THERAPEUTIC ROLE OF IL-37 IN SPINAL CORD INJURY

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1. Summary

Inflammatory response plays an essential role in protecting the body after injury or invasion by microorganisms. Inflammation must be highly regulated, otherwise, it may lead to tissue damage or even to inflammatory disease. In the nervous system, inflammatory response plays a crucial role in promoting axonal regeneration after injury, as has been consistently shown in several models of peripheral nerve injury. However, after spinal cord injury an inefficient control of the inflammatory response occurs, which exacerbates tissue damage (death neurons and axonal disruption), inefficiently clears myelin along the degenerating tracts, and becomes chronic.

IL-37 is unique in the IL 1 family in that this cytokine broadly limits innate and adaptive immunity. Transgenic mice for human IL-37 exhibit reduced disease severity in models of endotoxemia, acute lung injury, chemical colitis, myocardial ischemia, metabolic syndrome and sleep disturbance. However, whether IL-37 mediates anti-inflammatory actions in the central nervous system was unknown until this project.

We therefore investigated whether IL-37 mitigates the inflammatory response following SCI, and whether this led to reduced tissue damage and functional deficits. To carry out these experiments, we proposed the following aims:

1. To assess whether IL-37 suppresses inflammation in spinal cord injury.
2. To assess the involvement of IL-37 in minimizing tissue damage, functional deficits and development of neuropathic pain after spinal cord contusion injury in mice.
3. To assess whether IL-37 leads to axon regeneration following lesion to the spinal cord.

2. Results

SPECIFIC AIM 1. ASSESS WHETHER IL-37 SUPPRESSES INFLAMMATION IN SPINAL CORD INJURY

Since IL-37 is not expressed in the mouse, we studied the effects of IL-37 by generating a transgenic mouse that expresses the human form of IL-37 (hIL-37tg mouse). Using this mouse, we showed that there is undetectable/minimal expression of IL-37 in the spinal cord under physiological conditions. This low level is due to the instability sequence in human IL 37 that limits its lifetime. However, after the contusion injury, induction of IL 37 in the spinal cord parenchyma was observed. IL-37 expression profile occurred at 2 peaks of expression; after 12 hours and at day 3 post-injury, when the levels of IL-37 increased ~17 and ~35 fold, respectively. The early peak of IL-37 coincides with the induction of cytokines in the injured spinal cord (6-24 hours post-injury), whereas the latter correlates with the infiltration of blood monocytes (day 3).

We next sought to examine whether the early increase in IL 37 modulated gene expression of cytokines in the contused spinal cord. We observed that induction of IL-37 led to significant reduction in the expression of 23 out of the 32 cytokines in hIL-37tg mice, including an 80% reduction in the protein levels of IL-6.

Since cytokines regulate the recruitment and activation of immune cells into the injured spinal cord, we next evaluated the accumulation of inflammatory cells between WT mice and hIL-37tg mice following SCI. At day 1 post-injury, when granulocyte infiltration reaches peak levels, the spinal cord of hIL-37tg mice showed ~40% reduction in the number of granulocytes. There were no differences in the cell counts for activated microglia, blood-borne macrophages, CD4 and CD8 T cell, at this time point. However, seven days post-injury, when the accumulation of activated microglia and macrophages reaches peak levels in the injured spinal cord and accounts for ~80% of total immune cells, spinal cords from hIL-37tg mice exhibited significantly lower numbers of these two cell subsets. These results provides clear evidence on the marked anti-inflammatory actions of IL-37 in SCI.

SPECIFIC AIM 2. ASSESS THE INVOLVEMENT OF IL-37 IN MINIMIZING TISSUE DAMAGE, FUNCTIONAL DEFICITS AND DEVELOPMENT OF NEUROPATHIC PAIN AFTER SPINAL CORD CONTUSION INJURY IN MICE

We next assessed whether attenuating inflammation by IL-37 resulted in reduced functional deficits and tissue damage after SCI. hIL-37tg mice displayed significant improvement in locomotor recovery after SCI. There were significant improvements in BMS scores starting at 7 days post-injury (dpi), and remaining significantly enhanced for the following 3 weeks. At 28 dpi, all WT mice showed extensive ankle movement, but only 50% of these mice showed plantar paw placement but no weight-bearing stepping. In contrast, all hIL-37tg mice showed extensive ankle movement, plantar paw placement with weight support, and the majority displayed occasional stepping. Moreover, hIL-37tg mice performed ~2.5 fold faster locomotion on the treadmill than WT mice, further demonstrating the protective effect of IL-37 against functional loss in SCI.

We then assessed whether the improvement in motor skills of hIL-37tg mice was associated with reduced secondary tissue damage after SCI. Histological sections from the injury epicenter stained with luxol fast blue revealed enhanced myelin sparing in hIL-37tg compared to WT mice. Prevention of myelin loss by transgenic expression of IL-37 was evident at the lesion epicenter and at distances up to 800 μm caudal to the injury. Assessment of neuronal sparing in the ventral horns also demonstrated attenuated neuronal loss in hIL-37tg. Spinal cord sections stained against NeuN revealed that hIL-37tg mice had greater numbers of neurons at several regions rostral and caudal to the injury epicenter in hIL-37tg mice.

To assess a therapeutic use of IL 37 in SCI, we administered recombinant forms of human IL 37 (rIL 37) to determine possible beneficial effects in SCI. We first tested the IL-37 precursor (full-length of IL 37 isoform b), as previous studies have shown efficacy in vivo. We also administered a processed form of IL 37 with the N-terminus at valine 46. Since the blood-brain barrier prevents the entry of most molecules into the CNS, we infused rIL 37 into the lesion site 5 minutes after contusion injury, using a glass capillary (30μm diameter). We found that intraspinal injection of either full-length or processed rIL-37 significantly enhanced locomotor recovery in the BMS score, starting at day 10 post-

injury. At day 28, mice injected with saline had slight or extensive movement of the ankle. However, mice treated with full-length had greater locomotors skills, and showed extensive movement of the ankle and most of them also displayed plantar paw placement in at least one paw, but no stepping. Moreover, treatment with full-length rIL-37 increased in 50% the speed that mice were able to achieve on a treadmill further suggesting the beneficial effects of rIL 37 in preventing functional deficits after SCI.
We also assessed whether transgenic expression of IL-37 reduced neuropathic pain. Our data revealed that hIL-37tg mice showed reduced pain hypersensitivity to thermal stimuli after SCI, but did not reduce mechanical pain sensitivity.

These data demonstrate that IL-37 confers protection against locomotor deficits and tissue damage as well as reducing the effects of neuropathic pain to thermal stimuli.

SPECIFIC AIM 3. ASSESS WHETHER IL-37 LEADS TO AXON REGENERATION FOLLOWING LESION TO THE SPINAL CORD

Since infiltrating macrophages inhibit axonal outgrowth by releasing soluble factors and by cell-cell interaction, we sought to evaluate whether there was enhanced axonal regeneration in hIL-37tg mice after complete spinal cord transection. hIL-37tg and WT mice presented complete hind limb paralysis after the injury and lacked functional improvement at 10 weeks post-lesion. Histological assessment of sagittal spinal cord tissue sections revealed that transgenic expression of IL-37 did not led to regeneration of axons beyond the transection site. Similarly, axonal counts at rostral distances to the injury did not reveal any difference between hIL-37tg and WT mice, suggesting that IL-37 did not promote regeneration/sprouting of corticospinal axons.

3. Significant Statement

Spinal cord injuries often result in severely impaired locomotor, sensory and autonomic function. Although inflammation contributes to the physiopathology of spinal cord injury, several clinical trials of high doses of dexamethasone or methylprednisolone have not resulted in improved recovery of function. IL 37, a member of the IL 1 family, exerts

broad anti-inflammatory effects in several mouse models of inflammatory diseases. We report here that mice expressing human IL-37 exhibit reduced inflammation in the central nervous system and resulted in significantly improved functional disabilities following spinal cord injury. The administration of recombinant forms of human IL-37 enhances motor skills after spinal cord injury, suggesting that IL 37 could provide a new therapeutic approach to limit the harmful effects of inflammation in neurological conditions.

4. Publications

The following data, excluding the results obtained in the neuropathic pain studies, were recently published in *Proceedings of the National Academy of Sciences*, one of the journals with greatest impact factor in the scientific field.

Coll-Miro M, Francos-Quijorna I, Santos-Nogueira E, Torres-Espin A, Bufler P, Dinarello CA, López-Vales R (2016) Beneficial effects of IL 37 After Spinal Cord Injury in Mice. PNAS, 113:1411-1416 Impact Factor 9.8

These findings were also highlighted in the most important newspapers in Spain, including La Vanguardia, El Periódico, La Razón, ABC and El Mundo.