Chapter

The Role of Interleukins after Spinal Cord Injury

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Abstract

In skin wound healing the injured tissue goes through a normal progression, inflammation subsides and remodeling occurs. However after spinal cord injury inflammation persists and there is less progression into a regenerative/rebuilding phase. This inflammatory process after spinal cord injury is orchestrated by many cell types and numerous cytokines. Although there are several positive effects of inflammation after spinal cord injury, such as the removal of debris, the substantial upregulation of immune cells has been shown to contribute to neural degeneration. Several chemokines and cytokines including many interleukins are involved in guiding these immune cells to the lesion. While there are many inflammatory cytokines acting on these immune cells after SCI, there are also several anti-inflammatory interleukins that have shown beneficial effects in reducing inflammation. After SCI in a rat model, interleukin-10 and interleukin-19 have been shown to downregulate the synthesis of pro-inflammatory species including interleukin-1β and tumor necrosis factor-α, which resulted in a significant improvement in rat hind limb function. Also, interleukin-4 and interleukin-13 are related anti-inflammatory cytokines that regulate many aspects of inflammation and have also been shown to induce alternative macrophage activation. The differing and complex roles interleukins play, highlight their importance on the inflammation that persists after spinal cord injury. Here we review both the positive effects and negative effects that interleukins have during the multifaceted inflammation process following spinal cord injury.

Keywords: interleukins, spinal cord injury, inflammation, macrophages, microglia

1. Introduction

Spinal Cord Injury (SCI) is a devastating trauma and according to the National Spinal Cord Injury Statistical Center (NSCISC) there are approximately 294,000 people living with SCI in the United States [1]. After spinal SCI there is immediate cell death caused directly from the insult followed by a cascade of inflammation that leads to additional cell death and a much larger scar formation that impedes axonal regeneration [2, 3]. Although there are several positive effects of inflammation after SCI, the extensive infiltration of immune cells is a principal contributor to neural degeneration [4, 5]. These immune cells are guided to the lesion site from the periphery via many signaling cues including several interleukins (ILs) released by microglia, astrocytes, and peripheral macrophages within the lesion [5, 6].

Throughout the first hours after injury, polymorphonuclear leukocytes are the predominant infiltrating cells and over-activation of these cells causes tissue destruction through the release of significant amounts of neurotoxins including reactive oxygen species (ROS), reactive nitrogen species (RNS), chemokines, and enzymes [5, 7, 8]. Microglia, the resident macrophages, are also activated and migrate to the site of injury, proliferate, and transform from the ramified phenotype to amoeboid phagocytic cells [9]. These activated microglia and peripheral macrophages make up the majority of inflammatory cells present at the site of the lesion. Although in normal wound healing macrophages sequentially change and reduce inflammation, after SCI macrophages persist in an inflammatory state for prolonged periods resulting in progressive tissue degeneration [10, 11]. However these microglia/macrophages can be activated toward an anti-inflammatory phenotype and ILs are important signaling cues in the extracellular environment that help dictate this contrasting phenotype. The goal of this chapter is to examine the role ILs have on the dynamic inflammatory process that occurs after SCI.

2. Interleukins involved in inflammation after SCI

There are numerous known ILs and several of these ILs are shown to be involved in inflammation after SCI. Throughout this chapter we will discuss the ILs that have been investigated after SCI and whether their role is predominately inflammatory or anti-inflammatory. It is important to note, the role an IL plays after injury is not as simple as just inflammatory or anti-inflammatory. For many of the ILs there are multiple factors that determine whether they will have a beneficial role or a detrimental role, including the extent of initial injury, concentration of IL, other associated molecules in the injury, and the response of immune cells and glial cells [12, 13].

Although there is a broad spectrum of signaling molecules including cytokines, chemokines, and other reactive species after SCI, this chapter will just focus on ILs and only the ILs that are known to play a role in inflammation after SCI [14]. These ILs will be discussed in terms of the cell types that produce them, receptors they bind, cell types they target, timeline of upregulation, and ultimately their effect on inflammation after SCI.

2.1 Interleukin-1 family cytokines

IL-1α, IL-1β, and IL-33 are members of the IL-1 family that have been studied after SCI and all are predominantly inflammatory [15]. IL-1 is released via activated macrophages and microglia largely in response to disease, infection, or inflammatory events. IL-1 has two structurally and biologically similar isoforms, IL-1α and IL-1β [13]. These two isoforms share roughly 30% amino acid sequence homology and although they perform similar biological functions, IL-1β plays a more substantial role post-SCI [16, 17]. IL-1β has been shown to contribute to the exaggerated neuroinflammation following SCI that leads to secondary neural degeneration and cell death [16]. IL-1 signaling following SCI is diverse and complex, resulting in a recruitment of neurotoxins or immune system molecules that contribute to the inflammatory response [13].

The primary receptor for signaling of both IL-1 isoforms is the type-I interleukin-1 receptor (IL-1RI). IL-1 signaling is further regulated by a decoy receptor (IL-1RII) and a receptor antagonist (IL-1ra) [13]. The expression of these receptors mediates the inflammatory response to SCI and their mechanisms have been widely studied following SCI. After SCI in rats, IL-1R1 expression is elevated as

early as 4 hours, peaks at 8 hours to 1-day and remains elevated for 7 days post-injury [13]. Another study tested the role of IL-1ra as a regulatory molecule following SCI and observed that increased expression of IL-1ra suppressed IL-1β levels and increased locomotor function following SCI, suggesting that IL-1β and IL-1RI play critical roles in secondary tissue damage and impaired functional recovery post-SCI [16]. Likewise, administration of IL-1β suppressed the expression of IL-1ra following SCI indicating the regulatory nature of IL-1β interactions with its receptor antagonist [16]. Similarly, a study using IL-1 knockout mice observed a significantly smaller lesion area and improved locomotor function after SCI in comparison to wild-type mice [18].

The signaling cascade of the IL-1β/IL-1RI pathway is complex and yet to be completely understood, however it is widely understood that it stimulates the production of toxic intermediates that cause neural degeneration and cell death [13]. These toxic inflammatory mediators include prostaglandins, cyclooxygenase 2, and phospholipase A2 [13]. However, there are studies showing benefits of IL-1, where IL-1β null mice failed to remyelinate as rapidly as wild-type mice [19]. These different roles IL-1 plays are likely due to several factors including extent of injury as well as IL-1 concentration and timing of upregulation, but at present are not well understood.

Another member of the IL-1 family, IL-33, predominantly induces type-2 immune responses against allergens and infectious diseases [20]. IL-33 is upregulated in response to SCI and tends to localize in spinal cord astrocytes to reduce T cell infiltration and overexaggerated inflammation that leads to neuronal cell death [21]. IL-33 is classified as an alarm signal (alarmin) and is released by epithelial cells upon signals of cell or tissue death, but the exact *in vivo* mechanism of release is not fully understood [22]. After its release, IL-33 binds to ST2 receptors (IL-1RL1) that are present on multiple immune cells as an alert signal for immunologic and neurologic damage or inflammation [22].

One study that treated SCI injury in mice with administration of recombinant IL-33 indicated an attenuation of spinal cord encephalomyelitis progression and a significant decrease in neural tissue death, decrease in demyelination, and an overexaggerated astrocyte infiltration at the lesion site of the contused spinal cord [21]. These results yielded a significant increase in functional recovery and a dramatic decrease of the expression of TNF-α in the spinal cord for as long as 42 days post-SCI. In addition to suppression of pro-inflammatory cytokine release, IL-33 administration promoted the activation of anti-inflammatory M2 macrophage/microglia [21].

2.2 Interleukin-2 family cytokines

Cytokines from the IL-2 family, IL-2, IL-4, IL-7, IL-15, and IL-21, all share a common receptor subunit (gammac), which plays a major role in promoting and maintaining T lymphocyte populations [23]. IL-2 is a pro-inflammatory cytokine made up of four α helixes and is produced mainly by $CD4^+$ cells when activated. At an mRNA level, signals from T-cell receptor (TCR) and CD28 closely regulate the production of IL-2 [24]. After synthesis, IL-2 binds to a receptor complex, which consists of three subunits, IL-2Rα, IL-2Rβ, and the common γ-chain [24]. All three subunits are needed to achieve high affinity binding. These receptors are located on regulatory T cells and antigen-activated T lymphocytes [25]. To produce an IL-2-dependent response, IL-2 must be produced and IL-2R must be expressed within the same microenvironments [25].

IL-2 and its receptor, IL-2R, are crucial to maintaining the balance of the timing and adequacy of an immune response [26]. The primary role of IL-2 is to perpetuate

the proper response of memory T-cells to invading pathogens [27]. In addition, IL-2 is vital to the survival, as well as death, of lymphocytes, which has an effect on the development of the immune system. By properly maintaining the life of regulatory T cells (T reg) and activation-induced cell death, IL-2 is able to eliminate self-reactive T cells as a preventative measure against autoimmune diseases [27]. After SCI in a rat, IL-2 levels were significantly lower than intact controls from 3 days to 2 weeks post-SCI [14]. In addition, the interaction of IL-2 with its receptor after SCI contributes to the proliferation of T-helpers, which also have an effect on the proliferation of cytotoxic T cells, natural killer cells, lymphokine-activated killers, B cells, and macrophages [14].

IL-4 and IL-13 are related anti-inflammatory cytokines that regulate many aspects of inflammation and have also been shown to induce alternative macrophage activation (**Figure 1**) [28]. IL-4 is a cytokine that is involved in regulating immunity, and is secreted by Th2 cells, eosinophils, basophils, and mast cells [29]. IL-4 is also involved in allergic inflammation by utilizing Th2 lymphocytes, differentiated from Th cells, which can then be used in the production of effector cytokines [30]. IL-4 binds to its receptor IL-4Rα, and will dimerize with either γc (the common cytokine-receptor γ-chain) and produce the type-1 signaling complex, or with IL-13Rα1 and produce the type-2 signaling complex (**Figure 1**) [29, 31]. Although IL-4 has a major impact on immunity, it also affects cognition based on T-cells mediated by IL-4. When administered within a short period post injury, IL-4 exhibits anti-inflammatory effects; however, it can exert a pro-inflammatory response when macrophages possessing IL-4 are undergoing pro-inflammatory stimulation [29].

Lima et al. (2017) performed a study to understand the effect of the acute and sub-acute treatment using IL-4 on various populations of neural cells and on functional recovery *in vivo*. In the injured spinal cord, treatment using a systemic delivery of IL-4 (0.35 μg/kg) for 7 days, led to an upregulation of the anti-inflammatory

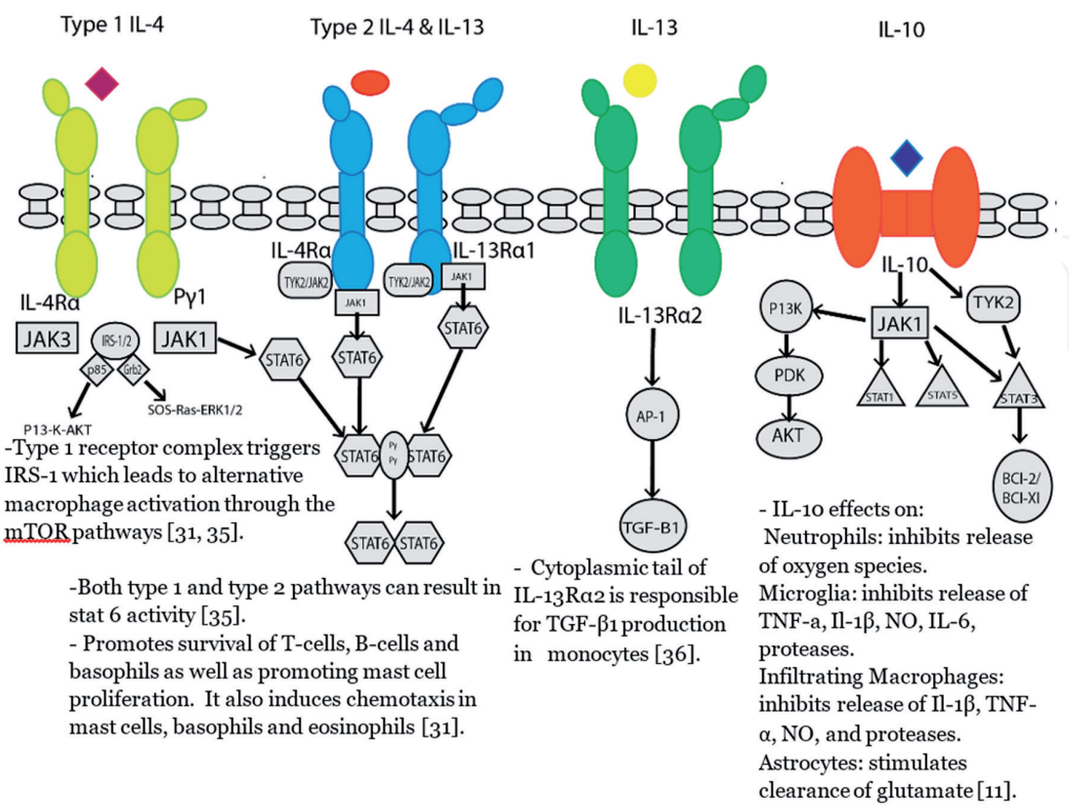

Figure 1.
IL-4, IL-13, and IL-10 pathways and effects.

IL-10 and a reduction in area of macrophage/microglia expressing inflammation markers CD11b and inducible nitric oxide synthase (iNOS) [32]. After systemic IL-4 treatment, they also observed an increase in the number of O4-positive cells (a marker for both type I and type II oligodendrocytes) and neuronal markers βIII-tubulin and NeuN, suggesting that IL-4 has a role in neuroprotection. This overall reduction in inflammation resulted in improved hind limb function in rats after SCI. Although they observed several positive effects, systemic IL-4 did not have an effect on the number of astrocytes or lesion size [32]. In another study a delayed intraspinal injection of IL-4 (100 ng of recombinant IL-4, 48 hours after injury) was given after a spinal cord contusion in mice [33]. The intraspinal injection of IL-4 resulted in an increase in microglia/macrophages expressing antigens characteristic of an anti-inflammatory M2 phenotype, reduced tissue damage, and improved hind limb function in mice after SCI [33]. These studies suggest that therapies using IL-4 could be a valuable treatment for improving function after SCI.

IL-13 is produced by different T cell subsets, dendritic cells, and activated Th2 cells [34]. Although the IL-13α2 receptor was originally thought to be a "decoy" receptor that serves as a neutralizer (**Figure 1**) [35], Fichtner-Feigl et al. showed a role for IL-13Rα2-mediated signaling that required the cytoplasmic tail of IL-13Rα2 in the production of transforming growth factor beta (TGF-β), an anti-inflammatory shown to down-regulate inflammatory cytokines, providing evidence for IL -13Ra2-mediated signaling (**Figure 1**) [31, 36]. Furthermore, after SCI it was shown that transplanted mesenchymal stem cells continuously expressing IL-13 improved functional recovery and decreased lesion size. In addition, IL-13 increased the amount of ARG-1-expressing macrophages [37].

IL-7 is a homeostatic cytokine that plays a key role in the survival of multiple immune cells and acts on lymphocytes [38]. The IL-7 receptor complex is composed of two chains, IL-7Rα and γc (the common cytokine-receptor γ-chain), which signal downstream to the JAK/STAT5 pathway, and assists in regulating the survival and development of immune cells [38]. IL-7 is produced by stromal cells in lymphoid organs and is necessary for T-cell development and their survival in the periphery [39].

After SCI in mice, IL-7 is promptly upregulated and displays as a strong chemotactic property for macrophages [40]. An intraspinal injection of IL-7 after SCI in mice, resulted in an increase in pro-inflammatory cytokines IL-1β, IL-6, and TNF-α, and a decrease in the anti-inflammatory cytokine IL-10 [38]. The increase in IL-7 also led to an increase in apoptosis, macrophage infiltration, and a decrease in hind limb function in mice after SCI [38]. Moreover, blocking the IL-7 receptor after SCI in mice, resulted in suppression of pro-inflammatory cytokines IFN-γ and TNF-α, an increase in IL-4 and IL-13, more macrophages expressing antigens characteristic of an anti-inflammatory M2 phenotype, an increase in spared white matter, and an improvement in hind limb function [40]. During SCI, the JAK/STAT5 pathway is activated, and IL-7 post-SCI also contributes to the activation of the JAK/STAT5 pathway, which upholds a crucial role in the inflammatory response and secondary damage [38]. When the JAK/STAT5 pathway was inhibited by pimozide, the effects of IL-7 discontinued, which emphasizes the relationship between the JAK/STAT5 pathway and IL-7 function [38]. Therefore, Yuan et al. (2019) concluded that the IL-7/JAK/STAT5 axis targeted by antagonists may represent a potential therapeutic treatment for SCI [38].

Similar to IL-2, IL-15 is a pro-inflammatory cytokine that is also part of the four α helix cytokine family. The main function of IL-15 is to provide a long-term immune response to invading pathogens by contributing to the homeostasis of natural killer cells and CD8+ memory T cells that express IL-2/IL-15Rβ and γc [27].

IL-15 has three receptors, IL-15Rα, IL-2Rβ, and γc, and shares two of the receptors with IL-2 (IL-2Rβ and γc) [41]. Although IL-15 has not been well studied after SCI, it has been shown to be involved in the development of neuropathic pain from nerve injury [42]. After sciatic nerve injury, IL-15 expression was observed in the spinal cord in astrocytes and microglia, and it is also present in neurons located in the dorsal and ventral horn [42].

IL-21 is a pleiotropic cytokine expressed by many immune cells including natural killer T cells and activated CD4+ T cells [43]. Similar to other inflammatory mediators, IL-21 is upregulated after SCI [44, 45]. Fu et al. (2017) studied peripheral blood-derived mesenchymal stem cells (PBMSCs) as a therapy for SCI and their role in the lesion microenvironment by analyzing the neuroprotection, differentiation, and immunoregulation of PBMSCs that were engrafted. When IL-21 was inhibited, a decrease in the secretion of IL-23a and IL-22 occurred [44]. When investigating the potential Th17/Treg-relative mechanism of PBMSCs therapy after SCI, Fu et al. (2017) discovered that the M1 macrophage migrated to lesion site and resulted in the pro-inflammatory secretion of IL-6 and IL-21, which led to CD4 + T cells differentiating into CD4 + IL17 + Th17 cells [44]. Furthermore it has been shown that IL-17 production is stimulated by the combination of IL-21 and TGF-β [45].

2.3 β common chain cytokines IL-3, and IL-5

The β common cytokine family, including IL-3 and IL-5, is defined by a shared receptor structure, comprising of a specific α chain and a common β chain that is essential for cytokine-specific receptor signaling [46]. IL-3 is a cytokine that is produced by activated T cell lymphocytes, which then induces the production of various hematopoietic cell types that are crucial to the immune response [47]. IL-5 is cytokine that is produced by hematopoietic and non-hematopoietic cells, including granulocytes, T cells, and natural helper cells [48]. IL-5 is also a mediator for eosinophilic inflammation by providing stimulation, differentiation, recruitment and activity of eosinophils. Due to their roles with eosinophils, IL-3 and IL-5 have been primarily studied in asthma, and their roles after SCI are not clear. However, both of them co-express in TH2 cells, which is a subset of CD4+ cells. These TH2 cells are characterized by the production of IL-4, IL-5, IL-10 and IL-13 and thus, may be beneficial in exerting anti-inflammatory effects after SCI [49, 50].

2.4 IL-6 and IL-11

IL-6 and IL-11 are grouped into one cytokine family because the receptor complex of each cytokine contains two of the signaling receptor subunit gp130 (**Figure 2**) [51]. For both IL-6 and IL-11 there are membrane bound receptors as well as soluble receptors and after ligand binding to either the membrane bound receptor or the soluble receptor, they form a complex with two gp130 receptors leading to Jak/STAT pathway signaling (**Figure 2**) [52, 53].

IL-6 is predominantly an inflammatory cytokine. After SCI in a mouse model, Pineau et al. observed IL-6 mRNA expression in astrocytes, microglia/ macrophages, and neurons, starting at 3 hours post-injury, peaking at 12 hours and continuing for 4 days post-injury [54]. Similarly after SCI in humans IL-6 is strongly upregulated. IL-6 levels in cerebrospinal fluid of SCI patients changed from undetectable (<4 pg./ml) in non-injured controls to an average of almost 30,000 pg./mL in the subset of patients with complete SCI [55]. Furthermore, the cerebrospinal levels of IL-6 correlated with the extent of spinal cord damage in humans, which demonstrates the importance of IL-6 after SCI.

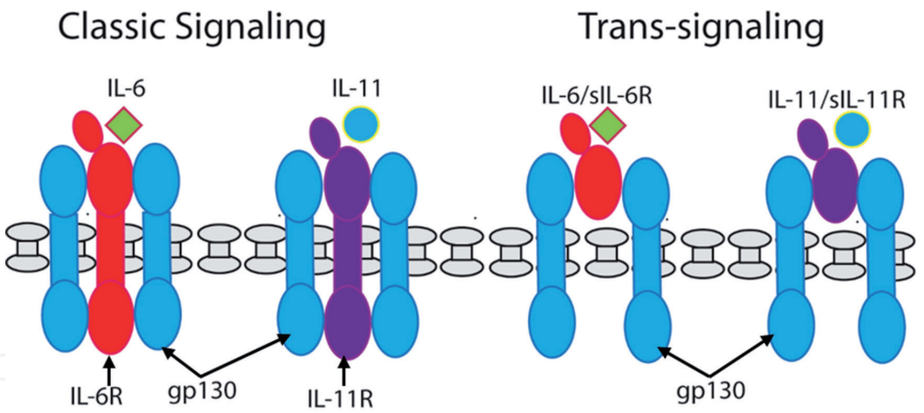

Figure 2.
Classic and trans-signaling. Cells that express the IL-6R or IL-11R will undergo classic signaling when IL-6 or IL-11 bind to the corresponding receptor, inducing gp130 dimerization and initiating intracellular signal transduction. Trans-signaling occurs when the ligand and soluble receptor complex (IL-6/sIL-6R or IL-11/sIL-11R) associate with gp130 inducing gp130 dimerization and initiating intracellular signal transduction.

IL-6 leads to recruitment of immune cells. Delivery of IL-6/sIL-6 receptor fusion protein to injury sites induced a sixfold increase in neutrophils and a twofold increase of macrophages/microglial [56]. Mice treated with an antibody against IL-6 receptor showed a reduction in neutrophil and monocyte/macrophage invasion [57, 58]. It was also shown that blocking IL-6 signaling after SCI reduces the damaging inflammatory activity by promoting the formation of alternatively activated M2 macrophages [59]. Taken together these data suggest IL-6 signaling is an activator of inflammation and a strong recruiter of immune cells after SCI.

After SCI astrocytes proliferate and migrate to the injury leading to a dense astroglial scar surrounding the lesion. It has been shown *in vitro* that IL-6 signaling acts on neural stem cells to induce their differentiation into astrocytes [60]. This was supported by several *in vivo* studies including, IL-6 knockout mice that showed suppression of astrogliosis following SCI [61], mice with an excessive expression of IL-6 and IL-6R showed abundant astrogliosis suggesting that astrocytes were selectively affected in these mice [62], and the development of astrogliosis was inhibited in mice given an IL-6 receptor blocker after SCI [58].

Several studies have shown that blocking IL-6 signaling improves functional recovery after SCI [57–59]. It has also been shown that delivery of IL-6/IL-6 receptor resulted in a four fold decrease in axon growth [56]. However there are studies showing that IL-6 is neuroprotective and aids in axonal regeneration [63, 64]. The differences in IL-6 effect may depend on the level of expression and timeline of IL-6 upregulation. The studies using an IL-6 blocker were performed in the sub-acute timeframe after SCI. In the sub-acute SCI, any neurotrophic effects of IL-6 appear to be overwhelmed by its proinflammatory features. Taken together, the aforementioned data demonstrates the importance of IL-6 after SCI. IL-6 upregulates inflammatory cytokines, recruits immune cells, effects macrophage phenotype, effects astrocyte activation, effects axonal regeneration, and effects functional recovery.

IL-11 has been shown to be primarily anti-inflammatory. Recombinant IL-11 administered to activated macrophages inhibited the production of pro-inflammatory cytokines TNF-α, IL-1β, IL-12, and nitric oxide production [65–67]. Furthermore, IL-11 has been shown to play an anti-inflammatory role in the airways for asthma [68], play a role in decreasing mucosal damage in inflammatory bowel disease [69], and importantly IL-11 has a neuroprotective role in multiple sclerosis [70]. Due to these anti-inflammatory roles, Cho et al. analyzed the role of IL-11 after SCI using IL-11Rα knockout mice [71]. In wild type mice, they observed

a significant upregulation in IL-11 with a peak gene expression 24 hours after injury and a significant upregulation of IL-11Rα at 3 and 7 days after SCI. Somewhat surprisingly, they did not observe significant differences in functional recovery or histopathology in IL-11Rα knockout mice as compared to wild type mice after SCI. The authors speculate that since "the peak in IL-11Rα expression is on the order of days after SCI, suggests that IL-11 signaling may not play as significant a role in the acute inflammatory response after injury, but more in the long-term sequelae such as oligodendrocyte survival". Maheshwari et al. used a cuprizone induced mouse model of demyelination in the central nervous system to analyze the effects of over-expression of IL-11 on demyelination/remyelination [72]. Overexpression of IL-11 was able to limit cuprizone-induced demyelination by reducing oligodendrocyte cell death, decrease microglial activation, and enhance spontaneous remyelination. Maheshwari's results further suggest that IL-11 likely plays a role in the long-term remyelination efforts after SCI and is not as involved in the sub-acute stage [72].

2.5 Interleukin-8 and interleukin-16

IL-8, also known as neutrophil chemotactic factor or CXCL8, primarily induces chemotaxis in neutrophils and granulocytes. IL-8 is a member of the chemokine family that acts on CXCR1 and CXCR2 receptors (il8ra and il8rb, respectively), which have been primarily studied on polymorphonuclear leukocytes. However many other cell types express these receptors including neurons [73]. Several studies have shown that IL-8 can be released by a wide variety of cells including monocytes endothelial cells, T lymphocytes, and macrophages [73]. After SCI in rat, GRO, the rat analogue of human IL-8, is strongly upregulated for at least 14 days and the upregulation of GRO strongly correlates with the extent of injury [14, 74, 75]. Furthermore IL-8 is upregulated in the cerebrospinal fluid of dogs and humans after SCI, and for humans the IL-8 levels are also shown to correlate with the extent of damage [55, 76, 77]. Although IL-8 clearly plays a role in neutrophil infiltration and overall inflammation after SCI, as shown by its significant upregulation, it has not been extensively studied after SCI.

IL-16 is a proinflammatory cytokine that is produced by mast and leukemic cells, fibroblasts, endothelial cells, granulocytes, dendritic cells, CD4+ and CD8+ T lymphocytes, monocytes, and microglial cells. IL-16 plays a role in the release of other proinflammatory cytokines (IL-1β, IL-6, IL-15, and TNFα), the increase of intracellular Ca++ or inositol-(1,4,5)-triphosphatase, and the translocation of protein kinase C [78]. These processes occur after IL-16 binds to the signal-transducing CD4 receptor molecule [79]. Moreover, IL-16 promotes lymphocyte migration and modulates apoptosis [80].

Following spinal cord injury, IL-16 plays a role in recruiting and activating inflammatory cells. Microglia that produce IL-16 migrate to the lesion site and other areas of significant neuronal damage [78]. Following neuroinflammation, it is suggested that IL-16 microglia are one of the first cells to respond [80]. In addition, macrophages with IL-16 remained present at the injury site for up to thirty days post injury, indicating long-term IL-16 function [78]. One study found that expression of IL-16 in microglia and macrophages is induced by the IL-12 p40 homodimer through IL-12Rβ1, but not IL-12 p70 [80]. Overall, the ability of IL-16 to quickly recruit microglia/macrophages to the lesion site following SCI results in increased neuronal damage and microvessel clustering [78].

2.6 Interleukin-10 family cytokines

Members of the IL-10 family of cytokines that have been studied after SCI include, IL-10, IL-19, IL-20, and IL-22 [81]. IL-10 is an anti-inflammatory cytokine that is produced by monocytes, B cells, dendritic cells, natural killer cells,

and T cells [82]. In leukocytes, IL-10 acts on both innate and adaptive immune cells with a wide range of immunomodulatory activities that suppress proliferation, cytokine secretion, and costimulatory molecule expression of proinflammatory immune cells. The IL-10 receptor consists of heterotetramer complex made of two IL-10R1 molecules, encoded by the IL10ra gene, and two IL-10R2 molecules, encoded by the IL10rb gene (**Figure 1**) [83]. IL-10 downregulates several pro-inflammatory cytokines and inflammatory species [11]. In addition, IL-10 can affect T cell and natural killer cell function indirectly and directly through connection with monocytes and macrophages. The overall impact of IL-10 is determined by the timing and site of its production, which are both affected by which cells are making IL-10. Since IL-10 production by one cell type affects the ability of other cells to make IL-10, IL-10-producing cells show potential to regulate each other [82].

Following SCI, IL-10 downregulates pro-inflammatory molecules IL-1β, IL-2, IL-6, TNF-α, IFN-γ, matrix metalloproteinase-9, nitric oxide synthase, myeloperoxidase, and reactive oxygen species. IL-10 also provides trophic support to neurons through downregulation of pro-apoptotic factors cytochrome c, caspase 3, and Bax, as well as upregulation of anti-apoptotic factors B cell lymphoma 2 (Bcl-2) and Bcl-2-associated X, B-cell lymphoma-extra large (Bcl-xl) (**Figure 1**) [11]. There have been several studies performed to test IL-10's therapeutic value as a treatment for SCI. Although these studies used a variety of different systemic and local methods to administer IL-10 after SCI, the majority of results showed strong positive effects from the IL-10. These positive effects after SCI include a reduction in pro-inflammatory molecules, macrophages expressing more antigens characteristic of an anti-inflammatory M2 phenotype, reduced lesion size, and an improvement in hind limb function [11, 84].

IL-19 is produced by monocytes and microglia, and binds to the IL-20 receptor complex, which consists of IL-20R1 and IL-20R2 chains [85]. Activated microglia upregulate IL-19 and express the IL-20 receptor complex [86]. It has also been shown that ablation of IL-19 in activated microglia increased the production of pro-inflammatory cytokines IL-6 and TNF-α, which demonstrates that IL-19 is predominately an anti-inflammatory cytokine in the central nervous system [86].

After SCI in mice, IL-19, IL-20R1 and IL-20R2 are upregulated [87]. In a series of four different experiments, mice with spinal cord injuries were treated with IL-19 [87]. As a result, Th2 cytokine synthesis was promoted, which polarized spinal microglial cells to an M2 phenotype. This helped resolve the inflammation, preserving myelin, neurons, and neuronal function. Overall, IL-19 attenuated macrophage accumulation, reduced protein levels of TNF-α and CCl2, promoted Th2 response and M2 macrophage activation, promoted angiogenesis by upregulating VEGF, upregulated HO-1 expression, and decreased oxidative stress in the injured region [87].

IL-20 is a proinflammatory cytokine that is predominately produced by monocytes and skin keratinocytes. IL-20 signals through both the IL-20R1/IL-20R2 heterodimer complex and the receptor complex composed of IL-22R1 and IL-20R2 [85]. Following spinal cord injury, IL-20 and its receptors are expressed in neurons, astrocytes, oligodendrocytes, and microglia in large amounts. IL-20 upregulates glial fibrillary acidic protein (GFAP), TGF-β1, TNF-α, MCP-1, and IL-6 expression, which stimulates astrocyte reactivation and migration [88]. As a result, glial scar border formation is enhanced. Moreover, IL-20 inhibits neuron outgrowth through upregulation of Sema3A/NRP-1 in PC-12 cells [88]. The overall result is irreversible neuronal loss and glial scar formation post-SCI. *In vivo*, anti-IL-20 mAb reduces the IL-20 inflammatory response, which improves motor and sensory functions, spinal cord tissue preservation, and reduces glial scar formation [88].

2.7 Interleukin-12 family cytokines

The IL-12 family is comprised of 4 members, IL-12, IL-23, IL-27 and IL-35 and each member is composed of α-subunit with a helical structure similar to type 1 cytokines and a β-subunit structurally related to the extracellular regions of Type 1 cytokine receptors [89]. However from this family of 4 cytokines, only the pro-inflammatory cytokine IL-12 has been assessed after SCI. IL-12 is produced by dendritic cells, macrophages, monocytes, neutrophils, microglia cells, and B cells [90]. The IL-12 receptor is made up of IL-12Rβ1 and IL-12Rβ2 chains [91]. IL-12 is a heterodimeric molecule, p70, formed from p40 and p35 chains. IL-12p70 is considered to be the biologically active cytokine that expresses nitric oxide synthase and TNF-α in microglia and macrophages. In T cells, p70 interacts with both IL-12Rβ1 and IL-12Rβ2. However, p70 treatment results in IL-16 mRNA inhibition due to inability to induce IL-16 promoter [80].

Yaguchi et al., administered IL-12 after SCI in mice and observed an increase in the number of activated macrophages and dendritic cells surrounding the lesion site and an increase in the expression of brain-derived neurotrophic factor adjacent to the injury. After IL-12 treatment, immunohistochemical analyses revealed that *de novo* neurogenesis and remyelination occurred. The mice treated with IL-12 also had a significant improvement in hind limb function [92].

2.8 Interleukin-17 family cytokines

IL-17 cytokines play important roles in both innate and adaptive immunity. IL-17A to IL-17F are highly conserved at the C terminus, and contain five spatially conserved cysteine residues that mediate dimerization [93, 94]. IL-17A and IL-17E have been identified and studied for the roles they play after SCI.

IL-17A is an important cytokine in regard to protective mechanisms against infectious diseases and inflammatory pathology within the immune system [95]. IL-17A is secreted by a multitude of cells including T cells, dendritic cells, and macrophages among others and binds to the A and C subunits of the IL-17 receptor to initiate signaling [95]. After SCI in rats, IL-17A is upregulated as early as 1 hour after injury, peaks at 24 hours, and remains above normal levels for at least 72 hours after injury [45]. This upregulation of IL-17 does appear to play a degenerative role on SCI recovery after a study was conducted using IL-17 knockout mice. IL-17 knockout mice showed increased locomotor function and decreased lesion size after SCI, which suggests that IL-17 expression regulates secondary degeneration of the neural tissue at the lesion site [96]. Recruitment of immune cells such as B cells, neutrophils, and dendritic cells were downregulated at 6 weeks following SCI [96].

Interleukin-25, also known as Interleukin-17E, is in the IL-17 family and binds to the heterodimer complex of IL-17A and IL-17-B receptor subunits. IL-25 has primarily been understood as a systemic type-2 inflammatory mediator that triggers significant helper T-cell expression and proinflammatory cytokine suppression, however its response following spinal cord injury is largely unknown. IL-25 is primarily derived from epithelial cells and macrophages in response to infection or inflammation and contributes to type-2 helper T cell (Th-2) activation [97]. Th2 cells are responsible for the release of anti-inflammatory cytokines IL-4, IL-5, and IL-13 which play a role in neural protection and regeneration against inflammation and neurotoxins [97].

The trafficking mechanism and inflammatory response of IL-25 post-SCI remains relatively unclear, but local injection of IL-25 into the lesion site post-SCI yields interesting and contradictory results. The local administration of IL-25 following spinal cord injury in 10-week old mice results in decreased locomotor

function, an increase in lesion size, and neuronal demyelination which contradicts the systemic immune response upon an IL-25 presence [97]. Interestingly, the systemic administration of IL-25 show ineffective results in regard to improved functional mobility following spinal cord injury. Microglia and astrocytes survival are also unaffected upon injection of IL-25 suggesting that IL-25 indirectly activates inflammatory molecules associated with these immune events [97]. These results raise questions about the precise role of IL-25 after SCI and possible therapeutic interventions using IL-25.

3. Conclusions

Although significant progress has been made in terms of spinal stabilization and medical care of patients after SCI, there has not been much progress made in terms of treatments for SCI to retain or regain the function that is lost. In order to design treatments for SCI, a better understanding of the inflammation process is crucial. As outlined in this chapter ILs are an intricate player in inflammation after SCI. For some of these ILs, there timeline of involvement and roles they play in inflammation has been defined. However, there is still much more research that needs to be completed to understand the roles many of these ILs play. Along with understanding the current ILs, there will assuredly be more signaling cues discovered that are involved after SCI.

Could inflammation be modulated to retain or regain a significant amount of function after SCI? This is a fundamental question that needs to be addressed. As highlighted in rodent models, such as what is observed in IL-17 knockout mice or treatments with anti-inflammatory cytokines, modulating inflammation is a promising approach for treating SCI. However it is important to realize that all variables including age, sex, level of injury, and force to cause the trauma, are controlled in these rodent models, and thus treating human SCI will be more challenging. These facts highlight the essential need to conduct more research on inflammation after SCI.

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