SCI is widely used because of its ease of use, low cost, reproducibility, and pathological features which closely mimic clinical cases of SCI in humans (Plemel et al., 2008; Marques et al., 2009). We hypothesize that precursor cell proliferation after a moderate compressive SCI in mice follows a specific temporal and spatial pattern for proliferation and cell fate. By labeling cells undergoing mitosis in response to injury with BrdU, we tracked precursor cell proliferation levels, and using immunohistochemistry for specific cell markers we determined cell fate and population dynamics, throughout the first 14 days after injury. We observed that proliferation was greater at the injury epicenter than in adjacent rostral and caudal spinal segments. Additionally, we found that at early time-points after injury, $BrdU^{+}$ cells mainly expressed microglial/macrophage and astrocytic markers, while at these same time-points in the control spinal cord, the mitotic cells predominantly expressed markers for mature oligodendrocytes and oligodendrocyte precursors.

EXPERIMENTAL PROCEDURES

Spinal cord compression injury

We anesthetized female Swiss Webster adult mice (25–35 g, 6–8 weeks old; Charles River, Wilmington, MA) with isoflurane (4% to induce and 2% to maintain; Phoenix Pharmaceutical, Inc., Burlingame, CA, USA). After disinfection of the dorsal area between the neck and hind limbs, we made a midline incision to expose the spinal column at the level of T8–T11, and a laminectomy on the 10th thoracic vertebra. We laterally compressed the spinal cord to a thickness of 0.35 mm and held for 15 s using one pair of modified forceps (Plemel et al., 2008). After injury, we sutured the muscles and closed the skin. Post-operatively, we administered saline and prophylactic Baytril (85 mg/kg/day; Bayer) and maintained the animals on an isothermic pad until alert and mobile. The analgesic we administered was pharmaceutical grade bupronepherine (0.05 mg/kg), given every 12 h post-operatively. We expressed the animals' bladders manually twice daily until the animals were capable of self-voiding. We continually evaluated animals for weight loss, dehydration, discomfort, infection, and autophagia, with appropriate veterinary care administered as needed. The experimental study was designed following National Institutes of Health (NIH) guidelines and with the approval of the University of California, Davis Institutional Animal Care and Use Committee (IACUC).

Behavioral testing

Basso Mouse Scale (BMS). The BMS for locomotion was performed as previously described (Basso et al., 1996).

Rotarod. Animals were placed on a Rotamex with a starting speed of 0 rpm. The speed increased by intervals of 0.5 rpm every 5 s and the time that the animal fell off was recorded.

Von Frey hair test. To test for sensory function after injury, we performed the von Frey hair test as previously described (Chaplan et al., 1994). The minimal amount of force, in grams, that elicited a withdrawal response was recorded.

Ethyl chloride test. A spray of ethyl chloride was applied to animal's hind paws. A score of 1 indicated no response; a score of 2 indicated withdrawal and licking of the paw, and sometimes a vocalization; a score of 3 demonstrated a marked response with withdrawal, jumping, and multiple vocalizations. The trial was repeated three times on each paw, with a time period of 5 min between each trial to prevent desensitization to the stimulus.

5-Bromodeoxyuridine labeling

To determine cell proliferation and fate during the acute phase of injury, we injected mice immediately after surgery with 50 mg/kg of body weight BrdU (Sigma) Q4 intra-peritoneally and then administered follow-up injections of BrdU every 12 h until perfusion 1, 3, 5, 9, or 14 days after injury.

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