Chapter 7

# Role of JAK-STAT Signalling on Motor Function Recovery after Spinal Cord Injury

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Additional information is available at the end of the chapter

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**Abstract**

JAK-STAT signalling is a main transduction pathway of cytokines and growth factors, which is involved in several biological processes including cell proliferation, cell differentiation, axon regeneration, apoptosis and inflammation. After spinal cord injury several cytokines activate the JAK-STAT pathway, thereby modulating several cell responses. In this chapter we discuss how regulation of this signalling pathway could improve motor recovery after injury by modulation of axon regeneration, neuroprotection, glial scar formation, demyelination and inflammatory response. Studies with gene over-expression, gene deletion and *in vitro* approaches will be discussed for understanding the cell-specific response to JAK-STAT signalling, with a focus on preclinical treatment with IL6-family cytokines, hematopoietic cytokines and IL10.

**Keywords:** cytokine, JAK-STAT, STAT3, axon regeneration, glial scar, inflammation

## 1. Introduction

Worldwide, an estimate of 180,000 cases of spinal cord injuries (SCI) occur yearly [1]. SCI results in the complete or partial loss of motor and sensory functions below the lesion site. This type of injury causes irreversible paralysis, chronic pain, loss of bladder, bowel and sexual function, amongst others dysfunctions, impairing quality and increasing the cost of life [2, 3].

The pathology of SCI in mammals starts with an acute phase during the first days of injury, which includes massive cell death and inflammatory response. The acute response is followed by a second phase during the first week after injury consisting of tissue replacement, where the loss of cells is replaced by a glial scar. After the second week it finalizes with a third

phase which continues for months involving chronic tissue remodelling, remyelination and circuit remodelling [4]. Although some spontaneous repair after SCI has been described in mammals including humans, it contributes poorly to motor and functional recovery. Progenitor cells proliferate and differentiate but mostly to glial cells while no neurogenesis occurs [5, 6]. Besides there is only a partial axon regeneration and circuit remodelling response that contribute to a limited compensatory recovery [2].

Several reviews have been published describing growth factors and cytokines that regulate cell response in SCI [2, 7, 8]. Advancements using these factors to improve spinal cord repair are being made, and preclinical treatments have been achieved by local delivery of growth factors and by physiological delivery with i.v. or i.p. injections (intravenous or intraperitoneal, respectively) [2]. Between the plethora of signalling pathways activated in response to tissue injury, the JAK-STAT signalling pathway is one of the main pathways that has been extensively studied because of its broad effect on the response to injury. In this chapter, we discuss the activation and role of JAK-STAT signalling in response to SCI and studies focused on modulating JAK-STAT to improve motor recovery. First, we briefly describe the JAK-STAT pathway components, followed by a discussion of its role in different cellular processes including: axonal regeneration, neuroprotection, glial response and its effects on the inflammatory response.

## 2. JAK-STAT signalling pathway

The JAK-STAT signalling pathway is involved in transmitting information from the extracellular milieu to gene promoters in the nucleus. The basic components of the JAK-STAT pathway are depicted in **Figure 1**. Several cytokines, growth factors and even hormones signal through the JAK-STAT pathway. Currently, there are 38 protein ligands and 36 cell surface receptor combinations have been described [9]. Besides the several combinations of protein ligands and receptor complexes, in mammals there are four JAK (Janus kinase) tyrosine kinases: JAK1, JAK2, JAK3 and Tyk2 (Non-receptor tyrosine-protein kinase TYK2); and seven STAT (Signal transducer and activator of transcription) transcription factors: STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b and STAT6 [9].

Activation of the JAK-STAT pathway begins after ligand binding to the receptor subunits, which forms homodimers, heterodimers or heteromultimers depending on the family receptor (**Table 1** presents ligands and receptors related to SCI). After multimerization, intracellular transduction is initiated through the recruitment of JAK kinases. JAKs phosphorylate receptor subunits and STAT transcription factors. After tyrosine phosphorylation, cytosolic STATs dimerize and are translocated to the nucleus to bind specific DNA regulatory sequences and regulate gene expression [10].

The JAK-STAT signalling pathway has different negative inhibitors. The most important inhibitor for preclinical studies is the classical negative feedback loop of suppressor of cytokine signalling (SOCS) proteins, which are target genes for STATs proteins and switch off JAK

proteins. Over-expression or deletion of SOCS3, one of the eight mammalian SOCS proteins, has been extensively used to modulate endogenous pathway activation [11–13].

Although it will not be discussed in this chapter, it has to be considered that ligands binding to receptor complexes also activate other intracellular signalling cascades besides the JAK-STAT pathway [10]. Phosphorylation of receptors induces activation of ERK1/2 and AKT pathways. Moreover, STATs can also be activated independently to the canonical JAK-STAT signalling pathway. Growth factor activation of RTKs (receptor tyrosine kinases) and NRTKs (non-receptor tyrosine kinases) can activate STATs. Hormone and chemokine binding to G protein–coupled receptors can also activate JAKs proteins to phosphorylate STATs [10].

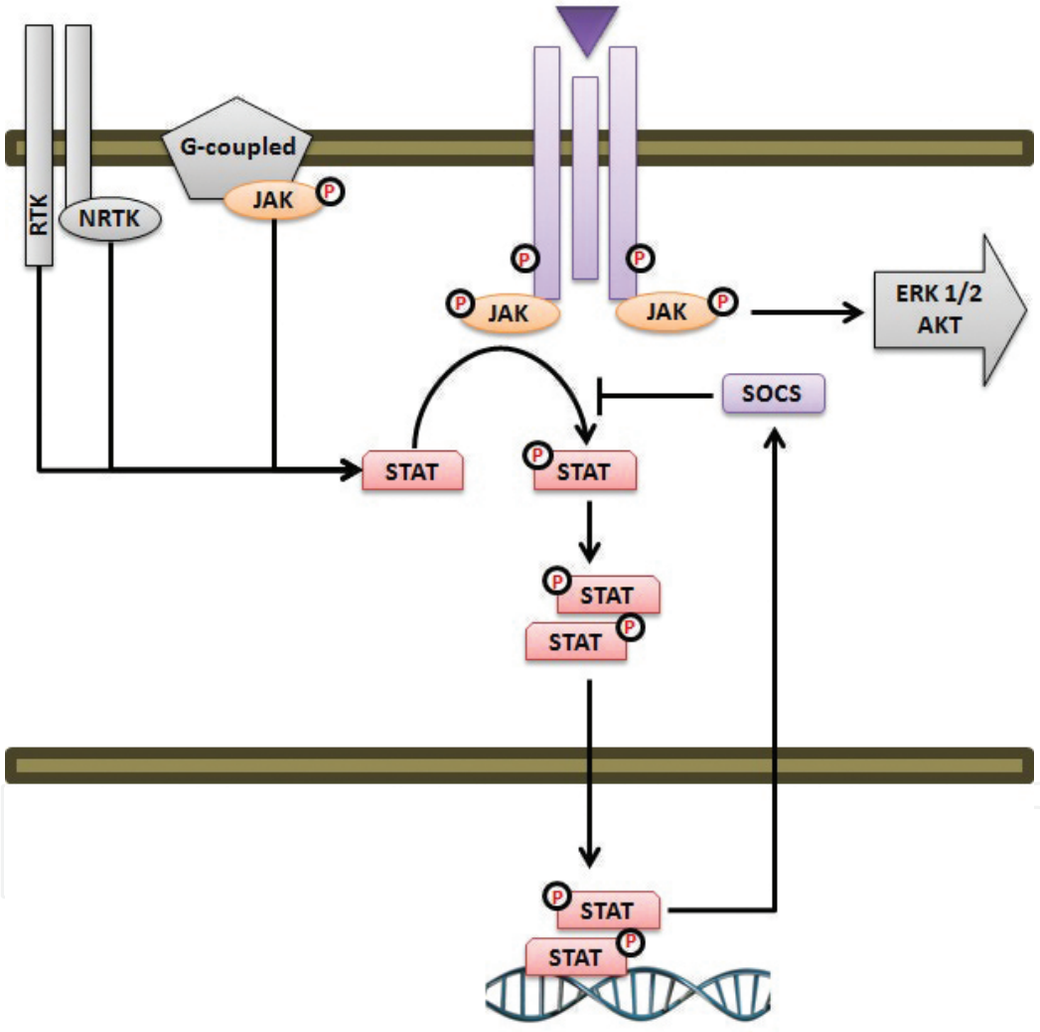


**Figure 1.** Basic components of the JAK-STAT signaling pathway. After binding of cytokines, receptors multimerize (violet boxes) recruiting to the membrane and activating JAK kinases (orange boxes) that initiate substrate phosphorylation (letter P). STAT transcription factors (pink boxes) are phosphorylated, dimerized and transported to the nucleus. STAT dimers regulate gene transcription. Among others, a classic target is SOCS that forms a negative feedback loop inhibiting JAK function. Alternative signaling pathways (grey boxes) for JAK-STAT are JAK-receptor complex activation of ERK1/2 and AKT pathways; STATs phosphorylation by RTKs, NRTKs or JAKs associated to G-coupled protein receptors.

## 3. JAK-STAT pathway activation in response to SCI

### 3.1. Cytokine expression in response to SCI

Before discussing the role of the JAK-STAT pathway in motor recovery, we will discuss the endogenous expression of cytokines and activation of STAT proteins in response to SCI. The upregulation of the IL6 family cytokines, IL6 (Interleukin 6), LIF (Leukemia inhibitory factor), OSM (Oncostatin M), IL11 (Interleukin 11) and CNTF (Ciliary neurotrophic factor) have been well characterized in response to SCI. Early IL6 and LIF upregulation has been detected in the acute phase after SCI, with a peak of expression around 6–12 hpi (hours post-injury) and basal levels at 4 dpi (days post-injury) [14, 15]. Different neural cell types contribute to IL6 and LIF expression. It has been shown that IL6 has a broad cell type expression, being detected in neurons, astrocytes and macrophages, while LIF is mainly expressed in meningeal astrocytes [14]. IL6 mRNA expression correlates with IL6 protein levels analyzed in another study, which detected an increase in IL6 concentration from 3 hours to 3 dpi [16]. In these studies the upregulation of cytokines occurs in the surrounding area of the lesion site, around 1 or 2 mm from the epicentre.

Early upregulation of OSM is also detected after SCI, with a strong peak at 6 hpi, but on the contrary to IL6 and LIF, upregulation of OSM is still detected until 1 mpi (months post-injury) [15]. IL11 and CNTF protein upregulation has also been detected after the acute phase. A steady increase in protein levels has been detected during the first week of injury for IL11 and during the first month for CNTF. In both cases no later times were analyzed to determine when the cytokines reached basal levels, therefore there is a possibility that they had an extended upregulation [17, 18]. IL11 cell expression has not been analyzed; on the contrary, it has been shown that CNTF has a chronic expression in glial cell types after spinal cord injury. CNTF has been detected in oligodendrocytes during the first month and in astrocytes up to 4 mpi [18, 19]. This chronic expression of CNTF is more spatially restricted than the early expressed cytokines IL6 and LIF, being detected only in the lesion borders [19].

| Cytokine family | Cytokine | Receptors | JAKs | STATs | Endogenous levels, preclinical or clinical trials |
|---|---|---|---|---|---|
| IL6 | IL6 | Gp130 – IL6R | Jak1 | STAT1-3 | U/P |
| | LIF | Gp130 – LIFR | Jak2 | STAT3 | U/P |
| | CNTF | Gp130 – LIFR – CNTFR | Tyk2 | STAT3 | U/P |
| | IL11 | Gp130 – IL11R | | STAT3 | U |
| | OSM | Gp130 – | | STAT3 | U/P |

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| Cytokine family | Cytokine | Receptors | JAKs | STATs | Endogenous levels, preclinical or clinical trials |
|---|---|---|---|---|---|
| | | LIFR | | | |
| Hematopoietic | G-CSF | CSF3R | Jak2 | STAT3 | P/C |
| | GM-CSF | CSF2Ra – βcR | Jak2 | STAT5a/b | P/C |
| | EPO | EpoR or EpoR-βcR | Jak2 | STAT5a/b | P/C |
| IL10 | IL10 | IL10Rα – IL10Rβ | Jak1 – Tyk2 | STAT1-3 | P |

**Table 1.** JAK-STAT ligands, receptors and transducers involved in spinal cord injury. Cytokines are shown with associated receptors and JAK-STAT components. It is shown if cytokines are up-regulated in response to SCI (U) or if they have been used in preclinical (P) or clinical (C) studies for spinal cord recovery.

### 3.2. JAK-STAT signalling in response to SCI

In concordance with the upregulation of cytokines in response to SCI, several studies have characterized JAK-STAT pathway activation in spinal cord cells. Consistent with the transient increase of IL6 concentration, in the same study it was detected an increase in gp130 dimerization and JAK1 phosphorylation [16]. As expected for the activation of the gp130/JAK1 axis, STAT1 and STAT3 are phosphorylated in response to SCI. pSTAT1 has an acute increase which reach basal levels at 2 dpi [20]. On the contrary, pSTAT3 has an extended increase which differs between studies. An increase in pSTAT3 has been detected up to 7 dpi in some studies [12, 16], while in another no basal levels were reached even after 2 weeks post-injury (wpi) [21]. Nevertheless, all studies agree that STAT3 has a longer activation than STAT1. Together with the temporal difference, protein localization suggests that STAT3 has a more prominent role in regulating gene expression than STAT1 in cell response. While pSTAT3 has been detected in neuron nuclei after spinal cord injury, pSTAT1 has been only detected in the cytoplasm [16, 20].

STAT3 activation has been characterized in several spinal cord cell types. In the acute injury, nuclear pSTAT3 is observed in neurons of the anterior horns [16] and transcriptional activity is also supported by the detection of SOCS3+ neurons [21]. Nuclear pSTAT3 and SOCS3 has been also detected in microglia/macrophage during the acute phase of injury [16, 21] and nuclear pSTAT3 in oligodendrocytes and astrocytes during the first week near the lesion site [12, 22]. Due to a prominent role in glial scar formation, spatial STAT3 activation on astrocytes has also been highly defined. It has been determined that during the first week pSTAT3+ astrocytes appear at the border of the lesion with elevated predominance on the first 500 μm near the injury [12, 22].

After the first week of injury, contusion models show chronic pSTAT3 signalling in glial cell types located in the lesion borders. pSTAT3 has been detected in oligodendrocyte precursor cells (OPCs) and oligodendrocytes at 2 wpi [18, 19]. This activation decreases from 1 to 5 wpi,

but at that time is still higher than on uninjured spinal cords [19]. pSTAT3+ astrocytes have been detected even further, at 12 wpi in the lesion borders [19].

## 4. Role of JAK-STAT in axon regeneration and collateral sprouting

To recover motor function after SCI, new connections have to be established after neuron death and axon degeneration [2]. These circuits can be established by two different cellular responses that should not be confused: Axon regeneration is the process where axons from severed neurons regrow from the injured tip or from a lateral growth distant to the injury (**Figure 2a-b**). On the contrary, collateral sprouting is a compensatory growth from undamaged axons initiated in response to injury (**Figure 2c**) [23].

While there is limited axon regeneration through the lesion area in response to complete SCI (**Figure 2a**), there is a compensatory remodelling on brain and spinal circuits when the spinal

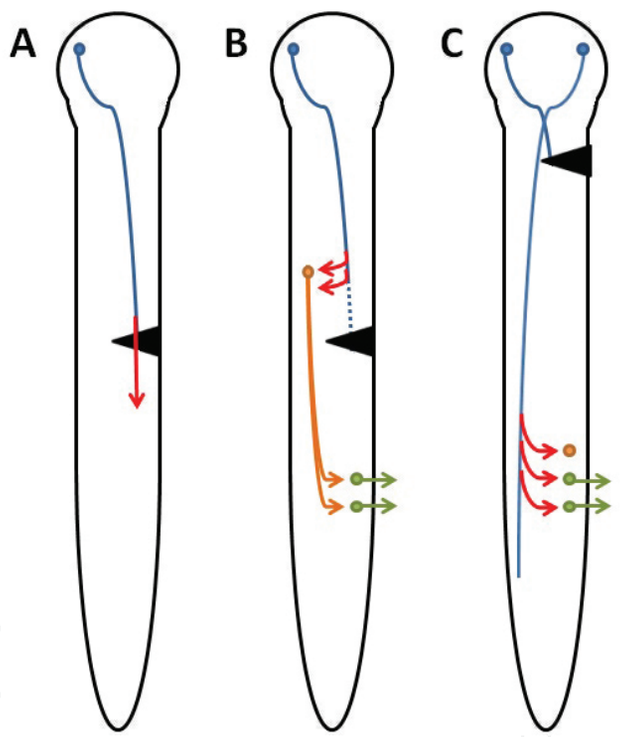


**Figure 2.** Axonal regeneration and collateral sprouting in spinal cord injury. Models used to evaluate axon regeneration and circuit remodeling, showing corticospinal tract (CST, in blue) as an example. Remodeling can be evaluated by axonal regeneration or collateral sprouting (both in red arrows) and new innervations can be connected to propiospinal neurons (PSNs, interneurons in orange) or motor neurons (in green). CNS injury is indicated as a darkened triangle in every model. **A**, In SCI models (usually contusion and hemisection) axonal regeneration of injured CSTs can be evaluated through and beyond the lesion area. **B**, in a hemisection model, innervation of new targets by injured CSTs can be evaluated. Regenerative axons from CSTs can innervate PSNs, which then connect to denervated motoneurons. **C**, in a unilateral pyramidotomy injury paradigm, collateral sprouting of uninjured CSTs to denervated PSNs and motor neurons can be measured. Anterograde, retrograde or retrograde trans-synaptic labelling are used depending of the injury model.

cord lesion is incomplete [2]. The corticospinal tract (CST) has been used as a model to study this recovery process. CST starts in the motor cortex and connects to spinal motor neurons, controlling voluntary motor function [23]. After spinal cord injury, CST circuit remodelling can be achieved by axon regeneration innervating long descendent interneurons that increase connection to motoneurons (**Figure 2b**) [24] or by collateral sprouting of uninjured axons to denervated motoneurons (**Figure 2c**) [25]. In this section we will discuss the role of JAK-STAT pathway on the promotion of axon regeneration and collateral sprouting after SCI. Briefly, we will discuss the results involving dorsal root ganglion (DRG) and optic nerve injury, to follow with studies of axon growth and collateral sprouting of the CSTs after SCI.

### 4.1. Role of JAK-STAT pathway in axon regeneration in SCI and other nerve injury models

#### *4.1.1. Axon regeneration after dorsal root ganglion or optic nerve axotomy*

The DRG model has been useful to study axon regeneration due to regenerative and non-regenerative branches. Sensory neurons from DRG target peripheral tissue with branches (i.e. sciatic nerve) that regenerate after axotomy. On the contrary, DRG neurons also target the dorsal column of the spinal cord, which do not regenerate after injury. This difference in the regenerative capacity has been partially related to the JAK-STAT pathway activation by studies on cytokine deficient mice, but conclusive results have been obtained in a study assessing the role of STAT3 in axon regeneration. While STAT3 deletion reduced axon growth of the severed sciatic nerve, STAT3 viral over-expression in the DRG improves axon regeneration of the non-regenerative nerve [26].

Similar to the activation of the JAK-STAT pathway in the DRG model, axon growth has been also promoted in the non-regenerative optic nerve. JAK-STAT activation by CNTF treatment or deletion of the SOCS3 negative feedback improved axon regeneration in optic nerve injury [11]. The studies in both injury models demonstrate that the JAK-STAT pathway, specifically the axis of STAT3/SOCS3, promotes axon regeneration. Therefore, a possible mechanism to improve motor recovery after SCI is through modulation of the JAK-STAT pathway to induce axon regeneration.

#### *4.1.2. Axon regeneration after the spinal cord injury*

A hemisection study showed that only a small subset of cortical neurons presented an increment in STAT3 and pSTAT3 levels after SCI, suggesting that on the contrary of peripheral nerves, in the spinal cord there is a lack of retrograde JAK-STAT activation [27]. As in DRG and optic nerve injury models, insufficient JAK-STAT activation could be related to the lack of axon regeneration. Several studies have shown that improvement of local extrinsic factors, such as cytokines administration, or improvement of intrinsic factors, such as the genetic modulation of the JAK-STAT pathway in neurons, improves axon regeneration after SCI, suggesting that modulation of JAK-STAT can be a bona fide target for designing novel therapies for spinal cord repair.

Administration of CNTF, G-CSF (granulocyte-colony stimulating factor), IL6 and LIF by intrathecal or i.p. injections results in improved axon regeneration through the lesion site and motor recovery after SCI. CNTF intrathecal injections during the first 10 days or administration in sodium-hyaluronate particles placed in the transection site have improved functional recovery after SCI [28, 29]. Although CST axon regeneration has not been assessed in CNTF treatment, a study showed increased retrograde labelling of the rubrospinal tracts [28]. Another study showed improved axonal sprouting through the lesion site, although it was not evaluated if these axons were from local neurons or descended from the brain [29].

Axon regeneration has also been assessed after spinal cord administration of G-CSF during 2 weeks after a hemisection [30]. CST axon regeneration was improved in the lesion site although no axon growth caudal to the injury was detected. The local G-CSF delivery also results in improvement of motor function and a similar result was observed in a transgenic mice line expressing G-CSF under the control of the MapKII promoter that is specifically activated in cortical and spinal cord neurons [30].

On the contrary to CNTF and G-CSF treatments, LIF has been administered daily by intraperitoneal injection since it is actively transported through the BBB with a mechanism involving the LIFR$\alpha$ receptor [31, 32]. LIF treatment for 14 days in a hemisection injury model improved the number of retrograde labelled CST and rubrospinal neurons. Improvement in motor recovery was assessed by RotaRod and Platform hang tests [32].

Although CNTF, G-CSF and LIF treatments showed an improvement in axon regeneration and motor function in rodents, in these studies the activation of the JAK-STAT pathway in cortical neurons was not assessed. Besides, these studies cannot distinguish if the improvement in axon regeneration and functional recovery is due to the modulation of the intrinsic program of cortical neurons or due to the modulation of other spinal cord cell types. Contrary to these studies, local IL6 administration has been shown to activate the JAK-STAT pathway in spinal and cortical neurons [33, 34]. IL6 intrathecal administration during the first week after a hemisection injury increased pSTAT3 levels in the spinal cord and in cortical neurons, while up-regulated the regeneration associated gene GAP-43 mRNA [34]. Although no motor recovery analyses were assessed, anterograde labelling showed an increase in the number of synapsin1+ CST axons near the lesion site [33].

The IL6 treatment suggests that cytokine delivery can promote the regenerative response of motoneurons by JAK-STAT pathway activation. In addition, specific STAT3 over-expression in the cortex has shown that this pathway can promote axon regeneration. After over-expression mediated by adenovirus injection in the cortex, pSTAT3 levels increased in cortical neurons and CST axonal regeneration improved through and beyond the lesion site of a hemisection [27].

### 4.2. Role of JAK-STAT in circuit remodelling by collateral sprouting

Although improvement in axon regeneration (**Figure 2a**) and motor recovery has been achieved by cytokine delivery, no relation has been established between the observed axon regeneration in the lesion site and the motor recovery. On the contrary, studies involving JAK-

STAT pathway and circuit remodelling by collateral sprouting (**Figure 2c**) have suggested that motor recovery is accomplished by innervation of uninjured axons to denervated motoneurons and interneurons.

| Cytokine | Treatment | Cell response | Motor recovery | References |
|---|---|---|---|---|
| Il6 | Acute by i.t. | Axon regeneration | Not assessed | [33, 34] |
| | Acute i.p., MR16-1 | Immune modulation | Improved | [66, 84, 85] |
| LIF | 10-14 days by i.p. | Axon regeneration<br>Glial modulation | Improved | [32, 60, 61] |
| CNTF | 10 days by i.p. or Hyaluronate | Axon regeneration<br>Glial modulation | Improved | [28, 29] |
| | SC viral expression | Collateral sprouting | Not assessed | [13] |
| OSM | Gel foam | Glial modulation | Improved | [15] |
| G-CSF | 2 weeks by i.t. | Axon regeneration<br>Neuroprotection | Improved | [30] |
| | Acute by subcutaneous, i.v. or i.t. | Neuroprotection<br>Glial modulation<br>Immune modulation | Improved | [40, 54-57, 81] |
| GM-CSF | Acute by i.v., i.t. or i.p. | Glial modulation | Improved | [54, 55, 64] |
| EPO | Acute by i.p. | Collateral sprouting<br>Glial modulation | Improved | [35, 58, 59] |
| IL10 | Acute by i.p. | Glial modulation<br>Immune modulation | Improved<br>Not improved | [77, 78] |
| | SC viral expression | Neuroprotection | Improved | [48, 49] |
| IL12 | Gel foam | Glial modulation<br>Immune modulation | Not improved | [82] |

**Table 2.** Pre-clinical studies with cytokine for motor recovery of SCI. Summary of JAK-STAT cytokine preclinical studies, for any cytokine it is shown administration timing and method. Time indicated as acute means a single dose at the moment of injury or daily doses from 1 to 5 dpi. Methods of administration are indicated as intrathecal (i.t.), intraperitoneal (i.p.), intravenous (i.v.) or subcutaneous injection; embedded on hyaluronate or gel foam placed in the lesion site; and by SC (spinal cord) viral expression. Contrary to the other studies, MR16-1 indicates a treatment with a neutralizing antibody against IL6 receptor.

Promotion of collateral sprouting by the hematopoietic cytokine EPO (Erythropoietin) has been assessed in a model of traumatic brain injury that results in denervation of the CST circuit. EPO i.p. injections during the acute phase resulted in increased collateral sprouting of uninjured CST fibers in the cervical and lumbar area and improved motor function [35]. Although it has not been shown that EPO activate the JAK-STAT signalling pathway in neurons, cortical viral infection has been useful to determine the role of the pathway in collateral sprouting. STAT3 viral over-expression in uninjured mice induced collateral

sprouting and innervations to propiospinal neuron (PSN, a type of interneuron) [27]. This viral expression was also assessed in a unilateral pyramidotomy model, which is an injury at the level of the medulla oblongata that severs half of the CST (**Figure 2c**) and it is useful to evaluate how collateral sprouting connects to spinal neurons. In this approach it was shown that STAT3 induced collateral sprouting, innervation of PSNs and motoneurons, and improvement in motor recovery which was assessed by behavioural and electrophysiological tests [27]. Cortical SOCS3 deletion also induced collateral sprouting in the pyramidotomy paradigm [13], suggesting that an endogenous JAK-STAT pathway activation promotes circuit remodelling. After finding a transient CNTF expression in denervated neurons caudal to injury, the same study showed that combination of cortical SOCS3 deletion and spinal cord CNTF over-expression improved collateral sprouting [13].

## 5. Role of JAK-STAT in local neuron response

In response to SCI the number of local motor and interneurons decrease with no neurogenesis to generate new neurons [2]. To improve motor recovery, promotion of axon regeneration and collateral sprouting should be accompanied by neuroprotective strategies, since number and dendrite distribution of local spinal neurons would be beneficial for circuit remodelling. We will start this section discussing *in vitro* studies related to the neuroprotective role of the JAK-STAT pathway, following *in vivo* studies with cytokine delivery and knockdown treatments after SCI.

### 5.1. Role of JAK-STAT in neuron survival

At a molecular level, studies in non-neural cells have shown that STAT transcription factors have different roles in apoptosis regulation. A comparative *in vitro* study of STAT1 and STAT3 showed that while STAT1 inhibits the expression of the anti-apoptotic genes Bcl-2 and Bcl-X, STAT3 promoted their expression [36]. STAT5 proteins, which are activated by a different set of cytokines (Table 1), also promote cell survival by Bcl-X upregulation [37].

*In vitro* studies have shown that the neuroprotective role of JAK-STAT cytokines is partly associated to STAT3 activation and expression of anti-apoptotic genes. IL10 activated STAT3 and promoted Bcl-2 and Bcl-xl expression in embryonic spinal cord neurons [38]. IL6 and OSM cytokines also activated STAT3 and promoted anti-apoptotic gene expression in cortical neurons and neuroblastoma cells [39]. In cultured cerebellar granule neurons, G-CSF also promoted STAT3 activation while increased Bcl-2 levels [40]. It should be considered that theses cytokines also activate the AKT pathway [39, 40]. Both inhibition of the JAK and AKT pathway decreased the neuroprotective effect, suggesting that probably both pathways contribute to the anti-apoptotic response [38, 40].

Related to the previous *in vitro* studies, *in vivo* studies in CNS injury suggest a protective role of STAT3 while an opposite role for STAT1. On a focal cerebral ischemia model, it was shown that STAT1 deficient mice presented a reduced infarct size and apoptotic cell number [41]. On the contrary, on a permanent cerebral ischemia model the IL6/STAT3 axis has been associat-

ed to positive outcomes. While the neutralization with an anti-IL6R antibody resulted in decreased STAT3 activation and increased lesion area, IL6 administration reduced the lesion size [42, 43]. Although these studies indicate a neuroprotective role of STAT3, specific cell type modulation should be assessed to differentiate the direct modulation on neuron response from indirect neuroprotection by modulation of glial and inflammatory cell types. At this moment, there is only one study where specific STAT3 deletion in neurons is analyzed in an *in vivo* CNS injury model. Mice with STAT3 deletion in neurons (using the Neurofilament L promoter) showed a decrease in motoneuron number after facial nerve injury and diminished expression of anti-apoptotic genes [44].

### 5.2. JAK-STAT modulation of neuronal protection in SCI

G-CSF and IL10 are cytokines with several studies showing beneficial outcomes in neuroprotection, inflammatory response and motor recovery after SCI [45, 46]. It has been shown that a pro-inflammatory response is negative for neuron survival [47]; therefore, in these cytokine treatments it is difficult to differentiate a direct modulation on neuronal survival and the effect on neuro-inflammation. Since it has been shown that spinal cord neurons express G-CSF and IL10 receptors [40, 48], we will discuss a small set of studies that focused in neuroprotection and in a following section we will discuss the modulation of the immune response in SCI.

G-CSF has been locally administrated to evaluate neuroprotection in SCI. Subcutaneous G-CSF treatment during the first five days after compression improved motor recovery, decreased apoptotic neurons in the acute phase and improved neuron number in the remodelling phase (6 wpi) [40]. Other studies with intrathecal administration of G-CSF for 2 weeks, commented before for assessing axonal regeneration, also showed neuroprotective results. This long-term treatment reduced apoptotic cell number and increased Bcl-xL expression in the spinal cord [30]. Two independent IL10 viral over-expression studies also improved motor function [48, 49] and one of these studies showed decreased pro-apoptotic protein levels and increased Bcl-2 levels, resulting in a higher neuron number [48]. Although it was shown by *in vitro* studies that G-CSF and IL10 treatments activate STAT3 in spinal neurons [40, 48], it has not been demonstrated that this activation occurs on *in vivo* treatments. *In vivo* analyses of IL10 over-expression only focused in neuronal NF-κβ activation [48]. Therefore, it is an open question if these cytokines promotes neuron survival by JAK-STAT activation or by other signalling pathway, as NF-κβ gene regulation.

Although by results of cytokine treatments the role of STAT3 in neuroprotection remains inconclusive, knockdown of the inhibitory protein SOCS3 has contributed to this proposition [21]. Local over-expression of shSOCS3 diminished SOCS3 mRNA levels. This knockdown increased pSTAT3 levels in a transection model and it decreased the Bax/Bcl-2 protein ratio, while improving neuron number near the lesion site [21]. A following study of the same group showed that SOCS3 knockdown increased dendrite growth in dorsal and ventral horns near the injury site [50].

Finally, consistent with the pro-apoptotic role of STAT1 on *in vitro* studies [36], STAT1 knockdown has shown positive outcomes after SCI. Administration of STAT1 siRNA reduced STAT1 protein levels in the spinal cord. Although no analysis of neuronal survival was

assessed, 1 day post-contusion the STAT1 knockdown mice had increased its Bcl-2 levels. siRNA treated mice also improved motor function assessed by BMS open-field score.

## 6. Role of JAK-STAT in glial response

In response to SCI, apoptosis of astrocytes and oligodendrocytes occurs in the acute phase of injury and then glial cells dynamically respond during weeks and months after injury. A glial hallmark of CNS injury is the glial scar, formed by reactive astrocytes, OPCs and meningeal cells [51]. This compact scar gathers around damaged tissue, inflammatory cells and fibroblasts. The glial scar is necessary to contain secondary injury, as disruption of the astrocyte scar with different transgenic models leads to increased cell death, demyelination and reduced functional motor recovery [12, 52, 53]. Although the glial scar is necessary for the containment of the lesion, reactive astrocytes express and secrete inhibitory molecules for axonal growth and sprouting, as chondroitin sulfate proteoglycans (CSPGs) [51]. Altogether with the glial scar formation, a demyelination process occurs during weeks after injury by oligodendrocyte apoptosis. Although mature oligodendrocytes do not proliferate to recover cell number, OPCs start to proliferate and differentiate and remyelination proceeds near the lesion area [6]. Accordingly to the glial response commented above, in this section we will discuss the following topics: Cytokine modulation of the glial response to reduce secondary damage, the regulation of reactive astrocyte and the neural stem progenitor cells (NSPCs) by the STAT3/SOCS3 axis.

### 6.1. Cytokines modulate glial response in SCI

Several studies with the hematopoietic and IL6-family cytokines have assessed astrocyte and oligodendrocyte responses with positive outcomes on tissue preservation and motor recovery. Acute administration of GM-CSF or G-CSF by i.p. or i.v. injections during the acute phase reduced lesion area, while increasing the spared myelin area and improving motor recovery after SCI [54–56]. Both cytokine treatments reduced NG2 levels (OPC marker) during the first week of injury [54, 55]. In one of these studies it was also shown that G-CSF up-regulated Bcl-xL and reduced apoptosis in oligodendrocytes [56]. Therefore, these results suggest that these cytokines maintain spared myelin by a protective mechanism and not by promotion of OPC proliferation.

GM-CSF and G-CSF also modulate the reactive astrocyte response during the first month after SCI, as seen by the reduction of GFAP levels and the CSPG neurocan [54, 55]. Another study with improvement in motor recovery by G-CSF intrathecal administration during the first day of injury also showed a reduction in the CSPG proteins neurocan and phosphocan [57]. Altogether with the previous studies, this last study suggest that G-CSF decrease the reactive gliosis because the treatment reduced TGF-β levels, a growth factor that promotes reactive gliosis; reduced vimentin levels, an astrocyte filament induced in glial scar; and presented less astrocyte proliferation [57]. Acute EPO i.p. injection also has similar results in astrocyte regulation after SCI. EPO treatment reduced lesion area and GFAP levels, while improving

preservation of myelin and motor recovery at 2 wpi [58]. A following study showed possible differences with GM-CSF and G-CSF on the OPC response. Although these cytokines reduce NG2 levels, it was shown that EPO treatment increased it at 4 wpi [59].

The IL6-family of cytokines also regulates glial response. LIF i.p. administration has been shown to reduce oligodendrocyte apoptosis [60, 61], although *in vivo* LIF signalling on oligodendrocyte is not clear. On one hand, one study found that SCI induced LIFRβ expression on oligodendrocytes and that LIF treatment induced pSTAT3 and pAKT in these cells [60]. On the contrary, an alternative study did not find LIFRβ expression on oligodendrocytes and suggested a LIF positive modulation of microglia [61]. Although studies with LIF i.p. treatment have not assessed the astrocyte response in SCI, it has been shown that this treatment incremented Nestin+ cell number near the lesion site [32]. CNTF treatment, previously commented for improved motor recovery and axon regeneration, also increased the density of astrocytes [28]. Finally, gel foam administration of OSM reduced the lesion area and preserved MBP (myelin basic protein), but on the contrary to the other IL6-family cytokines, it reduced GFAP levels near the lesion site. This glial modulation was accompanied by improved serotonergic fiber outgrowth and motor recovery [15].

### 6.2. STAT modulation of reactive astrocytes

Contrary to motoneuron response in SCI, astrocyte response has been studied with cell-specific deletions of STAT3 and SOCS3. Deletions on Nestin or GFAP expressing cells, two genes upregulated in reactive astrocytes, have elucidated the role of the JAK-STAT signalling pathway on reactive gliosis. Mice with Nestin:STAT3KO or GFAP:STAT3KO had an increased lesion area and decreased glial scar after 2 weeks of a contusive SCI [12, 22]. Consistent with the protective role of the glial scar, this was accompanied by increased demyelination and inflammatory response, altogether with a lack of motor recovery after injury. On the contrary, the deletion of the negative feedback in a Nestin:SOCS3KO mice had prolonged and increased pSTAT3 levels in response to SCI, resulting in reduced lesion area, early and increased glial scar surrounding this area, and improved motor recovery [12].

These *in vivo* studies demonstrated that the glial JAK-STAT signalling is necessary for secondary damage contention and further studies have elucidated the cellular mechanisms modulated by this pathway. First, it seems that JAK-STAT pathway is necessary for astrocyte survival in response to injury, as an *in vitro* study showed that GFAP:STAT3KO astrocytes had increased necrosis and protein release after a mechanical injury [62]. Besides cell survival, JAK-STAT controls gene expression associated to reactive gliosis. STAT3 controls the expression of glial filaments, as it is known that GFAP is a target gene for STAT3 [63] and in GFAP:STAT3KO mice, reduced levels of GFAP and vimentin were detected [22]. Cell morphology is also modulated by JAK-STAT pathway. In response to SCI, astrocytes near the lesion site change their cell morphology and orientation to form the glial scar. In GFAP:STAT3KO mice, astrocytes failed to change orientation and did not form the closed boundaries of the glial scar [53].

Another STAT transcription factor that controls astrocyte response is STAT5, which is activated by GM-CSF. As commented before, this cytokine reduced GFAP and CSPG levels

with an increase in motor recovery [55]. An *in vitro* study with astrocytes activated with TGF-β3, which up-regulates CSPG proteins, found that GM-CSF increased pSTAT5, pAKT and pRaf levels [64]. The cytokine also reduced TGF-β signalling and CSPGs expression. GM-CSF effects were blocked by JAK and PI3K inhibitors, suggesting that STAT5 and PI3K signalling could reduce the levels of axon inhibitory proteins secreted by reactive astrocytes [64]. Following studies should clarify if STAT5 has a similar role *in vivo*.

### 6.3. JAK-STAT modulation of neural stem progenitor cells

Adult neural stem progenitor cells (NSPCs) can generate new astrocytes and oligodendrocytes in homeostasis and in response to injury [5, 6]. NSPCs can also generate new neurons but only in some CNS specific areas, the dentate gyrus and the subventricular zone (SVZ) [65]. The role on the JAK-STAT pathway to modulate neurogenesis and gliogenesis is not clear and probably depends on the specific context, the cytokine types and cellular phenotype. At one hand, *in vitro* studies show that the JAK-STAT pathway induces gliogenesis in opposition to neurogenic differentiation, as seen in the blocking of IL6/STAT3 axis in NSPCs [66, 67]. On the other hand, there are some *in vivo* studies that have shown a JAK-STAT role for specific cytokines in adult NSPC proliferation and neurogenesis. In the dentate gyrus, CNTF and STAT3 deficient mice had reduced NSPC proliferation and neurogenesis [68]. Using cytokine injections and deficient mice it has been also shown that IL15 and IL10 regulate NSPC proliferation in the SVZ [69, 70].

Contrary to the neurogenesis of the dentate gyrus and the SVZ, in the spinal cord there is only gliogenesis. This cell differentiation occurs in three main cell types that proliferate in response to injury: ependymal cells, astrocytes and OPCs [6]. The role of the JAK-STAT pathway on proliferation and differentiation of ependymal cells and astrocytes has not been assessed, as transgenic mice with specific cell type deletion has been done only for Nestin and GFAP promoters, which are markers for both NSPCs and mature astrocytes. On the contrary, understanding on OPCs modulation has been done *in vivo* by NG2 knockout cells [71]. In response to injury, NG2+ cells proliferate and increase in number near the lesion site. NG2 proliferation is incremented in a NG2:SOCS3KO mice. On the contrary, in the NG2:STAT3KO mice no differences in NG2 cell number and proliferation were detected [71]. This could be explained by the modulation of OPC proliferation by other STAT transcription factors or by other pathways activated by JAK proteins (**Figure 1**), which are inhibited by SOCS3. JAK-STAT modulation of NG2 cell differentiation was also assessed in this study. Although STAT3 did not regulate OPC proliferation, in the same study it was shown that NG2:STAT3KO mice had reduced differentiation to mature oligodendrocytes during the first week of injury, but not at chronic phases (1mpi) [71].

## 7. Role of JAK-STAT in the inflammatory response

In response to injury neutrophils, macrophages and lymphocytes infiltrate to the spinal cord [72]. There are several studies where reduction of cell infiltration improves motor recovery

and tissue sparing [73, 74]. Even so, the reduction of functional recovery after SCI in mice with total ablation of infiltrated macrophages indicates that the inflammatory response is also necessary for proper recovery [75]. These differences could be explained taking into account that immune cells have different and opposite phenotypes. Microglia and macrophages, the main effectors during spinal cord inflammation, are capable of polarization that leads to either pro-inflammatory (M1 type) or anti-inflammatory cells (M2 type). While M1 macrophages participate in secondary damage as well as in axonal retraction observed after SCI, M2 macrophages are proposed to be protective and promote axonal growth [47]. Considering the role of cytokines in the regulation of the immune response, in this section we discuss studies where JAK-STAT signalling has been shown to reduce the inflammatory response or change macrophage phenotype for improvement in spinal cord functional recovery.

### 7.1. Anti-inflammatory cytokines

IL10 is a cytokine that has been studied in SCI for anti-inflammatory modulation and neuroprotection [46]. Endogenous IL10 expression has not been assessed in detail after SCI and it is only known that M2 infiltrated macrophages express Il10 surrounding the glial scar [75]. Using IL10 deficient mice it has been found that this cytokine is necessary for controlling the inflammatory response and apoptosis. After SCI, IL10 deficient mice have an increased expression of pro-inflammatory genes, increased levels of the pro-apoptotic proteins and decreased motor recovery [76]. Another study which replaced normal monocytes with IL10 deficient monocytes also resulted in reduced motor recovery, suggesting that this cytokine is necessary for the positive macrophage anti-inflammatory response [75]. IL10 treatments in SCI has resulted in motor recovery improvement by inflammatory and neuroprotective regulation. Acute i.p. administration of IL10 reduced lesion volume in two independent studies [77, 78]. But while one study showed that IL10 improved motor recovery and reduced TNF$\alpha$ expression in the injured spinal cord and in infiltrated macrophages [77], the other study did not find any functional recovery [78]. Although we previously commented other studies with motor recovery improvement by local viral over-expression of IL10 [48, 49], on those studies the inflammatory response was not assessed.

Another important anti-inflammatory cytokine is IL4, which signals by the STAT6 transcription factor. Although the neutralization of this cytokine by anti-IL4 antibody administration increased the inflammatory response, neither the IL4-neutralization treatment nor a study with STAT6 deficient mice found differences in motor recovery after SCI [79, 80]. These results indicate that Il4 signalling is not necessary for motor recovery. Further studies should test this cytokine in the promotion of the anti-inflammatory response in SCI.

In addition, there are some G-CSF studies which have shown immune modulation upon SCI. Previously commented studies for G-CSF modulation on glial cells also assessed immune response. Daily intravenous administration of G-CSF during the first 4 dpi showed that treatment not only improved motor recovery and oligodendrocyte protection, but also suppressed upregulation of the pro-inflammatory cytokines TNF$\alpha$ and IL1$\beta$ and reduced IL1$\beta$ + neutrophils infiltration [56]. Another treatment with G-CSF intrathecal injection during the first day reduced macrophage/microglia cell number and TNF$\alpha$ levels during the first 2 wpi

[57]. Although G-CSF is well known for neuroprotective modulation [45], only one study has elucidated a biological mechanism for G-CSF inflammatory modulation on SCI. While in this study was found an increase in microglial number at 7dpi in the G-CSF treated mice, *in vitro* analyses indicated that this cytokine induced a M2 microglial phenotype, reducing pro-inflammatory genes while inducing IL10 expression [81]. A further characterization should be done to demonstrate that G-CSF induces a M2 phenotype *in vivo*.

### 7.2. Pro-inflammatory cytokines

IL12 is a pro-inflammatory cytokine produced by macrophages and dendritic cells which signals via STAT4 [82]. Although it was shown that gel foam administration of IL12 regulated immune and OPC response, this treatment slightly improved motor recovery in C57BL/6 mice and did not improve function in BALB/c strain [83]. A similar result was found after SCI in STAT4 deficient mice, no improvement was found in comparison WT mice [79]. These studies suggest that IL12 signalling is not functionally important for motor recovery.

The IL6-cytokine family has shown different effects in inflammatory response after spinal cord injury. IL11 deficient mice have been assessed in SCI, with no significant differences in motor recovery or macrophage infiltration [17]. LIF treatment incremented Mac1 levels, a marker for macrophage/microglial, and IGF-1 expression on these cells [61], while gel foam administration of OSM reduced T-cell infiltration [15]. Although we previously discussed IL6 intrathecal administration for axon regeneration modulation [33, 34], IL6 is a pro-inflammatory cytokine which can be blocked to decrease inflammatory response. To avoid Il6 mediated immune response, a single i.p. administration of MR16-1, an anti-mouse IL6 receptor antibody, has been tested with positive outcomes. MR16-1 improved motor recovery after a contusive SCI [66, 84]. This treatment also reduced the lesion area, astrocyte proliferation and increased spared myelin area and neurofilament and serotonergic fibers near the lesion area [66, 85]. The mechanism underlying the MR16-1 improvement in tissue and functional recovery has been associated to microglial and infiltrated macrophage function. MR16-1 accelerated inflammatory resolution, as number of total macrophage/microglial began to decrease earlier in the treated group [85]. Further phenotypic characterization of immune cells lead to the discovery that in MR16-1 treated mice, the microglial cells had an increased phagocytic phenotype. It was also shown that macrophages had a predominant M2 phenotype and that anti-inflammatory cytokines were up-regulated while pro-inflammatory cytokines were down-regulated [84, 85].

## 8. Conclusion and clinical implications

Cytokine upregulation and JAK-STAT signalling activation are endogenous mechanism that are activated in response to SCI and can be used to improve motor recovery. Cell type specific and *in vitro* studies have identified the role of STATs modulation in spinal cord cells. STAT3 has been the most extensively studied STAT transcription factor in SCI, but promising results have been found in other STATs and further studies should continue to determine the roles of

STAT1 and STAT5 in SCI. Moreover, further studies with transgenic models should focus in other specific cell responses not studied up to now, like motoneuron and NSPC responses.

Several preclinical studies have shown positive outcomes for motor recovery and tissue sparing in JAK-STAT cytokine treatments (**Table 2**). Thus, modulation of the JAK-STAT signalling presents an opportunity to modulate neuron and glial response after an SCI in clinical settings. The hematopoietic cytokines have been already used in clinical studies for several pathologies, therefore are advanced in comparison with other JAK-STAT cytokines. Discussion of G-CSF and EPO treatments for SCI can be found in previous chapters [45, 86], while for GM-CSF there is one SCI clinical study finished which consisted in cytokine administration with transplant of bone marrow cells [87]. Although clinical studies have not been assessed for other cytokines in CNS trauma, there are clinical studies involving other pathologies that could be translated to SCI. The anti-inflammatory and neuroprotective mechanism of IL10 could also be assessed with a recombinant human IL10 that has been used for HIV infection and other several pathologies [46]. Emfilermin is a recombinant human LIF that has been tested, although with a lack of effectiveness, in clinical trials for embryo implantation and peripheral neuropathy [88, 89] that could also be used in SCI or other CNS diseases. Finally, tocilizumab, an anti-human IL6R, has shown positive outcomes in clinical studies for rheumatoid arthritis [90] and could be used for anti-inflammatory treatment in the spinal cord acute response.

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