Cytokine pathways regulating glial and leukocyte function after spinal cord and peripheral nerve injury

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A B S T R A C T

Injury to the nervous system causes the almost immediate release of cytokines by glial cells and neurons. These cytokines orchestrate a complex array of responses leading to microgliosis, immune cell recruitment, astrogliosis, scarring, and the clearance of cellular debris, all steps that affect neuronal survival and repair. This review will focus on cytokines released after spinal cord and peripheral nerve injury and the primary signalling pathways triggered by these inflammatory mediators. Notably, the following cytokine families will be covered: IL-1, TNF, IL-6-like, TGF-β, and IL-10. Whether interfering with cytokine signalling could lead to novel therapies will also be discussed. Finally, the review will address whether manipulating the above-mentioned cytokine families and signalling pathways could exert distinct effects in the injured spinal cord versus peripheral nerve.

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Contents

Introduction 62
Cytokines released after neural injury: production, receptor signalling pathways and immune responses 63
  IL-1 family members 63
  TNF family members 67
  TGF-β family members 68
  IL-10 family members 69
Cytokine effects on glia, endothelial cells and leukocytes and contribution of these cells to tissue damage, glial scarring and regeneration 69
  Glial cells 69
  Endothelial cells of the neurovascular unit 70
  Innate immune cells 71
Differences between the injured spinal cord and peripheral nerve 72
Conclusion 73
Acknowledgments 73
References 73

Introduction

Traumatic spinal cord injury (SCI) and peripheral nerve injury (PNI) cause damage to axons and their myelin sheaths. How axons respond to injury has been extensively studied for decades in a variety of organisms, ranging from invertebrates to non-human primates (Bradke et al., 2012). From these studies a main conclusion can be drawn: axonal regeneration is regulated by a multitude of intracellular and extracellular signals. Among which are several axon growth inhibitory molecules that have been found in myelin or associated with the scar (Burd et al., 2014; Cregg et al., 2014; Schwab, 2010). Wallerian degeneration (WD) and clearance of inhibitory myelin debris are delayed in the...
injured CNS compared to the injured PNS, which could be one of the main reasons why CNS regeneration is generally so poor (David and Lacroix, 2005). In addition, immune cells that phagocytose myelin debris are potentially the source of neurotrophic factors and anti-inflammatory molecules that may support the regenerative and repair processes. In support of this are studies that showed that CNS axons can grow in transplanted peripheral nerve segments (Barrette et al., 2008; David and Aguayo, 1981).

Another direct consequence of SCI is the destruction of neurons and glia at the site of lesion, which has been referred to as the primary mechanical lesion. Long-standing evidence suggests that a second wave of cell death follows the primary mechanical insult, leading to apoptosis of oligodendrocytes (OLs) and neurons (Crowe et al., 1997; Liu et al., 1997). However, it is important to note that they are many other suspected causes of secondary damage in the injured spinal cord, including ischemia, vascular damage, glutamate excitotoxicity, ionic dysregulation, and inflammation (for reviews, see (David and Lacroix, 2005; Donnelly and Popovich, 2008)). The topic of inflammation has received the greatest attention because immune cells are the main producers of free radicals, proteases, eicosanoids and cytokines, all of which are molecules that have been shown to be both capable of inducing cell death and myelin damage and of being expressed in the injured spinal cord within minutes to days of mechanical impact (Bao and Liu, 2004; Hains et al., 2001; Liu et al., 1998, 2000; Noble et al., 2002; Pineau and Lacroix, 2007; Rice et al., 2007; Wells et al., 2003). This time course fits well with the timing of secondary damage, suggested to occur between 4 h and 14 d post-SCI (Blight, 1985; Liu et al., 1997). The concept of secondary cell damage is at the center of the preclinical development of potential neuroprotective therapies, with many studies reporting reduced lesion size and/or increased spared tissue as a result of treatment (reviewed extensively by (Kwon et al., 2011)). An outstanding question has been why neurons projecting through peripheral nerves do not seem to be as sensitive to these potentially cytotoxic molecules. Indeed, peripheral nerve and spinal cord lesion sites seem to contain the same inflammatory cells and molecules, but perhaps not at the same timing, duration and expression levels. This subject will be addressed in the last part of this review.

In the CNS, as in the PNS, glia can be activated by several different types of signals and modulators, among which cytokines and neurotransmission-related compounds are considered to play major regulatory roles (Hanisch and Kettenmann, 2007). For example, microglia respond within minutes to ATP released after laser-induced SCI by extending their processes in order to rapidly shield the site of injury (Davalos et al., 2005; Nimmerjahn et al., 2005) (Fig. 1). Depending on the repertoire of receptors activated, microglia can either promote neuronal survival and regeneration or contribute to neuronal death (Hanisch and Kettenmann, 2007; Ransohoff and Perry, 2009). Although they do not physically respond as quickly as microglia (Farrar et al., 2012), astrocytes, the main cellular component of the glial scar, increase their GFAP expression, become hypertrophic, proliferate and migrate around the lesion site in a process generally referred to as reactive astrogliosis (Sofroniew, 2009). Several different mediators of reactive astrogliosis exist, among which are cytokines of the IL-1, TNF, IL-6-like, TGF-β, and IL-10 families (reviewed by (Sofroniew, 2009)). Importantly, the glial scar has been shown to exert both beneficial and detrimental effects after SCI (Brambilla et al., 2005, 2009; Faulkner et al., 2004).

Innate immune cells such as neutrophils and monocytes are rapidly recruited at sites of SCI (Beck et al., 2010; Fleming et al., 2006; Lee et al., 2011; Mawhinney et al., 2012; Pineau et al., 2010; Stirling and Yong, 2008; Thuawer et al., 2013). Evidence suggests that these cells could play a key role in both secondary damage and tissue repair after traumatic neural injury (Barrette et al., 2008; Kigerl et al., 2009; Shechter et al., 2009; Stirling et al., 2009). The apparent contradictions between these studies may be due to the fact that different subsets of immune cells have divergent effects, resulting in either neurotoxicity or regeneration in the injured spinal cord (David and Kroner, 2011). The effects of cytokines on glia and leukocytes and the contribution of these cells to tissue damage, glial scarring and regeneration will be explored in more detail in the second part of this review. One important area of focus will be how cytokines govern immune cell recruitment, activation and polarization in the context of injury, as this could explain some of the controversies and paradoxical effects of neuroinflammation in SCI.

This review begins with an overview of the principal families of cytokines for which a role in the pathophysiology of spinal cord and peripheral nerve injury has been established.

Cytokines released after neural injury: production, receptor signalling pathways and immune responses

IL-1 family members

The role of interleukin (IL)-1 family cytokines in the initiation and regulation of inflammation during infection and injury is well established. The family contains 11 members, the best characterized of which are IL-1α, IL-1β, IL-1 receptor antagonist (IL-1RA), IL-18, and IL-33 (Dinarello, 2009). IL-1α and IL-1β were the first two members of the family to be described. Despite the fact they share only 25–30% amino acid homology, they have a similar 3D structure and biological properties (Dinarello, 1991; Graves et al., 1990). Since they bind to the same receptor, IL-1 receptor type 1 (IL-1R1), with the same affinity and stimulate expression of similar downstream effectors, IL-1α and IL-1β have long been thought to trigger identical responses. However, Rothwell and colleagues have shown that the relative potencies of recombinant IL-1α and IL-1β vary depending on the context and the response being studied, thus suggesting that different mechanisms are likely to be involved in the various effects of IL-1 cytokines in the CNS (Andre et al., 2005; Anforth et al., 1998; Tsakiri et al., 2008). For example, the study by Anforth et al. showed that IL-1β is more effective than IL-1α at inducing fever when the cytokines are injected intracerebroventricularly (i.c.v.) (Anforth et al., 1998). Moreover, and in contrast to IL-1β, treatment with IL-1α failed to induce IL-6 production from glia and neurons, despite the fact that both forms of IL-1 were equally effective at increasing expression levels of chemokines such as CCL2/MCP-1, CXCL1/KC and IP-10 (Andre et al., 2005; Tsakiri et al., 2008). More recently, Rider et al. suggested that the precursor of IL-1α and mature IL-1β recruit different myeloid cells (neutrophils and macrophages, respectively) in response to hypoxia-induced cell death (Rider et al., 2011). It is therefore plausible that the two IL-1 cytokines signal through distinct signalling complexes and regulate expression of specific genes. Preliminary results in this direction, in which we compared the transcriptome of the injured spinal cord of IL-1α-knockout (KO), IL-1β-KO and wild-type (WT) mice during the acute phase of SCI using Affymetrix GeneChip microarrays, indicate that a few genes appear to be specifically regulated by IL-1α (D. Bastien and S. Lacroix, unpublished observation).

Interleukin-1α (IL-1α). IL-1α and IL-1β are synthesized as 31-kDa precursor proteins. However, only the IL-1α precursor (pro-IL-1α) is able to bind to IL-1R1, which suggests that this form is biologically active (March et al., 1985; Mosley et al., 1987). Proteases such as calpain (Carruth et al., 1991; Kobayashi et al., 1990), and more recently granzyme B, elastase and chymase (Afonina et al., 2011), were shown to cleave pro-IL-1α and yield a 17-kDa form. Initially, that cleavage was thought to have no effect on the biological activities of the cytokine, but recent studies have shown that the 17-kDa mature form of IL-1α has enhanced immunological potency compared to the proform (Afonina et al., 2011; Zheng et al., 2013). Zheng et al. also identified the IL-1 receptor type 2 (IL-1R2) as a regulator of IL-1α cleavage, being able to bind IL-1α and prevent calpain from processing the precursor cytokine. Despite this, evidence has shown that the proform has the ability to recruit leukocytes following its release from hypoxic...
cells (Rider et al., 2011). One unknown is whether the pro- and mature forms of IL-1α have the same biological functions.

IL-1α, like IL-33, high-mobility group box protein 1 (HMGB1), S100 proteins and heat-shock proteins (HSPs), belongs to a class of dual-function molecules capable of performing their functions in the nucleus as well as in the extracellular compartment. These molecules, also referred to as alarmins or danger-associated molecular pattern molecules (DAMPs) (see also Kigerl et al., 2014–in this issue), are constitutively expressed in the nucleus where they act as regulators of DNA transcription. However, once released into the extracellular space from necrotic cells upon tissue injury or infection, these molecules become powerful mediators of inflammation (Chan et al., 2012).

IL-1α has an N-terminal nuclear localization sequence (NLS), allowing it to translocate to the nucleus of cells, where it regulates transcription of proinflammatory genes (Bursyskova et al., 2004; Werman et al., 2004). During apoptosis, IL-1α remains associated with the chromatin and is retained in the apoptotic bodies, thus preventing the cytokine from triggering inflammation. Conversely, following necrosis, IL-1α dissociates from the chromatin and is released first in the cytoplasm and then in the extracellular milieu where it can initiate inflammation by binding the IL-1R1 on neighboring cells (Cohen et al., 2010; Luheshi et al., 2009). Accordingly, mice treated with antibody to IL-1α have reduced neutrophil recruitment in response to i.p. injection of necrotic cells when compared to mice treated with control antibody or antibody to IL-1β (Chen et al., 2007). This finding was confirmed by another in vivo study that showed that IL-1α released by dying cells, believed to be tissue-resident macrophages, triggers neutrophil recruitment through the CXCL1–CXCR2 axis in an IL-1R1-dependent manner (Eigenbrod et al., 2008).

In culture, IL-1α has been detected in multiple cell types (Dinarello, 2009). However, in the CNS, only resident microglia and infiltrating platelets have been confirmed as possible sources of IL-1α during inflammatory conditions, such as cerebral ischemia (Luhiš et al., 2011; Thornton et al., 2010). In the ischemic brain, microglia express IL-1α mRNA and protein as early as 4 h following reperfusion, mainly in regions exhibiting neuronal cell death (Luhiš et al., 2011). This time point precedes the reported infiltration of blood-derived immune cells and appearance of apoptotic neurons in this animal model (Broughton et al., 2009; Iadecola and Anrather, 2011). However, work with KO mice has revealed that both forms of IL-1, but not IL-1α

![Image](image-url)
or IL-1β alone, need to be knocked out to reduce ischemic infarct size (Boutin et al., 2001). These results highlight the possibility of compensatory changes in the IL-1 system and reinforce the need to carefully assess the individual and cumulative effects of cytokines of the IL-1 family.

Interleukin-1β (IL-1β). Because pro-IL-1β is unable to bind to IL-1R1, its enzymatic cleavage by caspase-1 is a required step in the production of a bioactive 17-kDa mature form of IL-1β. This proteolytic cleavage of pro-IL-1β depends on the assembly of large multiprotein complexes termed inflammasomes (Franchi et al., 2009; Lamkanfi and Dixit, 2012; Mariathasan and Monack, 2007; Martinon et al., 2009). Proteins encoded by the nucleotide-binding oligomerization domain and leucine-rich repeat (NLR) containing gene family form the central components of inflammasomes (see also Kigerl et al., 2014 in this issue). A question that had remained unanswered until recently is whether spinal cord resident cells express inflammasomes, and if so which cells influence inflammasome activation after SCI?

de Rivero Vaccari and colleagues were the first to demonstrate the existence of the NLRP1 inflammasome, consisting of NLRP1, caspase-1, caspase-11, XIAP, and ASC, an adaptor protein essential for caspase-1 recruitment and known to interact with several inflammasomes (Tschopp et al., 2003), in neurons of the normal rat spinal cord (de Rivero Vaccari et al., 2008). Neutralization of ASC in SCI mice reduced caspase-1 activation and IL-1β cleavage, and this was correlated with increased tissue sparing and functional recovery. More recently, we found that purinergic receptors of the P2X4-subtype are expressed in spinal cord neurons and regulate in a bioactive 17-kDa mature form of IL-1β (John et al., 2005). IL-1β promotes remyelination and repair (Mason et al., 2001). OPCs of IL-1β-KO mice exhibited a prominent delay in differentiation into mature OLs, which resulted in remyelination failure in these animals. Deletion of the IL-1β gene also led to decreased microglial and astrocytic expression of insulin-like growth factor (IGF-1), a factor known to stimulate proliferation, differentiation and survival of cells of the OL lineage (Barres et al., 1993; Mason et al., 2000). The multiple effects mediated by IL-1β in various cell types raise the interesting question of whether

### Table 1

Cytokine effects on glial, endothelial cells, leukocytes and neurons in the pathogenesis of spinal cord injury.

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>Cells</th>
<th>Cytokine-mediated effects</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td>Oligodendrocytes</td>
<td>↑ Remyelination; ↑ differentiation of OPCs into OLs</td>
<td>Mason et al. (2001)</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Endothelial cells</td>
<td>↑ Cell death</td>
<td>Ye et al. (2013); Allan et al. (2005); Zhu et al. (2012)</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Immune cells</td>
<td>↑ Endothelial-cell adhesion molecule expression; ↑ chemokine production; ↑ vascular stability</td>
<td>Pina et al. (2010)</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Glia</td>
<td>↑ Macrophage activation; ↑ Astrocyte activation; ↑ Microglia activation</td>
<td>Sato et al. (2012); John et al. (2005); Basu et al. (2004); Sato et al. (2012)</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Immune cells</td>
<td>Regulates T-cell differentiation (Th1 cells with IL-12; Th2 cells with IL-4)</td>
<td>Dinarello et al. (2012); Nakamishi et al. (2001)</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Glia</td>
<td>↑ Astrocyte/microglia activation and interaction</td>
<td>Myoshi et al. (2008)</td>
</tr>
<tr>
<td>IL-1β</td>
<td>TNF</td>
<td>↑ Apoptosis/demyelination</td>
<td>Chen et al. (2011)</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Endothelial cells</td>
<td>↑ Endothelial-cell adhesion molecule expression; ↑ BBB disruption</td>
<td>Allain and Rothwell (2001); Allain and Rothwell (2001)</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Immune cells</td>
<td>↑ Recruitment of innate immune cells</td>
<td>Bruce et al. (1996)</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Glia</td>
<td>↑ Microglia activation</td>
<td>Ackery et al. (2006); Caisha et al. (2005); Robin-Steel et al. (2012)</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Fas</td>
<td>↑ Apoptosis; ↑ axonal degeneration</td>
<td>Ackery et al. (2006); Caisha et al. (2005); Robin-Steel et al. (2012)</td>
</tr>
<tr>
<td>IL-1β</td>
<td>TGF-β</td>
<td>↑ Macrophage/microglia activation</td>
<td>Kohta et al. (2009)</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Glia</td>
<td>↑ Astroglial scar formation; ↑ fibrosis</td>
<td>Hella et al. (2011); Kohta et al. (2009); Laping et al. (1994); Logan et al. (1999)</td>
</tr>
<tr>
<td>IL-1β</td>
<td>IL-6</td>
<td>↑ OL differentiation; ↑ myelin production</td>
<td>Spoooren et al. (2011)</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Oligodendrocytes</td>
<td>↑ Neurogenesis; ↑ intrinsic growth capacity of sensory DRG neurons</td>
<td>Cafferty et al. (2004); Cao et al. (2006); Spoooren et al. (2011)</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Endothelial cells</td>
<td>↑ Endothelial-cell adhesion molecule expression; influences BBB maintenance</td>
<td>Spoooren et al. (2011)</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Immune cells</td>
<td>↑ TNF and IL-1β expression levels; ↑ innate immune cell recruitment; Mediates polarization of M1 macrophages</td>
<td>Romano et al. (1997)</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Glia</td>
<td>↑ Astrocyte differentiation from neural stem cells</td>
<td>Guerrero et al. (2012); Nakamura et al. (2005); Okada et al. (2004)</td>
</tr>
<tr>
<td>IL-1β</td>
<td>IL-10</td>
<td>↑ Neuronal survival</td>
<td>Thompson et al. (2013); Zhou et al. (2009a, 2009b)</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Immune cells</td>
<td>↑ Proinflammatory cytokine production</td>
<td>Genovese et al. (2009); Knoblach and Faden (1998); Thompson et al. (2013)</td>
</tr>
</tbody>
</table>
these responses are mediated by different intracellular signalling pathways.

Other than its effects on glia, IL-1β can also directly bind to IL-1R1 expressed on CNS endothelium to stimulate production of cytokines, chemokines, cell adhesion molecules (CAMs), PGs, NO, matrix metalloproteinases (MMPs); molecules that are involved in the recruitment and infiltration of leukocytes into the CNS parenchyma (Allan et al., 2005). Finally, although IL-1β does not seem to be directly toxic to healthy neurons, it can induce production of mediators that are highly neurotoxic through the neurons themselves or surrounding cells (Allan et al., 2005; Basu et al., 2004). Together, these studies demonstrate that IL-1β affects a large range of CNS cells and drives the expression of several molecules previously shown to play a key role in CNS injury and mechanisms of neurodegeneration and repair.

Interleukin-1 receptor type 1 (IL-1R1). IL-1R1, through which both IL-1α and IL-1β signal, possesses an extracellular domain together with a Toll-like/IL-1R (TIR) domain in the cytosolic compartment. It is part of the IL-1R/Toll-like receptor (TLR) superfamily (O’Neill and Dinarello, 2000). The other form of IL-1 receptor, IL-1R2, has a shorter intracellular domain believed to be biologically inert. Intracellular IL-1R2 regulates cell activation by preventing cleavage and activity of IL-1α (Zheng et al., 2013). IL-1R2 can also be cleaved from the cell membrane and released in the extracellular milieu, where it can bind IL-1 cytokines and prevent them from acting on IL-1R1 expressed on adjacent cells (Arend et al., 2008).

Activation of IL-1R1 by IL-1 cytokines leads to the recruitment of a sub-unit called IL-1 receptor accessory protein (IL-1RaCP). The complex composed of IL-1α/β, IL-1R1 and IL-1RaCP then allows the recruitment of the adaptor protein, myeloid differentiation factor 88 (MyD88). MyD88 subsequently recruits the IL-1 receptor-associated kinases (IRAKs) and tumor necrosis factor (TNF) receptor-associated factor 6 (TRAF6), to activate transcription factors such as nuclear factor-κB (NF-κB), mitogen-activated protein kinases (MAPKs) such as c-Jun N-terminal kinase (JNK), extracellular signal-regulated kinases 1 and 2 (ERK-1/2) and p38, and activator protein-1 (AP-1) (O’Neill, 2008) (Fig. 2). The importance of MyD88 has been documented by Adachi et al. who reported a complete loss of the characteristic immune responses normally mediated by IL-1R1 in MyD88-KO mice, such as IL-1-mediated cytokine production, acute phase proteins expression and T cell proliferation (Adachi et al., 1998). More recently, we found that the IL-1R1/MyD88 signalling pathway plays a major role in the recruitment of neutrophils and proinflammatory M1 monocytes after SCI by promoting the expression of chemokines such as CXCL1 and CXCL2 by astrocytes (Pineau et al., 2010). It is also important to know that IL-1RaCPβ, a splice isoform of IL-1RaCP with a variant TIR domain and whose expression is restricted to CNS neurons, has been shown to modulate neuronal responses to IL-1 (Nguyen et al., 2011; Smith et al., 2009). Although the neuron-specific function of IL-1RaCPβ is largely unknown, the C-terminal extension of AcPβ allows it to bind adaptor proteins and recruit them to the IL-1R1 receptor complex in an IL-1-dependent manner. Despite the fact that IL-1RaCPβ is able to associate with IL-1R1 in the

Fig. 2. Cytokine signalling pathways relevant to spinal cord and peripheral nerve injury. Schematic representation of the main cytokines, receptors and signalling pathways regulating neuroinflammation and immune responses after spinal cord and peripheral nerve injury. IL-1R1 and IL-1R1x are members of the IL-1R/TLR superfamily of receptors and thus share similar signalling pathways. Upon binding of their respective ligands, IL-1α, IL-1β (referred herein as IL-1) and IL-1β, they rapidly recruit a second receptor subunit in the cell membrane; IL-1RaCP for IL-1R1 and IL-1R1x for IL-1R1x. Formation of the receptor heterodimers then leads to the recruitment of MyD88, IRAKs and TRAF6. The ensuing cellular responses are primarily mediated by NF-κB- and MAPK-dependent gene expression. The effects of TNF and mTNF are mediated through binding with TNFR1 and TNFR2, respectively, which leads to the formation of complexes involving adaptor proteins of the TRADD, TRAF and FADD families. Depending on the composition of the receptor complexes formed and downstream signalling cascades, such as those mediated by NF-κB and MAPK, TNF will induce a broad spectrum of effects, including regulation of inflammatory gene expression and cell death and survival. The binding of Fas to FasR induces the recruitment of cytosolic signalling proteins such as FADD and pro-caspase-8, resulting in activation of apoptotic caspase cascade. The three TGF-βs mediate their action through the TGF-βRI-TGF-βRII complex, leading to the phosphorylation of SMAD transcription factors. IL-6 binds to IL-6R, leading to the formation of a receptor complex with gp130. The phosphorylation of gp130 by Jak kinases activates the canonical STAT family of signalling proteins. The effects of IL-10 are mediated via two receptor chains, IL-10R1 and IL-10R2, through activation of the JAK-STAT signalling pathway. All cytokine signalling pathways discussed lead to the transcription of genes involved in immune responses, scarring, cell death and tissue repair. Please refer to the core text of the review for the definition of abbreviations.
presence of IL-1, it should be pointed out that this association does not lead to the recruitment of MyD88 and IRAK4, and is therefore unable to mediate canonical IL-1 signalling [Smith et al., 2009]. Nguyen et al. further reported that IL-1α-induced, but not IL-1β-induced, p38 phosphorylation is significantly reduced in primary neuronal cultures from IL-1RαPb-KO mice (Nguyen et al., 2011). These results suggest an important role of IL-1RαPb in specific neuronal activities, but not all, in response to IL-1α.

Several studies suggested that IL-1R1 plays an important role in glial responses in brain and spinal cord injury. After penetrating stab injury to the brain, IL-1R1-KO mice exhibit reduced macrophage/microglia activation and astrogliosis around the lesion site (Basu et al., 2002b). The study also reported a decrease in IL-6, cyclooxygenase-2 (COX-2), and VCAM-1 mRNA expression. However, not all astrocytic responses that typically occur after CNS injury are compromised in mice lacking IL-1R1, as levels of chondroitin sulfate proteoglycans (CSPGs), tenascin, S100β and glutamate transporters (GLAST, GLT-1) remain unchanged (Lin et al., 2006). Therefore, some astrocyte properties do not seem to require the IL-1R1 pathway.

**Interleukin-1 receptor antagonist (IL-1RA).** Treatment of many diseases relies on blocking IL-1R1 signalling by way of administering its receptor antagonist, IL-1RA. Anakinra, the recombinant form of IL-1RA, is widely used in the clinical treatment of inflammatory diseases such as familial Mediterranean fever, rheumatoid arthritis, gout, and type 2 diabetes (reviewed in Dinarello, 2011; Dinarello et al., 2012). In the CNS after stroke, a single subcutaneous injection of IL-1Rα-dex reduced infarct volume by 33% in rats exposed to middle cerebral artery occlusion (MCAO) (Greenhalgh et al., 2010). More relevant for the clinical practice, IL-1RA administration at reperfusion after transient MCAO in a rat model reduced blood–brain barrier disruption, neutrophil infiltration, microglial activation and cytokine (IL-6) levels in the brain (Pradillo et al., 2012). Treatment with IL-1RA adsorbed into a gelatin sponge and applied on top of the contused spinal cord of rats promoted functional recovery for at least 4 weeks (Zong et al., 2012). Chronic intrathecal delivery of recombinant IL-1α also reduced apoptosis and caspase-3 activity in a contusion SCI rat model (Nesic et al., 2001). Together, these results suggest an important beneficial effect of IL-1Rα in the treatment of SCI and neuroprotective therapies with an inflammatory component.

**Interleukin-18 (IL-18).** Another molecule that possesses a similar structure to cytokines of the IL-1 family is IL-18. Like IL-1β, IL-18 is synthesised as an inactive 23-kDa protein that can be cleaved by caspase-1 into a bioactive 18-kDa mature form (Chayyur et al., 1997; Gu et al., 1997). Because of its role in the production of IFN-γ in Th-helper type 1 (Th1) cells, IL-18 is perhaps best known for its involvement in T-cell polarization towards a Th1 phenotype (Nakanishi et al., 2001). This skewing is due to a complex interplay between different immune cells and involves IL-18, IL-12 and the IL-18 receptor (IL-1R8). Somewhat surprisingly, IL-18 alone can stimulate Th2 responses such as production of the anti-inflammatory cytokines IL-4 and IL-13 (Nakanishi et al., 2001). Among its other functions, IL-18 also regulates production of CAMs, NO, cytolytic granules and cytokines such as IL-2, GM-CSF, IL-1β and TNF (Arend et al., 2008; Smith, 2011).

The biological functions of IL-18 are mediated through IL-1R8, a receptor of the IL-1R/TLR superfamily that is remarkably similar to the IL-1R complex (Arend et al., 2008; Smith, 2011). The IL-1R8 is composed of a ligand–binding chain, IL-18Rα (also known as IL-1R1 or IL-1R-related protein), and a signal-transducing chain, IL-1R8β (also referred to as IL-18R2 or IL-18R accessory protein). Once formed, this complex recruits different intracellular molecules such as MyD88, IRAKs and TRAF6, leading to activation of NF-κB and MAPK signalling pathways [JNK, ERK-1/2 and p38] (Thomassen et al., 1998) (Fig. 2).

Like IL-1β, IL-18 is believed to play a major role in several CNS diseases including neuropathic pain and SCI. IL-18 is involved in the induction of neuropathic pain that develops after ligation of the L5 spinal nerve; a model in which microglia from the spinal cord dorsal horn up-regulate their production of IL-18, which in turn signals to astrocytes through the IL-1R8–NF-κB pathway (Miyoshi et al., 2008). In the contusion SCI model, expression of the mature form of IL-18 is increased as early as 15–30 min post-injury and continues to rise until at least day 3 (de Rivera Vaccari et al., 2008). Expression of the mature form of IL-18 was further correlated with the assembly of a functional NALP1 inflammasome and caspase-1 activation in spinal cord neurons.

**TNF family members**

**Tumor necrosis factor (TNF).** Similar to IL-1, TNF has both neuroprotective and neurodegenerative effects in a number of CNS diseases and injuries (Siriam and O’Callaghan, 2007). TNF is synthesized as a 26-kDa membrane-bound precursor (mTNF) and cleaved into a 17-kDa mature form by the TNF-alpha converting enzyme (TACE; also known as ADAM17), which is expressed in the CNS (Karkkainen et al., 2000). Signalling pathways and biological effects of TNF are mediated through high affinity transmembrane receptors, namely TNF receptor type 1 (TNFR1) and TNFR2. They are both part of the TNFR superfamily and, like IL-1R1, signal through transcription factors such as NF-κB, JNK, ERK-1/2 and p38 MAPK, albeit through a different set of adaptor molecules (Cabal-Hierro and Lazo, 2012). TNFR1 is expressed in most CNS cell types and is the preferred target of soluble TNF, whereas TNFR2 is mainly expressed in microglia and endothelial cells and preferentially activated by mTNF. Following binding of TNF to TNFR1, which possesses a death domain (DD), two different signalling complexes can be formed: 1) Complex I involving TNFR-associated death domain (TRADD) and controlling expression of anti-apoptotic proteins that prevent cell death, and 2) Complex II involving both TRADD and FAS-associated death domain (FADD) and triggering cell death by way of apoptosis or programmed necrosis (also referred to as necroptosis) (Micheau and Tschopp, 2003). As opposed to TNFR1, TNFR2 lacks a functional DD and binds mTNF with higher affinity than sTNF. Its functions are mainly proinflammatory and anti-apoptotic, as it induces through TNFR-associated factors (TRAFs) several proinflammatory mediators downstream of NF-κB and AP-1 activation (Cabal-Hierro and Lazo, 2012; Santello and Volterra, 2012).

TNF is rapidly expressed (peak at 1 h) by microglia, astrocytes, OLs and neurons after SCI in both rats and mice (Pineau and Lacroix, 2007; Yan et al., 2001; Yune et al., 2003), whereas TNFR1 and TNFR2 are mainly found in astrocytes, OLs and neurons (Yan et al., 2003). Blockade of TNF during the acute phase of SCI in rats by means of systemic administration of etanercept (Enbrel), a TNF inhibitor functioning as a decoy receptor that binds to TNF, was shown to decrease neuronal and oligodendroglial apoptosis, reduce tissue damage and demyelination, and improve recovery of locomotor function (Chen et al., 2011). In support of the previous study, Genovese et al. have found that deletion of the TNFR1 gene or TNF blockade using infliximab (Remicade; an antibody directed against TNF) in SCI mice reduced inflammation, tissue damage and apoptosis, and improved functional recovery (Genovese et al., 2008). On the contrary, work done using animal models of stroke and epilepsy has revealed that TNF serves a neuroprotective function, as microglial activation was suppressed, oxidative stress increased and neuronal damage exacerbated in mice lacking both TNF receptors (Bruce et al., 1996). This result was recently confirmed by Lamberts et al. who showed that cortical infarction and behavioral deficits are exacerbated in TNF-KO mice after cerebral ischemia (Lamberts et al., 2009). This research went further by demonstrating that microglial-derived TNF exerts its neuroprotective properties through TNFR1. An interesting observation made by Bruce et al., however, is that injury-induced astrogliosis is apparently unaltered in mice lacking both TNFR1 and TNFR2. This suggests that TNF signalling is not critical for the astrocytic response to ischemia and epilepsy. Whether this holds true after SCI remains to be seen, especially when considering the discrepancies between the results obtained in the three models discussed.
above. Along these lines, transgenic TNF overexpression in SCI rats resulted in more activated astrocytes with longer processes and stronger GFAP staining in the border of the lesion (Chi et al., 2008). Despite exhibiting more apoptotic cells during the acute phase of SCI, tissue loss was reduced in TNF-transgenic rats compared to WT controls. Together, these results suggest a dual role for TNF in the pathophysiology of CNS injuries such as SCI. The protective or damaging outcome of TNF could depend on more than one factor, such as for example the duration of NF-κB activation, which we know is controlled by several regulatory proteins and may be affected by the redistribution of receptors between lipid rafts and non-raft regions of the plasma membrane (Lotocki et al., 2004). Hence, investigating these regulatory mechanisms will warrant the use time-specific and cell lineage-specific knockdown systems.

Fas ligand (Fasl). Fasl is a transmembrane protein that closely resembles to other TNF family members. It can directly act on adjacent cells or be released in a soluble form in the extracellular space upon cleavage by MMPs. Fasl signals through the Fas receptor (FasR, also known as CD95), one of the most studied members of the TNFR superfamily bearing a DD, also referred to as “death receptors” (reviewed in (Tibbetts et al., 2003)). The binding of Fasl to FasR induces the recruitment of cytosolic signalling proteins such as FADD and pro-caspase-8, ultimately resulting in apoptosis (Siegel et al., 2000) (Fig. 2). Also of relevance to this review is the fact that apoptosis triggered by Fasl–FasR interactions plays an important role in the regulation of various types of immune responses (Siegel et al., 2000).

In the context of SCI, protein expression for FasR has been detected in OLs, astrocytes and microglia, and expression correlated in time with markers of apoptosis (i.e. TUNEL, cleaved caspase-8, cleaved caspase-3) (Casha et al., 2001). Accordingly, mice harboring deletion of FasR have reduced loss of OL, but not neurons, and significantly improved axonal sparing, myelin preservation and locomotor recovery compared to WT after SCI (Cash a et al., 2005). FasR deficiency in SCI mice also caused a reduction in levels of activated caspase-3 and -9, together with the upregulation of anti-apoptotic proteins such as BCL-2 and BCL-XL (Yu et al., 2009). Using cell-specific Cre-lox mice, Letellier et al. have however found that the detrimental function of Fas, after SCI is due to its propensity to increase the migratory ability of neutrophils and macrophages at sites of injury, instead of by directly inducing apoptosis of FasR-bearing CNS cells (Letellier et al., 2010). Indeed, deletion of FasL in myeloid cells, but not of FasR in neural cells, led to improved functional recovery after SCI. Despite these conflicting results on the cellular mechanisms underlying the detrimental effects of Fasl/FasR in SCI, it should be emphasized that administration of soluble FasR (sFasR) in mice and rats, as a means to neutralize FasL, was proven to be an effective strategy to improve biological outcome; that is, improvements in cell survival, tissue sparing and integrity of descending axon tracts and functional recovery (Ackery et al., 2006; Robins-Steele et al., 2012).

Interleukin-6 (IL-6). IL-6 is a proinflammatory cytokine that is rapidly upregulated at the mRNA level during the acute phase of SCI (Bartholdi and Schwab, 1997; Pineau and Lacroix, 2007). We recently demonstrated that microglia/macrophages, astrocytes and neurons are the principal cellular source of IL-6 in the injured mouse spinal cord (Pineau and Lacroix, 2007). IL-6 exerts its biological functions by first binding to the IL-6 receptor (IL-6R). The IL-6/IL-6R complex then recruits glycoprotein 130 (gp130), allowing it to homodimerize and activate JAK-STAT pathway (Murakami et al., 1993) (Fig. 2).

Several studies have highlighted the importance of IL-6 in the initiation of inflammation and recruitment of innate immune cells after CNS injury. In particular, IL-6 can modulate the recruitment of neutrophils and macrophages by regulating chemokine expression (Romano et al., 1997). Using a gene therapy approach, we delivered a “hyper-IL-6” fusion molecule (consisting of IL-6 and the soluble IL-6R) at site of SCI. Hyper-IL-6 is known to be 100–1000 times more active than the separate molecules. We observed that the number of infiltrating neutrophils increased by six-fold (Lacroix et al., 2002). This was associated with a two-fold increase in lesion volume and a reduction in axonal regrowth inside grafts containing fibroblasts engineered to produce hyper-IL-6. IL-6 is also involved in the activation of astrocytes and microglia in the injured CNS (Penkowa et al., 1999). This was confirmed by Okada et al. who showed that treatment with an anti-IL-6R neutralizing antibody suppressed the development of the astroglial scar, reduced the infiltration of Mac-1 (CD11b/CD18)-positive myeloid cells and improved functional recovery (Okada et al., 2004). Another group found that treatment with the same function blocking antibody, MR16-1, shifted immune responsiveness towards an anti-inflammatory state, characterized by increased production of anti-inflammatory cytokines and the presence of more than normal numbers of M2 macrophages (Guerrero et al., 2012). Importantly, myelina was significantly more preserved and recovery of locomotor function enhanced in mice treated with the anti-IL-6R antibody. Besides its effects on immune and glial cells, IL-6 is known to enhance the intrinsic growth capacity of sensory DRG neurons after lesion of their peripheral branch axons (Cafferty et al., 2004; Cao et al., 2006), as well as to affect neurotransmitter release and neural activity (Spooren et al., 2011).

TGF-β family members

The TGF-β superfamily of ligands includes TGF-β1, TGF-β2 and TGF-β3, activins, bone morphogenetic proteins (BMPs) and growth and differentiation factors (GDFs) (Schmierer and Hill, 2007). They regulate a broad range of biological responses such as cell growth and differentiation, extracellular matrix molecule (ECM) production and cell survival. Because TGF-βs play an important role in the immune system (Li and Flavell, 2008), and because they have been the target of intensive research and clinical trials for a number of diseases in recent years (Akhurst, 2006; Akhurst and Hata, 2012), they will be the main focus here.

All three TGF-βs modulate their actions through a serine/threonine kinase receptor complex composed of the type 1 (TGF-βRI) and type 2 (TGF-βRII) receptors. The TGF-β molecule must first bind to TGF-βRII, which then recruits TGF-βRI to the complex and activates it by phosphorylation (Heldin et al., 1997). This, in return, results the phosphorylation of SMAD proteins by TGF-βRI and the propagation of the signal (Massague, 2000).

TGF-β molecules are known to regulate the expression of genes coding for cell-cycle regulators, differentiation factors, CAMs, and inflammatory mediators (Massague, 2000). Accordingly, they exert profound biological functions during development and neuropathology (Massague, 2000; Wyss-Coray and Mucke, 2002). Following traumatic SCI in rodents and humans, TGF-β1 and TGF-β2 are upregulated, but with different patterns of expression. While TGF-β1 was found to be associated with neurons and immune cells (Buss et al., 2008; Lagord et al., 2002; McTigue et al., 2000), TGF-β2 was mainly detected in reactive astrocytes forming the glial scar and small round CD68-positive cells that appeared to be infiltrating immune cells (Buss et al., 2008; Lagord et al., 2002). At first glance, these results suggest that TGF-β1 modulates neuronal and immune responses, whereas TGF-β2 regulates glial scarring and the production of ECMs such as collagen, fibronectin and CSPG. However, a number of studies indicate that it may be more complicated than that. First, the administration of TGF-β1 i.c.v. in rats has been reported to induce a glial scar (Laping et al., 1994; Logan et al., 1994; Morgan et al., 1995). Second, the neutralization of endogenous TGF-β1 by means of an i.c.v injection of anti-TGF-β1 antibody attenuated the formation of fibrous scar tissue, as verified using immunostaining for GFAP, fibronectin and laminin (Logan et al., 1994). Third, chronic inhibition of TGF-β1 suppressed glial scar formation and upregulated markers of microglia/macroage activation (iba1, ED1) in the injured rat spinal cord, a finding that was correlated with improved locomotor recovery (Kohta et al., 2009). Fourth, treatment of cerebral wounds with an
antibody specific to the active form of TGF-β2 also led to an attenuation of CNS scarring and inflammation (Logan et al., 1999). These results therefore suggest that substantial functional redundancy must be expected from members of the TGF-β family after CNS injury. Another explanation for the different outcomes in these studies may be the dosage, timing, duration and route of administration of the cytokine or antagonist treatment. Whatever the explanation, these data raise the possibility that anti-TGF-β therapies could be used as an effective anti-fibrotic therapy, thus limiting tissue scarring in CNS pathologies where astrocyte activation becomes excessive. This is exactly what the group of Frank Bradke has recently shown by giving the drug Taxol to SCI rats to dampen TGF-β1/SMAD signalling (Hellal et al., 2017). They reported that rats treated with Taxol had reduced scarring, increased regeneration and/or sprouting of 5-HT fibers, and improved functional recovery. Notably, Taxol was shown to reduce production of inhibitory molecules in both astrocytes and meningeal cells at the protein level.

**IL-10 family members**

IL-10 family members include IL-10, IL-19, IL-20, IL-22, IL-24, IL-26, IL-28A, IL-28B, and IL-29 (Ouyang et al., 2011; Sabat, 2010). Because most, if not all, of the published work on the subject of the IL-10 family of cytokines in SCI relates to IL-10, only the latter cytokine will be covered in this review.

**Interleukin-10 (IL-10).** IL-10 was identified by Mosmann and colleagues in 1989 and first given the name of cytokine synthesis inhibition factor (CSIF) (Fiorentino et al., 1989). As its name implies, one of its main functions is the negative regulation of proinflammatory cytokine production (e.g. IFN-γ, IL-1β, IL-2, IL-3, TNF, GM-CSF) in various cell types such as leukocytes and microglia (Balasingam and Yong, 1996; Basu et al., 2001; Fiorentino et al., 1989; Fuchs et al., 1996; Kremlev and Palmer, 2005; Takeda et al., 1999), making it one of the most potent anti-inflammatory cytokines. The biological effects of IL-10 are mediated via two receptor chains, IL-10R1 and IL-10R2, and by activating the JAK-STAT signalling pathway (Moore et al., 2001) (Fig. 2). STAT3 is the key transcription factor implicated in IL-10-mediated responses (Moore et al., 2001; Ouyang et al., 2011).

Although IL-10 may play distinct roles in injured tissue, its primary purpose is to suppress inflammatory cytokine production. This may help limit tissue damage that inflammation could inflict to bystander cells. Of particular relevance here, IL-10 was found to induce expression of anti-apoptotic proteins BCL-2 and BCL-X, and support survival of spinal cord neurons both in vitro and in vivo (Zhou et al., 2009a, 2009b). Notably, the study in which IL-10 was delivered close to sites of SCI using a gene therapy approach showed that the increased neuronal survival was accompanied by slight improvements in locomotion over 4 weeks (Zhou et al., 2009a). From a more therapeutic perspective, work from John Bethea’s laboratory has revealed that a single i.p. injection of IL-10 at 30 min post-SCI in rats was sufficient to improve recovery of hindlimb function (Bethea et al., 1999). It should be pointed out, however, that SCI rats that received two doses of the recombinant cytokines at 30 min and 3 days did not perform better than controls on the BBB locomotor test, thus suggesting once again the importance of timing when inflammation is concerned. Systemic injection of IL-10 also had neuroprotective effects in rat models of traumatic brain injury and spinal cord excitotoxicity (intraspinal injection of quisquausal acid) (Brewer et al., 1999; Knoblach and Faden, 1998; Plunkett et al., 2001). Deletion of the IL-10 gene in SCI mice increased levels of TNF, IL-1β, iNOS and myeloperoxidase (MPO) in the injured spinal cord (Genovese et al., 2009). This was correlated with an augmented number of apoptotic cells and worsening of hindlimb function at 30 days post-SCI. IL-10 may also be important for the beneficial effects of a certain subset of Ly-6C<sup>+</sup> CCR<sub>2</sub><sup>+</sup> CD11c<sup>+</sup> monocytes infiltrating the margins of spinal cord lesions and having anti-inflammatory and neuroprotective properties (Shechter et al., 2009). In particular, Shechter et al. showed that adoptive transfer of monocytes lacking IL-10 into SCI mice depleted in CD11c-expressing monocytes failed to reduce the lesion size and improve functional recovery to the extent of WT monocytes. In a subsequent publication, the same group reported that these infiltrating IL-10-producing monocyte-derived macrophages accumulate at the lesion border and apparently degrade inhibitory CSPGs associated with glial scar formation by producing MMP-13 (Shechter et al., 2011). However, further studies will be needed to clarify the phenotype of these immune cells and their functions, as well as the role of IL-10 in their overall impact on the SCI milieu. Whether these monocyte-derived macrophages behave the same way and can reproduce their beneficial effects in normal pathophysiological conditions, i.e., without irradiation or prior immune cell depletion, will also need to be addressed.

**Cytokine effects on glia, endothelial cells and leukocytes and contribution of these cells to tissue damage, glial scarring and regeneration**

The release of contents of necrotic cells and exposure of DAMPs, also referred to as danger signals, triggers the rapid response of glia, among which microglia appear to react the fastest (Fig. 1). This allows the activation of a cascade of inflammatory responses, mainly regulated by cytokines, which cause the activation of the neurovascular unit (NVU) and influx of innate immune cells at sites of neural injury. The inflammatory response is not only limited to the recruitment of circulating leukocytes and their activation, but also includes collateral responses from most if not all other cells present in the injury environment (Table 1). Here, we will focus our discussion on the three main types of glia (i.e. microglia, astrocytes and OLs), ECs of the NVU, and phagocytic innate immune cells (i.e. neutrophils and monocytes/macrophages). Special attention will be given to the in vivo role of these cells and their subsets in secondary tissue damage, glial scarring and regeneration in the context of neural injury.

**Glia cells**

Recent advancements in two-photon intravital microscopy (2P-IVM) enable scientists the opportunity to study dynamic physiological events within living tissues with minimal impact on cells. Researchers have thus been able to show that microglia are highly dynamic, constantly monitoring the nervous system in search of molecular evidence of homeostatic disturbance (Davalos et al., 2005; Nimmerjahn et al., 2005). In general, microglia residing in the normal adult mouse spinal cord seem to behave like those in the brain (Soulet et al., 2013), though there is no doubt that the plasticity of these cells is affected by their molecular environment (which we know varies greatly depending on regions and conditions). Following tissue injury or disease-induced damage, spinal cord microglia undergo rapid changes in cell shape, movements and migration ability (Davalos et al., 2012; Dibaj et al., 2010, 2011; Farrar et al., 2012; Fenrich et al., 2012; Nikic et al., 2011) (Fig. 1). It remains to be investigated whether cytokines are involved in these early changes, but many of the implicated molecules, such as purinergic pathways and NO (Dibaj et al., 2010), exert their effects through them (Pineau and Lacroix, 2009). The case of IL-18 is perhaps the best example of this, as cellular nucleotides released almost immediately after SCI and acting through P2X4 purinoreceptors mediate the release of mature IL-18 by CNS resident cells within minutes of the injury, resulting in the infiltration of innate immune cells from the blood (de Rivero Vaccari et al., 2012). It is also important to note that the P2X2-dependent release of mature IL-18 could promote cell motility indirectly by regulating the expression and release of other cytokines and chemokines (for review, see Inoue, 2006; Pineau and Lacroix, 2009). As their name implies, cytokines regulate cell movements, most often by the intermediary of chemokines, and are produced by activated microglia in response to SCI (Pineau and Lacroix, 2007). Cytokines have also been implicated in loss of neuronal and glial cells in both acute and chronic neurodegenerative disorders (Allan and Rothwell, 2001; Allan et al., 2005), as well as...
in regeneration of axons in the injured peripheral nerve (Nadeau et al., 2011).

Fate mapping studies in mice have recently revealed that microglia arise from erythro-myeloid precursors detected in the yolk sac during the embryonic stage of prenatal development (around days 8.5 to 9.5) rather than from postnatal hematopoietic stem cells (Ginhoux et al., 2010; Kierdorf et al., 2013; Schulz et al., 2012). Microglia are thus ontogenetically distinct from monocyte-derived macrophages. Discriminating infiltrating monocyte-derived macrophages from activated resident microglia in vivo has nevertheless been an arduous task, due to the fact that both cell types express common antigenic markers (David and Kroner, 2011; Gordon and Taylor, 2005). Microarray studies have however revealed that microglia, macrophages and monocytes express identical molecules but at different levels of expression (Bedard et al., 2007). One of these molecules is the fractalkine (CX3CL1) receptor, CX3CR1. In both mice and humans, CX3CR1 is highly expressed on microglia and a subset of patrolling monocytes referred to as M2, which will be discussed in a subsequent subsection of this review, but weakly expressed in M1 monocytes/macrophages (Geissmann et al., 2003; Harrison et al., 1998; Middler et al., 2007). The creation of mice expressing the fluorescent reporter enhanced GFP under the control of the endogenous Cx3cr1 locus, Cx3cr1-eGFP knock-in mice (Jung et al., 2000), has therefore been a valuable tool for studying microglia function in the healthy and diseased CNS, with one important limitation being that some monocytes and macrophages are also affected by the mutation. Keeping this limitation in mind, the Popovich laboratory has reported that deficiency in CX3CR1 promotes functional recovery after SCI (Donnelly et al., 2011). Lack of CX3CR1 signalling was shown to attenuate the capacity of microglia and monocyte-derived macrophages to produce proinflammatory cytokines and oxidative metabolites. This not only supports the idea that proinflammatory cytokines and oxidative metabolites are toxic to neural cells in the context of injury, but also implies that activation of CX3CR1 signalling in microglia and macrophages stimulates a proinflammatory response. Together with earlier work showing that grafting microglial cells into the injured spinal cord enhances neurite outgrowth (Rabchevsky and Streit, 1997), these data indicate that microglia might have protective as well as harmful effects after in SCI. It is only through the development of new approaches and technologies that we will understand all the intricacies of microglia and grasp the role they play in the normal and injured CNS.

The rapid release of a battery of inflammatory mediators by microglia after injury suggests that these cells could also influence the functions of other glial cells in their immediate environment, such as astrocytes and Ols. Astrocytes play a central role in brain and spinal cord homeostasis, in particular via their neurotransmitter recycling functions and ability to "seal" the CNS. The latter function of astrocytes, being expressed in the form of the glia limitans perivascularis, or in the form of the glial scar at its most extreme level of activation such as in response to tissue damage, prevents the introduction of pathogens into the CNS and controls attacks by immune cells (Sofroniew, 2009). Astrocytes contribute to inflammation by rapidly producing IL-1ß, TNF, and IL-6 (Minkiewicz et al., 2013; Pineau and Lacroix, 2007; Pineau et al., 2010). They also express receptors for many cytokines, including those recognizing cytokines of the IL-1, TNF, IL-6-like, TGF-ß and IL-10 families (John et al., 2003), giving rise to an autocrine and/or paracrine loop of astroglial activation. They must thus be considered among the first responders to injury, and NF-ßB and STAT3 seem to be central signalling hubs in this process. In this regard, it is noteworthy that selective inhibition of astroglial NF-ßB in transgenic mice reduces inflammation and lesion volume and improves locomotor recovery after spinal cord contusion (Brambilla et al., 2005). However, not all forms of astrocyte responses have a detrimental impact on tissue sparing in the context of injury. STAT3 signalling, for example, is a critical regulator of astrogliosis and scar formation limiting the spread of potentially aggressive immune cells (Herrmann et al., 2008; Okada et al., 2006). Conditional deletion of STAT3 from astrocytes resulted in increased lesion size and exacerbated demyelination, which correlated with a reduced motor recovery after SCI. STAT3 is a signal transducer of various cytokines including those of the IL-6-like and IL-10 families. This suggests that interventions designed to inhibit a specific cytokine or signalling pathway in astrocytes are most likely to provide effective long-term protection than blocking all astrocytic functions.

Like microglia and astrocytes, Ols are another important target of cytokines. As reviewed by MCTigue and Tripathi, Ols and OPCs are particularly vulnerable to inflammatory conditions that can arise from a traumatic injury to the CNS (McTigue and Tripathi, 2008). Indeed, a 50% loss of the OL population has been reported as early as day 1 post-SCI (Grossman et al., 2001). This could have important consequences on the early loss of motor function after SCI, as Ols are required for the maintenance of normal axon transport and an important source of growth factors and neurotrophic cytokines (Nave, 2010).

While Ols in the injured CNS respond to the loss of axonal contact and inflammation by undergoing apoptosis (Vargas and Barres, 2007), Schwann cells (SCs) in the injured PNS respond by entering cell cycle (Murinson et al., 2005). Furthermore, and in contrast to the well-established role of SCs in breakdown of the myelin sheets and phagocytosis of its debris in the injured peripheral nerve (Fernandez-Valle et al., 1995; Hirata and Kawabuchi, 2002; Reichert et al., 1994; Stoll et al., 1989), Ols have little or no capacity to clear myelin debris after CNS injury. This may contribute to explain why WD is extremely slow in the injured CNS compared to the PNS in both rodents and humans (George and Griffin, 1994; Miklossy and Van der Loos, 1991).

**Endothelial cells of the neurovascular unit**

The NVU of the CNS is a complex and dynamic structure composed of ECs, pericytes embedded in the endothelial basement membrane, perivascular macrophages, and the glia limitans perivascularis, consisting of the parenchymal basement membrane and a juxtaposed layer of astrocytic processes ("end-feet"). This structure bears the name of blood–brain barrier (BBB) or blood–spinal cord barrier (BSCB), depending on whether it is located in the brain or spinal cord. An (almost) equivalent of the BBB/BSCB exists in the PNS, and is called blood–nerve barrier (BNB). Like the BBB and BSCB, the BNB regulates the access of soluble mediators and leukocytes circulating in the blood to neural tissue parenchyma. Unlike its counterparts in the CNS, the BNB is believed to be more permeable because of the absence of glia limitans perivascularis, but this remains to be demonstrated experimentally. Since excellent reviews have described in detail the basic structure and functions of these three barriers (Bartanusz et al., 2011; Kanda, 2013; Muldoon et al., 2013; Zlokovic, 2008), we will focus here mainly on ECs forming the nonfenestrated endothelium and their response to neural injury.

Damage to the NVU and breakdown of the BSCB are common consequences of SCI (Balentine, 1978; Noble and Wrathall, 1988, 1989a, 1989b; Popovich et al., 1996). Of particular interest are studies that have linked vascular permeability with the evolution of secondary damage (reviewed by Tator and Fehlings, 1991, and revisited more recently by Fassbender et al., 2011). Permeability of the BSCB has been reported as early as 5 min following spinal cord contusion in rodents and primates (Dohrmann et al., 1971; Maikos and Shreiber, 2007). This enhanced permeability is clearly too early to be caused by the transmigration of blood-derived leukocytes, and is thus a direct consequence of physical disruption of the spinal cord vasculature due to mechanical injury (Maikos and Shreiber, 2007). However, mounting evidence suggests that activated resident and recruited immune cells may inflict further damage to the endothelium in the injured peripheral nerve and spinal cord (Gray et al., 2007; Lee et al., 2011). Although it may be expected that a certain number of leukocytes will passively enter injured tissue due to disruption of the microvasculature, active processes regulate the transmigration of immune cells. Leukocyte entry is a tightly coordinated process requiring cytokines and bind to different subsets of CAMs that are increased de novo in inflamed tissues (Ley et al., 2007). Interfering with the mechanisms by which inflammatory mediators
contribute to blood vessel dysfunction may therefore offer a unique opportunity to interfere with secondary events leading to enhanced neurological deficits. This was demonstrated by data showing that pharmacological or genetic inhibition of MMP activity after SCI prevented BSCB disruption and improved locomotor function (Lee et al., 2012a, 2012b; Noble et al., 2002). Lee et al. also found that the administration of a fluoroscein to SCI mice prevented the expression of proinflammatory cytokines (IL-1β, TNF, IL-6), reduced infiltration of myeloid cells, attenuated loss of tight junction molecules, and inhibited apoptotic cell death (Lee et al., 2012b). It should be emphasized that cytokines such as IL-1 and TNF can directly disrupt endothelial cell–cell interactions and trigger degradation of barrier function (Royall et al., 1989; Zhu et al., 2012). An interesting observation in the case of IL-1β is that its disruptive effect on endothelial barrier function is mediated through the IL-1R1-MyD88-ARNO-ARF6 cascade, a pathway independent of NF-κB (Zhu et al., 2012). A better understanding of the signaling pathways involved in the different functions of cytokines could therefore help target pathway-specific responses downstream of these receptors. An example would be the inhibition of vascular leak without interfering with immune-cell adhesion or other critical NF-κB-dependent responses.

Vessel regression and neovascularization are common features of spinal cord and peripheral nerve injury (Barrette et al., 2008; Benton et al., 2008; Blight, 1994; Dray et al., 2009). Such vascular remodelling processes are believed to be involved in the pathogenesis and recovery of traumatic neural injury. In support of the former are studies suggesting that OLs and neurons could be particularly sensitive to a constant blood supply due to their high-energy demand (Attwell et al., 2010). Accordingly, disruption of the endothelium has been regarded as one of the main causes of secondary damage after SCI (Fassbender et al., 2011; Mautes et al., 2000; Tator and Fehlings, 1991), although this idea has been challenged by Casella et al. who reported that neuronal death occurred before EC loss (and to a greater extent) in the vicinity of a contusion injury (Casella et al., 2006). Despite this correlative (not causal) evidence, several studies have shown that protecting the vascularized tissue led to improvements in tissue sparing and functional recovery after SCI (reviewed in (Fassbender et al., 2011)). One unknown is whether blood vessel regression and permeability may be necessary steps for neovascularization. This is important considering that neovessels might influence axonal regeneration in the injured PNS and CNS (Barrette et al., 2008; Dray et al., 2009).

**Innate immune cells**

Neutrophils and “classically activated” proinflammatory (M1) macrophages (Ly6Chi7/4+) are the first innate immune cells to respond, being already abundantly present in spinal cord parenchymal tissue at 12 h post-SCI (Pineau et al., 2010; Stirling and Yong, 2008). Their presence at sites of SCI is transient, peaking at 1–2 days before falling to lower levels at 4 days (Stirling and Yong, 2008; Thawer et al., 2013). Importantly, the time course of recruitment of these two cell populations at sites of peripheral (sciatric) nerve injury is identical to the one reported in the injured spinal cord in mice (Nadeau et al., 2011). The presence of these immune cell types during the chronic phase of injury may however differ between the CNS and PNS, as will be discussed in the last section of this review.

Neutrophils and macrophages are well recognized for their capacity to sterilize lesions by killing pathogens and their ability to clear cellular debris. Although they have long been considered as homogeneous cell populations, it is now well established that they show remarkable heterogeneity in terms of the molecules they express and functional properties. Monocyte and macrophage heterogeneity has been described first in humans and then more recently in mice, and implicated in pathologies such as cancer, diabetes, arthritis, obesity, atherosclerosis, infection, and neural injury (David and Kroner, 2011; Flavell et al., 2010; Shi and Pamer, 2011; Sica and Mantovani, 2012; Woollard and Geissmann, 2010). M1 and “alternatively activated” anti-inflammatory (M2) macrophages are commonly identified based on expression of cell surface markers, such as CD14 and CD16 in humans and Ly6C, CX3CR1 and CCR2 in mice (Geissmann et al., 2003; Gordon and Taylor, 2005). Monocyte-derived M1 macrophages are rapidly recruited to injured tissues via CCL2 and primarily involved in inflammation, proteolysis, and phagocytosis (Nahrendorf et al., 2007). M2 macrophages respond to a different chemokine, CX3CL1, and are implicated in both immune surveillance and the healing process (Nahrendorf et al., 2007). In the mouse spinal cord, like in the peripheral nerve, M1 and M2 are successively recruited at sites of injury (Kigerl et al., 2009; Nadeau et al., 2011; Thawer et al., 2013). In line with their reported effects in injured peripheral tissues, M1 macrophages present in the injured spinal cord produce proinflammatory mediators and are neurotoxic when co-cultured with primary neurons, whereas M2 macrophages have axon growth-promoting effects (Kigerl et al., 2009). An in vivo demonstration that M1 macrophages can kill neurons and contribute to secondary damage after SCI is however lacking, mainly because there is still no way to deplete these cells specifically. Likewise for the in vivo role of M2 macrophages, but the recent demonstration that mice lacking Nr4a (Nur77) developed fewer M2 monocytes and macrophages may enable such work to be performed in the future (Hanna et al., 2011, 2012). Another way to investigate the role of the latter cell subset could be using the cell depletion method recently developed by Miron et al. (Miron et al., 2013). The method is based on the concept that phagocytosis of clodronate-containing liposomes will induce apoptosis and relies on the accuracy of mannosylated clodronate liposomes that specifically bind the mannose receptor, also called CD206, a receptor whose expression is strongly upregulated after M2 polarization (Gordon, 2003; Martinez et al., 2006). Using this depletion strategy, Miron et al. unveiled that M2 macrophages/microglia are important for OL differentiation and remyelination (Miron et al., 2013). In the meantime, the closest a study has come to a direct assessment of the role of M1 macrophages in SCI is by depleting these cells in vivo by injection of liposome-encapsulated clodronate (Lee et al., 2011; Popovich et al., 1999). The study by Lee et al. demonstrated that preventing the recruitment of both neutrophils and monocytes, but not neutrophils or monocytes alone, is beneficial for functional recovery following SCI in mice (Lee et al., 2011). In contrast, in the study by Popovich et al., macrophage depletion alone was found sufficient to reduce spinal cord tissue damage and promote partial recovery of hindlimb function in SCI rats (Popovich et al., 1999). Thus, species differences might exist and could explain the need for dual depletion in mice. Combined with our earlier results showing that neutrophils and monocyte-derived M1 macrophages are recruited in the injured mouse spinal cord and peripheral nerve via a common signalling pathway (i.e. IL-1R1) (Nadeau et al., 2011; Pineau et al., 2010), these findings suggest a great level of redundant functions between these two populations of innate immune cells at least in mice.

Aside from their role in inflammation, recent SCI studies have attributed to Gr-1+ (Ly6G/Ly6C+) neutrophils and macrophages an important function in a variety of biological processes ranging from angiogenesis to wound healing and scar formation (Shechter et al., 2009, 2011; Stirling et al., 2009). It thus appears that the role of innate immune cells is more complex than previously thought and may also contribute to recovery. One finding shared by all these studies is the demonstration that the specialized functions of neutrophils and macrophages are dictated by cytokines present in the SCI environment. In turn, the cytokines produced by activated immune cells can regulate a variety of subsequent cell responses, including inflammation and immunity, BBB/BSCB maintenance and integrity, glial scar formation, demyelination and remyelination, and even neural cell death (see Table 1). We believe that this concept of heterogeneity in phenotype and function in the injured nervous system does not only apply to monocytes/macrophages, but also to neutrophils which are far more plastic than previously thought (for review, (Beyrau et al., 2012)). Recent work emerging from the cancer field has shown that neutrophils...
can be polarized towards a proinflammatory (N1) or anti-inflammatory (N2) phenotype, depending on the expression of TGF-β1 (Fridlender et al., 2009). The existence of different neutrophil subsets has previously been reported in various infection models (Tsuda et al., 2004), and has since been confirmed in several different systemic inflammatory diseases (Denny et al., 2010; Pillay et al., 2010, 2012). Whether N1 and N2 neutrophils can be found in the injured spinal cord and peripheral nerve remains an open question. The future identification of specific markers for the distinct populations of neutrophils will help answer these questions, and help study their role in secondary damage, scarring, regeneration and repair.

**Differences between the injured spinal cord and peripheral nerve**

It is interesting to note that, although the cells that reside in the normal spinal cord are to a large extent different from those of peripheral nerves, the temporal pattern of cytokine production and innate immune cell infiltration in these two systems is remarkably similar, at least during the first week of injury (Boivin et al., 2007; de Rivera Vaccari et al., 2012; Donnelly et al., 2011; Nadeau et al., 2011; Pineau and Lacroix, 2007; Stirling and Yong, 2008; Thawer et al., 2013). Assuming that excessive inflammation is one of the leading causes of secondary damage after neural injury, it may come as a surprise that inhibition of key proinflammatory cytokine pathways or depletion of innate immune CD11b+ cells has been repeatedly demonstrated to reduce repair and recovery after peripheral nerve injury (Barrette et al., 2008; Boivin et al., 2007; Nadeau et al., 2011). We are therefore led to ask why an apparently similar inflammatory response would be detrimental in the injured spinal cord, but beneficial in the injured peripheral nerve.

Following injury, severed proximal segments of axons that remain connected to their cell bodies retract over hundreds of micrometers (~300 μm on average) in a process referred to as “axonal dieback” or acute axonal degeneration (AAD) (Kerschensteiner et al., 2005). Axonal dieback is commonly observed in both the injured CNS and PNS and mediated by two distinct mechanisms. The initial phase of dieback occurs within 30 min of the axonal injury (Kerschensteiner et al., 2005; Knoferle et al., 2010), a rapid time-course that precedes the infiltration of circulating neutrophils and proinflammatory M1 monocytes and which thus suggests it is regulated by intrinsic neuronal mechanisms. This was confirmed by the demonstration that blocking calcium (Ca2+) release from intra-axonal Ca2+ stores or inhibiting calcium-dependant proteases such as calpain prevents AAD in the injured spinal cord and optic nerve (Kerschensteiner et al., 2005; Knoferle et al., 2010; Stirling et al., 2014b). The initial phase of AAD is followed by a delayed slow phase of axonal dieback mediated by macrophages infiltrating sites of injury and occurring between 2–4 days and 28 days after the lesion (Busch et al., 2009; Horn et al., 2008), thus supporting once again the concept that macrophages are playing an active role in various forms of secondary damage. Whether microglia contribute to SCI-induced axonal dieback is currently being debated, with recent iVM studies in favor and against this idea (Evans et al., 2014; Stirling et al., 2014a). Stimulation of microglia with the TLR2-specific agonist Pam2CSK4, but not zymosan, induced a protective mixed M1/M2 phenotype in microglia, which was associated with a reduction in secondary bystander damage to axons that were originally spared by the initial laser-induced SCI (Stirling et al., 2014a). Accordingly, TLR2-ko mice exhibited greater axonal dieback than control mice after laser-induced SCI and mildly reduced functional recovery after contusion SCI (Kigerl et al., 2007; Stirling et al., 2014a). This is in contrast to the situation occurring in the injured peripheral nerve where deficiency in TLR2 signalling was found to delay myelin debris clearance, axonal regeneration and recovery of locomotor function (Boivin et al., 2007). The differences in cell types present in each of these tissues could explain these different outcomes, taking into account that microglia and SCs, for example, express high levels of TLRs such TLR2 (Babcock et al., 2006; Karanth et al., 2006; Lee et al., 2006).

Neutrophils and M1 macrophages are considered to be toxic for neurons (Allen et al., 2012; Geremia et al., 2012; Kigerl et al., 2009; Lee et al., 2011). We find it particularly interesting that the initial phase of axon retraction precedes the infiltration of neutrophils and monocyte-derived M1 macrophages (CD45+ CD11b+ 7/4+ Ly-6G+ Ly-6C+ cells), a process that begins at around 6–12 h post-injury (Nadeau et al., 2011; Pineau et al., 2010; Stirling and Yong, 2008). It is also intriguing that these two cell types have almost completely disappeared from the injured spinal cord and peripheral nerve at 3–4 days (Nadeau et al., 2011; Pineau et al., 2010; Stirling and Yong, 2008). Three to 4 days post-injury is the time at which macrophages found at sites of neurodegeneration start to express genes and markers associated with pro-regenerative, anti-inflammatory M2-phenotype such as arginase-1 and CD206 (Kigerl et al., 2009; Nadeau et al., 2011). It is also the time at which peripheral nerve axons begin their attempt to regenerate across the injury site (Fu and Gordon, 1997). However, for an unexplained reason, the expression of M2 genes and protein markers is only transient after SCI and rapidly returns to preinjury levels by 7 days. In contrast, the expression of M1-related genes (e.g., CD16, CD32, CD86) is maintained, as such that after 28 days only M1 macrophages persist at sites of SCI (Kigerl et al., 2009). It has been proposed that the predominance of M1 macrophages and almost complete absence of M2 macrophages at sites of SCI could cause chronic inflammation, thus leading to secondary damage. This scenario is in contrast to what is thought to occur in the injured PNS, where it has been assumed that the expression of proinflammatory markers is rapidly decreased and stay decreased chronically. However, a formal demonstration of this is still lacking. This supposition, if true, could be explained by the increased expression of immunosuppressive cytokines such as TGF-β1 and IL-10, which were both found to be upregulated in the peripheral nerve but not in the spinal cord after injury (Beeri et al., 1998; Perrin et al., 2005). Other than cytokines, and as suggested by observations made in vitro and in vivo in the multiple sclerosis brain, the phagocytosis of myelin debris could be the factor conferring anti-inflammatory function (Boven et al., 2006). This would mean that the persistent expression of anti-inflammatory cytokines in the injured PNS is a direct consequence of the latter response, not the cause. This brings another question to mind: whether the long-lasting presence of M2 macrophages throughout the peripheral nerve distal stump is the main reason why regeneration is more efficient in the PNS than CNS?

The inhibitory influences of myelin debris persist for prolonged periods in the CNS and prevent axon regeneration. As explained above, this difference in the time required for myelin clearance in the CNS compared to the PNS may be due to differences in the immune response. The Rotshenker laboratory has established a strong correlation between the magnitude and timing of cytokine production and the initiation and progression of WD, and found that mice with abnormally slow WD express reduced levels of IL-18 and TNF (Beeri et al., 1998; Shamash et al., 2002). Together with a previous study that showed that microglia/macrophage activation is compromised in mice carrying a mutation in the IL-12 p40 gene (Li et al., 2001), these findings highlight the crucial role that cytokines may play in immune cell activation/recruitment, WD, and myelin clearance. This idea is further supported by our data showing that recruitment of innate immune cells and functional recovery after sciatric nerve lesion is impaired in the absence of IL-18 and TNF (Nadeau et al., 2011). The entry of blood-derived immune cells into restricted areas of the injured spinal cord, mostly limited to the core of the lesion, further suggests the possibility that production and/or release of proinflammatory cytokine expression may be defective in degenerating white matter tracts after SCI. Interestingly, injecting recombinant IL-1β or CCL2 and CCL3/MIP-1α into the injured mouse dorsal column triggered macrophage/microglial activation and myelin clearance. Neutralizing the same three cytokines/chemokines in the lesioned sciatic nerve via the administration of function-blocking antibodies suppressed the clearance of inhibitory myelin debris (Perrin et al., 2005). Taken together, these studies suggest...
that changes in the immune responses, which are dictated by the nature of the cytokines produced in the inflamed tissue, may explain the different responses to injury between the CNS and PNS.

Conclusion

In the recent years, we have learned a great deal about the complexity and heterogeneity of the immune cell response. We now know that immune cells are remarkably plastic and can be polarized towards different functional phenotypes depending on the cytokines and other signals (e.g. DAMPs, PAMPS) present in their immediate environment. This has been interpreted as indicating of the existence of different subsets of immune cells, playing a role in immune regulation, host defense and wound healing. We have also learnt that microglia are ontogenetically distinct from monocyte-derived macrophages, and that they are highly dynamic (rather than quiescent) and plastic. This new knowledge has enabled us to address key gaps in neuroimmunology, and to explain some of the discrepancies in results in the SCI field that had remained unexplained until recently. As knowledge and technology is evolving rapidly, it can be expected in years to come that the identification of the endogenous signals regulating immune cell phenotype and function will allow the creation of novel approaches to either trace/monitor or manipulate/target immune cells more specifically. It is expected that these advancements will eventually lead to the development of therapeutics aimed at regulating the entry of potentially harmful immune cells into the CNS or the activation these cell types to promote neuroprotective and regenerative effects. In this regard, anti-cytokines therapies seem particularly promising to achieve this goal.

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