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Protective Role of the Immune System in Spinal Cord Injury: Immunomodulation with Altered Peptide Ligands

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<http://dx.doi.org/10.5772/63221>

Abstract

Spinal cord injury (SCI) is a phenomenon characterized by damage to the spinal cord and nerve roots, resulting in loss of physiological activity below the lesion. Injury to the spinal cord activates a cascade of cellular and molecular reactions in which the immune system plays an essential role, as there is an uncontrolled immune response that endows further damage to neural tissue. However, the activity of immune system at the site of injury can be modified in order to obtain a neuroprotective environment and promote SCI recovery. This strategy has been designed under the light of the innovative concept “protective autoimmunity” (PA) and can be stimulated with the use of altered peptide ligands (APL). Adequate immunomodulation with APL can be obtained with the peptide A91, which is a safe synthetic peptide derived from the myelin basic protein (MBP) that has proven to be effective in preclinical research. Immunization with A91 is carried out with the objective of preventing further damage and promoting neuroprotection. This peptide has direct influence over SCI secondary mechanisms such as inflammation, lipid peroxidation, and apoptosis. Preclinical results suggest that immunization with A91 could be an effective treatment in the clinical field, providing a better quality of life to SCI patients.

Keywords: spinal cord injury, protective autoimmunity, A91, altered peptide ligand, immunization

1. Introduction

Spinal cord injury (SCI) is perhaps one of the most devastating conditions as it results in a disruption of motor, sensory, and autonomic functions, leading to permanent neurological

disability. According to the National Spinal Cord Injury Statistical Center (NSCISC), in 2014 over 276,000 people were suffering from SCI [1]. The incidence ranges over 12,500 new cases each year, with a prevalence of approximately 40 cases per million in the United States [2]. Epidemiological studies indicate that SCI has a higher incidence among male population, and people between 30 and 50 years old [3, 3]. Clinically, the most disabling outcomes of traumatic SCI are motor deficit and sensory loss. Nonetheless, due to autonomic dysfunction and depending on the level and severity of injury, SCI may also alter normal homeostasis and the respiratory, reproductive, urinary, and gastrointestinal systems [4–6]. Besides surgical intervention, the standard of care for SCI in several countries focuses on preventing shock and further damage with the use of methylprednisolone (MP), a synthetic glucocorticoid with anti-inflammatory properties. The National Acute Spinal Cord Injury Study (NASCIS) trials suggest that high-dose MP is effective for the management of acute SCI [5, 8]. However, further studies about NASCIS results indicate that the clinical benefits of the use of MP as a treatment for SCI are questionable [6, 10]. Acute MP therapy reduces cellular damage and secondary injury mechanisms but leads to risks of high-dose steroids [7]. For this reason, MP administration after SCI is still a controversial treatment, and it continues to be debated [8].

Another molecule that has been widely studied for the clinical treatment of SCI is Ganglioside, which is highly expressed in the cell membranes of the central nervous system (CNS). The synthetic form of this glycosphingolipid—monosialotetrahexosylganglioside (GM1)—was known for having anti-apoptotic, anti-excitotoxic, and neuroprotective properties [9, 14]. Therefore, several clinical trials were designed in order to test this drug on SCI [10]. In the most recent study, patients received NASCIS II doses of MP, and different doses of GM1 after the effect of MP was over. Although, GM1 treated patients presented a significant recovery, the beneficial effects related to the drug were inconsistent between different types of injuries and were lost during chronic SCI stages [11, 17]. These results suggest that GM1 has a limited effect and to the date, it is not an approved treatment for SCI [12].

Undoubtedly, the lack of an available treatment for SCI in the clinical field highlights the need to design new and safe therapies for injured patients. Nowadays, research is focused on targeting secondary SCI mechanisms with the objective of minimizing further damage, promoting regeneration and thus, improving functional recovery [13, 19]. One of the most important secondary injury mechanisms is the post-traumatic inflammatory response, which is roughly characterized by the presence of injury-dependent pro-inflammatory cytokines and infiltration of peripheral immune cells to the damaged area [14–22]. It was previously thought that the presence of this uncontrolled immune response in the CNS was harmful and pathological. However, other findings state a controversial theory: immune cells could play an essential role in neuroprotection and regeneration of the spinal cord after injury [15]. A better understanding of how inflammation mediates secondary injury suggests that suppressing all immune responses with the use of glucocorticoids is no longer a rational treatment approach. This information has led scientists to further investigate the beneficial effects of the immune system in several neurological conditions, including SCI [16]. Focusing on the beneficial mechanisms of the immune system after trauma has opened the doors for the design of a new therapeutic strategy. Nevertheless, some questions should first be answered. For instance, how

can the immune system be modulated to attain a protective microenvironment? What are the immune-related elements capable of providing the required immune-modulation? And how are they able to provide recovery after SCI?

2. Spinal cord injury pathophysiology

Damage to the spinal cord (SC) causes anatomical and functional deficits to the CNS, that result in the appearance of several long-term medical comorbidities. SCI is characterized by two different pathophysiological phases: primary and secondary injury [17]. Initial trauma to the SC—known as primary injury—is caused by a compressive or contusive mechanism that results in gross anatomical tissue disruption, and immediate hemostatic self-defense events that produce further damage to CNS structures. Direct impact to the SC leads to vascular disturbances such as hemorrhage, ischemia, edema, and hypoperfusion, resulting in tissue necrosis [18]. Hemorrhage and edema formation can raise the risk of developing increased parenchymal pressure and produce more tissue damage [19]. Also, reactive gliosis, demyelination, and axonal loss are often caused by immediate trauma and sustained compression to neural tissue [20]. Depending upon primary injury characteristics, there could be greater tissue damage and worse functional outcomes. Therefore, during this phase, treatment should focus on hemorrhage control to avoid necrosis and early decompression to stabilize intrathecal pressure.

As a consequence of primary injury, there is a cascade of biological reactions that occur minutes after injury and last for several weeks known as secondary injury [21]. This phase is quite complex, as it consists of the development of mechanisms like loss of ATP-dependent cellular functions, ion homeostasis imbalance, excitotoxicity, oxidative stress, lipid peroxidation, inflammation, and apoptosis [22]. There are several secondary mechanisms that are strongly related to the ischemic event observed in SCI. Ischemia produces a depletion of the intracellular amount of ATP, leading to a reduction in the energy-dependent cell function that preserve ion homeostasis [23]. Therefore, the sodium-potassium pump cannot execute its physiological activity, resulting in an elevated potassium (K^+) efflux, and a high influx of sodium (Na^+), calcium (Ca^{2+}), and chloride (Cl^-) into the cell. This homeostasis imbalance alters normal ion concentrations within the intracellular and extracellular spaces, producing a sustained membrane depolarization and a release of excitatory amino acid (EAA) neurotransmitters [24]. The pathological effect related to an increased concentration of EAA neurotransmitters, such as glutamate and aspartate, is known as excitotoxicity. This secondary injury mechanism is characterized by an overstimulation of the NMDA, kainate and AMPA glutamate receptors, which causes massive intracellular Ca^{2+} concentrations, resulting in a pathological neuronal excitation and cell death [25, 32]. Glial cells—especially oligodendrocytes—are very sensitive to excitotoxic damage because of their high expression of ionotropic glutamate receptors [26]. That is why an excessive glutamate accumulation related to SCI can produce oligodendrocyte death, and consequent white matter demyelination [27]. Also, because of glutamate excitotoxicity, there is an increased production of free radicals by reactive microglia, which contrib-

ute to lipid peroxidation (LP), and mitochondrial dysfunction [28, 36]. Therefore, this sustained toxic microenvironment is postulated to be one of the most detrimental secondary injury mechanisms related to SCI.

Oxidative stress accompanies secondary injury damage, and is mainly characterized by an increased mitochondrial production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) [29, 38]. The elevation in ROS and RNS concentration is closely related to the aforementioned high Ca^{2+} influx to the cells, as it stimulates free radical production [30]. Free radicals such as superoxide anion (O_2^-), nitric oxide (NO), and peroxynitrite (ONOO^-) create a toxic microenvironment by oxidizing nearby molecules producing neural energy failure, blood-brain barrier dysfunction, vascular reactivity, and potentiating inflammation [31, 41]. At high concentrations, these molecules can become cytotoxic and worsen secondary injury mechanisms [32]. Oxidative stress also influences excitotoxicity by exacerbating Ca^{2+} deregulation and thus, glutamate concentrations. The pathological production of ROS that arises after trauma causes oxidative damage, especially on lipids, originating LP. LP is characterized by producing a disruption in the normal structure of the polyunsaturated fatty acids in the cell membrane, such as arachidonic acid, and linoleic acid. In LP, the high concentration of free radicals, results in functional compromise and cell death [33, 43]. Also, structural damage to the cell membrane produces a reduction in the generation and transmission of the electrical potentials leading to synapse dysfunction [34]. Altogether these mechanisms potentiate apoptosis, which is a form of programmed cell death characterized by cell shrinkage, nuclear pyknosis, and chromatin aggregation in a stressful environment [35]. This deleterious event, (apoptosis) occurs after SCI by stimulation of the apoptosis-inducing factor (AIF) to the nucleus, or by direct mitochondrial disruption leading to a subsequent activation of caspase-3 signaling pathways [36, 47]. Evidence supports that secondary injury mechanisms contribute to delayed tissue damage, exacerbating damage, and limiting recovery after traumatic SCI.

2.1. Pathophysiological involvement of the immune system

The immune system has a pivotal and somehow controversial role within the pathophysiology of traumatic SCI. Immediately after trauma there is an activation of the inflammatory response that consists in the proliferation of resident microglia and astrocytes, a high concentration of pro-inflammatory molecules, and infiltration of peripheral immune cells to the site of injury (**Figure 1**). SCI induces the activation of a series of inflammatory stimuli leading to an increased concentration of cytokines and inflammatory cells that will determine the extent of secondary damage [37]. Evidence suggests that in the presence of an excitotoxic and inflammatory microenvironment, microglial cells differentiate into a M1 pro-inflammatory phenotype [38]. Under these conditions, activated microglia is capable of secreting interleukin 1β (IL- 1β), IL-6, tumor necrosis factor- α (TNF α), and macrophage colony-stimulating factor (MCSF) which are pro-inflammatory in nature [39].

A high free radical and cytotoxic substances secretion is more evident when there is microglial activation starting at day one, and increasing at 7 days post-injury [40]. That is why the immune response is closely related to LP, as these cells are capable of boosting ROS and NO concentrations, favoring oxidative stress, cell membrane dysfunction and thus, apoptosis [41, 50]. At

the same time, $\text{TNF}\alpha$ stimulates astrocyte proliferation and growth, leading to the formation of the glial scar within the chronic stages of SCI impeding axonal regeneration through the site of injury [42, 53]. Glial cells act immediately after trauma; they secrete pro-inflammatory molecules and promote inflammation, favoring the appearance of secondary injury mechanisms. In spite of the above mentioned deleterious effects, microglial—especially when differentiated into an M2 phenotype (IL-10 and TGF- β)—and astrocyte activation could produce a beneficial effect through a high production of growth factors like brain derived neurotrophic factor (BDNF), and neurotrophin-3 (NT-3), essential for tissue repair [43]. Also, these cells are capable of expressing glutamate transporters that help reducing harmful

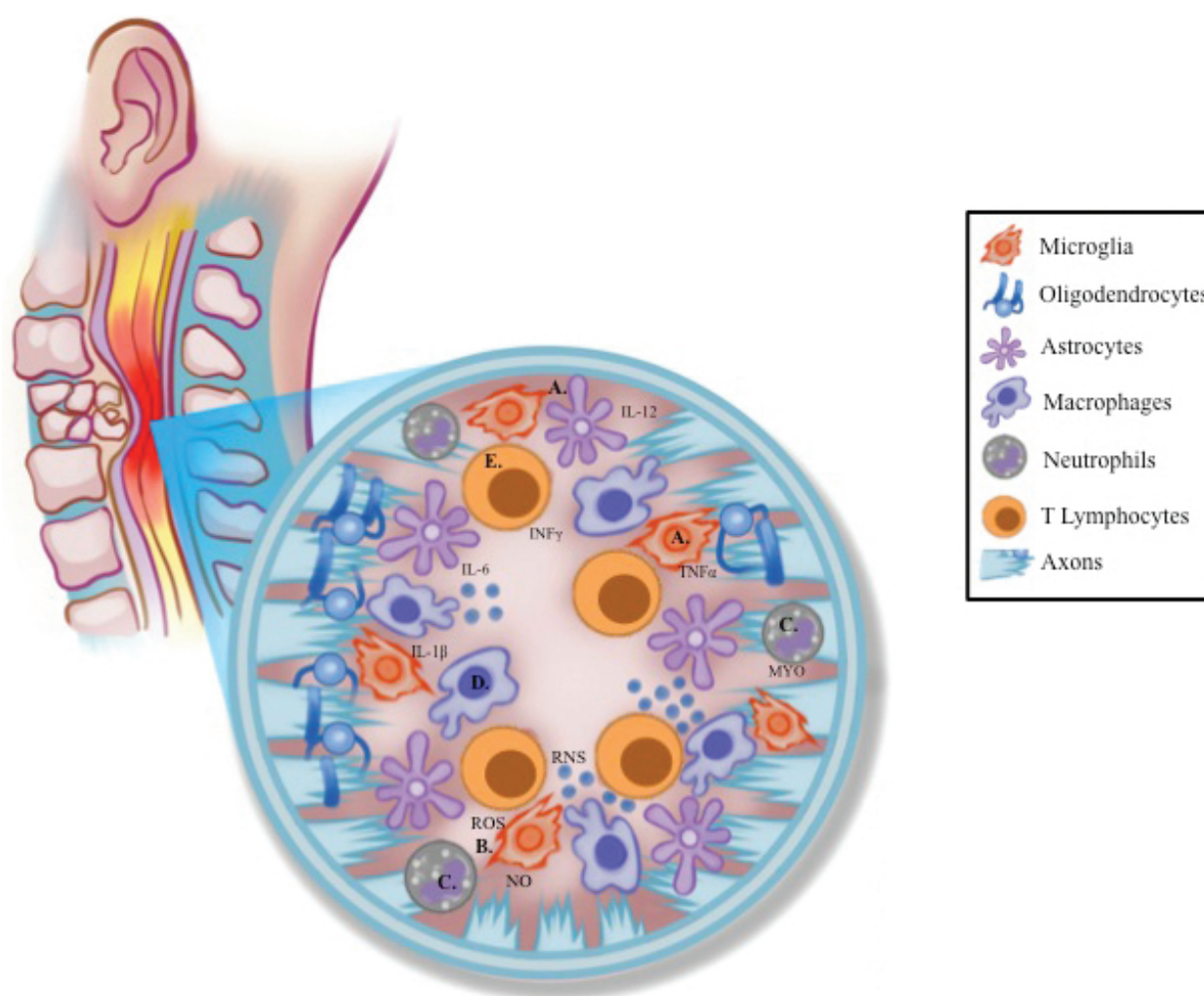


Figure 1. Cellular immune response in SCI. A. M1 microglial differentiation, astrocyte activation, and IL-1 β , IL-6, and TNF α secretion. B. Increase in the concentration of ROS, NO, and RNS. C. Peripheral infiltration of neutrophils and myeloperoxidase secretion. D. Macrophages arise from peripheral tissue and activate the adaptive immune response. E. Infiltration of T-lymphocytes to the site of injury and INF γ secretion. Abbreviations: IL, interleukin; RNS, reactive nitrogen species; ROS, reactive oxygen species; NO, nitric oxide; TNF α , tumor necrosis factor- α ; INF γ , interferon- γ ; MYO, myeloperoxidase.

concentrations, leading to a reduction in excitotoxicity [44]. Evidence suggests that even though the immune system is considered to be completely pathological in nature, it can also provide beneficial effects for SCI repair.

In normal conditions, inflammatory reaction leads the response to a pathological outcome, promoting damage and spinal cord degeneration. The severity of SCI determines the intensity of the inflammatory response and the glial reaction to SCI. Glial cells are well distributed within the CNS, and have the ability to proliferate and migrate to the site of injury. In response to injury there is a high glial secretion of cytokines and chemokines—such as IL-1, IL-6, and TNF α —that allows migration of peripheral immune cells [45]. TNF α stimulates the expression of adhesion molecules like endothelial intracellular adhesion molecule -1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) altering the blood-brain barrier permeability and inducing a peripheral infiltration of immune cells to the CNS (**Figure 1**). Cell infiltration to the CNS is considered the principal factor for tissue disruption and sustained neural damage. The first peripheral cells to arrive at the site of injury are neutrophils [46]. These cells have the ability to phagocyte and clear debris, but they also secrete ROS and RNS, as well as other pathological proteolytic and oxidative enzymes like myeloperoxidase, producing greater tissue damage [47, 58]. At the same time, macrophages arise from resident microglia or from peripheral monocytes with the objective of removing cell debris and stimulating angiogenesis [48]. These cells play an essential role in the immune response, as they help in the activation and reclusion of the adaptive immune system cells. Microglia, astrocytes and dendritic cells may act as antigen-presenting cells (APC), leading to T lymphocyte activation, proliferation, and infiltration to the site of injury [49]. Lymphocytes recognize the signal and proliferate creating large numbers of clones, specific to the antigen being presented by the APC. At 3 days post-injury, there is an evident infiltration of T-cells to the CNS, these cells determine the intensity and continue on modulating the immune response to trauma [50]. In SCI, the presence of T-lymphocytes is considered detrimental as they secrete a Th1 cytokine profile including interferon gamma (IFN γ) [51, 63]. IFN γ is a pro-inflammatory cytokine capable of inducing free radical production, increasing IL-6, IL-12, IL-1 β , and TNF α concentrations, and activating apoptotic-signaling pathways [52–65]. These events represent the immune activity within the acute phases of SCI and if it is not well controlled it could turn into a chronic and degenerative immune response.

When inflammation is modulated, i.e., we control the intensity of inflammatory response, the type, the action, and the arrival of immune cells, we could expect a change in the final outcome. With this regard, there is a strong evidence suggesting that lymphocytes could also have favorable activity, as they are capable of synthesizing several growth factors, like BDNF, NT3, and nerve growth factor (NGF) [53–68]. These molecules are known for being capable of promoting a protective and regenerative environment for CNS disorders [54].

To the date, it is clear that the immune response appearing after SCI could be pathological if it is not well controlled at the onset of SCI [55]. In a short period of time, this uncontrolled response leads to the extensive recognition of CNS peptides, proteins, lipids, or nucleic acids by the immune system [56]. This interaction between immune cells and CNS constituents—like the MBP—promotes the activation of lymphocytes and thereby, the possible development

of an autoimmune response [57, 72, 73]. As a result, higher levels of demyelination are noted, leading to loss of sensitive and motor synapses. Also, several studies have identified that B cells secrete self-reactive antibodies and pro-inflammatory cytokines, promoting autoimmune activity [58, 75]. Therefore, the immune response elicited after SCI is considered to be one of the most important secondary injury mechanisms, as it plays an essential role in stimulating the appearance of a neurotoxic microenvironment after injury. However, further studies about the immune system and its relationship with CNS damage suggest that it is not completely pathological, as it has protective and regenerative properties [59–78].

3. Protective autoimmunity

The reactive immune response against self-constituents appearing after injury has been widely studied, as it can be an excellent target in the design of new therapies for SCI treatment. It was previously thought that the presence of immune activity was detrimental and it had to be suppressed [60–81]. However, more recent findings suggest that such response and the infiltration of peripheral immune cells to the site of injury is a phenomenon destined to protect and restore the CNS after trauma. The phenomenon capable of inducing this beneficial actions has been termed protective autoimmunity (PA) and is a mechanism in which the adaptive immune cells—especially lymphocytes that recognize self-constituents and potentiate an autoreactive response—help maintain tissue integrity in SCI. Researchers have demonstrated that PA is genetically encoded, and it is a physiological phenomenon linked to the inflammatory activity in the CNS, capable of providing protection in several neurodegenerative disorders [61, 83]. The immune system plays an essential role in tissue restoration, angiogenesis, and is capable of increasing functional recovery in CNS trauma [62]. However, in normal conditions the intense inflammatory response appearing after injury overshadows the beneficial effects of PA. For that reason, it was thought that boosting PA after injury could promote the beneficial instead of deleterious effects of the immune response to injury. In an attempt to test this hypothesis, researchers performed a passive transfer of T-cells specific to MBP, and demonstrated that it reduced tissue damage and improved motor recovery in rats with SCI [63, 86]. These studies suggested that the delayed adaptive immune response to injury and the low concentration of autoreactive T-cells are the reasons why PA is not evident, but a higher and earlier presence of these cells could potentiate the beneficial effects of PA over SCI.

Shortly after researchers envisioned that active immunization could elicit a higher proliferation and migration of autoreactive (antigen specific) T-cells to the CNS increasing the action of PA. Therefore, immunization with natural CNS components could help activate this response [64]. In line with this motion, several studies were performed indicating that, immunization with MBP can modulate the immune response, potentiate PA, and provide neuroprotection to the injured tissue [65, 88, 89]. In spite of the encouraging results, immune modulation with neural constituents increases the risk of developing an autoimmune disease such as experimental autoimmune encephalomyelitis (in rodents; EAE) or multiple sclerosis (in humans; MS) [66, 91]. For that reason, neural constituents were studied with the objective of creating a peptide

capable of stimulating PA and reducing the risk of developing an autoimmune disease [67, 93]. These experiments led to the creation of altered peptide ligands (APL), which are synthetic peptides with changes in specific amino acid residues, critical for T-cell receptor (TCR) binding [68]. In the normal immune response, the MBP has agonist properties as it interacts with the TCR, leading to lymphocyte differentiation toward a Th1 phenotype [69, 96, 97]. With the objective of altering TCR recognition, a specific amino acid substitution makes APL become partial agonists or antagonists capable of deviating the immune response [70, 98]. That is why APL are able to cause lymphocyte anergy or their differentiation to an anti-inflammatory Th2 phenotype. This way, APL can alter the natural response of the immune system after SCI, by being able to change the whole Th1 (pro-inflammatory) cytokine profile toward a Th2 (anti-inflammatory) profile. Therefore, by altering the immune response, APL represent a good therapeutic approach for the treatment of SCI and other neurodegenerative diseases [71, 99].

3.1. Modulation of protective autoimmunity with the use of altered peptide ligands

A safe and effective way of increasing PA is through immunization with APL [72]. This strategy allows a change in the interaction with the TCR from an agonist to a partial agonist switching the response toward a Th2 cytokine pattern [73]. To create APL, identification of the essential residues of MBP for an acquired immune response to self-determinants was investigated. Evidence indicates that the amino acid sequence including the 87 to 99 residues is the most encephalitogenic portion of the MBP and that it is essential for TCR recognition [74, 102]. This amino acid sequence was fundamental for the creation of several APL and the evaluation of their effect over the immune response [75]. Altering the amino acid sequence by substituting each residue of the encephalitogenic region of the MBP with alanine, led to the discovery of this group of APL [76, 104]. While trying to identify an ideal peptide to promote PA, several APL derived from MBP encephalitogenic epitopes like G91, A96, or A97, along with A91 were tested as therapeutic strategies for CNS trauma. These peptides were capable of controlling the MBP peptide induced autoimmune reaction by altering the MBP specific T-cell responses [77]. Also, these APL demonstrated to be able of providing significant protection by reducing neuronal loss [78]. More importantly, they were limited the extent of the immune secondary injury mechanisms by inducing changes in the cytokine secretion profile of T-cells and enhanced the recovery of motor activity [79, 99, 103]. Studied APL showed different levels of neuroprotection, however, the conclusion of these conducted studies was that the APL A91 provided the best therapeutic effects without the risk of an autoimmune response [80].

Evidence has demonstrated that lysine at the position 91 is an essential residue of the MBP p87–99 for the development of a Th1 immune response. With this respect, it has been shown that when the amino acid 91 (lysine) is replaced with glycine (G91), the peptide which is non-encephalitogenic, regulates the proliferative response and modifies the cytokine secretion profile (toward a Th2 profile) of encephalitogenic MBP 87–99 reactive T-cells [81, 99, 103, 104]. The substitution of lysine at the position 91 for the amino acid alanine led to the creation of the APL: A91. This APL counteracts the production of pro-inflammatory cytokines, generating a microenvironment with anti-inflammatory features [82, 103, 104]. A91 (amino acid sequence:

VHFFANIVTPRTP) is a safe synthetic non-encephalitogenic peptide, capable of inhibiting the development of autoimmune disease while maintaining neuroprotection [83, 104]. This APL (A91) has proven to be an effective TCR partial agonist capable of modulating the immune response after CNS injury, and increasing the beneficial effects of PA. Immunization with A91 peptide down regulates Th1 activity and increases the levels of a Th2 cytokine pattern (IL-4 and IL-10) creating an anti-inflammatory microenvironment [84, 105, 106].

3.2. The altered peptide ligand A91 as a potential treatment for spinal cord injury

To increase neuroprotection, A91 was designed to boost PA and to act directly over secondary mechanisms in SCI. Immunization with A91 has been tested as a subcutaneous injection, which has resulted to be an effective and minimally invasive route of administration. The use of this strategy in preclinical studies indicates that active immunization with A91 with a single dose of 150–200 µg improves neurological recovery. It is important to note that in order to avoid the risk of an autoimmune disease while maintaining neuroprotection, immunization with myelin-associated antigens should be fully controlled. A study evaluating the dose and therapeutic window of A91 indicates that beneficial effect of this peptide lies between 10 minutes up to 72 hours after SCI [85]. Further studies also indicate that A91 could be considered as a prophylactic therapeutic vaccine since its administration before SCI could provide high levels of neuroprotection and motor recovery [86]. These results could be of relevant benefit as an approach to provide with prophylactic measures to patients sustaining invasive spinal surgery procedures.

On the other hand, with the objective of evaluating the effect of A91 immunization in the presence of the gold standard treatment for SCI (MP) another study was carried out. The results of this investigation indicated that when these two treatments were administered at the same time, the beneficial properties of A91 were abolished [87]. However, the extensive therapeutic time window of A91 enables immediate MP administration and immunization with A91 up to 48 hours later [88, 109]. This approach has given the possibility of rescuing the beneficial effects elicited by A91, as animals subjected to this combined strategy, present neuroprotection and a higher motor recovery. These results also allow envisioning the possible clinical application of this therapy with no risk of avoiding the therapeutic effects theoretically provided by MP. In the search of increasing the neuroprotective effect of A91, double immunization has also been evaluated [89]. However, unexpectedly a higher concentration of this peptide eliminates the beneficial aftermath of the therapy [90]. Studies in our laboratory have also demonstrated that A91 can be applied at different SCI stages and still be effective. Our investigation suggests that adequate immunization must be performed immediately after injury or during the acute phase [91, 109]. Moreover, studies including vaccination of A91 in chronic SCI are now being conducted, and results have demonstrated to be profitable (Unpublished data).

APL were designed to specifically target the immune-related secondary injury mechanisms in order to attain neuroprotection, promote regeneration, and thus improving motor and sensory recovery in SCI. In line with this, previous studies have shown that immunization with A91

peptide produces an adequate T-cell proliferation characterized by a Th2 phenotype, where the production of IL-4 and IL-10 is increased [92, 110]. Additionally, A91 specific T-cells are capable of producing BDNF, which could be linked directly with the functional recovery appearing after immunization [93]. This anti-inflammatory and permissive microenvironment controls the inflammatory response elicited by SCI, reducing some of the main harmful phenomena developed by inflammation. For instance, it has been demonstrated that A91-immunization is capable of inhibiting LP, which is closely related to the action of immune system and it is one of the most aggressive phenomena related to SCI [94]. LP is present immediately after injury reaching its maximum peak at 4–5 h and has a second increase between 24 hours and 5 days [95]. With this regard, a study conducted to evaluate the impact of A91-immunization on LP showed that A91 is able to reduce the concentration of ROS at the site of injury having a strong impact over the second peak of this phenomenon [96]. A further study indicated that A91-immunization counteracts the production of nitric oxide (NO) and down regulates the expression of the gene encoding for nitric oxide synthase (iNOS) [97]. These are some of the beneficial mechanisms that explain, at least in part, the effect of this strategy on LP.

Apoptotic cell death is another of the main destructive phenomena triggered after SCI. This phenomenon is activated by inflammatory cytokines, free radicals, excitotoxic agents, and increased levels of intracellular calcium [98]. After SCI, neurological recovery depends mainly on the extent of neuronal loss and the functionality of the residual neural tissue. Numerous studies have shown that many neurons die as a consequence of apoptosis. Therefore, regulating apoptotic cell death might play an important role in the neurological recovery following SCI [99, 116]. Recent investigations on the field found that immunizing with A91 decreases caspase-3 activity and TNF α concentrations, reducing the number of apoptotic cells, which is directly correlated with functional improvement after injury [100].

Altogether, the aforementioned observations provide clear evidence on the mechanisms by which A91-immunization exerts its beneficial effects. Besides reducing secondary injury mechanisms, A91 peptide has also proven to prevent tissue damage, as immunized animals presented a higher number of myelinated axons and survival of rubrospinal neurons compared to controls [101, 109, 110]. These results were consistent throughout several SCI preclinical studies. Also it was noted that motor recovery had a direct correlation with neuronal survival, myelin preservation, and apoptosis reduction in treated groups [102, 107, 109, 110, 114, 117, 118]. As a consequence of these encouraging results, we have envisioned the possibility of combining this strategy with others that have also shown beneficial effects [103, 117, unpublished data]. For that reason, A91-immunization was administered along with glutathione monoethyl ester (GSHE), which is an anti-oxidant capable of accelerating the immune response and providing neuroprotection [104, 117]. The results of this study showed that after a contusive or a compressive SCI, this combination induced better motor recovery, higher number of myelinated axons, and better rubrospinal neuron survival than immunization alone. These results open an interesting scenery for clinical studies.

Finally, in order to consider A91-immunization for being used at clinical settings, it is of relevance to contemplate vaccine safety. With this regard, immunizing with A91 shows no

signs of autoimmune disease development, possibly due to its low affinity to major histocompatibility complex (MHC) molecules [105]. Furthermore to evaluate vaccine safety, the clinical appearance of treated animals was assessed, and no weight variation or other clinical data of EAE in immunized animals was detected [106]. A91 has proven to be effective in several studies conducted at different time points showing the stability of the vaccine in promoting recovery. Preclinical results of studies evaluating vaccine efficacy indicate that this therapy could be possibly applied to SCI patients and improve their recovery and quality of life.

4. Concluding statement

Injury to the spinal cord stimulates the appearance of innate and adaptive immune responses, which could participate in either the pathogenesis or healing responses to trauma. The immune system should not be suppressed; instead, it must be modulated to attain its beneficial effect. That is why the use of immunosuppressant drugs like MP in the clinical field no longer seems a rational treatment. As a physiological hemostatic self-defense mechanism, PA is an essential mechanism to be considered for the pathophysiology and treatment of SCI. Boosting PA with the use of APL is needed in order to increase the functional recovery in the immune related neurodegenerative diseases. In SCI, immunization with the APL A91 has proven to reduce part of the immune-related secondary injury mechanisms without the risk of developing an autoimmune response. Preclinical results suggest that this therapy could be an effective treatment for SCI recovery, as it is closely related to a higher motor improvement, which is the most evident deficit in SCI patients. However, further studies related to the use of APL in SCI are needed to translate this therapy to the clinical field. For instance, we have to ensure that immunization with this peptide does not cause any side effect (i.e. hypersensitivity or autoimmunity). Additionally, further experiments should be performed in order to find out the best adjuvant to be used in humans, even the investigation should be directed to elucidate if the use of adjuvants is really necessary. Finally, the dosing of the peptide as well as the schedule of administration at clinical settings should also be investigated.

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