Chapter 1: Introduction

1.1 Overview

Central nervous system (CNS) trauma, in the form of spinal cord injury (SCI), is a debilitating disorder, which in most serious cases leads to paralysis and loss of normal function below the level of injury (Moon and Bunge, 2005). In the United States alone, the annual incidence of SCI is estimated at 40 cases per million of population, consisting of approximately 12,000 new cases each year (NSCISC, 2009). In Australia, this is seen to a lesser extent with 12.4 cases per million of population or approximately 300-400 new cases each year (Cripps, 2004). In addition, depending on the level and severity of the lesion, SCI creates further complications that influence the quality of life of those affected; for instance, some common problems include “paralysis, sensory loss, intractable pain, pressure sores, and urinary and other infections” (reviewed in (Rossignol et al., 2007).

SCI is a devastating condition resulting in considerable neuronal injury, loss of tissue, often manifested in functional deficits consistent with limited repair (Schwartz and Yoles, 2006). In humans, SCI has been regarded as a daunting goal and although progress on animal models of injury offer a realistic objective (Waxman and Kocsis, 1997), axonal regeneration post SCI remains only partially understood (Buss et al., 2007). The life expectancy of SCI sufferers remains to escalate as new strategies improving SCI treatments continue to emerge (Wyndaele and Wyndaele,

2006). Although, it is important to mention that life expectancy is lower in persons injured as children as compared to those injured as adults (Shavelle et al., 2007). Unfortunately, despite advances in neuro-regenerative research for SCI, the most notably used pharmacological agent used in the clinic is methylprednisolone, which effectiveness is limited to the timing of the injury (Bracken et al., 1997; Braughler et al., 1987; Gimenez y Ribotta et al., 2002). This post injury treatment helps decrease the pro-inflammatory response believed to be deleterious to the organism, however, the contents of this thesis highlight the complexity of the SCI and discuss the beneficial potential of inflammation in the repair of injured nerves.

Here, we explore several mechanisms underlying axonal CNS regeneration after spinal injury and discuss important experimental concepts of recent findings, which validate extensive progress toward a better understanding of the injury.

1.2 General Study Aim

This thesis examines possible mechanism/s underlying axonal CNS regeneration of primary afferent neurons in the dorsal column of the spinal cord following a peripheral nerve lesion (refer to section 1.4.5 for more details). Briefly, this type of lesion was first described as part of an *in vivo* study by Peter Richardson and Valerie Issa in 1984, where they demonstrated the intrinsic capability for mature injured neurons to regenerate through the CNS environment (Richardson and Issa, 1984),

known to be inhibitory for regrowth (David and Aguayo, 1981). Regeneration, in this experimental model was focused on the dorsal root ganglion (DRG) neurons and on the fact that anatomically DRG neurons extend processes both centrally and peripherally (Chong et al., 1999). Given this, as long as the peripheral DRG branch was lesioned prior to injury of the CNS branch, axonal regeneration of the CNS branch was possible (Richardson and Issa, 1984). Interestingly, at present the exact mechanism/s mediating this regenerative response has not been fully elucidated. Although, it has been proposed that the inflammatory cascade (mainly macrophage cells) in the DRG following peripheral axotomy, is a likely contributor to the CNS regeneration observed (Lu and Richardson, 1991).

The injury model used in this study relies on the regenerative concept of CNS nerves investigated by Richardson and Issa (1984) and it was chosen for the following reasons: a) it is based on the intrinsic regenerative capability for adult, injured CNS neurons to regenerate; b) it allows for axonal regeneration through the CNS environment without the application of any exogenous growth-promoting agent to stimulate regrowth; c) the amount of regeneration has been shown to be limited to some nerves; d) there is evidence that the inflammatory response might be playing a crucial role; and e) the regenerative mechanism/s underlying this response has yet to be fully characterised.

In light of the current information regarding this model of CNS regeneration, this introductory chapter consists of a brief overview of some of the challenges involved in achieving CNS regeneration and a final discussion regarding the role of inflammatory cells after CNS injury.

1.3 Problem: CNS Regeneration

In contrast to the peripheral nervous system (PNS), severed axons in the adult mammalian CNS fail to regenerate (Ramón y Cajal, 1928). This inability to spontaneously regenerate following injury often results in loss of motor and sensory function below the injury site. However, since the scientific demonstration that mature CNS neurons were capable of regeneration (David and Aguayo, 1981), many other groups have attempted to maximise the amount of axonal CNS regeneration through a variety of techniques (discussed below). Firstly, we begin this section with a brief discussion on the fundamental differences between the central and peripheral nervous system in relation to myelin clearance and phagocytosis, followed by some common misconceptions regarding the immune privileged status of the CNS. Finally, we highlight experimental evidence from scientists in field indicating that at least in some cases, CNS regeneration although limited can be improved depending on the physical, biochemical and cellular changes that occur in the CNS environment after injury.

1.3.1 Central versus Peripheral Nervous System

While mature injured neurons in the CNS fail to regenerate, in the PNS this does not apply and it might be explained by glial differences between these two systems (reviewed in (Vargas and Barres, 2007). The CNS glia comprises astrocytes, microglia and oligodendrocytes and the PNS glia comprises only of Schwann cells.

In the PNS, as well as in the CNS axonal degeneration can be observed within days after injury with both systems/compartments experiencing immune cell infiltration, largely consisting of inflammatory cells such as macrophages (Popovich et al., 2002; Schwartz et al., 1999a). This inflammatory response has been considered an important process in wound healing and has been suggested to require active communication between the injured tissue and the recruited immune cells necessary for tissue repair (Kodelja et al., 1997; Mantovani et al., 2005). However, the timing and functionality of inflammatory cells in the degradation of myelin and removal of debris after injury has been suggested to differ between these two compartments (reviewed in; (Trivedi et al., 2006; Vargas and Barres, 2007). In fact, the degradation and removal of debris from the injury site is cleared within days in the PNS, while in the CNS, this process is prolonged for weeks (Hughes et al., 2002). This delayed response was closely studied in transected lumbar dorsal root fibers, which normally extend through both the PNS and CNS, validating a delay in onset myelin clearance from the CNS microenvironment as compared to the PNS, where macrophages have easier access to degenerating nerves (George

and Griffin, 1994). Similarly, Lawson et al (1994) found that inflammatory responses to Wallerian degeneration following optic or sciatic nerve injury resulted in the poor clearance of myelin debris from the CNS as observed in the PNS (Lawson et al., 1994). This was attributed to the synergistic functionality of Schwann cells and phagocytic cells such as macrophages in the PNS, whilst the myelin producing cells of the CNS, namely oligodendrocytes, appeared to contribute minimally to Wallerian degeneration (Lawson et al., 1994; Ludwin, 1990).

It is therefore clear that the PNS, has not only the ability to mount a robust inflammatory response but it is also able to rapidly clear degenerating axonal and myelin debris. This, as discussed by others might be due to the infiltration of macrophage cells, probably recruited by cytokines such as tumour necrosis factor-alpha (TNFα) during Wallerian degeneration which do so “without affecting the extent of myelin damage or phagocytosis” (Liefner et al., 2000). In addition, other macrophage secreted factors including interleukin-1 (IL-1) has also been shown to participate during nerve repair process (LaFleur et al., 1996; Manson et al., 2001; Mason et al., 2001), together with stimulation of Schwann cell proliferation (Salzer and Bunge, 1980) and interaction with extracellular matrix components during the Wallerian degeneration process, promote neurite outgrowth (Agius and Cochard, 1998).

Concomitantly, macrophage cells also up-regulate apolipoprotein E, known to participate in the recycling of lipids needed for redistribution and

metabolism in nerve repair, noted to occur during the later stages of Wallerian degeneration when a higher number of phagocytes could be detected (Stoll et al., 1989). Macrophages have been described to facilitate debris removal and support neurite outgrowth by secreting neurotrophic factors such as nerve growth factor (NGF) (Barouch et al., 2001b; Heumann et al., 1987; Moalem et al., 2000) and brain derived neurotrophic factor (BDNF) (Bouhy et al., 2006; Schulte-Herbruggen et al., 2005).

Interestingly, an added complexity to axonal regeneration has been documented in experimental evidence investigating the effect of myelin taken from either PNS or CNS, found both to be inhibitory for axonal growth (Bahr and Przyrembel, 1995). However, the inhibitory properties of myelin on neurite growth can be attenuated by laminin, a Schwann cell derived product, present in the PNS but not in the CNS (David et al., 1995). On a separate study investigating the effects of myelination in the CNS and PNS, found that inhibition of certain regulators such as myosin II, impairs PNS myelination although in the CNS inhibition of this regulator is necessary for myelin formation (Wang et al., 2001).

Given that myelin derived from normal and axotomised peripheral nerves is inhibitory in nature for axonal regrowth, suggests that these inhibitors are present in both CNS and PNS compartments (Bahr and Przyrembel, 1995). This led Vargas and Barrens (2007) to propose that the robustness and rapidity of myelin debris clearance from the PNS might be a likely

contributor to the regenerative ability observed in peripheral nerves (reviewed in (Vargas and Barres, 2007). The efficient debris clearance in the PNS has been described as part of the functioning of the immune response, particularly in regards to phagocytosis by macrophage cells (reviewed in (Trivedi et al., 2006), a factor noted to occur faster in the PNS, as opposed to a 3-fold delay observed in the CNS (George and Griffin, 1994). This has been attributed to poor phagocytic recruitment during Wallerian degeneration in the CNS, as compared to the PNS supported by increased adhesion molecule expression on vascular endothelial cells (Castano et al., 1996). Interestingly, other reports indicate that macrophage functionality might be related to the timing of their activation (Lazarov-Spiegler et al., 1998b; Yin et al., 2003), phenotype of activation (Schwartz et al., 2006a; van Rossum et al., 2008) and phagocytic capabilities (Lazarov-Spiegler et al., 1998b; Zeev-Brann et al., 1998). Taken together, it would seem advantageous to minimise the prolonged exposure of injured axons to degenerating CNS myelin debris, however, the CNS does not favour resealing of axotomised axons, whereas the PNS does (Ahmed et al., 2001). This, as suggested by Ahmed and colleagues (2001), is another likely contributor to the ability of PNS neurons to regenerate following injury, because the maintenance of sealed central processes may be indicative of a constant supply of neurotrophic factors to the injured neuron (Ahmed et al., 2001).

Interestingly, the inflammatory response after injury not only varies between the PNS and CNS but there are published reports, which

describe intra-compartmental variation within the CNS. Notably, there is evidence indicating that injury to the brain as compared to the spinal cord results in greater inflammatory response in the latter compartment (Batchelor et al., 2008; Schnell et al., 1999b). Differences in intra-compartmental responses to injury as suggested by Popovich and colleagues (2002), “may be due to unique mechanisms controlling inflammation in these compartments” (Popovich et al., 2002). Although advances in neuroimmunology have led to the understanding regarding the process of inflammation, findings such as those aforementioned reiterate the importance, complexity and vast knowledge yet to be deciphered about inflammation. Overall, even though we have only briefly discussed some of the differences between CNS and PNS, it is clear that direct comparison between compartmental functionality remains to be further investigated.

1.3.2 Introduction to Spinal Cord Injury

The spinal cord, in a simplistic way, is a bridge-like structure conveying neural messages between the CNS and the PNS. For this reason, traumatic injury to the spinal cord results in varying degrees of motor, sensory and autonomic loss of function below the site of injury.

Progress to repair the injured spinal cord has shed some new light into the possible mechanisms for improving the outcome after injury. Here we describe some of the problems associated with injury to the mammalian spinal cord, as well as a brief description into some of the strategies used

to overcome these limitations (refer to Table 1 for a brief summary on the events of secondary neuronal injury following SCI).

1.3.2.1 CNS Regeneration

Since the time of Ramon y Cajal in 1928, it was believed that adult CNS neurons were not able to regenerate, often forming retraction bulbs, which Cajal described as 'abortive regeneration' (Ramón y Cajal, 1928). Consequently, there was little interest in conducting research on CNS regeneration until the publication of a prominent study by David and Aguayo in 1981, which demonstrated that using a peripheral nerve graft, transected axons of spinal cord neurons could elongate through the graft (David and Aguayo, 1981). Briefly, using a sciatic nerve bridge positioned extraspinally between the medulla and the thoracic spinal cord, a large number of regenerated neurons were found, providing evidence that many of the axons within the grafts had originated in the CNS (David and Aguayo, 1981). This study not only confirmed that CNS axons were intrinsically capable of regeneration, but also indicated that the CNS environment limits axonal regeneration of injured neurons.

Evidence of the growth-promoting properties of peripheral nerve (PN) grafts in the regeneration of injured CNS neurons was further investigated by Aguayo's laboratory in 1987, where the concept of CNS regeneration, in this case of mature retinal ganglion cells (RGCs), following optic nerve injury was challenged. Briefly, by the removal of the optic nerve and connecting it with a PN graft joining the eye to the superior colliculus,

RGCs were found to be not only able to extend through the length of the graft but also make synapses in the CNS (Vidal-Sanz et al., 1987). Experiments such as the aforementioned, validated the notion that intrinsically mature injured CNS neurons were capable of regeneration, consequently shifting research interest to CNS myelin, myelin-associated molecules and the glial scar, as major components limiting the regeneration of injured CNS neurons (discussed below).

1.3.2.1.1 Myelin

An understanding of axonal inhibition came from two reports in 1988 by Caroni and Schwab, providing evidence that in the CNS, myelin could actively inhibit growth and prevent regeneration of matured injured neurons. The first study showed that CNS myelin, derived from oligodendrocytes, could inhibit neurite growth *in vitro* (Caroni and Schwab, 1988a). The second study also conducted *in vitro*, showed that this inhibitory activity could be neutralised with a monoclonal antibody (IN-1) generated against protein fractions expressed on the surface of oligodendrocytes and the myelin they produce (Caroni and Schwab, 1988b). The extent of the inhibitory properties of CNS myelin was confirmed in a follow up study in 1990, where it was reported that the *in vivo* use of the IN-1 antibody promoted axon regeneration in the lesioned spinal cord (Schnell and Schwab, 1990). This study was the first to provide evidence that an antibody-mediated approach could be used to block the axon growth inhibitors associated with myelin.

At a later time, a protein defined as Nogo was identified as the antigen of the IN-1 antibody, existing in three isoforms detected in several CNS regions, including optic nerve, spinal cord and cerebral cortex (Chen et al., 2000; GrandPre et al., 2000; Prinjha et al., 2000). Prior to the identification of Nogo, *in vitro* studies described another myelin protein currently known as myelin-associated glycoprotein (MAG), to also be a potent inhibitor of neurite outgrowth (McKerracher et al., 1994) in developing cerebellar as well as matured DRG neurons (Mukhopadhyay et al., 1994). In relation to an injured system, *in vitro* evidence suggests the majority of inhibitory activity can be accounted from MAG secreted from damaged white matter (Tang et al., 2001). Yet if regeneration were to occur, neurites will most likely be geometrically found growing parallel to the longitudinal axis of white matter tracts (Pettigrew and Crutcher, 1999). Currently, there are several other known CNS inhibitors of axon regeneration including oligodendrocyte-myelin glycoprotein (Wang et al., 2002), semaphorin 4D (Moreau-Fauvarque et al., 2003) and ephrin B3 (Benson et al., 2005).

Interestingly, in the developing CNS, the capacity for nerve regeneration is more pronounced than what is observed in the adult CNS, and this coincides with the onset of myelin formation (Keirstead et al., 1992; Saunders et al., 1998; Treherne et al., 1992). Indeed, prevention of oligodendrocyte development and myelination by repeated x-irradiation of newborn rats resulted in the elongation of injured fibres, consequently attributed to a myelin-free CNS environment (Savio and Schwab, 1990). However, even in the mature CNS, treatment with the IN-1 antibody has

been shown to result in functional improvement in the presence of myelin (Thallmair et al., 1998; Z'Graggen et al., 1998).

The aforementioned studies conducted *in vitro* and *in vivo*, restate evidence for the inhibitory potential of myelin and its associated proteins, a factor that until now has generated limited regeneration of injured CNS nerves. However, in addition to the role of myelin in restricting neurite outgrowth, other type of inhibitors to axonal growth also exists. These are associated with the post-traumatic scarring formed at the injury site, forming physical barriers to CNS regeneration (discussed below).

1.3.2.1.2 Physical Barriers

In mammalian CNS, another major barrier to axon regeneration is the glial scar that forms at the injury site (McKeon et al., 1991; Rudge and Silver, 1990). This scar as reviewed by Silver and Miller, contributes to the inhibitory environment of the injured CNS and serves as a physical and biochemical barrier to axonal regeneration (Silver and Miller, 2004) (refer to Fig. 1 for a schematic representation of a lesioned spinal cord).

In the adult CNS, soon after injury, inhibitory molecules secreted by reactive astrocytes and together with the formation of a glial scar formed at the injury site obstructs axonal growth (Mansour et al., 1990). Indeed, in knock-out animals for major proteins of astrocyte cytoskeleton, such as glial fibrillary acidic protein (GFAP) and vimentin, demonstrated improved axonal plasticity observed in anatomical and functional recovery after SCI,

attributed to a reduction in astroglial presence (Menet et al., 2003). Therefore, typically the glial scar is often associated with the formation of 'dystrophic end bulbs' representing regenerative failure (Ramón y Cajal, 1928). Aside from the physical barrier created by the reactive astrocytes, there are also other known biochemical inhibitory molecules present in the extracellular matrix that are expressed after injury, including chondroitin sulfate proteoglycan (CSPG), keratin sulfate proteoglycan, NG2, and cytotactin/tenascin (Camand et al., 2004; Jones et al., 2002; Lemons et al., 1999; McKeon et al., 1991; Tang et al., 2003; Zuo et al., 1998).

The scar formation primarily consists of reactive astrocytes and proteoglycans, however, it seems reasonable that if the injury compromises the dura mater, invading cells will also be found at the lesion site. The predominant cells that enter the lesion site are macrophages, and these cells are capable of reducing the inhibitory properties of the CNS white matter with their cellular persistence associated with strain-specificity (David et al., 1990; Popovich et al., 1997). These cells have been reported to have beneficial as well as detrimental effects following CNS tissue damage/repair and their controversial role will be discussed in more detail later.

After CNS injury, the role of astrocytes can be viewed from two different perspectives, one where they hinder axonal outgrowth (as aforementioned) and the other where they contribute to re-establishment of the blood brain barrier (BBB), reduction in immune cell infiltration, tissue

disruption and increased motor dysfunction (Faulkner et al., 2004). In other words, in the regenerative system even though astrocytic ablation proved useful during axonal growth, the overall progress in repair and wound healing process in the aforementioned factors is restricted (Bush et al., 1999). Therefore, the ratio of benefit versus damage cannot be overlooked. Interestingly, the topical application of steroids such as glucocorticocoids at the site of CNS injury, reduced the density of cellular infiltrates which “could enhance the overall degree of axon regeneration” (Li and David, 1996), perhaps by neutralization of inhibitory factors like chondroitin sulfate (Moon et al., 2001) and/or by concomitant stimulation of the neuronal cell body and injured axon to extend beyond the injury site (Lu et al., 2004). Some of these concepts will be explored next as potential avenues for improvement of axonal regeneration in the matured CNS.

1.4 *Therapeutic Avenues for CNS Regeneration*

Experimental therapies for SCI attempt to augment the amount of regeneration and growth of injured axons in the adult CNS. As aforementioned, there are several inhibitors of axonal growth present in the CNS environment that contribute to the failure of axonal regeneration in the adult spinal cord (reviewed in (Schwab and Bartholdi, 1996), some of which have been associated to a cascade of secondary tissue damage (Fitch et al., 1999). Therefore, for regeneration of injured nerves to occur in the CNS, inhibitors to axonal growth must be either bypassed or eliminated from the CNS milieu.

Here, we discuss some of the potential therapeutic avenues for CNS regeneration including: peripheral nerve grafts/transplants/artificial scaffolds, growth factors, cell transplants, vaccination with CNS homogenates and the pre-conditioned lesion (refer to Table 2 for a brief description of rodent studies of promising human interventions for SCI repair).

1.4.1 Peripheral Nerve Grafts/Transplants/Scaffolds

The utilisation of peripheral nerves as a means to stimulate regeneration is logical considering their regenerative capability in the PNS environment and it is clear that their degree of axonal regeneration in peripheral neurons is more pronounced than that of CNS axons (Guth, 1974). This important PNS property has been demonstrated with the use of transplanted peripheral nerves used in the injured CNS as bridges to bypass the lesion epicentre, where at least some CNS axons regenerated when provided with this growth supportive environment (David and Aguayo, 1981). Thereafter, experimental attempts to promote CNS axonal regeneration continued to investigate the use of PNS segments as grafts to circumvent the CNS environment and stimulate regeneration into these grafts (Aguayo et al., 1981; Benfey and Aguayo, 1982; Richardson and Issa, 1984).

The use of these grafts led to the successful regeneration of CNS nerves (David and Aguayo, 1981), however this resulted in limited functional improvement (Richardson et al., 1982). The regenerative power of injured

nerves has been validated via the administration of conditioning lesions resulting in increased regeneration of nerves in the PNS (Sjoberg and Kanje, 1990) as well as in the CNS (Oudega et al., 1994; Qiu et al., 2005; Richardson and Issa, 1984). As an effort to increase the functional recovery, some of the aforementioned techniques have been combined with various therapies (including anti-inflammatory drugs, vertebral wiring, growth factors, olfactory cells, fibrin glue and acidic fibroblast growth factor) to stimulate regeneration of CNS axons into, through and beyond grafts (Andrews and Stelzner, 2004; Blits et al., 2000; Cheng et al., 1996; Cheng and Olson, 1995).

An important factor to consider in SCI, is that "the presumed lack of trophic support to axotomised CNS neurons provided the rationale for the exogenous application of trophic factors to stimulate regeneration" (reviewed in (Plunet et al., 2002). In fact, modified peripheral nerves grafts expressing neurotrophic factors have been found to improve axonal regeneration (Blits et al., 1999) and recovery of hind limb function after SCI (Blits et al., 2000). Indeed, many experiments have used a combination of strategies to obtain significant recovery after CNS injury such as the fetal spinal cord transplants (Broude et al., 1999; Coumans et al., 2001), autologous PN grafts (Yick et al., 1999). For example, a technique using pre-degenerated peripheral nerve graft, a conditioning lesion and administration of neurotrophins showed that axonal regeneration beyond the scope of the graft is possible under the right neuro-nourishment conditions (Oudega and Hagg, 1996, 1999).

There is now evidence that neurotrophic factors such as BDNF can promote regeneration of adult CNS axons (Xu et al., 1995; Ye and Houle, 1997), which has been shown to increase the expression of regeneration-associated genes (RAGs) such as growth-associated protein 43 (GAP-43) and T-alpha Tubulin-1 (Kobayashi et al., 1997). More importantly, this intrinsic capacity for axonal regeneration in the presence of these RAGs has been shown to remain active even 1-year after injury, which "holds promise to individuals with spinal cord injuries" (Kwon et al., 2002).

Furthermore, the use of intercostal nerve transplants has also been shown to lead to partial recovery after complete spinal cord transection in rodents (Cheng et al., 1996), in non-human primates (Levi et al., 2002) and even in clinical patients with reported sensory and motor recovery of function below the injury site (Oppenheim et al., 2009). Transplantation studies using autologous grafts are advantageous to the organism, given that such strategies promote "CNS axon regeneration and functional repair and are in part attractive because autologous grafts reduce the risk of immune rejection" and prevent the need for immunosuppression (reviewed in (Moon and Bunge, 2005). In experimental animal models of SCI, the implementation of grafts and transplants provide valuable information about SCI regeneration especially when using autologous material, yet further improvements towards increased functional recovery, at least in high order non-human primates is needed after complete SCI injury.

In addition, advances in bioengineering and the fabrication of artificial

scaffolds have propelled science to a new stratum in medical research. Some of which include: non-immunogenic polymers (Moore et al., 2006), collagen hydrogels (Marchand et al., 1993), neurogel (King et al., 2003), fibronectin mats (Woerly et al., 2001), carbon filaments (Khan et al., 1991) and nitrocellulose implants (Houle and Ziegler, 1994). The nature of these scaffolds can be advantageous to the regenerating system since they can also be used to deliver neurotrophins and help axons grow in a linear trajectory (Stokols and Tuszynski, 2006). It is essential to reiterate that all these strategies have been designed to stimulate and support the regeneration of injured CNS neurons by modifying their surrounding microenvironment.

1.4.2 Growth Factors

The beneficial properties of growth factor proteins and their ability to stimulate axonal growth after SCI has been an area of interest in several studies (Blesch et al., 1999; Kobayashi et al., 1997; Lu et al., 2001; Ruitenberg et al., 2003; Schnell et al., 1994; Tuszynski et al., 1996; Xu et al., 1995). However, for the purpose of this thesis we will mainly focus on the family of growth proteins known as, neurotrophins (NT), with special reference to a highly investigated NT molecule known as brain-derived neurotrophic factor (BDNF).

Generally, the expression of growth factors and their receptors is altered after injury often resulting in significant neuronal atrophy. This change neuron-NT tropism has been previously investigated in normal as well as

in injured neurons (Tuszynski et al., 1996). Interestingly, it has been reported that blocking of BDNF antibodies in uninjured or axotomised CNS neurons, increased neuronal death of lesioned neurons as compared to controls and this was attributed to the endogenous BDNF support to neurons (Giehl et al., 1998). This injury-induced neuronal death has been demonstrated to be completely prevented by the exogenous application of BDNF to injured corticospinal neurons (Giehl and Tetzlaff, 1996).

In addition, injury-activated immune cells such as microglia and macrophages are believed to be involved in CNS axonal sprouting process via NT production and secretion by these cells (Batchelor et al., 1999). The guidance of these axonal sprouts might be the result of the trophic gradient found along the wound margin, although as observed in other CNS injuries, once axonal sprouts reach the injury epicenter growth is halted (Batchelor et al., 2002). As previously discussed, this inhibition in axonal growth is most likely due the presence of neurite inhibitory molecules present at the lesion. The poor axonal elongation at the injury site, has prompted the exploration of experimental approaches investigating the regenerative capability of injured axons by the exogenous application of BDNF via an osmotic mini-pump, demonstrating some degree of neuroprotection (Namiki et al., 2000), functional recovery in groups receiving a combination of NT and fetal spinal cord transplants (Coumans et al., 2001) or in groups grafted with genetically modified cells secreting NT also stimulated sprouting and some CNS regeneration as long as NT nourishment is continuously provided to injured axons (Tobias

et al., 2003).

Interestingly, NT have a close interaction with the immune system and this provides alternative therapeutic strategies that could potentially be used to treat SCI (Kerschensteiner et al., 2003). For example, immune cells such as activated lymphocytes (T cells) can synthesize and release NT, such as nerve growth factor (NGF) (Ehrhard et al., 1993; Santambrogio et al., 1994). Which together with the secretion of biologically active BDNF from B cells and monocytes, could provide a beneficial neuroprotective effect within the CNS (Kerschensteiner et al., 1999) and might have critical implications for immuno-regulatory therapies. However, it should also be mentioned that even though BDNF has been closely related to neuroprotection, in inflammatory conditions such as Multiple Sclerosis (MS) immune cell infiltration could also produce damaging effects (Stadelmann et al., 2002). In fact, essential immune functions such as macrophage recruitment into the inflammatory lesion are regulated by NGF (Flugel et al., 2001). The role of immune cells as sources of NT has generated a lot of interest given that cellular infiltrates like B cells, macrophages, and T cells are all recruited after injury (Barouch and Schwartz, 2002). For example, treatment of motoneurons with inflammatory cytokines resulted in detrimental effects when neurons were deprived of NT produced by immune cells recruited to the CNS (Hammarberg et al., 2000). This suggests a beneficial role of immune cells in protecting injured tissue.

In essence, the evidence that immune cells can actively contribute to the survival of injured neurons in the CNS has been a difficult bridge to cross, given the classical belief that the CNS is an immunologically privileged site. However, it is becoming clear that cross-talk between the CNS and immune system has significant implications for CNS repair and is crucial to consider when designing clinical therapies (Lotan and Schwartz, 1994) especially in inflammatory conditions such as MS (Kerschensteiner et al., 2003). Therefore, the role of NT in the nervous system and the immune system is neither limited to each system but rather shared by each (Hohlfeld, 2007).

1.4.3 Cell Transplants

The inhibitory nature of the CNS environment for axonal growth after injury, allowed for the investigation of alternative strategic avenues like cellular transplantation to create a more permissive environment for neurite outgrowth in the CNS. A variety of cell-based approaches have been translated clinically to contribute to the repair of damaged pathways using neural and non-neural tissue elements as previously reviewed (Reier, 2004). Some of these approaches include Schwann cells (Guest et al., 1997; Menei et al., 1998), olfactory ensheathing glial cells (Li et al., 1997; Ramer et al., 2004), fibroblasts (Tobias et al., 2003; Wu et al., 2008), stem cells (Liu et al., 2000; McDonald et al., 1999), fetal spinal cord (Broude et al., 1999) and even multifunctional immune cells such as macrophage cells have emerged as possible candidates (Bomstein et al., 2003; Lazarov-Spiegler et al., 1996; Rapalino et al., 1998; Schwartz et al.,

1999a). There are numerous cell types currently being used in research to stimulate growth of injured CNS neurons, with some including genetically modified cellular transplant, however, due to the contents of this thesis, we will only briefly highlight some of the beneficial properties of macrophage cells.

Note that the beneficial attributes of macrophage cells will be discussed in more detailed later in this chapter under the section titled “role of inflammatory cells in CNS injury”.

1.4.3.1 Macrophage Cells

It is noteworthy that the characterisation between microglia (also known as CNS macrophages) and peripheral macrophage cells has led to conflict between studies given that commonly used antibodies for antigenic identification cannot guarantee complete distinction between these cells (Guillemin and Brew, 2004). However, morphological characteristics have been employed by researchers as an attempt to distinguish between microglia (stellate appearance), compared to infiltrating blood monocyte (smooth surfaces) classified as macrophage cells (reviewed in (Carson et al., 2006). Therefore, for simplicity, cells testing positive against cluster differentiation 68 (CD68) immunostaining will hereafter be referred to as macrophage cells throughout this thesis, which might reflect a mixed population of the aforementioned cells.

The need to change the inhibitory property of the adult CNS environment, where normally nerve regeneration of injured nerves is restricted, has taken advantage of the beneficial features of macrophage cells to encourage CNS regeneration. Constraints such as limited macrophage response during Wallerian degeneration in the CNS as compared to the PNS, where this response occurs more robustly, interrupts with the regenerative process in the CNS (Avellino et al., 1995). Which has been attributed to the ability of PNS injured nerves to attract these immune cells as they are needed for repair and regrowth (Hirschberg and Schwartz, 1995). Evidence for this comes from studies conducted *in vitro*, where macrophages co-cultured *ex vivo* with PNS tissue, and implanted into the lesion site of spinal cord, promoted the regeneration of CNS fibres and partial functional recovery (Lazarov-Spiegler et al., 1996; Rapalino et al., 1998). This role of macrophage cells is consistent with earlier observations describing the potential of these cells to change the non-growth supporting property of the CNS into a growth-permissive (David et al., 1990). Interestingly, *ex vivo* pre-incubation of macrophage cells with PNS segments was found to be more beneficial than CNS exposed macrophages as indicated by their phagocytic activity (Lazarov-Spiegler et al., 1996). Although, it is important to mention that the beneficial capacity of these cells might be dependent upon the number of transplanted macrophages (Rapalino et al., 1998).

1.4.4 Vaccination with CNS Homogenate

As aforementioned, there have been many attempts aimed at bypassing

the injured CNS environment. Here, we describe an attractive approach using antibodies against axon growth inhibitors as a way to neutralise the effects of myelin and myelin-associated inhibitors. This was first demonstrated using the monoclonal antibody IN-1 that when introduced into injured adult spinal cords, it promoted regeneration (Bregman, 1995; Schnell et al., 1994; Schnell and Schwab, 1990). This approach, although successful in neutralising CNS inhibitors, resulted in limited regeneration of CNS fibres. Therefore, it became evident that other CNS inhibitors also required to be simultaneously blocked in order for regeneration to occur.

An ideal approach, as suggested by David and Ousman (2002), would be to deliver “a cocktail of monoclonal antibodies against each of the inhibitors” in myelin, a difficult task to accomplish given the lack of identification of all CNS inhibitors (David and Ousman, 2002). For this reason, the novel approach tested by Huang and colleagues (1999), involved the simultaneous blocking of all axon growth inhibitor by recruiting the animals’ own immune system to produce antibodies against the inhibitors in myelin, using myelin-rich tissue (i.e. spinal cord) as the immunogen (Huang et al., 1999). Their results indicated that the antibodies produced by myelin-rich vaccinated animals were not only able to cross the BBB and bind to inhibitors associated with myelin, but were also able to stimulate axon regeneration over long distances (Huang et al., 1999). Furthermore, sera from these myelin-rich treated animals were able to block myelin-derived growth inhibition *in vitro*, supporting the idea that antibodies mediate this effect (Huang et al., 1999).

The idea that an antibody-mediated approach could block myelin-derived growth inhibition was further investigated by Ellezam and colleagues (2003), where they evaluated the possibility that myelin-rich vaccination could promote regeneration of retinal ganglion cells (RGCs). Their findings indicated that axonal regeneration in myelin-rich treated animals significantly improved as a result of the vaccination (Ellezam et al., 2003). In addition, their observations were consistent with previous results by Huang et al and supported the concept that antibodies obtained through this vaccination regime are able to bind to white matter *in vivo* and can be utilized *in vitro* to overcome the inhibitory effects of myelin, thus "indicating that the effect on regeneration is antibody-mediated" (Ellezam et al., 2003). These authors further suggested that the observed beneficial effects on CNS regeneration might be attributed to the binding of antibodies on neuronal epitopes present on non-growth permissive molecules. These studies highlighted the importance of recruiting the immune system in CNS regeneration and reiterate the complexity of these systems. Certainly, a much greater understanding is needed to comprehend this relationship, which will be explored in the upcoming chapters.

1.4.5 Pre-Conditioned Lesion

The ability of CNS neurons to regenerate under certain conditions was demonstrated by David and Aguayo in 1981, where it was confirmed and clarified that the axonal environment played an important role for the

axon's ability to regrow (David and Aguayo, 1981). Consequently, it became evident that the response of the DRG neuronal cell body played a critical role in regeneration of CNS afferent neurons (Richardson and Issa, 1984).

Briefly, as previously mentioned DRG neurons have both peripheral and central processes that project into peripheral nerves and the spinal cord, and this provides a unique opportunity to study specific changes that underlie axonal CNS regeneration (Chong et al., 1999). The pioneering experiment of Richardson and Issa (1984), demonstrated that DRG neurons could regenerate their central axons into peripheral nerve transplants (inserted into the dorsal column of the spinal cord) only if the peripheral DRG axon had been previously cut (Richardson and Issa, 1984). The extent of CNS regeneration, although limited in the number of fibre elongation, was an exciting proof-of-concept proposing intrinsic ability for CNS neurons to regenerate. This procedure is now known as a pre-conditioning lesion for its ability to increase axonal regeneration by "conditioning" DRG neurons.

These observations suggested that the acquired regenerative competence of DRG neurons depended on some form of cell body activation in response to peripheral axotomy, ascribed to the inflammatory response and gene expression of DRG neurons (Broude et al., 1997; Lu and Richardson, 1991). The former response was investigated by Lu and Richardson (1991), where intraganglionic injections of either an

inflammatory agent and/or macrophage cells, resulted in the mimicking of events caused by the peripheral axotomy, consequently promoting axonal regeneration of the injured CNS DRG branch (Lu and Richardson, 1991). This regenerative trigger correlated with the increased expression and transport of growth-associated protein (GAP-43) observed in DRG neurons (Schreyer and Skene, 1993). Conversely, injury to DRG central branch alone does not result in a GAP-43 upregulation, and this is consistent with the poor regenerative capability of injured CNS neurons.

It is important to note the clinical impracticality of performing a conditioning lesion to stimulate CNS regeneration in humans. Therefore, our aim is to understand the underlying mechanism/s of the pre-conditioned lesion model in relation to its regenerative CNS trigger and to further investigate its relationship to inflammation, especially to ubiquitous immune cells such as macrophage cells.

1.5 Role of Inflammatory Cells After CNS Injury

Immune responses, especially inflammation, are considered part of the host-defense repertoire not only in situations requiring elimination of foreign pathogens but are also involved in the process of tissue repair. However, as reviewed by Correale and Villa (2004) the prolonged inflammatory exposure coupled to uncontrolled persistence of inflammation can be harmful to the living organism as recognised in MS (Correale and Villa, 2004). The beneficial and detrimental inflammatory effect occurs depending on the phenotypic activation of immune cells such

as lymphocytes and macrophages, as they are not only able to produce anti-inflammatory molecules but also pro-inflammatory molecules, capable of producing neurotoxic effects in neurons (reviewed in (Carson et al., 2006).

Inflammation is mediated by the cellular and humoral components of the immune system and has been described as a neuroprotective response to injury or destruction of tissues (Hohlfeld, 2007). In the CNS, the inflammatory response following SCI has been shown to begin rapidly following cord trauma and this involves the robust activation of immune cells (both resident and recruited) at different times post injury (Popovich et al., 1997). This normal response to injury was nicely reviewed by Schwab and Bartholdi is often accompanied by the release of anti- and pro-inflammatory regulators, variations in vascular permeability, oedema, as well as the classic activation and infiltration of resident and recruited inflammatory cells (Schwab and Bartholdi, 1996).

It is important to acknowledge that after axonal injury (i.e. in either the PNS or CNS) there are several factors contributing to or mediating the immunological response to injury, however, for simplicity and to strengthen our argument, we will mainly focus on inflammatory cells (i.e. macrophages) throughout this thesis.

1.5.1 Macrophage Controversy (Detrimental vs Beneficial Effects)

The presence of inflammatory cells (i.e. macrophages) within the CNS compartment has traditionally been regarded as pathological for some of these main reasons: (i) these cells are able to exert beneficial as well detrimental effects *in situ* with no clarity as to how these effects can be regulated (Bethea, 2000); (ii) these cells have been negatively perceived whenever present in the CNS due to the immune-privileged condition of the CNS (Carson et al., 2006; Popovich et al., 2002).

Within the last decade or so, the involvement of macrophages as detrimental factors has been progressively modified from experimental evidence indicating that a well-controlled immune response can be beneficial to the repair of the injured organism (Hauben and Schwartz, 2003; Hendrix and Nitsch, 2007; Moalem et al., 1999; Rapalino et al., 1998; Yin et al., 2003). This evidence has identified inflammatory cells as a 'double edge sword' due to their dual-role in tissue repair (Bethea, 2000; Wyss-Coray and Mucke, 2002). It is also conceivable that given what it is known now about these cells, suppression of the right type of inflammatory response could alter the recovery state of the injured tissue (refer to Fig. 2 for a brief comparison of factors dictating the balance between detrimental and beneficial effects in CNS repair following trauma).

In the forthcoming section we will briefly describe several examples outlining the dichotomy in macrophage activity and how these changes

affect CNS injury. Some of the events explained hereafter include: vascular permeability, remyelination, and T cell activation.

1.5.1.1 Vascular Permeability

Trauma to the CNS, for example in the form of SCI resulted in a robust inflammatory reaction followed by changes in vascular permeability (Bao et al., 2004; Tator and Fehlings, 1991). Reduction in immune cell recruitment after SCI has been reported to contribute to the attenuation in vascular permeability, as well as tissue damage and significant improvement in functional recovery (Noble et al., 2002). Normally, cellular infiltration into the CNS compartment is highly regulated and restricted, however, injury to the CNS disrupts the BBB integrity resulting in a cascade of events altering the status of the CNS (Pachter et al., 2003). These events initiated by peripherally derived immune cells such as neutrophils result in tissue destruction and enlargement of the lesion (Dusart and Schwab, 1994), which is closely followed by via the release of inflammatory mediators from macrophages, recruited after 1-2 days and peaking at 5-7 post injury (Blight, 1992). The acute inflammatory phase and widespread activation and recruitment of inflammatory cells after CNS trauma consequently leads to an up-regulation and release of pro-inflammatory cytokines such as TNFα and IL-1β (reviewed in (Donelly and Popovich, 2008), described to increase vascular permeability (Schnell et al., 1999a); a factor reported to occur after an inflammatory response from trauma (Andersson et al., 1992; Perry et al., 1993; Wedmore and Williams, 1981). Interestingly, the topical application of NT such as BDNF onto the

spinal cord before or shortly after injury significantly reduces vascular permeability and SCI pathology (Sharma, 2003). Therefore, the effects of vascular permeability based on these studies, highlight a detrimental role for inflammatory cells after CNS injury.

1.5.1.2 Remyelination

Demyelination after axonal injury has been previously shown to be mediated by infiltrating macrophages cells, driven by axonal breakdown (Bruck et al., 1995). For this reason, clinical treatments using high-dosage anti-inflammatory steroid treatment at the acute phase of SCI reported beneficial effects (Bracken et al., 1997), indirectly suggesting a negative effect of inflammation in the CNS. However, the use of anti-inflammatory corticosteroids on spinal cord repair contributed to the inhibition of oligodendrocytes (myelinating cells of the CNS), consequently affecting remyelination, altering the release of trophic factors like BDNF (Chari et al., 2006), as well modifying macrophage functionality such as in the suppression of inflammatory mediators (Russo-Marie, 1992). Similarly, there is evidence indicating that the selective depletion of macrophages in the acute remyelination phase, also results in the functional impairment of oligodendrocytes, which is an essential component in demyelinating conditions (Kotter et al., 2001) and during CNS repair.

An obvious explanation of the role of macrophages in promoting remyelination, may be their requirement for phagocytosis of myelin debris following CNS trauma (reviewed in (Vargas and Barres, 2007). In fact, *in*

vitro evidence indicates that CNS myelin and not PNS myelin can inhibit the maturation of oligodendrocyte progenitors (Robinson and Miller, 1999), thus consistent with observations describing impairement of these cells *in vivo*, resulting in poor remyelination (Kotter et al., 2006). The involvement of macrophage cells in remyelination is crucial for oligodendrocyte progenitor maturation, synthesis of new myelin (Hohlfeld, 2007) and for the increased rate of remyelination associated with rapid macrophage recruitment (Hinks and Franklin, 2000). Based on these observations the inflammatory macrophage response following CNS injury also plays a beneficial role in remyelination.

1.5.1.3 T Cell Activation

Following the primary CNS insult (e.g. SCI), a sequence of secondary degenerative cascade of events exacerbate tissue destruction as well as functional loss of intact neurons that survived the initial trauma, manifested as neurological deficits (Blight, 1994). This has been typically attributed to the inflammatory response to the initial injury and to the release of inflammatory mediators (Dusart and Schwab, 1994). Several studies have shown that reducing inflammation decreases secondary degeneration and functional deficit after SCI (Gonzales et al., 2003; Young, 1993), especially when treatment is administered in the acute injury phase (Ditor et al., 2006). For example, neutralisation of chemokines, such as CXCL10, which is a potent T cell chemoattractant, significantly reduced inflammation, promoted revascularisation and functional recovery of the injured spinal cord (Gonzales et al., 2003; Gonzalez et al., 2007), as well as enhanced

angiogenesis and axonal sprouting (Glaser et al., 2004; Glaser et al., 2006). The extent of recovery was ascribed to the reduced migration and activation of T cells and most importantly to their close interaction with macrophage cells (Bucky Jones et al., 2005).

Indeed, T cell-mediated immunity in the form of adoptive transfer has been found to participate in the local immune response promoting the recovery from SCI, especially when this treatment was delayed 1-week after injury (Hauben et al., 2000). The potential for T cell immune regulatory effects in the injuried CNS has been demonstrated to be neuroprotective even after optic nerve injury (Moalem et al., 1999). This T cell-mediated neuroprotective response can be reduced by the loss of these cells in animals with neonatal thymectomy (Yoles et al., 2001). However, the extent of T cell participation in the CNS needs to occur in a 'tightly regulated' manner to maximise the beneficial aspects of the immune response, as well as minimising the potentially destructive effects that these cells can exert (Schwartz and Kipnis, 2005). Neuroprotection, might be correlated with the activation of the T cell-mediated response as well as the regulation of antigen presenting cells like macrophages (Shaked et al., 2004). Based on these observations, it seems like T cells offer a beneficial role for CNS repair, however, their role in the CNS has also been described to be detrimental to the organism, such as in the animal models of MS (Aharoni et al., 2005).

1.5.2 Macrophage Involvement in Nerve Repair/Regeneration

Macrophages are multifunctional cells and have the ability to exert detrimental as well as beneficial effects (Bethea and Dietrich, 2002; Schwartz et al., 2006a). Interestingly, it was recently demonstrated that boosting or modulating the functionality of macrophage cells in a SCI model, via the intraperitoneal injection of regulatory cytokine (granulocyte-macrophage colony stimulating factor, GM-CSF) significantly improved locomotor activity in injuried rats (Bouhy et al., 2006); which provides a minimally invasive strategy for CNS repair that is clinically relevant. The importance of macrophage cells has also been described in wound repair, vascularisation and angiogenesis (Kodelja et al., 1997). However, the limited capability of the CNS compartment to mount a robust inflammatory response after injury has often been regarded as detrimental during axonal regenerative attempts.

Hereafter, we will briefly discuss some of the contributions of macrophage cells in CNS nerve regeneration and highlight their usefulness after trauma, especially in relation to their activation, exogenous application and their functional depletion.

1.5.2.1 Macrophage Activation and Exogenous Application

The relationship between macrophage activation, manifested through phagocytosis, revealed that pre-incubation of these cells with PNS segments enhanced their phagocytic activity, which was suppressed by

the pre-exposure of these cells to CNS segments (Lazarov-Spiegler et al., 1996). The pre-treatment of injured CNS nerves with the beneficial macrophage phenotype resulted in the regrowth of these nerves, which was correlated with the spread of macrophage cells, phagocytic activity and extent of myelin clearance (Lazarov-Spiegler et al., 1998b). Therefore, based on these observations, the failure of injured CNS axons to regenerate might be associated with the inability of the CNS environment to mount an adequate inflammatory response, suggesting that rapid myelin clearance from the CNS might promote axonal regeneration *in situ* (reviewed in (Vargas and Barres, 2007).

This inability to efficiently recruit inflammatory cells to the site of injury in the CNS has been circumvented by the exogenous application of macrophages into the CNS. Increasing the number of macrophage cells at the injury site has certainly proven efficacious in the stimulation of CNS regeneration in *in vivo* studies using exogenous application techniques such as co-transplantation of microglia cells with fetal spinal cord tissue (Prewitt et al., 1997), skin-coincubated macrophages (Bomstein et al., 2003) and cellular grafts of autologous macrophage cells (Franzen et al., 1998; Rapalino et al., 1998). The beneficial functionality of these cells in the support of axonal CNS regrowth has been attributed to the elimination of growth-inhibiting myelin products, most likely by phagocytosis of debris resulting from the trauma and/or by the secretion of regulatory cytokines and NT (Batchelor et al., 1999; Dougherty et al., 2000; Franzen et al., 1998; Prewitt et al., 1997).

Moreover, for the effects by the exogenous application of macrophage cells to be of benefit to the injured tissue, the site, number and timing of macrophage activation have been reported to be of importance (Schwartz and Yoles, 2006). It is believed that under the appropriate stimulation, inflammatory cells express a phenotype required for CNS repair, as long as they avoid commitment to a destructive phenotype (Schwartz et al., 2006a). However, this is rather challenging to control given that macrophage cells actively participate in the good and bad outcomes in inflammation and are commonly regarded as having split personalities (Duffield, 2003; Gordon, 2003). A proof-of-concept example, where macrophage cells might be expressing a beneficial phenotype was provided in 2003, evident in the significant regeneration of RGCs, determined by the timing of macrophage activation (Yin et al., 2003).

In fact, to complicate this neuroimmune interaction even more, the synthesis of regulatory cytokines by macrophage cells has been suggested to be mediated by a subpopulation of macrophages that is able to produce both pro- and anti-inflammatory cytokines (Calvo et al., 2005). The realization that macrophages are needed for CNS repair is becoming more and more interesting with conflicting evidence provided for their beneficial and detrimental effects. However, as suggested by Fujiwara and Kobayashi “therapeutic interventions targeting macrophages and their products may open new avenues for controlling inflammatory diseases” (Fujiwara and Kobayashi, 2005). Although, it is obvious that in order for

the use of inflammatory cells to be clinically available as alternative treatments, this intricate relationship must be better understood.

1.5.2.2 Macrophage Depletion

An understanding into the diverse functionality of macrophage cells has been demonstrated by the selective depletion of these cells *in vivo* (Van Rooijen and Kesteren-Hendrikx, 2003). After SCI, a detrimental role for immune cell infiltration into the CNS has been associated with secondary degeneration (Blight, 1985). Studies investigating the role of macrophage depletion after SCI have described a decrease in post traumatic tissue loss and an improvement in locomotion ability (Popovich et al., 1999). Indeed, initiation of secondary degeneration in regards to SCI cavity enlargement and glial scarring, which counteract axonal regeneration, has been attributed to macrophage activation; a factor that might be modulated by the administration of anti-inflammatory agents and thereby reducing secondary CNS damage (Fitch et al., 1999). Similarly, the accompanying presence of T cells at the injury site has also been demonstrated to contribute to the damaging effects resulting from the initial injury, however, a reduction in T cell recruitment has been shown to ameliorate tissue preservation and reduction in functional deficit (Gonzalez et al., 2003). These findings indicate that immune cell infiltration into the CNS injury site provide harmful outcomes to the organism and these need to be suppressed or at least controlled to some degree to improve chances of recovery.

This dichotomy of inflammatory cell functionality reiterates the significance of understanding how these cells operate *in vivo*, so that its beneficial effects can be translated into clinical settings. It is noteworthy that the use of therapies administered after CNS injury, which inhibit or suppress the inflammatory response, could also be worsening the condition. For example, the importance of macrophages for efficient CNS remyelination was evident after their depletion during the acute phase of remyelination, resulting in significant oligodendrocyte impairment (Kotter et al., 2001). In fact, these authors further suggested that suppression of the inflammatory response to injury might be undesirable to the regenerative attempts that follow demyelination. It was later discovered that myelin debris acted as an inhibitor of oligodendrocyte precursor cell differentiation, which is an important process in the remyelination phase (Kotter et al., 2006). Conversely, as aforementioned in the PNS, macrophages promote tissue repair and regeneration, yet macrophage depletion in experimental autoimmune neuritis (EAN), an animal model of peripheral nerve inflammation, suppressed the severity of the disease (Jung et al., 1993). It is therefore clear that the multi-functionality of these cells provides conflicting evidence in the repair of CNS tissue and this specific cellular functionality that will be discussed in chapter 4 of this thesis.

1.5.3 Final Overview and Clinical Problem

Injury to the CNS such as in the case of SCI is a devastating condition given that injured CNS nerves do not spontaneously regenerate as compared to nerves in the PNS (Ramón y Cajal, 1928). This

compartmental difference in regenerative ability has been attributed to the intrinsic properties of the PNS, such as being able to mount an adequate inflammatory response to injury due to a lack of immune-privileged status (Kodelja et al., 1997), the presence of Schwann cells as well as the rapid removal of myelin debris clearance after injury by macrophages (Hughes et al., 2002; Vargas and Barres, 2007). In the CNS, together with the absence of these factors observed in the PNS, there is a biochemical and physical barrier that deters injured CNS neurons from regenerating (Silver and Miller, 2004). This is commonly associated with the formation of the glial scar and the inhibitory effects of myelin and its associated proteins, all of which inhibit axonal regeneration.

In order to bypass these barriers for axonal growth to occur, alternative strategies to promote CNS regeneration have been utilised including the use of peripheral nerve grafts/transplants/scaffolds, growth factors, cell transplants, and regulation of the immune system (vaccination experiments). Yet, even though many of these approaches have shed light into the mechanisms underpinning CNS regeneration, these findings are still at the early stages. In fact, one half of the scientist community agree that the inherent restriction of inflammatory effectors in the CNS make this terrain handicap for regeneration (Lazarov-Spiegler et al., 1998a) and the other half believe that inflammation in the CNS is deleterious to the organism, thus providing conflicting views for the role of cells such as macrophages in inflammation and tissue repair (Bethea, 2000). Accordingly, the uncontrolled characteristics of macrophage cells make

the use of these cells problematic as part of any formal treatment for CNS injuries (Donelly and Popovich, 2008).

In the past decade, “despite advances in medical and surgical care, the current clinical therapies for spinal cord injury are largely ineffective” (Eftekharpour et al., 2008). There is still a paramount need to protect the remaining, uninjured neural tissue from detrimental secondary degeneration events. This might be mediated by the release of NT from immune cellular mediators in, around and near the site of damage, consequently providing support against further neuronal damage and encouraging the remyelination process (Hohlfeld, 2007). Certainly, the involvement of immune cells in neuro-regenerative therapies is pivotal for CNS repair, renewal and regeneration given the emerging evidence describing a close relationship between the immune and nervous system (Kerschensteiner et al., 2003; Lotan and Schwartz, 1994). Moreover, in the search for effective clinical treatments for SCI, via the reduction in the extent of inflammatory repertoire, is notably methylprednisolone, with the limiting factor being, that treatment administration needs to occur in the acute injury phase (Amador and Guest, 2005; Bracken et al., 1997; Braughler et al., 1987).

1.6 Hypotheses

The order of the following proposed questions is in reference to their presentation in each of the result chapters throughout this thesis. In

addition, the rationale for each of these questions is addressed at the end of each respective introductory chapter.

Here, using the pre-conditioned lesion as the injury model, we investigated whether:

A) Does the immune response following the use of sciatic nerve homogenate as a vaccine, affect the regeneration of primary afferent neurons in the CNS of pre-conditioned animals (Chapter 2)?

B) Do the effects from a pre-conditioning peripheral lesion result in the regeneration of injured neurons in other CNS locations (Chapter 3)?

C) Does macrophage depletion affect the regenerative trigger characteristic of the pre-conditioned lesion (Chapter 4)?

Note that the term ‘pre-conditioned’, will be regularly used within the context of this thesis and it relates to a pre-conditioning peripheral nerve injury (i.e. sciatic nerve axotomy) performed 7 days prior to CNS lesion (i.e. spinal cord injury, unless otherwise stated).

It is also important to mention that each of the chapters in this thesis has been individually structured and formatted as manuscripts to be submitted for future publications.

1.7 Reference List

Agius, E., Cochard, P., 1998. Comparison of neurite outgrowth induced by intact and injured sciatic nerves: a confocal and functional analysis. J Neurosci. 18, 328-338.

Aguayo, A., David, S., Bray, G., 1981. Influences of the glial environment on the elongation of axons after injury: transplantation studies in adult rodents. J Exp. Biol. 95, 231-240.

Aharoni, R., Arnon, R., Eilam, R., 2005. Neurogenesis and neuroprotection induced by peripheral immunomodulatory treatment of experimental autoimmune encephalomyelitis. J Neurosci. 25, 8217-8228.

Ahmed, F., Ingoglia, N., Sharma, S., 2001. Axon resealing following transection takes longer in central axons than in peripheral axons: implications for axonal regeneration. Exp. Neurol. 167, 451-455.

Amador, M., Guest, J., 2005. An appraisal of ongoing experimental procedures in human spinal cord injury. J Neurol. Phys. Ther. 29, 70-86.

Andersson, P., Perry, V., Gordon, S., 1992. The acute inflammatory response to lipopolysaccharide in CNS parenchyma differs from that in other body tissues. Neurosci. 48, 169-186.

Andrews, M., Stelzner, D., 2004. Modification of the regenerative response of dorsal column axons by olfactory ensheathing cells or peripheral axotomy in adult rat. Exp. Neurol. 190, 311-327.

Avellino, A., Hart, D., Dailey, A., MacKinnon, M., Ellegala, D., Kliot, M., 1995. Differential macrophage responses in the peripheral and central nervous system during Wallerian degeneration of axons. Exp. Neurol. 136, 183-198.

Bahr, M., Przyrembel, C., 1995. Myelin from Peripheral and Central Nervous System is a Non-Permissive Substrate for Retinal Ganglion Cell Axons. Exp. Neurol. 134, 87-93.

Bao, F., Chen, Y., Dekaban, G., Weaver, L., 2004. Early anti-inflammatory treatment reduces lipid peroxidation and protein nitration after spinal cord injury in rats. J Neurochem. 88, 1335-1344.

Barouch, R., Kazimirsky, G., Appel, E., Brodie, C., 2001b. Nerve growth factor regulates TNF-alpha production in mouse macrophages via MAP kinase activation. J Leukoc. Biol. 69, 1019-1026.

Barouch, R., Schwartz, M., 2002. Autoreactive T cells induce neurotrophin production by immune and neural cells in injured rat optic nerve: implications for protective autoimmunity. FASEB J 16, 1304-1306.

Batchelor, P., Liberatore, G., Wong, J., Porrit, M., Frerichs, F., Donnan, G., Howells, D., 1999. Activated macrophages and microglia induce dopaminergic sprouting in the injured striatum and express brain-derived neurotrophic factor and glial cell line derived neurotrophic factor. J Neurosci. 19, 1708-1716.

Batchelor, P., Porritt, M., Martinello, P., Parish, C., Liberatore, G., Donnan, G., Howells, D., 2002. Macrophages and microglia produce local trophic gradients that stimulate axonal sprouting toward but not beyond the wound edge. Mol. Cell Neurosci. 21, 436-453.

Batchelor, P., Tan, S., Wills, T., Porrit, M., Howells, D., 2008. Comparison of inflammation in the brain and spinal cord following mechanical injury. J Neurotrauma 25, 1217-1225.

Benfey, M., Aguayo, A., 1982. Extensive elongation of axons from rat brain into peripheral nerve grafts. Nature 296, 150-152.

Benson, M., Romero, M., Lush, M., Lu, Q., Henkemeyer, M., Parada, L., 2005. Ephrin-B3 is a myelin-based inhibitor of neurite outgrowth. Proc. Natl. Acad. Sci. 102, 10694-10699.
Bethea, J., 2000. Spinal cord injury-induced inflammation: a dual-edged sword. Prog. Brain Res. 128, 33-42.
Bethea, J., Dietrich, W., 2002. Targeting the host inflammatory response in traumatic spinal cord injury. Curr. Opin. Neurobiol. 15, 355-360.
Blesch, A., Uy, H., Grill, R., Cheng, J., Patterson, P., Tuszynski, M., 1999. Leukemia inhibitory factor augments neurotrophin expression and corticospinal axon growth after adult CNS injury. J Neurosci. 19, 3556-3566.
Blight, A., 1985. Delayed demyelination and macrophage invasion: a candidate for secondary cell damage in spinal cord injury. Cent. Nerv. Syst. Trauma 2, 299-315.
Blight, A., 1992. Macrophages and inflammatory damage in spinal cord injury. J Neurotrauma 9, S83-91.
Blight, A., 1994. Effects of silica on the outcome from experimentall spinal cord injury: implications of macrophages in secondary tissue damage. Neurosci. 60, 263-273.
Blits, B., Dijkhuizen, P., Boer, G., Verhaagen, J., 2000. Intercostal nerve implants transduced with an adenoviral vector encoding neurotrophin-3 promote regrowth of injured rat corticospinal tract fibers and improve hindlimb function. Exp. Neurol. 164, 25-37.
Blits, B., Dijkhuizen, P., Carlstedt, T., Poldervaart, H., Schiemanck, S., Boer, G., Verhaagen, J., 1999. Adenoviral vector-mediated expression of a foreign gene in peripheral nerve tissue bridges implanted in the injured peripheral and central nervous system. Exp. Neurol. 160, 256-267.
Bomstein, Y., Marder, J., Vitner, K., Smirnov, I., Lisaey, G., Butovsky, O., Fulga, V., Yoles, E., 2003. Features of skin-coincubated macrophages that promote recovery from spinal cord injury. J Neuroimmunol. 142, 10-16.
Bouhy, D., Malgrange, B., Multon, S., Poirrier, A., Scholtes, F., Schoenen, J., Franzen, R., 2006. Delayed GM-CSF treatment stimulates axonal regeneration and functional recovery in paraplegic rats via an increased BDNF expression by endogenous macrophages. FASEB J 20, E493-E502.
Bracken, M., Shepard, M., Holford, T., Leo-Summers, L., Aldrich, E., Fazl, M., Fehlings, M., Herr, D., Hitchon, P., Marshall, L., Nockels, R., Pascale, V., Perot, P., Piepmeier, J., Sonntag, V., Wagner, F., Wilberger, J., Winn, H., Young, W., 1997. Administration of Methylprednisolone for 24 or 48 Hours or Tirilazad Mesylate for 48 Hours in the Treatment of Acute Spinal Cord Injury: Results of the Third National Acute Spinal Cord Injury Randomized Controlled Trial JAMA 277, 1597-1604.
Braughler, J., Hall, E., Means, E., Waters, T., Anderson, D., 1987. Evaluation of an intensive methylprednisolone sodium succinate dosing regimen in experimental spinal cord injury. J Neurosurg 67, 102-105.
Bregman, B., 1995. Recovery from spinal cord injury mediated by antibodies to neurite growth inhibitors. Nature 378, 498-501.
Broude, E., McAtee, M., Kelley, M., Bregma, B., 1999. Fetal spinal cord transplants and exogenous neurotrphic support enhance c-jun expression in mature axotomized neurons after spinal cord injury. Exp. Neurol. 155, 65-78.

Broude, E., McAtee, M., Kelley, M., Bregman, B., 1997. c-Jun expression in adult rat dorsal root ganglion neurons: differential response after central or peripheral axotomy. Exp. Neurol. 148, 367-377.
Bruck, W., Bruck, Y., Maruschak, B., Friede, R., 1995. Mechanisms of macrophage recruitment in Wallerian degeneration. Acta Neuropathol. 89, 363-367.
Bucky Jones, T., Hart, R., Popovich, P., 2005. Molecular control of physiological and pathological T cells recruitment after mouse spinal cord injury. J Neurosci. 25, 6576-6583.
Bush, T., Puvanachandra, N., Horner, C., Polito, A., Ostenfeld, T., Svendsen, C., Mucke, L., Johnson, M., Sofroniew, M., 1999. Leukocyte infiltration, neuronal degeneration, and neurite outgrowth after ablation of scarforming, reactive astrocytes in adult transgenic mice. Neuron 23, 297-308.
Buss, A., Pech, K., Kakulas, B., Martin, D., Schoenen, J., Noth, J., Brook, G., 2007. Growth-modulating molecules are associated with invading Schwann cells and not astrocytes in human traumatic spinal cord injury. Brain 130, 940-953.
Calvo, C., Amigou, E., Desaymard, C., Glowinski, J., 2005. A pro- and an anti-inflammatory cytokine are synthesised in distrint brain macrophage cells during innate activation. J Neuroimmunol. 170, 21-30.
Camand, E., Morel, M., Faissner, A., Sotelo, C., Dusart, I., 2004. Long-term changes in the molecular composition of the glial scard and progressive increase of serotoninergic fibre sprouting after hemisection of the mouse spinal cord. Eur. J Neurosci. 20, 1161-1176.
Caroni, P., Schwab, M., 1988a. Two Membrane Protein Fractions from Rat Central Myelin with Inhibitory Properties for Neurite Growth and Fibroblast Spreading. J Cell Biol. 106, 1281-1288.
Caroni, P., Schwab, M., 1988b. Antibody Against Myelin-Associated Inhibitor of Neurite Growth Neutralizes NonPermissive Substrate Properties of CNS White Matter. Neuron 1, 85-96.
Carson, M., Doose, J., Melchior, B., Schmid, C., Ploix, C., 2006. CNS immune privilege: hiding in plain sight. Immunol. Rev. 213, 48-65.
Castano, A., Bell, M., Perry, V., 1996. Unusual aspects of inflammation in the nervous system: Wallerian degeneration. Neurobiol. Aging 17, 745-751.
Chari, D., Zhao, C., Kotter, M., Blakemore, W., Franklin, R., 2006. Corticosteroids delay remyelination of experimental demyelination in the rodent central nervous system. J Neurosci. Res. 83, 594-605.
Chen, M., Huber, A., van der Haar, M., Frank, M., Scnell, L., Spillman, A., Christ, F., Schwab, M., 2000. Nogo-A is a myelin-associated neurite outgrowth inhibitor and an antigen for monoclonal antibody IN-1. Nature 403, 434-439.
Cheng, H., Cao, Y., Olson, L., 1996. Spinal cord repair in adult paraplegic rats: partial restoration of hind limb function. Science 273, 510-513.
Cheng, H., Olson, L., 1995. A new surgical technique that allows proximodistal regeneration of 5-HT fibers after complete transection of the spinal cord. Exp. Neurol. 136, 149-161.
Chong, M., Woolf, C., Haque, N., Anderson, P., 1999. Axonal regeneration from injured dorsal roots into the spinal cord of adult rats. J Comp. Neurol. 410, 42-54.
Correale, J., Villa, A., 2004. The neuroprotective role of inflammation in nervous system injuries. J Neurol. 251, 1304-1316.
Coumans, J., Lin, T.-S., Dai, H., MacArthur, L., McAtee, M., Nash, C., Bregma, B., 2001. Axonal regeneration and functional recovery after complete spinal cord

transection in rats by delayed treatment with transplants and neurotrophins. J Neurosci. 21, 9334-9344.
Cripps, R., 2004. Spinal cord injury, Australia, 2002-03. Injury research and statistics series 22, 1-17.
David, S., Aguayo, A., 1981. Axonal elongation into peripheral nervous system "bridges" after central nervous system injury in adult rats. Science 214, 931-933.
David, S., Bouchard, C., Tsatas, O., Giftochristos, N., 1990. Macrophages can modify the nonpermissive nature of the adult mammalian central nervous system. Neuron 5, 463-469.
David, S., Braun, P., Jackson, D., Kottis, V., Mckerracher, L., 1995. Laminin Overrides the Inhibitory Effects of Peripheral Nervous System and Central Nervous System Myelin Derived Inhibitors of Neurite Growth. J Neurosci. Res. 42, 594-602.
David, S., Ousman, S., 2002. Recruiting the immune response to promote axon regeneration in the injured spinal cord. Neuroscientist 8, 33-41.
Ditor, D., Bao, F., Chen, Y., Dekaban, G., Weaver, L., 2006. A therapeutic time window for anti-CD11b monoclonal antibody treatment yielding reduced secondary tissue damage and enhanced behavioral recovery following severe spinal cord injury. J Neurosurg. Spine 5, 343-352.
Donelly, D., Popovich, P., 2008. Inflammation and its role in neuroprotection, axonal regeneration and functional recovery after spinal cord injury. Exp. Neurol. 209, 378-388.
Dougherty, K., Dreyfus, C., Black, I., 2000. Brain-derived neurotrophic factor in astrocytes, oligodendrocytes, and microglia/macrophages after spinal cord injury. Neurobiol. Dis. 7, 574-585.
Duffield, J., 2003. The inflammatory macrophage: a story of Jekyll and Hyde. Clin. Sci. 104, 27-38.
Dusart, I., Schwab, M., 1994. Secondary cell death and the inflammatory reaction after dorsal hemisection of the rat spinal cord. Eur. J Neurosci. 6, 712-724.
Eftekharpour, E., Karimi-Abdolrezaee, S., Fehlings, M., 2008. Current status of experimental cell replacement approaches to spinal cord injury. Neurosurg. Focus 24, E19.
Ehrhard, P., Erb, P., Graumann, U., Otten, U., 1993. Expression of Nerve Growth Factor and Nerve Growth Factor Receptor Tyrosine Kinase TrK in Activated CD4-positive T-Cell Clones. Proc. Natl. Acad. Sci. 90, 10984-10988.
Ellezam, B., Bertrand, J., Dergham, P., Mckerracher, L., 2003. Vaccination stimulates retinal ganglion cell regeneration in the adult optic nerve. Neurobiol. Dis. 12, 1-10.
Faulkner, J., Herrmann, J., Woo, M., Tansey, K., Doan, N., Sofroniew, M., 2004. Reactive astrocytes protect tissue and preserve function after spinal cord injury. J Neurosci. 24, 2143-2155.
Fitch, M., Doller, C., Combs, C., Landreth, G., Silver, J., 1999. Cellular and molecular mechanisms of glial scarring and progressive cavitation: in vivo and in vitro analysis of inflammation-induced secondary injury after CNS trauma. J Neurosci. 19, 8182-8198.
Flugel, A., Matsumuro, K., Neumann, H., Klinkert, W., Birnbacher, R., Lassmann, H., Otten, U., Wekerle, H., 2001. Anti-inflammatory activity of nerve growth factor in experimental autoimmune encephalomyelitis inhibition of monocyte trans-endothelial migration. Eur. J Immunol. 31, 11-22.

Franzen, R., Schoenen, J., Leprince, P., Joosten, E., Moonen, G., Martin, D., 1998. Effects of macrophage transplantation in the injured adult rat spinal cord: a combined immunocytochemical and biochemical study. J Neurosci. Res. 51, 316-327.
Fujiwara, N., Kobayashi, K., 2005. Macrophages in inflammation. Curr. Drug Targets Inflamm. Allergy 4, 281-286.
George, R., Griffin, J., 1994. Delayed macrophage responses and myelin clearance during Wallerian degeneration in the central nervous system: the dorsal radiculotomy model. Exp. Neurol. 129, 225-236.
Giehl, K., Schutte, A., Mestres, P., Yan, Q., 1998. The survival-promoting effect of glial cell line-derived neurotrophic factor on axotomized corticospinal neurons in vivo is mediated by an endogenous brain-derived neurotrophic factor mechanism. J Neurosci. 18, 7351-7360.
Giehl, K., Tetzlaff, W., 1996. BDNF and NT-3, but not NGF, prevent axotomy-induced death of rat corticospinal neurons in vivo. Eur. J Neurosci. 8, 1167-1175.
Gimenez y Ribotta, M., Gaviria, M., Menet, V., Privat, A., 2002. Strategies for regeneration and repair in spinal cord traumatic injury. Prog. Brain Res. 137, 191-212.
Glaser, J., Gonzalez, R., Perreau, V., Cotman, C., HS, K., 2004. Neutralization of the chemokine CXCL10 enhances tissue sparing and angiogenesis following spinal cord injury. J Neurosci. Res. 77, 701-708.
Glaser, J., Gonzalez, R., Sadr, E., Keirstead, H., 2006. Neutralization of the chemokine CXCL10 reduces apoptosis and increases axon sprouting after spinal cord injury. J Neurosci. Res. 84.
Gonzales, R., Glaser, J., Liu, M., Lane, T., Keirstead, H., 2003. Reducing inflammation decreases secondary degeneration and functional deficit after spinal cord injury. Exp. Neurol. 184, 456-463.
Gonzalez, R., Glaser, J., Liu, M., Lane, T., Keirstead, H., 2003. Reducing inflammation decreases secondary degeneration and functional deficit after spinal cord injury. Exp. Neurol. 184, 456-463.
Gonzalez, R., Hickey, M., Espinosa, J., Nistor, G., Lane, T., Keirstead, H., 2007. Therapeutic neutralization of CXCL10 decreases secondary degeneration and functional deficit after spinal cord injury in mice. Regen. Med. 2, 771-783.
Gordon, S., 2003. Alternative activation of macrophages. Nat. Rev. 3, 23-35.
GrandPre, T., Nakamura, F., Vartanian, T., Strittmatter, S., 2000. Identification of the Nogo Inhibitor of Axon Regeneration as a Reticulon Protein. Nature 403, 439-444.
Guest, J., Rao, A., Olson, L., Bunge, M., Bunge, R., 1997. The Ability of Human Schwann Cell Grafts to Promote Regeneration in the Transected Nude Rat Spinal Cord. Exp. Neurol. 148, 502-522.
Guillemin, G., Brew, B., 2004. Microglia, macrophages, perivascular macrophages, and pericytes: a review of function and identification. J Leukoc. Biol. 75, 388-397.
Guth, L., 1974. Axonal regeneration and functional plasticity in the central nervous system. Exp. Neurol. 45, 606-654.
Hammarberg, H., Lidman, O., Lundberg, C., Eltayeb, S., Gielen, A., Muhallab, S., Svenningsson, A., Lindå, H., van der Meide, P., Culheim, S., Olsson, T., Piehl, F., 2000. Neuroprotection by encephalomyelitis: rescue of mechanically injured neurons and neurotrophin production by CNS-infiltrating T and natural killer cells. J Neurosci. 20, 5283-5291.

Hauben, E., Butovsky, O., Nevo, U., Yoles, E., Moalem, G., Agranov, E., Mor, F., Leibowitz-Amit, R., Pevsner, E., Akselrod, S., Neeman, M., Cohen, I., Schwartz, M., 2000. Passive or Active Immunization with Myelin Basic Protein Promotes Recovery from Spinal Cord Contusion. J Neurosci. 20, 6421-6430.
Hauben, E., Schwartz, M., 2003. Therapeutic Vaccination for Spinal Cord Injury: Helping the Body to Cure Itself. TRENDS in Pharmacol Sci 24, 7-12.
Hendrix, S., Nitsch, R., 2007. The role of T helper cells in neuroprotection and regeneration. J Neuroimmunol. 184, 100-112.
Heumann, R., Lindholm, D., Bandtlow, C., Meyer, M., Radeke, M., Misko, T., Shooter, E., Thoenen, H., 1987. Differential regulation of mRNA encoding nerve growth factor and its receptor in rat sciatic nerve during development, degeneration, and regeneration: role of macrophages. Proc. Natl. Acad. Sci. 84, 8735-8739.
Hinks, G., Franklin, R., 2000. Delayed changes in growth factor gene expression during slow remyelination in the CNS of aged rats. Mol. Cell Neurosci. 16, 542-556.
Hirschberg, D., Schwartz, M., 1995. Macrophage recruitment to acutely injured central nervous system is inhibited by a resident factor: a basis for an immune-brain barrier. J Neuroimmunol. 61, 89-96.
Hohlfeld, R., 2007. Does inflammation stimulate remyelination? J Neurol. 254, 1/47-41/54.
Houle, J., Ziegler, M., 1994. Bridging a complete transection lesion of adult rat spinal cord with growth factor-treated nitrocellulose implants. J Neural Transplant Plast. 5, 115-124.
Huang, W., Mckerracher, L., Braun, P., David, S., 1999. A therapeutic vaccine approach to stimulate axon regeneration in the adult mammalian spinal cord. Neuron 24, 639-647.
Hughes, P., Wells, G., Perry, V., Brown, M., Miller, K., 2002. Comparison of matrix metalloproteinase expression during Wallerian degeneration in the central and peripheral nervous systems. Neurosci. 113, 273-287.
Jones, L., Yamaguchi, Y., Stallcup, W., Tuszynski, M., 2002. NG2 is a major chondroitin sulfate proteoglycan produced after spinal cord injury and is expressed by macrophages and oligodendrocyte progenitors. J Neurosci. 22, 2792-2803.
Jung, S., Huitinga, I., Schmidt, B., Zielasek, J., Dijkstra, C., Toyka, K., Hartung, H., 1993. Selective elimination of macrophages by dichloromethylene diphosphonate-containing liposomes suppresses experimental autoimmune neuritis. J Neurol. Sci. 119, 195-202.
Keirstead, H., Hasan, S., Muir, G., Steeves, J., 1992. Suppression of the onset of myelination extends the permissive period for the functional repair of embryonic spinal cord. Proc. Natl. Acad. Sci. 89, 11664-11668.
Kerschensteiner, M., Gallmeier, E., Behrens, L., Klinkert, W., Kolbeck, R., Hoppe, E., Stadelmann, C., Lassmann, H., Wekerle, H., Hohlfeld, R., 1999. Activated Human T Cells, B Cells and Monocytes Produce Brain-Derived Neurotrophic Factor (BNDF) in vitro and in Brain Lesions: A Neuroprotective Role of Inflammation? J Exp. Med. 189, 865-870.
Kerschensteiner, M., Stadelmann, C., Dechant, G., Wekerle, H., Hohlfeld, R., 2003. Neurotrophic cross-talk between the nervous and immune systems: implications for neurological diseases. Annu. Neurol. 53, 292-304.

Khan, T., Dauzvardis, M., Sayers, S., 1991. Carbon filament implants promote axonal growth across the transected rat spinal cord. Brain Res. 541, 139-145.
King, V., Henseler, M., Brown, R., Priestley, J., 2003. Mats made from fibronectin support oriented growth of axons in the damaged spinal cord of the adult rat. Exp. Neurol. 182, 383-398.
Kobayashi, N., Fan, D., Giehl, K., Bedard, A., Wiegand, S., Tetzlaff, W., 1997. BDNF and NT-4/5 prevent atrophy of rat rubrospinal neurons after cervical axotomy, stimulate GAP-43 and Talpha1-tubulin mRNA expression, and promote axonal regeneration. J Neurosci. 17, 9583-9595.
Kodelja, V., Muller, C., Tenorio, S., Schebesch, C., Orfanos, C., Goerdt, S., 1997. Differences in angiogenic potential of classically vs alternatively activated macrophages. Immunobiol. 197, 478-493.
Kotter, M., Li, W., Zhao, C., Franklin, R., 2006. Myelin impairs CNS remyelination by inhibiting oligodendrocyte precursor cell differentiation. J Neurosci. 26, 328-332.
Kotter, M., Setzu, A., Sim, F., Van Rooijen, N., Franklin, R., 2001. Macrophage depletion impairs oligodendrocyte remyelination following lysolecithin-induced demyelination. Glia 35, 204-212.
Kwon, B., Liu, J., Messerer, C., Kobayashi, N., McGraw, J., 2002. Survival and regeneration of rubrospinal neurons 1 year after spinal cord injury. Proc. Natl. Acad. Sci. 99, 3246-3251.
LaFleur, M., Underwood, J., Rappolee, D., Werb, Z., 1996. Basement membrane and repair of injury to peripheral nerve: defining a potential role for macrophages, matrix metalloproteinases, and tissue inhibitor of metalloproteinases-1. J Exp. Med. 184, 2311-2326.
Lawson, L., Frost, L., Risbridger, J., Fearn, S., Perry, V., 1994. Quantification of the mononuclear phagocyte response to Wallerian degeneration of the optic nerve. J Neurocytol 23, 729-744.
Lazarov-Spiegler, O., Rapalino, O., Agranov, G., Schwartz, M., 1998a. Restricted inflammatory reaction in the CNS: a key impediment to axonal regeneration? Mol. Med. Today 4, 337-342.
Lazarov-Spiegler, O., Solomon, A., Schwartz, M., 1998b. Peripheral Nerve-Stimulated Macrophages Simulate a Peripheral Nerve-Like Regenerative Response in Rat Transected Optic Nerve. Glia 24, 329-337.
Lazarov-Spiegler, O., Solomon, A., Zeev-Brann, A., Hirschberg, D., Lavie, V., Schwartz, M., 1996. Transplantation of activated macrophages overcomes central nervous system regrowth failure. FASEB J 10, 1296-1302.
Lemons, M., Howlans, D., Anderson, D., 1999. Chondroitin sulphate proteoglycan immunoreactivity increases following spinal cord injury and transplantation. Exp. Neurol. 160, 51-65.
Levi, A., Dancausse, H., Li, X., Duncan, S., Horkey, L., Olivera, M., 2002. Peripheral nerve grafts promoting central nervous system regeneration after spinal cord injury in the primate. J Neurosurg. Spine 96, 197-205.
Li, M., David, S., 1996. Topical glucocorticocoids modulate the lesion interface after cerebral cortical stab wounds in adult rats. Glia 18, 306-318.
Li, Y., Field, P., Raisman, G., 1997. Repair of adult rat corticospinal tract by transplants of olfactory ensheathing cells. Science 277, 2000-2002.
Liefner, M., Siebert, H., Sachse, T., Michel, U., Kollias, G., Bruck, W., 2000. The role of TNF-alpha during Wallerian degeneration. J Neuroimmunol. 108, 147-152.

Liu, S., Qu, Y., Stewart, T., Howard, M., Chakrabortty, S., Holekamp, T., McDonald, J., 2000. Embryonic stem cells differentiate into oligodendrocytes and myelinate in culture and after spinal cord transplantation. Proc. Natl. Acad. Sci. 97, 6126-6131.
Lotan, M., Schwartz, M., 1994. Cross talk between the immune system and the nervous system in response to injury: implications for regeneration. FASEB J 8, 1026-1033.
Lu, P., Blesch, A., Tuszynski, M., 2001. Neurotrophism without neurotropism: BDNF promotes survival but not growth of lesioned corticospinal neurons. J Comp. Neurol. 436, 456-470.
Lu, P., Yang, H., Jones, L., Filbin, M., Tuszynski, M., 2004. Combinatorial therapy with neurotrophins and cAMP promotes axonal regeneration beyond sites of spinal cord injury. J Neurosci. 24, 6402-6409.
Lu, X., Richardson, P., 1991. Inflammation near the nerve cell body enhances axonal regeneration. J Neurosci. 11, 972-978.
Ludwin, S., 1990. Oligodendrocyte survival in Wallerian degeneration. Acta Neuropathol. 80, 184-191.
Manson, J., Suzuki, K., Chaplin, D., Matsushima, G., 2001. Interleukin-1beta promotes repain of the CNS. J Neurosci. 21, 7046-7052.
Mansour, H., Asher, R., Dahl, D., Labkovsky, B., Perides, G., Bignami, A., 1990. Permissive and non-permissive reactive astrocytes: immunofluorescence sstudy with antibodies to the glial hyaluronate-binding protein. J Neurosci. Res. 25, 300-311.
Mantovani, A., Sica, A., Locati, M., 2005. Macrophage polarization comes with age. Immunity 23, 344-346.
Marchand, R., Woerly, S., Bertrand, L., Valdes, N., 1993. Evaluation of two cross-linked collagen gels implanted in the transected spinal cord. Brain Res. Bull 30, 415-422.
Mason, J., Suzuki, K., Chaplin, D., Matsushima, G., 2001. Interleukin-1beta promotes repain of the CNS. J Neurosci. 21, 7046-7052.
McDonald, J., Liu, X., Qu, Y., Liu, S., Mickey, S., Turetsky, D., Gottlieb, D., Choi, D., 1999. Transplanted embryonic stem cells survive, differentiate and promote recovery in injured rat spinal cord. Nat. Med. 5, 1410-1412.
McKeon, R., Schreiber, R., Rudge, J., Silver, J., 1991. Reduction of neurite outgrowth in a model of glial scarring following CNS injury is correlated with the expression of inhibitory molecules on reactive astrocytes. J Neurosci. 11, 3398-3411.
McKerracher, L., David, S., Jackson, D., Kottis, V., Dunn, R., Braun, P., 1994. Identification of Myelin-Associated Glycoprotein as a Major Myelin-Derived Inhibitor of Neurite Growth. Neuron 13, 805-811.
Menei, P., Montero-Menei, C., Whittemore, S., Bunge, R., Bunge, M., 1998. Schwann Cells Genetically Modified to Secrete Human BDNF Promote Enhanced Axonal Regrowth Across Transected Adult Rat Spinal Cord. Eur. J Neurosci. 10, 607-621.
Menet, V., Prieto, M., Privat, A., Gimenez y Ribotta, M., 2003. Axonal plasticity and functional recovery after spinal cord injury in mice deficient in both glial fibrillary acidic protein and vimentin genes. Proc. Natl. Acad. Sci. 100, 8999-9004.

Moalem, G., Gdalyahu, A., Shani, Y., Otten, U., Lazarovici, P., Cohen, I., Schwartz, M., 2000. Production of neurotrophins by activated T cells: implications for neuroprotective autoimmunity. J Autoimm. 15, 331-345.
Moalem, G., Leibowitz-Amit, R., Yoles, E., Mor, F., Cohen, I., Schwartz, M., 1999. Autoimmune T cells protect neurons from secondary degeneration after central nervous system axotomy. Nat. Med. 5, 49-55.
Moon, L., Asher, R., Rhodes, K., Fawcett, J., 2001. Regeneration of CNS axons back to their target following treatment of adult rat brain with chondroitinase ABC. Nat. Neurosci. 4, 465-466.
Moon, L., Bunge, M., 2005. From animal models to humans: strategies for promoting CNS axons regeneration and recovery of limb function after spinal cord injury. J Neurol. Phys. Ther. 29, 55-69.
Moore, M., Friedman, J., Lewellyn, E., Mantila, S., Krych, A., Ameenuddin, S., Knight, A., Lu, L., Currier, B., Spinner, R., Marsh, R., Windebank, A., Yaszemski, M., 2006. Multiple-channel scaffolds to promote spinal cord axon regeneration. Biomaterials 27, 419-429.
Moreau-Fauvarque, C., Kumanogoh, A., Camand, E., Jaillard, C., Barbin, G., Boquet, I., Love, C., Jones, E., Kikutani, H., Lubetzki, C., Dusart, I., Chedotal, A., 2003. The transmembrane semaphorin Sema4D/CD100, an inhibitor of axonal growth, is expressed on oligodendrocytes and upregulated after CNS lesion. J Neurosci. 23, 9229-9239.
Mukhopadhyay, G., Doherty, P., Walsh, F., Crocker, P., Filbin, M., 1994. A novel role for myelin-associated glycoprotein as an inhibitor of axonal regeneration. Neuron 13, 757-767.
Namiki, J., Kojima, A., Tator, C., 2000. Effect of brain-derived neurotrophic factor, nerve growth factor, and neurotrophin-3 on functional recovery and regeneration after spinal cord injury in adult rats. J Neurotrauma 17, 1219-1231.
Noble, L., Donovan, F., Igarashi, T., Goussev, S., Werb, Z., 2002. Matrix metalloproteases limit functional recovery after spinal cord injury by modulation of early vascular events. J Neurosci. 22, 7526-7535.
NSCISC, 2009. National spinal cord injury statistical center report, 1-2.
Oppenheim, J., Spitzer, D., Winfree, C., 2009. Spinal cord bypass surgery using peripheral nerve transfers: review of translational studies and a case report on its use following complete spinal cord injury in human. Experimental article. Neurosurg. Focus 26, E6.
Oudega, M., Hagg, T., 1996. Nerve growth factor promotes regeneration of sensory axons into adult rat spinal cord. Exp. Neurol. 140, 218-229.
Oudega, M., Hagg, T., 1999. Neurotrophins promote regeneration of sensory axons in the adult rat spinal cord. Brain Res. 818, 431-438.
Oudega, M., Varon, S., Hagg, T., 1994. Regeneration of adult rat sensory axons into intraspinal nerve grafts: promoting effects of conditioning lesion and graft predegeneration. Exp. Neurol. 129, 194-206.
Pachter, J., de Vries, H., Fabry, Z., 2003. The blood-brain barrier and its role in immune privilege in the central nervous system. J Neuropath. Exp. Neurol. 62, 593-604.
Perry, V., Andersson, P., Gordon, S., 1993. Macrophages and inflammation in the central nervous system. TINS 16, 268-273.
Pettigrew, D., Crutcher, K., 1999. White Matter of the CNS Supports or Inhibits Neurite Outgrowth In Vitro Depending on Geometry. J Neurosci. 19, 8358-8366.

Plunet, W., Kwon, B., Tetzlaff, W., 2002. Promoting axonal regeneration in the central nervous system by enhancing the cell body response to axotomy. J Neurosci. Res. 68, 1-6.
Popovich, P., Guan, Z., McGaughy, V., Fisher, L., Hickey, W., Basso, D., 2002. The neuropathological and behavioral consequences of intraspinal microglia/macrophage activation. J Neuropath. Exp. Neurol. 61, 623-633.
Popovich, P., Guan, Z., Wei, P., Huitinga, I., Van Rooijen, N., Stokes, B., 1999. Depletion of hematogenous macrophages promotes partial hindlimb recovery and neuroanatomical repair after experimental spinal cord injury. Exp. Neurol. 158, 351-365.
Popovich, P., Wei, P., Stokes, B., 1997. Cellular inflammatory response after spinal cord injury in sprague-dawley and lewis rats. J Comp. Neurol. 377, 443-464.
Prewitt, C., Niesman, I., Kane, C., Houle, J., 1997. Activated macrophage/microglial cells can promote the regeneration of sensory axons into the injured spinal cord. Exp. Neurol. 148, 433-443.
Prinjha, R., Moore, S., Vinson, M., Blake, S., Morrow, R., Christie, G., Michalovich, D., Simmons, D., Walsh, F., 2000. Inhibitor of Neurite Outgrowth in Humans. Nature 403, 383-384.
Qiu, J., Cafferty, W., McMahon, S., Thompson, S., 2005. Conditioning injury-induced spinal axon regeneration requires signal transducer and activator of transcription 3 activation. J Neurosci. 25, 1645-1653.
Ramer, L., Au, E., Richter, M., Liu, J., Tetzlaff, W., Roskams, A., 2004. Peripheral olfactory ensheathing cells reduce scar and cavity formation and promote regeneration after spinal cord injury. J Comp. Neurol. 473, 1-15.
Ramón y Cajal, S., 1928. Degeneration and Regeneration of the Nervous System. Oxford University Press, London.
Rapalino, O., Lazarov-Spiegler, O., Agranov, E., Velan, G., Yoles, E., Fraidakis, M., Solomon, A., Gepstein, R., Katz, A., Belkin, M., Hadani, M., Schwartz, M., 1998. Implantation of stimulated homologous macrophages results in partial recovery of paraplegic rats. Nat. Med. 4, 814-821.
Reier, P., 2004. Cellular transplantation strategies for spinal cord injury and translational neurobiology. NeuroRx. 1, 424-451.
Richardson, P., Issa, V., 1984. Peripheral injury enhances central regeneration of primary sensory neurones. Nature 309, 791-793.
Richardson, P., McGuinness, U., Aguayo, A., 1982. Peripheral nerve autografts to the rat spinal cord: studies with axonal tracing methods. Brain Res. 237, 147-162.
Robinson, S., Miller, R., 1999. Contact with central nervous system myelin inhibits oligodendrocyte progenitor maturation. Develop. Biol. 216, 359-368.
Rossignol, S., Schwab, M., Schwartz, M., Fehlings, M., 2007. Spinal cord injury: time to move? J Neurosci. 27, 11782-11792.
Rudge, J., Silver, J., 1990. Inhibition of neurite outgrowth on astroglial scars in vitro. J Neurosci. 10, 3594-3603.
Ruitenberg, M., Plant, G., Hamers, F., Wortel, J., Blits, B., Dijkhuizen, P., Gispen, W., Boer, G., Verhaagen, J., 2003. Ex vivo adenoviral vector-mediated neurotrophin gene transfer to olfactory ensheathing glia: effects on rubrospinal tract regeneration, lesion size, and functional recovery after implantation in the injured rat spinal cord. J Neurosci. 23, 7045-7058.
Russo-Marie, F., 1992. Macrophages and the glucocorticoids. J Neuroimmunol. 40, 281-286.

Salzer, J., Bunge, R., 1980. Studies of Schwann cell proliferation. I. An analysis in tissue culture of proliferation during development, Wallerian degeneration, and direct injury. J Cell Biol. 84, 739-752.
Santambrogio, L., Benedetti, M., Caho, M., Muzaffar, R., Kulig, K., Gabell, N., Hochwald, G., 1994. Nerve growth factor production by lymphocytes. J Immunol. 153, 4488-4495.
Saunders, N., Kitchener, P., Knott, G., Nicholls, J., Potter, A., 1998. Development of walking, swimming and neuronal connections after complete spinal cord transection in the neonatal opossum, Monodelphis domestica. J Neurosci. 18.
Savio, T., Schwab, M., 1990. Lesioned Corticospinal Tract Axons Regenerate in Myelin-Free Rat Spinal Cord. Proc. Natl. Acad. Sci. 87, 4130-4133.
Schnell, L., Fearn, S., Klassen, H., Schwab, M., Perry, V., 1999b. Acute inflammatory responses to mechanical lesions in the CNS: differences between brain and spinal cord. Eur. J Neurosci. 11, 3648-3658.
Schnell, L., Fearn, S., Schwab, M., Perry, V., Anthony, D., 1999a. Cytokine-induced acute inflammation in the brain and spinal cord. J Neuropath. Exp. Neurol. 58, 245-254.
Schnell, L., Schneider, R., Kolbeck, R., Barde, Y., Schwab, M., 1994. Neurotrophin-3 enhances sprouting of corticospinal tract during development and after adult spinal cord lesion. Nature 367, 170-173.
Schnell, L., Schwab, M., 1990. Axonal regeneration in the rat spinal cord produced by an antibody against myelin-associated neurite growth inhibitors. Nature 343, 269-272.
Schreyer, D., Skene, J., 1993. Injury-associated induction of GAP-43 expression displays axon branch specificity in rat dorsal root ganglion neurons. J Neurobiol. 24, 959-970.
Schulte-Herbruggen, O., Nassenstein, C., Lommatzsch, M., Quarcoo, D., Renz, H., Braun, A., 2005. Tumor necrosis factor-alpha and interleukin-6 regulate secretion of brain-derived neurotrophic factor in human monocytes. J Neuroimmunol. 160, 204-209.
Schwab, M., Bartholdi, D., 1996. Degeneration and regeneration of axons in the lesioned spinal cord. Physiol. Rev. 76, 319-370.
Schwartz, M., Butovsky, O., Bruck, W., Hanisch, U., 2006a. Microglial phenotype: is the commitment reversible? TINS 29, 68-74.
Schwartz, M., Kipnis, J., 2005. Protective autoimmunity and neuroprotection in inflammatory and noninflammatory neurodegenerative diseases. J Neurol. Sci. 233, 163-166.
Schwartz, M., Lazarov-Spiegler, O., Rapalino, O., Agranov, I., Velan, G., Hadani, M., 1999a. Potential Repair of Rat Spinal Cord Injuries Using Stimulated Homologous Macrophages. Neurosurg. 44, 1041-1046.
Schwartz, M., Yoles, E., 2006. Immune-based therapy for spinal cord repair: Autologous macrophages and beyond. J Neurotrauma 23, 360-370.
Shaked, I., Porat, Z., Gersner, R., Kipnis, J., Schwartz, M., 2004. Early activation of microglia as antigen-presenting cells correlates with T cell-mediated protection and repair of the injured central nervous system. J Neuroimmunol. 146, 84-93.
Sharma, H., 2003. Neurotrophic factors attenuate microvascular permeability disturbances and axonal injury following trauma to the rat spinal cord. Acta Neurochir Suppl 86, 383-388.
Shavelle, R., DeVivo, M., Paculdo, D., Vogel, L., Strauss, D., 2007. Long-Term Survival After Chilhood Spinal Cord Injury. J Spinal Cord Med. 30, S48-S54.

Silver, J., Miller, J., 2004. Regeneration beyond the glial scar. Nat. Rev. Neurosci. 5, 146-156.
Sjoberg, J., Kanje, M., 1990. The initial period of peripheral nerve regeneration and the importance of the local environment for the conditioning lesion effect. Brain Res. 529, 79-84.
Stadelmann, C., Kerschensteiner, M., Misgeld, T., Bruck, W., Hohlfeld, R., Lassmann, H., 2002. BDNF and gp145trkB in multiple sclerosis brain lesions: neuroprotective interactions between immune and neuronal cells? . Brain 125, 75-85.
Stokols, S., Tuszynski, M., 2006. Freeze-dried agarose scaffolds with uniaxial channels stimulate and guide linear axonal growth following spinal cord injury. Biomaterials 27, 443-451.
Stoll, G., Mueller, H., Trapp, B., Griffin, J., 1989. Oligodendrocytes but not astrocytes express apolipoprotein E after Injury of Rat Optic Nerve. Glia 2, 170-176.
Tang, S., Qiu, J., Nikulina, E., Filbin, M., 2001. Soluble myelin-associated glycoprotein released from damaged white matter inhibits axonal regeneration. Mol. Cell Neurosci. 18, 259-269.
Tang, X., Davies, J., Davies, S., 2003. Changes in distribution, cell associations, and protein expression levels of NG2, neurocan, phosphacan, brevican, versican V2, and tenascin-C during acute to chronic maturation of spinal cord scar tissue. J Neurosci. Res. 71, 427-444.
Tator, C., Fehlings, M., 1991. Review of the seondary injury theory of acute spinal cord trauma with emphasis on vascular mechanisms. J Neurosurg 75, 15-26.
Thallmair, M., Metz, G., Z'Graggen, W., Raineteau, O., Kartje, G., Schwab, M., 1998. Neurite growth inhibitors restrict plasticity and functional recovery following corticospinal tract lesions. Nat. Neurosci. 1, 124-131.
Tobias, C., Shumsky, J., Shibata, M., Tuszynski, M., Fischer, I., Tessler, A., Murray, M., 2003. Delayed grafting of BDNF and NT-3 producing fibroblasts into the injured spinal cord stimulates sprouting, partially rescues axotomized red nucleus neurons from loss and atrophy, and provides limited regeneration. Exp. Neurol. 184, 97-113.
Treherne, J., Woodward, S., Varga, Z., Ritchie, J., Nicholls, J., 1992. Restoration of conduction and growth of axons through injured spinal cord of neonatal opossum in culture. Proc. Natl. Acad. Sci. 89, 431-434.
Trivedi, A., Olivas, A., Noble-Haeusslein, L., 2006. Inflammation and spinal cord injury: Infiltrating leukocytes as determinants of injury and repair processes. Clin. Neurosci. Res. 6, 283-292.
Tuszynski, M., Gabriel, K., Gage, F., Suhr, S., Meyer, S., Rosetti, A., 1996. Nerve growth factor delivery by gene transfer induces differential outgrowth of sensory, motor, and noradrenergic neurites after adult spinal cord injury. Exp. Neurol. 137, 157-173.
Van Rooijen, N., Kesteren-Hendrikx, E., 2003. "In vivo" depletion of macrophages by liposome-mediated "suicide". Methods Enzymol. 373, 3-16.
van Rossum, D., Hilbert, S., Strabenburg, S., Hanisch, U., Bruck, W., 2008. Myelin-phagocytosing macrophages in isolated sciatic and optic nerves reveal a unique reactive phenotype. Glia 56, 271-283.
Vargas, M., Barres, B., 2007. Why is Wallerian degeneration in the CNS so slow? Annu. Rev. Neurosci. 30, 153-179.

Vidal-Sanz, M., Bray, G., Villegas-Perez, M., Thanos, S., Aguayo, A., 1987. Axonal regeneration and synapse formation in the superior colliculus by retinal ganglion cells in the adult rat. J Neurosci. 7, 2894-2909.
Wang, H., Tewari, A., Einheber, S., Salzer, J., Melendez-Vasquez, C., 2001. Myosin II has distinct functions in PNS and CNS myelin sheath formation. J Cell Biol. 154, 137-146.
Wang, K., Koprivica, V., Kim, J., Sivasankaran, R., Guo, Y., Neve, R., He, Z., 2002. Oligodendrocyte-myelin glycoprotein is a Nogo receptor ligand that inhibits neurite outgrowth. Nature 417, 941-944.
Waxman, S., Kocsis, J., 1997. Spinal cord repair: progress towards a daunting goal. Neuroscientist 3, 263-269.
Wedmore, C., Williams, T., 1981. Control of vascular permeability by polymorphonuclear leukocytes in inflammation. Nature 289, 646-650.
Woerly, S., Pinet, E., de Robertis, L., Van Diep, D., Bousmina, M., 2001. Spinal cord repair with PHPMA hydrogel containing RGD peptides (NeuroGel). Biomaterials 22, 1095-1111.
Wu, J., Huang, W., Tsai, Y., Chen, Y., Cheng, H., 2008. Nerve repair using acidic fibroblast growth factor in human cervical spinal cord injury: a preliminary Phase I clinical study. J Neurosurg. Spine 8, 208-214.
Wyndaele, M., Wyndaele, J., 2006. Incidence, prevalence and epidemiology of spinal cord injury: what learns a worldwide literature survey? Spinal Cord 44, 523-529.
Wyss-Coray, T., Mucke, L., 2002. Inflammation in Neurodegenerative Disease-A Double-Edge Sword. Neuron 35, 419-432.
Xu, X., Guenard, V., Kleitman, N., Aebischer, P., Bunge, M., 1995. A combination of BDNF and NT-3 promotes supraspinal axonal regeneration into Schwann cell grafts in adult rat thoracic spinal cord. Exp. Neurol. 134, 261-272.
Ye, J., Houle, J., 1997. Treatment of the chronically injured spinal cord with neurotrophic factors can promote axonal regeneration from supraspinal neurons. Exp. Neurol. 143, 70-81.
Yick, L., Wu, W., So, K., Yip, H., 1999. Peripheral nerve graft and neurotrophic factors enhance neuronal survival and expression of nitric oxide synthase in Clarke's nucleus after hemisection of the spinal cord in adult rat. Exp. Neurol. 159, 131-138.
Yin, Y., Cui, Q., Irwin, N., Fischer, D., Harvey, A., Benowitz, L., 2003. Macrophage-derived factors stimulate optic nerve regeneration. J Neurosci. 23, 2284-2293.
Yoles, E., Hauben, E., Palgi, O., Agranov, E., Gothilf, A., Cohen, A., Kuchroo, V., Cohen, I., Weiner, H., Schwartz, M., 2001. Protective autoimmunity is a physiological respnse to CNS trauma. J Neurosci. 21, 3740-3748.
Young, W., 1993. Secondary injury mechanisms in acute spinal cord injury. J Emerg. Med. 11, 13-22.
Z'Graggen, W., Metz, G., Kartje, G., Thallmair, M., Schwab, M., 1998. Functional recovery and enhanced corticofugal plasticity after unilateral pyramidal tract lesion and blockade of myelinassociated neurite growth inhibitors in adult rats. J Neurosci. 18, 4744-4757.
Zeev-Brann, A., Lazarov-Spiegler, O., Brenner, T., Schwartz, M., 1998. Differential effects of central and peripheral nerves on macrophages and microglia. Glia 23, 181-190.

Zuo, J., Neubauer, D., Dyess, K., Ferguson, T., Muir, D., 1998. Degradation of chondroitin sulfate proteoglycan enhances the neurite-promoting potential of spinal cord tissue. Exp. Neurol. 154, 654-662.