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Galectin-1 in injured rat spinal cord: Implications for macrophage phagocytosis and neural repair

Andrew D. Gaudet *, David R. Sweet [1], Nicole K. Polinski [2], Zhen Guan, Phillip G. Popovich *

Center for Brain and Spinal Cord Repair, The Ohio State University, Room 670, Biomedical Research Tower, 460W. 12th Ave., Columbus, OH 43210, USA
Department of Neuroscience, Wexner Medical Center, The Ohio State University, Room 670, Biomedical Research Tower, 460W. 12th Ave., Columbus, OH 43210, USA

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ABSTRACT

Galectin (Gal)-1 is a small carbohydrate-binding protein and immune modulatory cytokine that is synthesized locally at the site of peripheral nerve injury. In this environment, Gal1 can promote regeneration of injured peripheral axons, in part by modifying the function of macrophages recruited to the site of injury. Unlike in injured peripheral nerves, macrophages do not promote axon regeneration in the injured central nervous system (CNS), perhaps because Gal1 levels are not regulated appropriately. Because the dynamics and cellular localization of endogenous Gal1 have not been rigorously characterized after CNS injury, we examined the spatio-temporal distribution of Gal1 in rat spinal cords subjected to a standardized contusion injury. Whereas Gal1 was not expressed in uninjured spinal cord, it was significantly upregulated after SCI, especially within the lesion core. Gal1 was expressed in ~40% of lesion-localized macrophages at 3–28 days post-injury (dpi), and in ~45% of astrocytes in the lesion border at 7–28 dpi. Most lesion-localized Gal1+ macrophages did not express the phagocytosis marker ED1, and Gal1+ cells contained less phagocytosed lipids. These data suggest that time- and location-dependent regulation of Gal1 by macrophages (and astrocytes) could be important for modulating phagocytosis, inflammation/gliosis, and axon growth after SCI.

1. Introduction

Galectin-1 (Gal1) is a 14.5 kDa carbohydrate-binding protein (lectin) that promotes regeneration of injured peripheral nervous system (PNS) axons (Gaudet et al., 2005, 2011; McGraw et al., 2004a, 2004b, 2005; Rabinovich et al., 2007). Gal1 enhances PNS regeneration indirectly by acting on non-neuronal cells, particularly macrophages (Horie et al., 2004). Gal1 function is controlled by redox state: when oxidized, Gal1 is a monomer that has little lectin activity and acts similar to a cytokine (as opposed to the dimeric reduced form) (Inagaki et al., 2000). Oxidized Gal1 likely drives axon regrowth by regulating macrophage inflammatory signaling, accumulation, and phagocytosis (Echigo et al., 2010; Gaudet et al., 2009; Horie et al., 2004).

After spinal cord injury (SCI), blood monocytes infiltrate the lesion site then differentiate into inflammatory (M1) macrophages that can exacerbate pathology (secondary damage) (Donnelly and Popovich, 2008; Kigerl et al., 2009). In contrast to macrophages that respond to peripheral tissue damage, inflammatory macrophages in SCI lesions do not phagocytose efficiently and these cells persist indefinitely at sites of injury (Gaudet et al., 2011; Greenhalgh and David, 2014; Herbert et al., 2004; Kigerl et al., 2009; Martinez et al., 2009; Martini et al., 2008; Mosser and Edwards, 2008). These features of CNS macrophages have been implicated in regeneration failure after SCI (McPhail et al., 2004; Pruss et al., 2011; Stirling et al., 2004; Vallieres et al., 2006).

Data from animal models indicate that Gal1 could influence recovery after SCI. Exogenous Gal1 may improve recovery after SCI in mice, possibly through altering astrocyte physiology (Han et al., 2011) or improving axon plasticity (Quinta et al., 2014). Transplantation of Gal1-expressing neural stem cells also improves anatomical and behavioral recovery after SCI in marmosets (Yamane et al., 2010). In these latter studies, Gal1 was added to the injured spinal cord, suggesting that the spatiotemporal regulation of endogenous Gal1 after SCI is not optimal for regulating inflammation or creating a growth permissive environment. Indeed, Gal1 is expressed at low levels in the uninjured adult CNS (Akazawa et al., 2004). Rubrospinal neurons, which do not mount an effective regenerative response, fail to upregulate Gal1 at two weeks post-SCI (McGraw et al., 2004b).

Abbreviations: BDNF, brain-derived neurotrophic factor; CNS, central nervous system; ECM, extracellular matrix; Gal1, galectin-1; GFAP, glial fibrillary acidic protein; −IR, −immunoreactivity; PNS, peripheral nervous system; SCI, spinal cord injury

* Corresponding authors at: Center for Brain and Spinal Cord Repair, The Ohio State University, Room 670, Biomedical Research Tower, 460W. 12th Ave., Columbus, OH 43210, USA.
E-mail addresses: gaudet.6@osu.edu (A.D. Gaudet), popovich.2@osu.edu (P.G. Popovich).
[1] Current address: School of Medicine, Case Western Reserve University, Cleveland, OH, USA.
[2] Current address: Translational Science and Molecular Medicine, Michigan State University, Grand Rapids, MI, USA.

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Here, we perform the first comprehensive quantitative analysis of Gal1 expression and cellular localization after SCI in rat. We show that Gal1 mRNA and protein increase significantly in the lesion epicenter during the first week post-SCI with persistent expression evident in cells within and adjacent to the injury site. In the lesion core, Gal1 is upregulated in macrophages/microglia, mainly those that are non-phagocytic (OX42 +/ED1 −). The inverse relationship between expression of Gal1 and ED1 (a lysosome associated protein) corresponded with fewer Gal1 + cells containing phagocytosed lipids at the peak of the inflammatory response (at 14 dpi). In the lesion border, Gal1 is not significantly elevated in microglia; rather, Gal1 is increased in reactive astrocytes of the glial scar. These data suggest that time- and location-dependent regulation of Gal1 by macrophages (and astrocytes) could be important for modulating phagocytosis, inflammation/gliosis, and axon growth after SCI.

2. Results

2.1. Galectin-1 mRNA and protein are upregulated transiently at the SCI epicenter

To determine how Gal1 mRNA and protein expression change after SCI, spinal cord segments were isolated from naïve, sham, or SCI rats at different times post-injury. Gal1 mRNA expression was significantly increased in the lesion epicenter at 3 days post-injury (dpi) (203% higher than uninjured; Fig. 1a). Gal1 protein exists in an equilibrium between monomeric and dimeric forms. Both Gal1 types increased after SCI (Fig. 1b); dimeric Gal1 increased 360% at 7 dpi, whereas monomeric Gal1 was increased at 7 and 14 dpi compared to uninjured control tissue (270% and 360% higher than uninjured, respectively). Therefore, a monophasic increase in expression of both Gal1 mRNA and protein occurs at the lesion epicenter between 7 and 14 d post-SCI.

2.2. Galectin-1 increases in cells and matrix surrounding the spinal contusion lesion

Immunohistochemistry was used to document the temporal and spatial distribution of Gal1 immunoreactivity (IR) in cells throughout intact and injured spinal cord (Fig. 2). Compared with uninjured spinal cord (Fig. 2k), Gal1-IR increased significantly in lesioned tissue by 7 dpi (Fig. 2a–e, l) then continued to increase throughout the rostro-caudal extent of the lesion until 14 dpi (Fig. 2l) after which Gal1-IR decreased toward baseline/uninjured control levels at 28 dpi (Fig. 2f–j, l). Gal1-IR was significantly increased in cells and tissue directly adjacent to the lesion (glial scar) at all timepoints (Fig. 2i (inset), m, n).

In uninjured spinal cords, primary afferent terminals in the superficial dorsal horn, motor neurons in the ventral horn and axons throughout the white matter were Gal1-IR (Fig. 3a′–d′). By 7 dpi, most Gal1-IR was found in inflammatory cells, predominantly macrophages (Fig. 3e′–h′), although Gal1-IR also increased in fusiform cells, presumably astrocytes (arrowheads, Fig. 3f′, f″; see also Fig. 5), nearby the lesion and in central canal ependyma (arrows, Fig. 3g′, g″).

2.3. Galectin-1 immunoreactivity is increased and sustained in a subset of macrophages/microglia within the lesion epicenter

Although Gal1 was undetectable in microglia in the uninjured spinal cord (Fig. 4a, b), Gal-IR increased markedly in activated OX42 + macrophages/microglia after SCI (Fig. 4c–j). A high density of Gal1-IR cells was found in the lesion epicenter at all timepoints examined. To quantify Gal1 expression in lesion-localized macrophages/microglia, OX42 + cells within the lesion epicenter were circled, then area and average Gal1-IR intensity per macrophage were quantified. The OX42 antibody recognizes CD11b/c (Tamatani et al., 1991). Compared with microglia in uninjured tissue, macrophages/microglia in the lesion had significantly higher average Gal1-IR intensity at all timepoints (Fig. 4k, l).

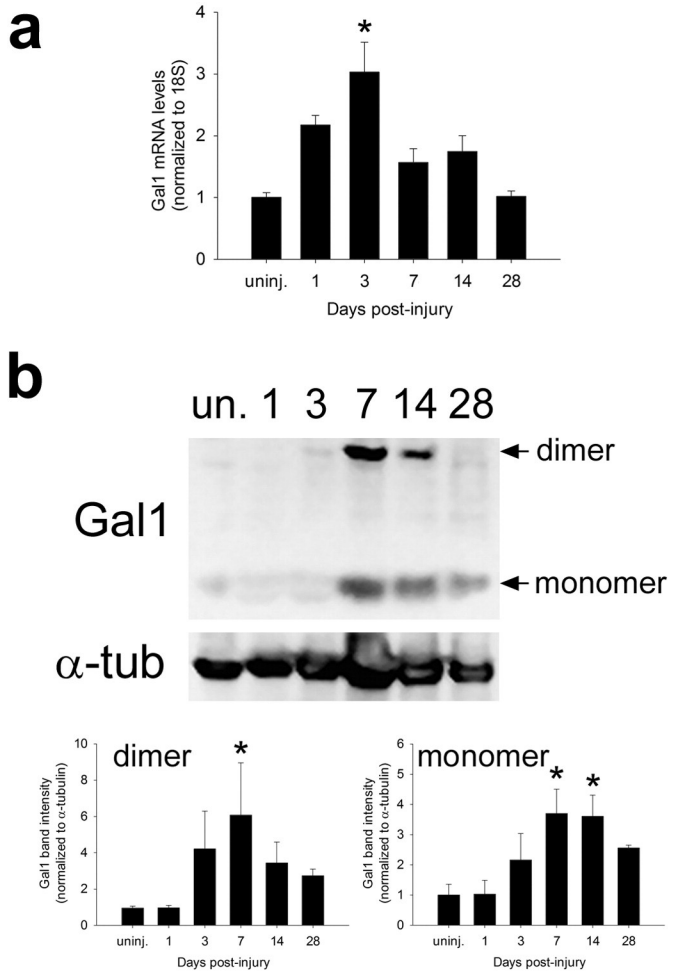

Fig. 1. Gal1 mRNA and protein increased in the lesion epicenter after moderate SCI. (a) Gal1 mRNA levels, assessed using qRT-PCR, increased 200% at 3 dpi compared to uninjured spinal cord. (b) Gal1 protein, assessed using Western blots, also increased after SCI: Gal1 dimer (~29 kDa) increased significantly at 7 dpi, while expression of Gal1 monomer (14.5 kDa) increased at 7 and 14 dpi compared with uninjured spinal cord. Gal1 bands were normalized to α-tubulin (α-tub) expression; uninj (uninjured). *$p < 0.05$ vs. uninjured.

Although macrophage density and size changed over time (macrophages in 3 dpi lesions were more sparsely distributed and smaller than those at later times), 30–40% of macrophages were Gal1 + at all post-injury timepoints examined (compared to 1.2% of microglia in uninjured spinal cord). At later times post-injury (14, 28 dpi), Gal1-IR was reduced and more diffuse in large multi-nucleated macrophages with phagocytic inclusions (Fig. 4g–j). These data indicate that activated macrophages/microglia in the lesion express higher levels of Gal1 than do parenchymal microglia, and that the proportion of Gal1 + macrophages in the lesion remains constant between 3–28 dpi.

2.4. Astrocytes, but not microglia, express galectin-1 in the lesion border

As shown in Fig. 1, Gal1-IR was increased in cells in the glial scar. Confocal microscopy was used to reveal the identity of Gal1-expressing cells in the penumbral tissue (Fig. 5). OX42 + parenchymal microglia, even those closer to the lesion, express low levels of Gal1 (Fig. 5a–f, m). Conversely, OX42 + cells associated with blood vessels, presumably perivascular microglia or newly recruited monocytes, were Gal1 + (asterisk, Fig. 5d–f). Overall, Gal1-IR in glial scar OX42 + cells was significantly lower than in lesion-localized microglia/macrophages (Fig. 5m).