● **PERSPECTIVE**

Spinal cord injury: potential neuroprotective therapy based on neural-derived peptides

Spinal cord injury (SCI) is a degenerative disease that causes an important neurological impairment, it is a medically complex and life-disrupting condition. SCI has a devastating impact on international health systems due to its irreversible consequences and lack of efficient treatment, affecting mostly young population within their productive years. Global SCI incidence in high-income countries is 40 to 80 new cases per million per year, based on quality country-level incidence studies of SCI from all causes (Chen et al., 2013). The pathophysiology of SCI is described as a biphasic event, consisting of a primary and secondary phase of injury. The primary phase involves the initial mechanical injury, which disrupts axons, blood vessels, and cell membranes. This is followed by a secondary injury phase involving vascular dysfunction, edema, ischemia, excitotoxicity, electrolyte shifts, free radical production, delayed apoptotic cell death, and inflammation. Inflammatory response is one of the main phenomena participating in tissue degeneration; however, recent studies have demonstrated that modulation instead of inhibition of this prominent event promotes neuroprotection after SCI (Know et al., 2004).

Immunomodulation of inflammatory response: Inflammatory reaction has been cataloged as a quintessential destructive mechanism after SCI, in fact, it has been assumed that, in order for treatments to be efficient, the activation of the immune system must be restrained to counter neural destruction and favor tissue regeneration. Nevertheless, relevant studies from Michal Schwartz and others have shown that, modulation of immune response could be a better therapeutic strategy to protect and regenerate neural tissue (Hauben et al., 2003; Ibarra et al., 2004; Schwartz et al., 2014). Immunization with neural-derived constituents, such as myelin basic protein (MBP), has shown to modulate immune response and provide beneficial effects after SCI. However, the use of MBP confers a potential risk to induce an autoimmune response due to its immunogenic characteristics (Constantinescu et al., 2011). Therefore, MBP's principal antigenic sequence (amino acids 87–99) was changed to create A91 peptide, which is not considered to induce an autoimmune response (Gaur et al., 1997). A91 peptide solely differs in position 91, in which lysine is substituted by alanine, and it is considered a non-autoimmune neural derived peptide (Hauben et al., 2000).

Neuroprotective effect of A91-immunization after SCI: Once presented to T helper lymphocytes (CD4⁺), A91 moderates the inflammatory process in the acute phase of SCI by inducing Th2 lymphocytes, an anti-inflammatory phenotype, considered to be a partial agonist that triggers a low intensity response in comparison to other antigens (Hauben et al., 2000). The cytokines secreted by A91-activated lymphocytes are mainly interleukin 4 (IL-4) and interleukin 10 (IL-10). In addition, it has been observed that immunization with A91 *in vivo* and *in vitro* stimulates the production of neurotrophic factors which are essential for the correct function of the CNS. Moreover, they contribute to tissue regeneration and neurogenesis, and stimulate the formation of new synaptic connections (Martiñon et al., 2012, 2016).

Production of IL-4 and IL-10 reduces reactive oxygen species (ROS). High levels of IL-10 not only abate the synthesis of tumor necrosis factor-α (TNF-α) in macrophages, but also participate in the inhibition of lipid peroxidation (Zhang et al.,

2015). On the other hand, IL-4 diminishes the synthesis of interferon γ (IFN-γ), a cytokine that spurs macrophages and microglia to acquire a proinflammatory M1 phenotype. In addition, IL-4 favors the production of arginase, which eliminates arginine and, consequently, reduces the production of nitric oxide (NO•) by inducible nitric oxide synthase (iNOS). Thus, there is a less amount of nitrates in the lesion region inhibiting the formation of reactive nitric species (RNS) (Schwartz et al., 2006).

A91-immunization has conferred neuroprotective effects in models of SCI. For instance, recent experiments showed that immunization with A9-peptide reduces lipid peroxidation, one of the main destructive phenomena developed after SCI (Ibarra et al., 2010). In the same way, it was demonstrated that this therapy induces a significant decrease of iNOS gene expression and thus, of NO• (Garcia et al., 2012). Subsequent studies in rats with SCI demonstrated that A91-immunization is capable of reducing the number of apoptotic cells. This reduction was accompanied by a significant decrease in caspase-3 activity and TNF-α concentrations (Rodríguez-Barrera et al., 2013).

In order to establish a prophylactic therapy for SCI, the effect of A91-immunization was assessed by immunizing with the peptide prior to SCI. Immunization induced a significant increase in the survival of rubrospinal and ventral horn neurons. These observations correlated with a significant improvement in motor recovery (Ibarra et al., 2013). Finally, a very recent study showed that A91-immunization can exert its beneficial actions even at chronic stages of injury. With this regard, it was observed that vaccination with A91 (immediately after SCI) induces a long-term production of brain derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) (observed at chronic stages of SCI), and a substantial improvement in motor recovery (Martiñon et al., 2016).

Synergism of A91-immunization with other drugs: Methylprednisolone (MP) is currently the only approved acute treatment available for patients with SCI. Due to the promising results of A9-immunization, it was of interest to find out if this potential therapy would be beneficial in MP-treated individuals. The study indicated that MP administered immediately after SCI, if given concomitantly with vaccination, wipes out the benefit of the latter. Nevertheless, if MP is given immediately after the injury and the vaccination 48 hours later, MP does not detract from the beneficial effect A91-immunization (Ibarra et al., 2004). Similar results were obtained when A91-immunization was administered conjointly with cyclosporine A (CsA), which has shown to exert also neuroprotective effects (Ibarra et al., 2003). Although both compounds (MP and CsA) -per separate-own beneficial effects on SCI, (*i.e.*, lipid peroxidation inhibition), they possess an antagonistic effect on A9-immunization, mostly as a consequence of their immunossupressive properties. In another study, A91-immunization was combined with glutathione monoethyl ester (GME), an antioxidant agent that by itself, has demonstrated to exert neuroprotective effects after SCI (Guízar-Sahagún et al., 2005). In this case, the experiments showed that after SCI, combination therapy induced better motor recovery, a higher number of myelinated axons, and better rubrospinal neuron survival than immunization alone. GME contributes to the elimination of ROS, reducing lipid peroxidation levels without interfering with A91's therapeutic effect (Martiñon et al., 2010). Thus, the combination of A91 and GME proved to have superior effects when administered immediately after SCI or even if applied within the first 72 hours after injury (Martiñon et al., 2007; del Rayo Garrido et al., 2013).

A91-immunization as a treatment for severe spinal cord injury: Once proven the therapeutic effect of A91 immunization for moderate SCI, some studies took place to determine its role in

Table 1 Studies on A91-immunization therapy after spinal cord injury (SCI)

References	A91-immunization results
A91 immunization alone	
Ibarra et al., 2010	Reduces lipid peroxidation levels
Garcia et al., 2012	Decrease of inducible nitric oxide synthase (iNOS) gene expression and nitric oxide (NO•) production
Martiñon et al., 2012	In animals with moderate SCI, immunization with A91 promotes T-cell proliferation, which then leads to an increase of interleukin 4 (IL-4) and brain derived neurotrophic factor (BDNF) in the site of lesion
Rodríguez-Barrera et al., 2013	Reduces the number of apoptotic cells and decreases caspase-3 activity and tumor necrosis factor-α (TNF-α) concentrations
Ibarra et al., 2013	Induces a significant increase in the survival of rubrospinal and ventral horn neurons, correlating this with a significant improvement in motor recovery
Martiñon et al., 2016	Induces a long-term production of BDNF and neurotrophin-3 (NT-3) and a substantial improvement in motor recovery at chronic stages of SCI
A91 immunization with other drugs	
Ibarra et al., 2004	The beneficial effect of A91 immunization was avoided if applying in combination with Methylprednisolone or Cyclosporin A, however, there is a therapeutic window after SCI that accommodates A91-immunization and methylprednisolone treatment
Martiñon et al., 2007	A91-immunization combined with glutathione monoethyl ester, induced a better motor recovery, a higher number of myelinated axons and better rubrospinal neuron survival than immunization alone

severe SCI. Despite the immediate administration of A91 after injury, motor recovery was not significant between immunized and control groups. The irrelevant effect of A91 in severe contusion is explained by the excessive liberation of catecholamine and cortisol after SCI, thus, it leads to an immunosuppressing syndrome, which impairs lymphocyte proliferation, besides of causing apoptosis. In animals with moderate SCI, immunization with A91 promotes T-cell proliferation, which then leads to an increase of IL-4 and brain derived neurotrophic factor in the site of lesion. In rats subjected to either severe contusion or complete transection, concentrations of these molecules were barely detected. These results were reflected in a poor recovery of motor function (Martiñon et al., 2012, 2016).

The opposite effects of A91 observed in severe and moderate models were also analyzed exploring gene expression. Quantitative PCR proved that in moderate lesion, IL-6, IL-12, IL-1β, IFN-γ, TNF-α were decreased, while the expression of IL-10, IL-4, and insulin-like growth factor 1 (IGF-1) was increased. This cytokine pattern explains, in some way, the neuroprotective effect of A91 immunization after moderate injury (unpublished data). On the other hand, in the case of severe injury, there is an increase of IL-12 and IFN-γ which could explain the lack of motor recovery. The effect of different doses and timings of A91 immunization is being currently studied in severe SCI.

Conclusions: Immunization with A91 peptide has shown to exert neuroprotective effects after moderate SCI. This approach is attained by reducing ROS and RNS. Moreover, it can promote the production of anti-inflammatory molecules, and neurotrophic factors that contribute to enhance motor recovery and neuron survival (**Table 1**).

In spite of the promising results, a range of new experiments should be designed before A91 therapy can be translated to hu-
mans. Studies regarding A91 immunization in chronic SCI and its use with others adjuvants, or the use of other neural-derived peptides such as Copolymer 1 constitute future objectives in our laboratory.

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