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ACUTE GRANULOCYTE MACROPHAGE-COLONY STIMULATING FACTOR TREATMENT MODULATES NEUROINFLAMMATORY PROCESSES AND PROMOTES TACTILE RECOVERY AFTER SPINAL CORD INJURY

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Abstract—Neuroinflammation is known to play a key role in the prognosis of functional recovery after spinal cord injury (SCI). The involvement of microglial and mast cells in early and late stages of inflammation has been receiving increasing attention. This study was aimed at determining the influence of a pro-inflammatory cytokine, the granulocyte macrophage-colony stimulating factor (GM-CSF), on microglia and mast cell activation, glial scar formation and functional recovery following SCI. Rats were randomly injected with saline or GM-CSF one hour after a C4–C5 mediolateral hemisection. To assess functional impairment and recovery, the rats were subjected to sensorimotor tasks for one month. Then, responses evoked by forepaw stimulation in the primary somatosensory cortex were recorded. We also quantified the changes in GM-CSF, IL-1β, IL-6 and BDNF levels, the gliosis and lesion volume as well as microglial and mast cell density, and mast cell surface. Our findings show that GM-CSF promotes cortical reactivation and recovery of tactile abilities, whereas it does not influence motor performances. A transient decrease in pro-inflammatory cytokines after GM-CSF treatment was also observed, whereas the endogenous GM-CSF level was unchanged. While the beneficial role of GM-CSF in reducing glial scar is confirmed, our findings reveal that neuroinflammatory events mediated by microglial and mast cells as well as the alteration of IL-1β and IL-6 levels are paralleled with an improvement in tactile recovery. These mechanisms could limit the duration and intensity of homeostatic imbalance and promote the plasticity of spared tissues.

Key words: spinal cord injury, granulocyte macrophage-colony stimulating factor, neuro-inflammation, functional recovery, forepaw representation, somatosensory cortex.

INTRODUCTION

Approximately 80% of spinal cord injuries (SCIs) are caused by trauma such as motor vehicle, work and sport-related accidents, but they may also result from violent aggression or falls. The resulting damage is due to several types of spinal lesions such as torsion, section, compression, ischemia or hemorrhage. The incidence of acute SCI ranges between 25 and 59 new cases per year per million inhabitants in the United States (Devivo, 2012). Among all SCI cases, the level of cervical SCIs has been steadily increasing over recent decades. SCIs are most prevalent in the young population between the ages of 15 and 35 years and secondarily in the elderly (Devivo, 2012). The primary injury, restricted to the lesion site, is characterized by mechanical trauma, whereas secondary damage involves multiple mechanisms such as hemorrhage, ischemia, excitotoxicity and inflammation in spared spinal cord tissues (David et al., 2012). Although considerable effort has been dedicated to rehabilitative methods, the severe impairment of sensorimotor functions induced by SCI shows limited recovery in humans, as the primary tissular damage and secondary degeneration are largely irreversible. Hence, it is critical to limit the main detrimental agents of neuronal dysfunction and loss to allow favorable physiological conditions for recovery.

Long-lasting functional deficits depend on the completeness and spinal level of the injury, as well as on post-injury treatment and care. Spontaneous tissular reorganization can lead to either limited recovery or damaging changes. These spontaneous alterations are mediated by neuroplasticity mechanisms, which include synaptic rearrangement, axonal sprouting and changes in neural cell properties and interactions. In patients with complete SCI, the plasticity of the central nervous system (CNS) does not functionally compensate for the disruption of spinal pathways, although neuromodulating the spinal cord circuitry, e.g. with epidural stimulation, can promote functional recovery (Harkema et al., 2011; Angeli et al., 2014). In contrast, following incomplete SCI, sensorimotor functions can be partly re-established by intensive training (Dietz and Fouad, 2014). Interestingly, we found that after cervical spinal cord hemisection, functional recovery of tactile sensitivity was correlated with the extent of re-mapping in the primary somatosensory cortex representation of the impaired forelimb in rats

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Abbreviations: CNS, central nervous system; CSF, cerebrospinal fluid; GFAP, Glial Fibrillary Acidic Protein; GM-CSF, granulocyte macrophage-colony stimulating factor; PBS, phosphate-buffered saline; SCI, spinal cord injury.

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trained on a forced-locomotion device (Martinez et al., 2009b). However, the available evidence indicates that inadequate training can lead to maladaptive events such as neuropathic pain or spasticity (Adams and Hicks, 2005; Gwak et al., 2012). Functional recovery can thus be promoted by both limiting the secondary damage and reinforcing neuroplasticity. In human patients, these therapeutic strategies are systematically associated with pharmacological treatment aimed at reducing neurodegenerative and inflammatory events.

Since the intact CNS is an immune-privileged organ, any damage caused to its tissue leads to dramatic changes in cellular and molecular interactions defined as neuroinflammation (Bechmann and Woodroofe, 2014). Inflammation that takes place early after SCI is known to play a key role in the prognosis of functional recovery. However, recent studies have highlighted the dual aspect of inflammation, i.e. harmful or beneficial for recovery after CNS damage (Crutcher et al., 2006; Chan, 2008; Wee Yong, 2010; Finnie, 2013). The neuroinflammatory mechanisms involve the resident immune cells such as microglia, the leptomeningal mast cells and the peripheral immune cells such as neutrophils, hematogenous macrophages and lymphocytes. The neuroinflammatory events include phenomena such as astrocyte activation and alteration of the vascular compartment (Carson et al., 2006; Sinescu et al., 2010). They are paralleled with fenestration of the brain blood barrier at the endothelial level, which allows the recruitment of peripheral immune cells. Moreover, compared with the brain, spinal cord vascularization exhibits an increased permeability to cytokines, while the number of transporter molecules, and tight and adherence junction proteins is reduced (Bartanusz et al., 2011).

Among the cells that contribute to these inflammatory mechanisms, microglial and mast cells have been receiving increasing attention, largely because of the recent identification of their crucial role in several wound healing processes. These two types of cells are involved in both early and delayed stages of neuroinflammation. They receive pro-inflammatory signals and enhance inflammatory processes by developing complex interactions with each other and with neural cells (Skaper et al., 2013; Zhang et al., 2016). Mast and microglial cells also exert beneficial effects on wound healing. As antigen-presenting cells, they both exhibit early phagocytic functions known to enhance neuronal and glial debris clearance and to potentiate axonal regrowth. In addition, the available evidence suggests that mast cells reduce chronic inflammation after trauma by releasing proteases and histamine from intra-cytoplasmic granules. This degranulation has been shown to have protective effects on the injured tissue via the release of chymase. This protease limits astrogliosis and T-cell infiltration probably via the degradation of inflammatory-associated cytokines by the mMCPA4 chymase (Hendrix et al., 2013; Nelissen et al., 2014). Microglial cells show other beneficial effects, such as remyelination, by modifying their own gene expression during early demyelination phases and by reshaping the structural features of synaptic connections in response to injury (Olah et al., 2012; Miyamoto et al., 2013). These mechanisms mediate a significant improvement in sensorimotor functions (Donnelly and Popovich, 2008; Tsai, 2012; Redondo-Castro et al., 2013; Nelissen et al., 2014).

In this context, some studies have focused on the effect of the granulocyte macrophage-colony stimulating factor (GM-CSF), a pro-inflammatory cytokine strongly involved in the proliferation and maturation of myeloid progenitors and precursors. Some studies have highlighted its neuroprotective effects after SCI by preventing programed neuronal death (Schäbitz et al., 2008), inducing neurite growth and enhancing production of neurotrophic factors via monocyte-derived macrophages as well as microglial cells (Bouhy et al., 2006), maintaining structural and axonal integrity and repressing production of axonal regrowth inhibitor proteins in glial scar (Huang et al., 2009). Furthermore, other studies have shown that early and/or delayed injection of GM-CSF can promote sensorimotor recovery after SCI (Bouhy et al., 2006; Kim et al., 2013).

Considering the beneficial role of GM-CSF through its significant influence on the modulation of inflammatory processes, our aim was to characterize the functional recovery and the associated biochemical and cellular changes related to an acute GM-CSF injection after spinal hemisection. In order to elucidate the GM-CSF functional effect, we studied the recovery of sensorimotor functions assessed by specific behavioral tests during the post-lesional period and the final reorganization of somatosensory cortical representations. Since these functional recoveries may be influenced by plasticity and neuroinflammation processes, we also investigated the effect of GM-CSF on these processes by determining the early changes in BDNF, IL-1β and IL-6 cytokine levels as well as delayed microglial and mast cell activation.

EXPERIMENTAL PROCEDURES

All experiments were conducted in full conformity with Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. The principal investigator (J-M. Brezun) is authorized by license number 01007.01, delivered by the French Ministry of Food, Agriculture and Fisheries. This study has been reviewed and approved by the Neuroscience ethics committee N° 71.

EXPERIMENTAL GROUPS

Three-month-old male Wistar rats (n = 78) weighing 350–400 g were used in this study and assigned randomly to three experimental groups: sham-operated rats (Sh), rats with C4–C5 medio-lateral spinal cord hemisection (HS) and HS rats treated with GM-CSF one hour after the lesion (GC).

Ten animals per group were subjected to behavioral evaluations. Then, five animals per group were used for electrophysiological cortical mapping. All these rats (n = 30) were perfused with a 4% paraformaldehyde