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Spinal cord injury therapies in humans: an overview of current clinical trials and their potential effects on intrinsic CNS macrophages

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Introduction: Macrophage activation is a hallmark of spinal cord injury (SCI) pathology. CNS macrophages, derived from resident microglia and blood monocytes, are ubiquitous throughout the injured spinal cord, and respond to signals in the lesion environment by changing their phenotype and function. Depending on their phenotype and activation status, macrophages may initiate secondary injury mechanisms and/or promote CNS regeneration and repair.

Areas covered: This review provides a comprehensive overview of current SCI clinical trials that are intended to promote neuroprotection, axon regeneration or cell replacement. None of these potential therapies were developed with the goal of influencing macrophage function; however, it is likely that each will have direct or indirect effects on CNS macrophages. The potential impact of each trial is discussed in the context of CNS macrophage biology.

Expert opinion: Activation of CNS macrophages is an inevitable consequence of traumatic SCI. Given that these cells are exquisitely sensitive to changes in microenvironment, any intervention that affects tissue integrity and/or the composition of the cellular milieu will undoubtedly affect CNS macrophages. Thus, it is important to understand how current clinical trials will affect intrinsic CNS macrophages.

Keywords: macrophage, microglia, neuroinflammation, neurotrauma, spinal cord injury

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1. Introduction

Traumatic spinal cord injury (SCI) occurs following mechanical compression or rupture of the spinal cord. Although protective devices and preventative measures have helped to reduce the risk of SCI, few options exist for minimizing the cell death and loss of function caused by trauma-induced ischemia, apoptosis, excitotoxicity and inflammation [1]. Because most (if not all) of these secondary injury mechanisms can be initiated and/or coordinated by cellular and humoral mediators of the immune system, it is important to understand how current or planned therapies influence inflammatory cascades, even if inflammation is not the designated therapeutic target. Such considerations are perhaps best centered on activated microglia and monocyte-derived macrophages (collectively referred to as CNS macrophages). Indeed, CNS macrophages are a pathological hallmark of all forms of mammalian SCI [1,2].

Once activated, CNS macrophages exert conflicting effects on CNS tissue; they can facilitate regeneration and repair through the release of trophic cues and/or

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Article highlights.

- CNS macrophages are a pathological hallmark of all forms of mammalian spinal cord injury (SCI).
- Macrophages can change function in response to new environmental stimuli and adopt pathological and pro-regenerative phenotypes in the injured CNS.
- There are currently 20 ongoing clinical trials testing neuroprotective, regenerative, or cell transplantation/replacement therapies for SCI.
- All current clinical trials have the potential to alter macrophage function either directly or indirectly.
- The more we understand about CNS macrophages and how current therapies affect these cells, the greater the potential for improving function after SCI.

This box summarizes key points contained in the article.

growth factors or contribute to secondary cell death by releasing toxins, proteases and cytokines [3-12]. These divergent effects of CNS macrophages were recently shown to be associated with unique molecular phenotypes that are regulated by cues in the lesion environment [13]. Macrophage effector functions are dynamic and can change in response to new stimuli in the lesion microenvironment [14,15]. It is therefore important to consider how different therapies will alter the microenvironment and influence CNS macrophages after SCI.

The goal of this review is to highlight current SCI clinical trials that test therapies designated as neuroprotective, axon regenerative or cell transplantation/replacement then discuss how these therapies could influence macrophage activation and function. Federal and privately-funded double-blinded clinical trials are the primary focus. Pre-clinical/basic science interventions will be discussed briefly. For systematic reviews of current preclinical data for SCI therapies see [16-18]. For a review of previous clinical trials and ongoing, but not double-blinded trials, see [19]. ClinicalTrials.gov was used as the primary source to identify clinical trials. Data gathered from clinicaltrials.gov are used in accordance with the terms and conditions of the website and the ClinicalTrials.gov ID numbers (CTID) are reported when available.

2. Neuroprotective therapies

Neuroprotective therapies attempt to promote the survival of neurons, glia and endothelia that are not immediately destroyed by mechanical trauma. Many pre-clinical neuroprotective strategies have emerged as putative clinical therapies.

2.1 Current neuroprotective therapies

Currently, there are six neuroprotective therapies being tested in clinical trials for SCI. Five are drug treatments (methylprednisolone, minocycline, erythropoietin, Riluzole, and HP-184) and one trial is evaluating the effects of systemic hypothermia. Methylprednisolone has already been tested in clinical trials and is currently used as a 'standard of care' comparator for newer therapies in two clinical trials (see umbilical cord mononuclear cell and erythropoietin trials below). The neuroprotective effects of methylprednisolone have been reviewed previously [19-21].

Minocycline, a second-generation tetracycline derivative, has neuroprotective and anti-inflammatory effects [22]. Several studies have shown it to be a promising candidate for treating SCI, especially since minocycline is already used in humans for other indications and can readily cross the blood–brain barrier [17]. However, recent studies in rat models of SCI failed to observe a neuroprotective effect of minocycline [23,24]. Whether benefits will be observed in people is not clear. One ongoing trial, sponsored by the University of Calgary, is testing the safety and tolerance of minocycline in acute SCI patients [25] (CTID: NCT00559494).

Erythropoietin (EPO) is a hematopoietic growth factor involved in erythropoiesis and is produced mainly by the kidney. Independent studies have shown that EPO also acts as a neurotrophic factor and is important for neural development [26]. The neuroprotective potential of EPO could be attributed to its ability to reduce apoptosis and inflammation and promote vascular repair (reviewed in [26]). These effects would presumably benefit the injured spinal cord; however, the use of EPO in rodent pre-clinical SCI models has produced conflicting data. Gorio *et al.* [27] were the first to report the neuroprotective and anti-inflammatory effects of EPO in preclinical SCI models but since then, a number of independent studies, including one that was a formal replication, failed to show any benefit of EPO or its derivatives [28-30]. An ongoing Phase III clinical trial is comparing acute EPO administration to methylprednisolone in patients with spinal shock [31] (Niguarda Hospital, Italy; CTID: NCT00561067). The estimated completion date was October 2010, but no updates are available.

The excess accumulation of intra-axonal sodium (Na^+) ions leads to cytotoxic cell edema, intracellular acidosis and reverse activation of the Na^+–Ca^{2+} exchanger; all have been implicated as Na^+-dependent mechanisms of secondary injury of spinal cord white matter [32-36]. In experimental models of SCI, selective blockade of Na^+ channels is neuroprotective [33,37,38]. Two ongoing clinical trials will determine whether blocking Na^+ channels can facilitate recovery after SCI in humans. Sanofi-Aventis sponsored a Phase II trial examining oral HP184 (a Na^+/K^+ channel blocker) in patients with chronic SCI [39] (CTID: NCT00093275). The trial was completed in 2008 and anecdotal reports by trial participants suggest that HP184 improves motor and autonomic function [40] however, no official data have been released or published. An independent Phase I trial will test the safety and pharmacokinetics of Riluzole in acute SCI [41] (CTID: NCT00876889). Preclinical data indicate that Riluzole is more effective than other Na^+ channel blockers at promoting

recovery after SCI [37]. The Riluzole clinical trial started in April 2010 but no data are currently available.

Experimental and clinical data indicate that mild or moderate hypothermia may be a promising neuroprotective therapy for acute SCI (reviewed in [42]). Hypothermia may work by reducing cellular metabolic demand, excitotoxicity, vascular permeability, edema and/or inflammation [42]. A recent Phase I trial showed enhanced conversion rates from American Spinal Injury Association (ASIA) A to B, C or D (~ 43%) without adverse complications in a small cohort of cervical SCI patients that received moderate endovascular cooling [43]. Details about the cooling techniques applied to human SCI were published [44] and a Phase II trial is pending [45].

2.2 The role of macrophages in neuroprotective therapies

Pre-clinical data from independent laboratories indicate that blocking or attenuating intraspinal accumulation of monocyte-derived macrophages (MDM) promotes neuroprotection and enhances recovery after SCI [46-52]. Intravenous injection of liposome-encapsulated clodronate selectively depletes MDMs and macrophages in spleen and liver [46]. When applied to rodent models of SCI, clodronate liposomes reduce lesion cavitation and axonal retraction at the site of injury [46,52]. The efficacy of macrophage depletion can be enhanced using combination therapies. For example, clodronate liposomes were recently shown to synergize with Rolipram, a phosphodiesterase inhibitor with neuroprotective and axon growth promoting capabilities [51]. Post-injury injection of anti-CD11d antibodies, specific for integrins found on MDMs (and neutrophils), also limits recruitment of MDMs to the injured spinal cord and improves motor, sensory and autonomic function [48,53,54]. Because clodronate liposomes and anti-CD11d antibodies are being tested clinically for other indications, such as psoriasis, cancer and osteoporosis (CTIDs: NCT00877097 [55], NCT00009945 [56], NCT00249808 [57]) it is surprising that neither has been tested in clinical trials for SCI.

Since limiting the infiltration and activity of MDMs can dramatically affect the outcome from SCI and because most neuroprotective therapies can directly affect MDM and tissue macrophage (e.g., microglia) function, it is important to consider how existing neuroprotective therapies could affect post-SCI macrophage responses.

Methylprednisolone is a synthetic glucocorticoid with potent anti-inflammatory effects. When applied in pre-clinical models of SCI, methylprednisolone reduces neutrophil and MDM influx to sites of injury and limits inflammatory signaling cascades [58,59]. However, the high doses of methylprednisolone used in animals and people can cause adverse systemic reactions that could obscure any neuroprotective anti-inflammatory effects afforded by methylprednisolone. For example, high dose methylprednisolone increases the incidence of steroid myopathy and interstitial pulmonary edema [60-62]. The anti-inflammatory and neuroprotective effects can be improved and the adverse reactions avoided by locally administering methylprednisolone to the spinal cord. This was achieved recently via intraspinal delivery of nanoparticles loaded with methylprednisolone [63].

In addition to its antibiotic properties, minocycline has anti-inflammatory effects. When applied to models of SCI, minocycline significantly reduces microglia and macrophage activation [64]. These effects are associated with increased survival of oligodendrocytes, reduced lesion pathology, decreased pain-like behavior and improved neurological function [64-69]. *In vitro*, minocycline has been shown to inhibit neurotoxic nitric oxide release from glutamate-activated microglia [70]. Therefore, the observed effects associated with minocycline treatment after SCI could be an indirect consequence of minocycline improving neuron and glial survival by limiting the toxic properties of activated MDMs and microglia [66-68].

This same relationship holds for hypothermia treatment. After SCI, neutrophil influx and morphological indices of microglial activation are reduced by systemic or epidural hypothermia [71-73]. These changes occur in parallel with improvements in neurological function; however, a direct cause-effect relationship has not been confirmed.

Voltage gated sodium channels have been detected on macrophages and expression of these channels increases as macrophages become activated [74]. In experimental autoimmune encephalomyelitis (EAE, an experimental autoimmune disease of the brain and spinal cord) sodium channel blockers reduce inflammatory cell accumulation in the spinal cord by 75% and reduce phagocytosis, a functional property of microglia/macrophages [75]. Future studies should determine the effects of Riluzole and other sodium channel blockers on microglia/macrophage function after SCI.

3. Regenerative therapies

Trauma to the spinal cord causes *de novo* synthesis of axon-growth-inhibitory molecules at and nearby the site of injury. When growing axons encounter these molecules, intracellular signaling cascades are initiated that halt the advance of growth cones and in most cases, the growth cones collapse and the axons 'die-back' [76,77]. To date, most experimental and clinical research has focused on understanding the growth-inhibitory role of myelin proteins [e.g., myelin-associated glycoprotein (MAG), myelin oligodendrocyte glycoprotein (MOG) and neurite outgrowth inhibitor (Nogo)] and components of extracellular matrix, namely chondroitin sulphate proteoglycans (CSPGs) [78-80].

The goal of most regenerative therapies is to devise novel ways to overcome these extrinsic barriers to endogenous repair in an effort to improve functional recovery. Presently, two approaches are being tested in clinical trials: i) application of monoclonal antibodies to neutralize inhibitory myelin proteins and ii) pharmacological manipulation of growth-inhibitory signaling pathways.

3.1 Current regenerative therapies

Two clinical trials are testing the effects of exogenous anti-myelin antibodies. The first, sponsored by Novartis [81] (CTID: NCT00406016), is an international multi-center trial with centers in the USA, Germany, Spain, Canada and Switzerland. In these centers, the feasibility and safety of prolonged intrathecal or bolus injections of ATI355, an anti-Nogo-A antibody, is being tested. Expected completion of these trials is July 2011. The second trial, sponsored by GlaxoSmithKline [82] (CTID: NCT00622609), examined safety, tolerability, pharmacokinetics and immunogenicity of various doses of intravenous GSK249320, an anti-MAG monoclonal antibody, in healthy volunteers. The Phase I safety trial was completed in April 2009 without any serious adverse effects (for more details see [83]). GSK249320 is being tested in Phase II trials as a treatment for stroke [84] (CTID: NCT00833989).

Chondroitinase ABC (chABC) is a bacterial enzyme that cleaves CSPGs. When applied to experimental models of SCI, chABC attenuates inhibitory CSPG gradients and enhances axon regeneration [85]. Although the safety and efficacy of chABC preclinical data in experimental SCI is compelling [18], especially when combined with other interventions [86-91], chABC has not been applied to human SCI. However, other types of chondroitinases have been tested clinically (and more trials are ongoing) as treatments for visual disorders without serious side effects [92]. Based on the therapeutic potential of chABC, we mention it here and discuss it below in the context of macrophage activation.

Cethrin® and lithium are two drugs that have been used to block signaling pathways that are associated with axon growth inhibition after SCI. Both drugs may also confer neuroprotection [93,94]. Cethrin (BA-210), an antagonist of the Rho GTPase, has been tested in two clinical trials sponsored by Alseres Pharmaceuticals. The first study (CTID: NCT00500812) was completed in February 2009 [95]. Interim results indicate that Cethrin improved motor recovery without serious adverse events [96]. A second, larger Phase II trial was initiated in 2008 [97] (CTID: NCT00610337) but was prematurely terminated because of insufficient funds [98].

Lithium blocks glycogen synthase kinase 3β (GSK-3β), a serine/threonine kinase that inhibits axon growth [99]. Lithium-mediated inhibition of GSK-3β was shown to increase the regenerative potential of CNS axons in animal models of SCI [94]; however, recent data indicate that robust suppression of GSK-3 also can impair axon regeneration [100]. Based on earlier data, oral lithium was (or is currently being) tested in three clinical trials. A Phase I study [101] was initiated in 2007 by the China Spinal Cord Network (CTID: NCT00431171) to determine plasma levels of lithium after oral administration in patients with chronic SCI. Results from this study helped in the design of two larger Phase II trials, also sponsored by the China Spinal Cord Network. The first [102] (CTID: NCT00750061), started in 2008, tested the safety and efficacy of oral lithium in chronic SCI patients. Results from that study are pending. Because lithium was also found to promote proliferation and differentiation of transplanted stem cells in experimental SCI models [103] a second trial [104] was started in 2010 (CTID: NCT01046786) with the goal of combining oral lithium with intraspinal transplantation of umbilical cord blood mononuclear cells (see below for review of stem cell trials). That study is in progress.

3.2 The role of macrophages in regenerative therapies

In response to SCI, macrophages reportedly increase their expression of MAG and Nogo receptors [105]. Activation of the Nogo receptor (NgR) regulates immune cell motility and it has been hypothesized that Nogo or MAG in intact or newly synthesized myelin repels inflammatory macrophages via NgR activation [106,107]. Thus, in the injured spinal cord, Nogo–NgR interactions could represent an endogenous anti-inflammatory mechanism for limiting the spread of potentially injurious macrophages beyond the site of injury. If so, the efficacy of anti-Nogo antibody trials could be limited by a concurrent increase in intraspinal migration of macrophages into spared tissue segments [106]. Indeed, *de novo* synthesis of NgR expression on macrophages seems to correlate with enhanced phagocytosis and clearance of myelin in injured peripheral nerves – a presumed prerequisite for successful axonal regeneration [108]. Additional research is needed to determine the functional significance of engaging NgRs on CNS macrophages.

Enzymatic digestion of CSPGs [e.g., via chondroitinase ABC (chABC)] or other extracellular matrix (ECM) proteoglycans can increase the axon-growth-permissiveness of the injured CNS microenvironment. However, these enzymatic therapies may also improve axon plasticity, sprouting or regeneration through indirect effects on microglia and macrophages [109]. Indeed, disaccharides from cleaved CSPGs promote the activation of an anti-inflammatory microglial phenotype *in vitro* [110]. It is not known if this also occurs *in vivo*; however, chABC prevents macrophage-mediated axonal retraction in the injured spinal cord [111]. After SCI, retraction of injured CNS axons is associated with an inflammatory 'M1' macrophage phenotype whereas anti-inflammatory 'M2' macrophages promote axon growth through CSPG barriers [13,53]. The interplay between CNS macrophages and degradation products of the ECM and glial scar is poorly understood but should be considered given that clinical trials using chABC or similar enzymes are inevitable.

Cethrin promotes neuroprotection and axon regeneration in part through the ability of one of its active components, BA-210, to block Rho GTPase activity [93,112]. In macrophages, Rho GTPases affect a number of pro-inflammatory functions, mostly through the regulation of NF-κB and downstream signaling intermediates [113]. After SCI, Rho activation is increased in macrophages throughout the injury site [114]. Whether intraspinal macrophage Rho GTPase activity predicts a pro-inflammatory phenotype is not known;

however, pro-inflammatory macrophages contribute to a range of processes associated with CNS injury and repair including axonal dieback, sensory afferent sprouting and myelin phagocytosis [13,52,115]. To optimize efficacy and facilitate the interpretation of future pre-clinical and clinical trials, it will be important to consider how Cethrin or other Rho antagonists influence macrophage phenotype and function.

In macrophages, GSK3 regulates the balance of pro- and anti-inflammatory cytokine synthesis [116]. When applied to macrophages, lithium inhibits GSK3 and promotes the differentiation of an M2 macrophage phenotype, even in the presence of pro-inflammatory stimuli [117,118]. As described above, M2 macrophages could be neuroprotective and indeed, GSK3 inhibitors minimize inflammatory toxicity in hippocampal slice cultures [118]. In experimental models of SCI, lithium reduces macrophage accumulation in the injured spinal cord suggesting that lithium also may influence the migration and survival of microglia or monocyte-derived macrophages [103]. Consistent with this hypothesis, long-term exposure to lithium induces macrophage apoptosis [119]. Collectively, these data suggest that in addition to influencing axon growth, lithium also may have profound immune modulatory effects after SCI.

4. Cell replacement therapies

A variety of cell-based clinical trials have been completed or are underway to test the feasibility and safety of using different cell types to repair the injured spinal cord. Specifically, autologous cells: macrophages and olfactory ensheathing cells; and stem cells: bone marrow, embryonic or umbilical cord blood-derived, are being developed as therapeutics for spinal cord injury.

4.1 Autologous cells: macrophages (ProCord) and olfactory ensheathing cells

The scientific rationale and design of the ProCord trials, sponsored by Proneuron Biotechnologies, have been reviewed in detail previously [120,121]; therefore, only a summary will be provided here. ProCord is a proprietary procedure whereby autologous macrophages are isolated from individuals with SCI then are activated by co-incubating the cells with autologous skin biopsies. Once activated, the macrophages are surgically introduced into the injured spinal cord. Preclinical data suggest that this approach produces macrophages that confer neuroprotection and/or improve plasticity/regeneration in the injured spinal cord [122]. The results of the Phase I trial indicate that the cell preparation and surgical technique are feasible and are without adverse effects [120]. A larger Phase II trial [123] was initiated in 2003 (CTID: NCT00073853); however, the trial was suspended prematurely in 2006, reportedly because of financial limitations. A recent paper documents issues of pragmatics, patient recruitment and demographics for this trial [124]. It is not known if or how the transplanted macrophages affected spinal cord structure or function or if transplanted cells altered the intrinsic macrophage response. The latter data cannot be determined from the Phase II trial; however, analyses of safety and efficacy data are in progress and will be reported separately [Dr Dan Lammartse, pers. commun.].

Olfactory ensheathing cells (OECs) are myelinating cells found in close proximity to axons in the olfactory bulbs. OECs have the functional and phenotypical characteristics of astrocytes and Schwann cells. In pre-clinical models of SCI, transplantation of OECs improves functional recovery, axon growth/regeneration, remyelination and neuroprotection (for a review see [125]). Although the feasibility of OEC transplantation in humans with SCI has been reported [126,127], this approach remains controversial (see below and [125]). Clinicaltrials.org lists one current clinical trial [128] sponsored by the Wroclow Medical University (Poland; CTID: NCT01231893). The purpose of this Phase I study is to assess the safety and feasibility of autologous OEC transplantation into individuals with complete SCI. Patients are currently being recruited for this study.

4.2 Stem cells from bone marrow, umbilical cord blood or embryonic tissue

Preclinical studies show that transplantation of bone marrow cells or umbilical cord blood improves anatomical and functional recovery after SCI (for review see [129]). Results of previous Phase I/II clinical trials indicate that transplantation, through lumbar puncture, of bone marrow cells is safe and feasible in people with SCI [130]. Clinicaltrials.gov lists four trials examining the effects of bone marrow stem cell (BMSC) transplantation after SCI. The first (CTID: NCT00816803), sponsored by Cairo University, involved transplantation of autologous BMSCs into chronically injured spinal cord [131]. Studies were completed in December 2008 and preliminary reports from a small cohort of patients (n = 18) indicate positive effects without evidence of tumorigenicity [132]. The second trial, sponsored by the Translational Research Informatics Center in Japan (CTID: NCT00695149) is testing safety and efficacy of cell delivery via lumbar puncture with the goal of restoring sensory and motor function after SCI [133]. Results from a single case study indicate that the approach is safe and feasible and that modest improvements were observed [134]. The estimated completion date for this trial is Dec. 2010. The third trial, sponsored by TCA Cellular Therapy, LLC (Louisiana, USA; CTID: NCT01162915) began in July 2010 [135]. The purpose of this Phase I study is to determine the safety of intrathecal infusion (via lumbar puncture) of BMSCs in individuals with SCI below C5. The fourth, conducted by International Stemcell Services Ltd (India; CTID: NCT01186679), examined the safety and efficacy of autologous bone marrow derived stem cell transplantation [136]. Cells were either directly transplanted into the spinal cord of chronically injured individuals or injected intrathecally into patients with acute and subacute injuries. The study was completed in August of 2010 and according to the company

website 'safety (was) established' [137]; however, the data have not been peer reviewed and no results have been posted at clinicaltrials.gov.

Another trial, sponsored by the China Spinal Cord Injury Network (CTID: NCT01046786), is examining the feasibility, safety, efficacy and optimal dose of umbilical cord blood mononuclear cell transplants, used alone or in combination with methlypredinsolone or oral lithium in patients with chronic SCI [138]. Details of the trial design have been published but no data are available [139]. A case study reports morphometric and physical improvements without adverse effects following umbilical cord stem cell transplantation into a patient with a chronic (20 years) SCI [140].

Transplanted glial-restricted precursor cells promote neuroprotection, remyelination and functional recovery in a preclinical model of SCI (reviewed in [141]). Based on those data, an FDA-approved Phase I clinical trial, sponsored by Geron Corp. (CA, USA), was initiated to evaluate the safety of GRNOPC1, a proprietary human embryonic stem cell-derived oligodendrocyte progenitor cell line. GRNOPC1 will be intraspinally injected into people with acute (7 – 14 days post injury), neurologically complete spinal cord injuries (CTID: NCT01217008) [142]. This is the first FDA-approved trial to test the therapeutic potential of embryonic tissue for SCI. Patient enrollment is in progress.

Cellular transplantation therapies are becoming big business abroad. Beike Biotechnology in China and the XCell-Center, a private regenerative medicine clinic in Germany, offer stem cell-based services as a treatment for SCI. Some individual case reports from groups not associated with these companies have reported positive effects with transplanted stem cells in individuals with SCI [143-145]. These companies also cite positive results in patients receiving their cells; however the data have not been peer-reviewed [19,146]. Accordingly, many members of the international scientific community remain skeptical about the safety and efficacy of these and other transplantation therapies [19,147-150].

4.3 The role of macrophages in stem cell therapies

Stem cell transplants may modulate post-SCI inflammation. The precise mechanisms by which this occurs have not been defined but several reports indicate that macrophage accumulation is reduced along with intraspinal expression of pro-inflammatory cytokines [151-153]. It is possible that stem cells (derived from bone marrow or umbilical cord blood) promote repair in part by changing macrophage phenotype and function. The same is potentially true for OECs. Intraspinal transplantation of OECs into injured rats alters the macrophage response to SCI [154]. Interestingly, indexes of pro-inflammatory macrophage activation are increased acutely but decreased chronically with OEC administration [154]. As discussed above, environmental cues shape the phenotype and function of macrophages [14,15] and cues that reduce pro-inflammatory macrophage activation or that increase the prevalence of alternatively activated (M2) macrophages could promote neuroprotection and axon regeneration [13].

In human umbilical cord blood, high concentrations of IL-4, IL-10 and IL-13 exist [155]. These anti-inflammatory cytokines promote alternative (i.e., M2) macrophage activation. When purified from blood then injected intravenously, umbilical cord blood cells (UCBCs) also increase plasma levels of IL-10, IL-13 and IL-4 and reduce the secretion of pro-inflammatory cytokines (e.g., TNF-α, IL-12) from splenocytes [155,156]. Intravenous injection of UCBCs into mice with a chronic form of experimental Alzheimer's disease increases anti-inflammatory cytokines in the brain [156]. This suggests that injecting stem cells into animals or people with chronic SCI could alter cytokine expression in the CNS and therefore the phenotype and function of macrophages, even after damage to the blood–brain barrier has been repaired.

Bone-marrow derived stem cells have similar effects. In a model of stroke, human BMSC (hBMSC) increase the ratio of IL-4:INF-γ or IL-4:TNF-α creating a lesion environment in which an alternative microglial phenotype develops [157]. *In vitro* data indicate that BMSC release various factors that promote alternative macrophage activation or that decrease the production of pro-inflammatory mediators by macrophages [158-160].

The effect of GRNOPC1 cells on macrophages has not been evaluated; however, other embryonic stem cells have been shown to affect inflammation after SCI. Specifically, macrophage infiltration is reduced following intravenous administration of undifferentiated cells or intraspinal injection of glial-restricted precursor cells in rodents [161,162]. Further work is needed to determine how macrophages are affected by GRNOPC1 cells.

5. Conclusions

Spinal cord injury elicits a robust inflammatory response that is comprised predominantly of activated resident microglia and newly recruited monocyte-derived macrophages. These cells persist indefinitely at the site of injury and change their phenotype and function in response to signals in the lesion microenvironment. Such changes occur spontaneously as a consequence of delayed secondary degeneration or repair. Additional changes are expected following therapeutic intervention. Indeed, all neuroprotective, pro-regenerative or cell replacement therapies that are currently being tested in clinical trials for SCI are intended to change the composition and dynamics of the lesion environment. Therefore, each intervention has the potential to influence the activation status, phenotype and function of CNS macrophages (**Figure 1**). To enhance clinical research outcomes and improve recovery for individuals with SCI, it will be important to consider how these interventions affect CNS macrophages.

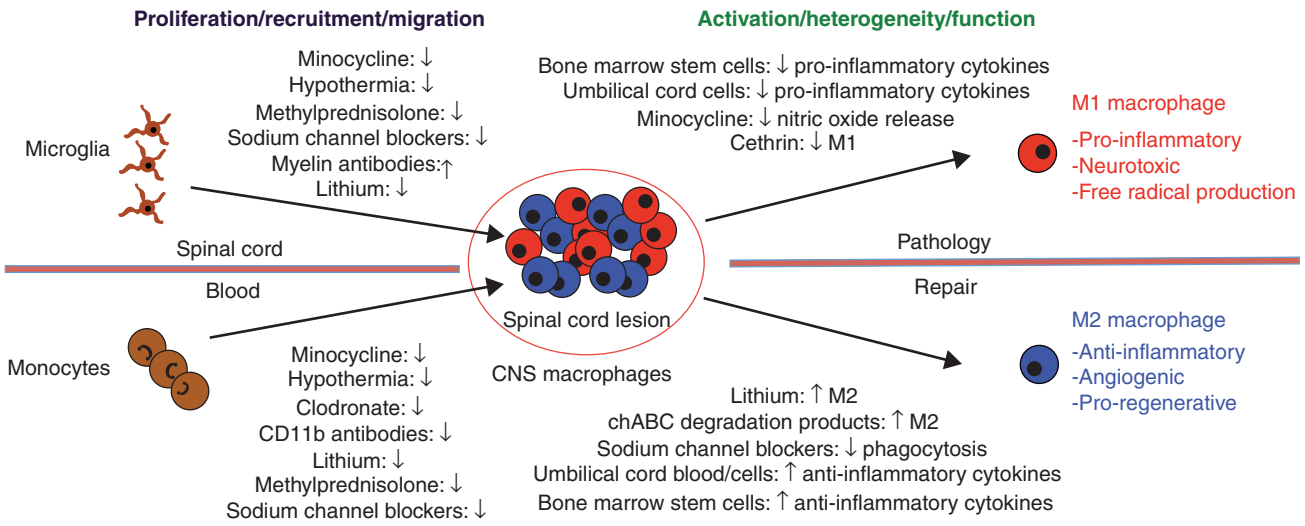

Figure 1. Therapies being tested in clinical trials of spinal cord injury (SCI) undoubtedly affect different aspects of CNS macrophage function. Proliferation/recruitment/migration: Traumatic SCI causes endogenous CNS microglia to proliferate then migrate to the site of injury. After a few days, monocytes infiltrate the injury site from the blood where they differentiate into macrophages. A variety of pre-clinical therapies that have proven efficacious may exert their effects in part by regulating the continuum of microglia/monocyte migration and/or activation. Listed are examples of different manipulations and their presumed effects on microglia/macrophages (up/down arrow implies activation or suppression) Activation/heterogeneity/function: Different environmental cues at the site of injury will cause macrophages to adopt unique phenotypic and functional properties. Macrophages with pro-inflammatory/neurotoxic properties (M1 macrophages) and those with anti-inflammatory/pro-reparative (M2 macrophages) properties exist early after injury [13]. These functions are dynamic and can change in response to new stimuli, including various therapeutic interventions that are being tested in clinical trials. Please see text for description of specific therapeutic approaches.

6. Expert opinion

Macrophages are found throughout the acute and chronically injured spinal cord. Their proximity to and ability to interact with and influence all cells in the nervous system confers upon them priority status when planning and interpreting different types of therapeutic intervention. Indeed, macrophages express a diverse repertoire of receptors that signal regenerative and neurotoxic effector functions. Often times, these seemingly dichotomous functions are elicited in parallel at sites of injury/inflammation [12] and the signaling pathways that control injurious or reparative functions are also used by neurons and glia. This exemplifies the need to understand the diversity of receptors and signaling pathways that are activated in macrophages after SCI so that researchers can anticipate the effects of a given therapy on macrophage function. For example, lithium affects GSK activity. Suppression of neuronal GSK3 is associated with increased regenerative potential; however, suppression of GSK3 in macrophages may impair their ability to self-regulate proinflammatory signaling cascades that culminate in the production of neurotoxic molecules.

The complexity of macrophage functional diversity is compounded by the fact that spinal cord injury elicits a CNS macrophage response comprised of at least two distinct macrophage subsets, that is resident microglia and blood monocytes (**Figure 2**). Furthermore, there is tremendous diversity within each of these macrophage pools. For example, at least two distinct monocyte subsets are known to infiltrate the injured spinal cord [13,163] and the temporal sequence of their entry and activation in the tissue is not fully understood (**Figure 2**). However, as we grow to understand that functional heterogeneity defines the macrophage response after SCI, scientists should rely less on the potential meaning of more or less staining with generic macrophage phenotypic markers (i.e., Mac1/OX-42 (anti-CD11b), ionized calcium-binding adapter molecule 1 (Iba1), ED-1/CD68) in tissue sections. Indeed, less labeling for CD11b in response to treatment *X* may predict less tissue damage, but if the treatment affects signaling pathways that are mostly active in reparative CNS macrophages (e.g., M2 cells), the reduction in CD11b could have adverse effects on resolution of inflammation and tissue repair.

Future studies should begin to define the different macrophage subpopulations that occupy sites of injury and nearby spinal segments, proximal and distal to the lesion. Within these regions, we also need to know which ligands and receptor cascades elicit macrophage activation after SCI. The ligands may exist in the tissue, blood or cerebrospinal fluid.

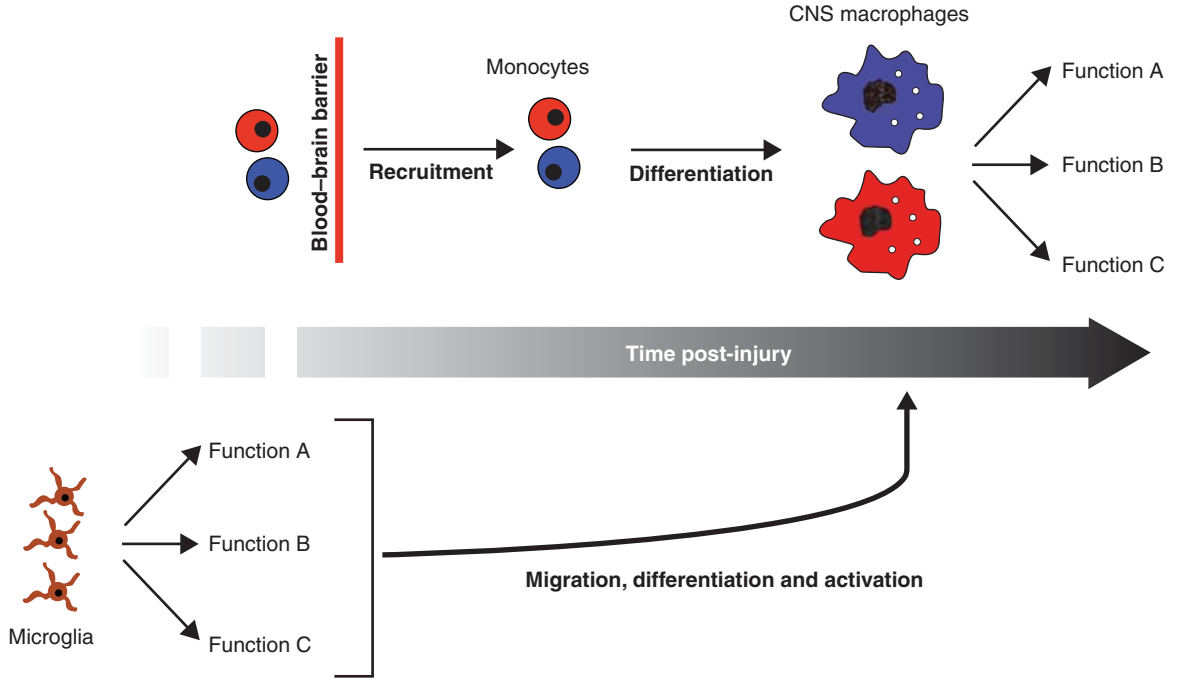

Figure 2. Macrophage functional heterogeneity after spinal cord injury (SCI). Microglia and monocyte-derived macrophages comprise the bulk of CNS macrophages after SCI. The temporal progression of activation and distribution of these different cell populations is unique with microglia dominant early after SCI. Monocyte-derived macrophages infiltrate after a period of days then persist indefinitely after SCI. The mechanisms underlying the continuum of activation of each of these cell populations creates unique challenges for interpreting the effects of therapeutics. Indeed, drugs, cell-based therapies and rehabilitation can affect the progression of activation, migration and/or differentiation of CNS macrophages.

Currently, the identity and hierarchical regulation of the endogenous ligands that control the phenotypic and functional diversity of macrophages is unknown. Drugs, cell-based therapies or rehabilitation therapies may influence many of these ligands.

At least one ongoing pilot clinical study [164] will determine how SCI influences circulating levels of an endogenous protein that can affect microglia/macrophage activation. Specifically, scientists and clinicians at the North Shore Long Island Jewish Health System (NY) are measuring circulating levels of macrophage migration inhibitory factor (MIF) after SCI [164]. Should this trial identify MIF as a biomarker that predicts changes in macrophage activation after SCI, it could serve as an important diagnostic tool for correlating the effects of therapies with the dynamics of macrophage phenotype and function.

As we learn more about macrophage heterogeneity and hierarchical signaling cascades and develop bioassays to assess macrophage function after injury, designer drugs may be developed that can affect CNS cells without affecting macrophages. Alternatively, it may be advantageous to selectively manipulate macrophages prior to, in parallel with or after delivery of a neuroprotective, regenerative or cell-based therapy. The more we understand about CNS macrophages and how current therapies affect these cells, the greater the potential for improving function after spinal cord injury.

Declaration of interest

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