Experimental Neurology 271 (2015) 390–400

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Research Paper

Lack of galectin-3 improves the functional outcome and tissue sparing by modulating inflammatory response after a compressive spinal cord injury

Klauss Mostacada [a,c], Felipe L. Oliveira [b], Déa M.S. Villa-Verde [d], Ana Maria Blanco Martinez [b,*]

[a] Laboratório de Neurodegeneração e Reparo, Instituto de Ciências Biomédicas, Universidade Federal do Rio de Janeiro, Avenida Carlos Chagas Filho, 373, CCS, Cidade Universitária, Rio de Janeiro, RJ21941-902, Brazil
[b] Laboratório de Proliferação e Diferenciação Celular, Instituto de Ciências Biomédicas, Universidade Federal do Rio de Janeiro, Avenida Carlos Chagas Filho, 373, CCS, Cidade Universitária, Rio de Janeiro, RJ21941-902, Brazil
[c] Laboratório de Neurodegeneração e Reparo, Faculdade de Medicina, Departamento de Patologia, HUCFF, Universidade Federal do Rio de Janeiro, Avenida Carlos Chagas Filho, 373, CCS, Cidade Universitária, Rio de Janeiro, RJ21941-902, Brazil
[d] Laboratório de Pesquisas sobre o Timo, Instituto Oswaldo Cruz, Fiocruz, Avenida Brasil, 4365, Pavilhão Leônidas Deane, sala 510, Manguinhos, Rio de Janeiro, RJ21040-360, Brazil

ARTICLE INFO

Article history:
Received 3 June 2015
Received in revised form 4 July 2015
Accepted 7 July 2015
Available online 14 July 2015

Keywords:
Spinal cord injury (SCI)
Galectin-3
Neuroinflammation
Neutrophils
Microglia
Macrophages
Mice

ABSTRACT

Spinal cord injury (SCI) is a traumatic event that results in motor, sensitive or autonomic function disturbances, which have direct impact on the life quality of the affected individual. Recent studies have shown that attenuation of the inflammatory response after SCI plays a key role in the reestablishment of motor function. Galectin-3 is a pleiotropic molecule belonging to the carbohydrate-ligand lectin family, which is expressed by different cells in different tissues. Studies have shown that galectin-3 induces the recruitment and activation of neutrophils, monocytes/macrophages, lymphocytes and microglia. Thus, the aim of this study was to evaluate the effects of the lack of galectin-3 on the functional outcome, cellular recruitment and morphological changes in tissue, after SCI. C57BL/6wild-type and galectin-3 knockout mice were used in this study. A vascular clip was used for 1 min to generate a compressive SCI. By BMS we detected that the Gal-3$^{-/-}$ presented a better functional outcome during the studied period. This finding is related to a decrease in the injury length and a higher volume of spared white matter at 7 and 42 days post injury (dpi). Moreover, Gal-3$^{-/-}$ mice showed a higher number of spared fibers at 28 dpi. Because of the importance of the inflammatory response after SCI and the role that galectin-3 plays in it, we investigated possible differences in the inflammatory response between the analyzed groups. No differences in neutrophils were observed 24 h after injury. However, at 3 dpi, the Gal-3$^{-/-}$ mice showed more neutrophils infiltrated into the spinal tissue when compared with the WT mice. At this same time point, no differences in the percentage of the CD11b/Arginase1 positive cells were observed. Remarkably, Gal-3$^{-/-}$ mice displayed a decrease in CD11b staining at 7 dpi, compared with the WT mice. At the same time, Gal-3$^{-/-}$ mice presented a more prominent Arginase1 stained area, suggesting an anti-inflammatory cell phenotype. Taken together, these results demonstrated that the lack of galectin-3 plays a key role in the inflammatory process triggered by SCI, leading to better and early recovery of locomotor function.

1. Introduction

Spinal cord injury (SCI) is a traumatic event that results in sensitive, motor and autonomic dysfunction that has direct impact on the quality of life of affected individuals (Tetreault, 2014). Understanding of the changes in the spinal cord environment after a trauma is necessary, in order to develop strategies that aim to promote proper functional recovery. During the last few decades, evidence has accumulated showing that the inflammatory response elicited after SCI plays a pivotal role during the course of the initial trauma (Caroleo et al., 2001; David and Kroner, 2011; Elkabes and Black, 1996; Gensel and Zhang, 2015; Kigerl et al., 2009; Lee et al., 2010, 2011; Ousman and Kubes, 2012; Popovich et al., 1999; Shechter et al., 2009; Stirling et al., 2009; Taoka et al., 1997).

Neutrophils are the first cells recruited to the inflamed tissue, reaching their peak level 24 h after injury and decreasing to a non-significant level 7 days post injury (dpi) (Stirling and Yong, 2008). Although the precise function of neutrophils after SCI is still unknown, there is supportive data showing that neutrophils can play both

* Corresponding author at: Laboratório de Neurodegeneracão e Reparo, Instituto de Ciências Biomédicas, Universidade Federal do Rio de Janeiro, Avenida Carlos Chagas Filho, 373, CCS, Cidade Universitária, Rio de Janeiro, RJ 21941-902, Brazil.
E-mail addresses: klaussmostacada@histo.ufrj.br (K. Mostacada), felipe@histo.ufrj.br (F.L. Oliveira), dvv@ioc.fiocruz.br (D.M.S. Villa-Verde), martinez@histo.ufrj.br (A.M.B. Martinez).

http://dx.doi.org/10.1016/j.expneurol.2015.07.006
0014-4886/

detrimental and beneficial roles after a spinal trauma (Stirling et al., 2009; Taoka et al., 1997). These cells secrete large amounts of pro-inflammatory cytokines (IL-1β, 1IL-6 and TNF-α) and chemokines (MIP-1, MCP-1 and IL-8) (Jin et al., 2010), amplifying the inflammatory response, leading to deleterious effects by the recruitment of inflammatory cells and/or release of proteolytic enzymes such as metalloproteinases and elastase (Korkmaz et al., 2010; Mautes et al., 2000; Noble et al., 2002; Semple et al., 2013; Yong, 2005).

Unlike neutrophils, microglia/macrophages (macrophages) have been extensively studied, but their beneficial/detrimental functions after SCI are still under debate. Macrophages are able to produce and secrete large amounts of neurotrophic factors in vitro and in vivo (Caroleo et al., 2001; Elkabes and Black, 1996). However, when activated, these cells are able to secrete pro-inflammatory cytokines such as TNF-α, IL-1, free radicals, nitrogen metabolites, reactive oxygen species and proteases (David and Kroner, 2011; Gensel and Zhang, 2015; Kigerl et al., 2009; Popovich et al., 1999). Depletion of macrophages has shown to improve functional recovery after SCI in rats (Gris et al., 2004; Popovich et al., 1999). Nevertheless, these cells are part of an orchestrated inflammatory repertoire and their differentiation into harmful cells or into restorative cells, depends on signals present in the injured tissue (David and Kroner, 2011; Gensel and Zhang, 2015; Kigerl et al., 2009), thus, leading to a concept of M1 (classic activated macrophages) and M2 (alternatively activated macrophages) phenotypes, which can exert both pro- and anti-inflammatory functions, respectively (Gordon, 2003).

Among the molecules that have effects on inflammation, galectins have raised great interest in studies related to nervous system disorders. Galectin-3 is a pleiotropic multifunctional molecule involved in cell activation and chemotaxis, cell growth and differentiation, apoptosis, cell cycle, cell adhesion. It assumes an important position as an inflammatory regulator (Dumic et al., 2006), exerting its effects on innate and on adaptive immune cells (Almkvist and Karlsson, 2002; Farnworth et al., 2008; MacKinnon et al., 2008; Nieminen et al., 2005; Oliveira et al., 2009; Sano et al., 2003; Villa-Verde et al., 2002). In vitro, galectin-3 induces the expression of L-selectin and IL-8 by neutrophils, suggesting that it can modulate neutrophil migration and activation (Nieminen et al., 2005). Galectin-3 is also involved in the recruitment of monocytes/macrophages from the blood stream to the inflamed tissue through interactions with laminin and fibronectin, but the underlying mechanisms are not fully understood (Danella Polli et al., 2013). Studies with different inflammation models have revealed that the lack of galectin-3 decreases the mobilization and activation of neutrophils and macrophages in tissues, with consequent reduction in the expression of pro-inflammatory cytokines and chemokines (Alves et al., 2010, 2012; Brand et al., 2012; Nieminen et al., 2005; Rotshenker et al., 2008; Sano et al., 2003). Furthermore, studies have demonstrated that galectin-3 influences macrophage polarization into either the M1 or M2 phenotype (MacKinnon et al., 2008).

In the context of the nervous system, galectin-3 has recently gained attention and its function in health and disease continues to be a subject of debate. Reichert et al., (1994) showed that OLA (slow Wallerian degeneration) mice presented fewer macrophages infiltrated into the tissue and correlated this finding with a decrease in galectin-3 expression and deficient tissue debris clearance, in a sciatic nerve transection model. On the other hand, with the use of galectin-3 knockout mice (Gal-3$^{-/-}$), our group has shown that after a sciatic nerve injury, the lack of galectin-3 speeds up the Wallerian degeneration process, which was closely linked to an increase in macrophage recruitment and pro-inflammatory mediators production (Mietto et al., 2013; Narciso et al., 2009). After SCI, galectin-3 gene expression is upregulated in the injured spinal cord (Pajoohesh-Ganji and Byrnes, 2011) and its inhibition through modified citrus pectin (MCP) administration, improved the functional outcome and tissue sparing in a model of contusive SCI (Pajoohesh-Ganji et al., 2012). The results of the cited study suggest that the inhibition of galectin-3 leads to an anti-inflammatory microglial function, but up to now there is no information about the inflammatory response after SCI using Gal-3$^{-/-}$ mice. Here, we sought to investigate the inflammatory response in wild type and Gal-3$^{-/-}$ mice after a compressive SCI, focusing on the myeloid cell linage.

2. Material and methods

2.1. Animals and surgical procedures

Seventy-eight female C57BL/6 background, wild type and galectin-3 knockout mice (8–10 weeks old — Fundação Osvaldo Cruz, Brazil), were anesthetized with ketamine (100 mg/kg) and xylazine (15 mg/kg). The T9 lamina was removed and the spinal cord received a compressive latero-lateral injury using a vascular clip (30 g compressive force, Kent Scientific Corporation, USA) for 60s as described previously (Marques et al., 2009, 2014). The incision was closed in layers and 1 mL of saline solution was administered subcutaneously to prevent dehydration, 3 times/day during the survival time. The bladder was manually expressed 3 times/day until the bladder reflex was restored. Animals were housed five per cage in a controlled environment (12 h dark–light cycle) with food and water ad libitum. No inflammatory signs were observed in the skin wound or in urine analysis. All the procedures were approved by the Animal Care Committee from "Centro de Ciências da Saúde, Universidade Federal do Rio de Janeiro" — Protocol Number DHEICB061.

2.2. Behavioral measures

2.2.1. Open field locomotor test

Motor recovery was assessed using a standardized open field locomotor rating scale Basso Mouse Scale (BMS — Basso et al., 2006). Before SCI, mice were acclimated to the open field environment, for one week in a single daily session of 4 min. After SCI, mice were evaluated during daily a testing period of 4 min, from 0 to 7 days and weekly from 7 to 42 days post injury, by a pair of raters blinded to the genotype.

2.2.2. Ladder rung walking task

Forty-two days after injury, mice were assessed by the ladder rung walking task, a qualitative and quantitative test to measure motor function, as described by Metz and Wishaw (2009). Briefly, mice were submitted to walk along a horizontal ladder on which the spacing of rungs is variable and periodically changed. The apparatus consists of two side walls made of clear acrylic and metal rungs (3 mm diameter) to create a randomly spaced floor with a minimum distance of 1 cm between the rungs (Metz and Wishaw, 2009). Mice were recorded by means of a high definition Microsoft webcam, during the crossing of the rung ladder from one side to the other, 3 times for each different side (left and right). For this test, we were not able to perform comparisons between mouse strains, due to the poor functional recovery presented by the wild type mice (see the Results section; supplementary material S1).

3. Histology

At the different time points (24 h, 3, 7, 28 and 42 dpi), mice were transcardially perfused with 0.1 M phosphate buffered saline (PBS; pH 7.4) followed by 4% paraformaldehyde. The entire spinal cord was dissected and post-fixed for 24 h in 4% paraformaldehyde, cleaved into a 1 cm segment centered from the injury epicenter, rinsed overnight in 0.1 M PBS and cryoprotected in 30% sucrose before being. The block was serially sectioned into 10 μm slices on a Leica CM1860 cryostat and the slices were collected on StarFrost slides (Knittel StarFrost, GE).