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IMMUNE CELLS AND STEM CELLS IN SPINAL CORD INJURY: DEFINING SPINAL CORD INJURY ASSOCIATED MICROGLIA

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On the cover: 1474 CD45^{+} immune cells represented using the first- and second components of a t-distributed stochastic neighbor embedding (tSNE) dimensionality reduction. Centroids (not illustrated) were estimated for each time point of evaluation (healthy, 0.5 hour, 2 hours, 6 hours, 24 hours, 36 hours, 3 days, 7 days, 21 days, 90 days) post spinal cord injury (SCI) by calculating the mean position for the first- and second component for all cells within each condition. The Euclidian distance in two-dimensional space was calculated between each cell and each centroid and the cell was assigned to the centroid to which the Euclidian distance was the shortest. Each cluster formed by the procedure was assigned a separate color. Arrow indicates the direction of time and transformation of microglia from homeostasis (hMicroglia) into disease-associated microglia (DAM) in SCI. Illustrations of microglial cells are scattered across the background. Illustrations of microglial cells and arrow (color modified) are created by Smart Servier Medical Art (https://smart.servier.com) and are licensed under CC BY 3.0.

Immune Cells and Stem Cells in Spinal Cord Injury: Defining Spinal Cord Injury Associated Microglia

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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ABSTRACT

Traumatic spinal cord injury (SCI) commonly results from falls, sport activities or traffic accidents and most often results in lost sensory, motor and/or autonomic functions below the level of injury. The cellular- and immune response following SCI is complex and has implications for regeneration and recovery. The immune cell response, and the microglial response in particular, has not yet been investigated at single-cell resolution. Furthermore, several questions remain concerning the importance of histocompatibility of transplanted stem cells, their cellular response and their contribution to functional recovery following SCI. This thesis investigated stem cell transplantation as a therapeutic approach for SCI as well as disease-specific transformations of immune cells, focusing on microglia, following SCI.

In the ***first project*** we investigated the causality between transplantation of neural progenitor cells (NPCs) and recovery of hind limb locomotor function following SCI. Ventricular-subventricular zone (V-SVZ) derived NPCs were transplanted into the injury epicenter of rats subjected to severe contusion SCI. The NPCs were investigated in terms of differentiation, transcriptional profile, effect on neuroinflammation and causal contribution to recovery of hind limb locomotor function. We found that there is causality between transplantation of NPCs and recovery of hind limb locomotor function and that this correlates with their differentiation into oligodendrocytes, enhancement of myelination and suppression of neuroinflammation. In the ***second project*** we investigated the importance of histocompatibility of mesenchymal stem cells (MSCs) transplanted into SCI. Syngeneic- or allogeneic mouse bone marrow derived MSCs were transplanted into the injury epicenter of mice subjected to severe contusion SCI and the immune- and inflammatory response as well as the contribution to functional recovery was investigated. We found that syngeneic MSCs activate macrophages alternatively and enhance neuronal survival, which correlates with suppression of inflammation and enhancement of hind limb function. Thus, the histocompatibility of transplanted MSCs is of importance for their therapeutic potential in SCI. In the ***third project*** we investigated the cellular response of MSCs transplanted into SCI. Mouse bone marrow derived fluorescent MSCs were transplanted into the injury epicenter during the acute phase of SCI in mice and isolated one week later. The global transcriptional profile, phenotype and fate of the MSCs was investigated. We found that MSCs transplanted into SCI adopt immune-cell like characteristics by up-regulating expression of genes and surface markers associated with immune cells and immune system functions (phagocytosis/endocytosis, production of cytokines/chemokines). In the ***fourth project*** we investigated disease-specific transformations of immune cells in SCI at single cell resolution. SCI was induced in mice and $CD45^{+}$ immune cells were isolated from the spinal cord at various time points and subjected to single-cell RNA sequencing. The gene expression analysis was supplemented with histological evaluation as well as a depletion model of microglia. We found that following degeneration, demyelination or trauma to the CNS homeostatic microglia undergo a distinct temporal transformation resulting in a disease-associated subtype of microglia, which persists in the chronic phase of the injury and has a beneficial role for functional recovery.

In conclusion, stem cell therapy for SCI shows a great deal of potential but is, at the moment, not sufficient or efficient enough to restore function to a near-normal level. Moreover, following SCI microglia undergo disease-associated transformations, which persists in the chronic phase of the injury, and contribute to functional recovery.

LIST OF SCIENTIFIC PAPERS

included in the thesis

I. **Adult neural progenitor cells transplanted into spinal cord injury differentiate into oligodendrocytes, enhance myelination, and contribute to recovery**
*Sankavaram SR, **Hakim R**, Covacu R, Frostell A, Neumann S, Svensson M, Brundin L.*
Stem Cell Reports. 2019 May 14;12(5):950-966.
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PMID: 31031190

II. **Syngeneic, in contrast to allogeneic, mesenchymal stem cells have superior therapeutic potential following spinal cord injury**
***Hakim R**, Covacu R, Zachariadis V, Frostell A, Sankavaram S, Svensson M, Brundin L.*
J Neuroimmunol. 2019 Mar 15;328:5-19.
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PMID: 30551037

III. **Mesenchymal stem cells transplanted into spinal cord injury adopt immune cell-like characteristics**
***Hakim R**, Covacu R, Zachariadis V, Frostell A, Sankavaram SR, Brundin L, Svensson M.*
Stem Cell Res Ther. 2019 Apr 3;10(1):115.
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IV. **Permanently re-programmed microglia in spinal cord injury contribute to functional recovery**
***Hakim R**, Zachariadis V, Sankavaram SR, Han J, Harris R, Brundin L, Enge M, Svensson M.*
Manuscript

ADDITIONAL CONTRIBUTIONS

not included in the thesis

V. **Adjuvant stereotactic radiosurgery reduces need for retreatments in patients with meningioma residuals.**
*Frostell A, **Hakim R**, Dodoo E, Sinclair G, Ohlsson M, Förander P, Milovac B, Brundin L, Svensson M.*
World Neurosurg. 2016 Apr;88:475-482.
doi: 10.1016/j.wneu.2015.10.062.
PMID: 26546994

VI. **A review of the segmental diameter of the healthy human spinal cord.**
*Frostell A, **Hakim R**, Thelin EP, Mattsson P, Svensson M.*
Front Neurol. 2016 Dec 23;7:238.
doi: 10.3389/fneur.2016.00238.
PMID: 28066322

VII. **Health-related quality of life dynamics 2 years following aneurysmal subarachnoid hemorrhage: a prospective cohort study using EQ-5D.**
*von Vogelsang AC, Thelin EP, **Hakim R**, Svensson M.*
Neurosurgery. 2017 Oct 1;81(4):650-658.
doi: 10.1093/neuros/nyx059.
PMID: 28368438

VIII. **The national incidence of PML in Sweden, 1988-2013.**
*Iacobaeus E, Burkill S, Bahmanyar S, **Hakim R**, Byström C, Fored M, Olsson T, Brundin L, Montgomery S.*
Neurology. 2018 Feb 6;90(6):e498-e506.
doi: 10.1212/WNL.0000000000004926.
PMID: 29321229

IX. **Recovery of voice after reconstruction of the recurrent laryngeal nerve and adjuvant nimodipine.**
*Mattsson P, Frostell A, Björck G, Persson JKE, **Hakim R**, Zedenius J, Svensson M.*
World J Surg. 2018 Mar;42(3):632-638.
doi: 10.1007/s00268-017-4235-9.
PMID:29282507

X. **Epidemiology of NMOSD in Sweden from 1987 to 2013: A nationwide population-based study.**
*Jonsson DI, Sveinsson O, **Hakim R**, Brundin L.*
Neurology. 2019 Jul 9;93(2):e181-e189.
doi: 10.1212/WNL.0000000000007746.
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CONTENTS

LIST OF ABBREVIATIONS

BBB	Basso, Beattie and Bresnahan
BMS	Basso Mouse Scale
CFU	Colony-Forming Unit
CNS	Central Nervous System
CPM	Counts Per Million
DAM	Disease-Associated Microglia
DTR	Diphtheria Toxin Receptor
FDR	False Discovery Rate
GO	Gene Ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
MSC	Mesenchymal Stem Cell
NPC	Neural Progenitor Cell
NSC	Neural Stem Cell
RMS	Rostral Migratory Stream
RNAseq	RNA sequencing
SCI	Spinal Cord Injury
scRNAseq	Single-cell RNA sequencing
V-SVZ	Ventricular-Subventricular Zone

INTRODUCTION

Spinal cord injury

The spinal cord relays information between the body and the brain

The spinal cord extends from the base of the brainstem along the vertebrae in a caudal direction and is a part of the central nervous system (CNS). It is protected by the meninges, the cerebrospinal fluid (CSF) located in the subarachnoid space, fibrous- and adipose tissue located in the epidural space as well as by vertebrae. The CNS, including the spinal cord, is composed out of gray- and white matter. The cell bodies and the dendrites of neurons as well as interneurons and glial cells reside in the gray matter while the white matter is mainly made up of myelinated axons. Ascending tracts (dorsal column, lateral- and ventral spinothalamic) in the white matter contain afferent fibers which carry sensory information (crude- and fine touch, pain, temperature, proprioception) entering the spinal cord through the dorsal root. The cell bodies of the afferent axons are located in the dorsal root ganglion. Descending tracts (ventral- and lateral corticospinal, reticulospinal, rubrospinal, vestibulospinal, tectospinal) in the white matter contain efferent axons, which exit the spinal cord on the ventral side and mediate motor function by innervation of muscles. The dorsal and ventral roots merge and form one common spinal nerve in the periphery [1].

External forces are not the only cause of spinal cord injury

A disruption in the spinal cords ability to relay information between the cerebrum and the body is an injured spinal cord. This functional deficit stems from injury to the sophisticated and fragile system and network of cells, supportive tissue and axons. The initial disruption, which is termed the *primary injury*, can be classified as traumatic or non-traumatic. Traumatic spinal cord injury (SCI) is defined as an injury occurring from external forces resulting in a horizontal dislocation of vertebrae causing mechanical damage to neuronal- and glial cells, axons and blood vessels. The most common sources of external forces are traffic accidents, falls and sport activities. Non-traumatic SCI stems from disease processes including neurodegeneration, vascular lesions, tumors and infections [2, 3].

Young men have the highest risk for spinal cord injury

With reliable data available from only a few countries the incidence- and prevalence of SCI has proven difficult to measure and to generalize across geographical regions. The incidence in countries such as Sweden, Finland, France, Ireland, Fiji, Turkey, Spain and Australia varies between 10-20 cases per million individuals. On the contrary, countries such as Romania, Spain and Greenland have an incidence rate of 20-30 cases per million individuals [4] with USA, Portugal and New Zeeland taking the lead with around 40-50 cases per million individuals [4, 5]. Highest prevalence of SCI is found in the USA at about 900 individuals per million and lowest in France and Finland at about 250 individuals per million [4]. Eight out of ten patients with a SCI is a male [6] between 15-30 years of age [7]. About half of the SCIs involve the cervical spinal cord while only about one third of the SCIs affect the thoracic spinal cord [2, 6].

The secondary injury is the culprit

Following the primary injury of SCI, the injury continues to develop and is termed the secondary injury. The secondary injury is further divided into the acute phase (0-2d), subacute phase (3-14d), intermediate phase (15d-6m) and the chronic phase (>6m). Pro-inflammatory cytokines such as IL-1β and TNF-α are secreted during the acute phase of SCI, which contributes to inflammation but also to formation of edema. The newly formed edema increases the intra-parenchymal pressure, adds to, and extends the primary injury by inducing a mechanical pressure on the tissue [2, 8]. During the acute phase of SCI the blood vessels are disrupted by thrombosis, bleeding and/or vasospasm which results in insufficient supply of oxygen and nutrients (i.e. ischemia) to the spinal cord [9]. Moreover, the blood-spinal cord barrier is compromised resulting in infiltration of inflammatory cells (granulocytes, T/B-cells and macrophages) from the periphery which in combination with the negative impact of the ischemia eventually causes cells to undergo necrosis [8, 10, 11]. Due to the hostile conditions during the subacute phase neurons and glial cells experience disruption in their balance of Na^+, K^+ and Ca^{2+} ions. Elevated levels of intracellular Ca^{2+} in neurons can result in necrosis or mitochondrial dysfunction, thus further developing the SCI [12, 13]. Neurons undergoing necrosis release glutamate into the parenchyma [14]. The glutamate activates kainite, AMPA- and NMDA-receptors on other neurons which in combination with their reduced ability to regulate their sodium levels due to dysfunction of ATP-dependent sodium pumps results in them undergoing necrosis [2, 15-17]. Neural cells undergoing necrosis release large amounts of ATP, potassium and DNA, which activates microglia. The activated microglia in combination with other immune cells phagocytose neurons and oligodendrocytes undergoing apoptosis as well as myelin debris [18]. Infiltrating macrophages and lymphocytes contribute to apoptosis of neurons while glial cells accelerate the inflammatory response [2, 18]. The phagocytizing cells release free radicals which contribute to the necrosis of neural cells by mediating damage to DNA and oxidation of proteins and lipids [19]. Thus, apoptosis of glial- and immune cells as well as neural cells in a SCI environment result in a loss of neurons and demyelination of axons [20]. Hence, the death of neural cells as well as the lack of proper blood supply during the acute- and subacute phase adds significantly to the primary injury, and the secondary injury thereby probably exceeds the extent of the primary injury.

During the chronic phase of SCI lost neurons and oligodendrocytes are replaced by a cystic cavity formed by macrophages, extracellular fluid and connective tissue [21]. Reactive astrocytes in combination with extracellullar matrix (ECM) proteins (proteoglycans, tenascin, NG2) form a glial scar, which surrounds the cystic cavity [22, 23]. Stromal cells formed from pericytes and fibroblasts can add to the glial scar. The cystic cavity in combination with the glial scar surrounding it forms a mechanical- and biochemical barrier for axons to regenerate from the cranial to the caudal side of the SCI [2, 22, 24, 25]. However, the role of the glial scar is mainly to encapsulate the injury area, the inflammatory cells and the injury-associated molecules and thereby reduce their effect on the surrounding tissue [26, 27]. During this phase axons undergo Wallerian degeneration. Moreover, myelin-associated molecules (Nogo-A, MAG, proteoglycans) which are debris and bi-products from myelin degradation, and substances released from dying oligodendrocytes can inhibit neurite outgrowth and axonal regeneration [28]. Nogo-A is expressed mainly by oligodendrocytes and inhibits neurite outgrowth when it attaches to its receptor on the membrane of the neuron [29]. MAG is present in myelin and is originally produced by oligodendrocytes [30]. Proteoglycans are closely related to the formation of the glial scar and are expressed by oligodendrocyte precursors, astrocytes and meningeal cells [31].

Moreover, myelin debris present in the injury epicenter prevents the joint action of macrophages, lymphocytes and astrocytes required for re-myelination of axons. The glial scar itself also has a detrimental effect on the myelination through secretion of inhibitory factors [28, 32]. Hence, there is a mechanical barrier formed by the cystic cavity and the glial scar, which inhibits neurite- and axonal outgrowth. The glial scar and secreted substances also form a biochemical barrier by limiting the myelination of axons. The lack of functional axons result in a lack of functional recovery, be it motor, sensory or autonomic functions.

The spinal cord, and brain, undergo modifications due to the plasticity of the CNS

Attempts at endogenous regeneration is mediated by neural precursors found in the central canal of the spinal cord as well as by oligodendrocyte progenitors which differentiate into neurons and astrocytes/oligodendrocytes respectively [33, 34]. Moreover, the spinal cord has an endogenous plasticity which can be enhanced by physical activity [35]. This plasticity also extends to collateral sprouting of long-tract axons and interneurons [36, 37]. Plasticity following SCI can also have negative consequences and result in spasticity, autonomic dysreflexia and pain [38, 39]. Pain is most likely a consequence of abnormal sprouting of calcitonin gene-related peptide fibers in combination with hyper-excitability of neurons in the dorsal horn [40]. Additionally, the cerebral cortex responds to SCI and is reorganized. Regions that innervate limbs and body parts, which have lost their innervation due to the SCI, are taken over by regions that are innervating regions that are not affected by the SCI [41-43]. The enhanced plasticity in the spinal cord following SCI is mediated by lower levels of GABA-mediated inhibition [44] which might also explain the cortical plasticity observed in patients with SCI and may result in an inability to perform voluntary movements previously possible [45].

Spinal cord injuries are classified using imaging and international guidelines

The level of the SCI is reported with regards to the spinal cord levels, and not the vertebrae, affected. A computed tomography scan is usually implemented to visualize fractures in the vertebral column while magnetic resonance imaging is used for characterization of injuries to the spinal cord, nerve roots, ligaments and other surrounding soft tissue structures [46, 47]. A SCI in the clinical setting is evaluated using the International Standards for Neurological Classification of Spinal Cord Injury [48] which is comprised of three parts: I) American Spinal Injury Association (ASIA) motor score, II) ASIA sensory score and III) ASIA Impairment Scale grade. The scoring can and should be used as a measure of development during treatment in relation to the condition at admission. The SCI is further classified into complete- and incomplete injuries. Incomplete injuries can be subdivided into central cord syndrome (bladder dysfunction, motor dysfunction in upper but not lower limbs and sensory loss) [49], Brown-Séquard syndrome (loss of fine touch, proprioception and vibration on the same side as the injury and loss of sensation for pain- and temperature on the opposite side) and anterior- and posterior syndrome [50].

The spinal column, respiration and circulation have to stabilized

Unfortunately, at the moment, there is no curative treatment for SCI. In addition, patients suffering from a SCI also have to worry about short- and long term adverse effects of their injury. In the acute phase of SCI, a patient may encounter either spinal- or neurogenic shock. Spinal chock is a complete lack of motor, sensory and autonomic function below the injury level and is characterized by hypo-reflexia early on and hyper-reflexia later on. However, the end of spinal chock is not always easy to determine [2, 51]. Neurogenic shock is caused by a loss of sympathetic innervation, which results in reduced vascular tone leading to hypotension and bradycardia. Patients suffering from high thoracic- or cervical injuries are especially at risk since the sympathetic nerves cannot be reached [52]. Management includes avoidance of hypotension in order to ensure adequate perfusion of the spinal cord. The oxygen saturation in the blood is to be maintained high and formation of deep venous thrombosis should be actively counteracted [53]. The usage of methylprednisolone sodium succinate (corticosteroid) has been actively debated but is a potential option in the individualized treatment of the patient [2]. The drug acts by maintaining blood flow, keeping the blood-spinal cord barrier intact, inhibits lipid peroxidation and reduces inflammation [54]. Decompressive surgery results in realignment of the vertebrae and thereby reduces the pressure on and ischemia in the spinal cord [55]. Surgical decompression enhance functional recovery and reduce the extent of the secondary injury in both animal models as well as in humans [55, 56]. Moreover, early decompressive surgery seems to be beneficial in relation to delayed surgery [57]. Hence, in the acute phase of SCI the cranio-spinal axis should be immobilized in order to limit the extent of the injury and the respiratory, cardiac and haemodynamic functions should be closely monitored and managed in order to limit the extent of the secondary injury and to enhance recovery [58].

A large variety of treatment approaches are being investigated

Several types of approaches for treatment and management of SCI are currently being evaluated in basic- and clinical research. One such approach is administration of neuroprotective agents. These therapies aim to reduce the extent of the secondary injury by reducing inflammation, enhancing growth and by blocking signals inhibiting regeneration. Agents such as IL-10, minocycline, non-steroidal anti-inflammatory drugs (NSAID) and atorvastatin are undergoing evaluation. IL-10 is an anti-inflammatory cytokine with the ability to reduce production and release of TNF-α from astrocytes in the CNS [59]. Minocycline is an agent that can inhibit microglial activation, reduce pro-inflammation and apoptosis of oligodendrocytes [60, 61]. NSAIDs such as indomethacin and ibuprofen have been evaluated with the intent to reduce the secondary injury [1]. Administration of atorvastatin (cholesterol reducing drug) has resulted in reduced inflammation and suppression of astrocyte- and microglial activation. Sodium channel blockers such as Riluzole or NMDA antagonists (magnesium) have been evaluated in order to counteract the excitotoxicity by reducing activation of sodium-channels in neurons [1, 62]. Erythropoietin, which enhances proliferation- and differentiation of erythrocytes, can enhance regeneration and differentiation of cholinergic neurons following traumatic brain injury and is currently evaluated for SCI [63]. Trials with anti-Nogo-A antibodies are aiming at enhancing axonal regeneration and have proven potential for regeneration and myelination of axons [64, 65]. Chondroitin sulfate proteoglycans (CSPGs) have a significant inhibitory effect on the regeneration of axons [66]. Administration of chondroitinase ABC that have the potential to degrade CSPGs can enhance axonal regeneration [67]. Neurotrophic factors (NGF, NT-3,

BDNF and GDNF) which mediate survival, growth, guidance and plasticity in the CNS have been administrated in various ways following SCI to promote axonal regeneration and axonal sprouting [68, 69]. bFGF has also been successfully administrated and been able to reduce excitotoxicity but can also contribute to angiogenesis in the spinal cord [70]. Previously, agents such as thyrotropin-releasing hormone, monosialotetrahexosylganglioside and naloxone have been evaluated without any convincing results [1].

Another approach is cellular transplantation which aims at replacing lost tissue and modulating the preserved tissue [71]. Several types of stem cells have been investigated: olfactory ensheathing cells (OECs), embryonic stem cells (ESCs), oligodendrocyte precursor cells, Schwann cells, neural stem cells (NSCs) and mesenchymal stem cells (MSCs). Several beneficial modulations have been observed such as improved locomotor and hind limb recovery as well as axonal regeneration and re-myelination [71]. NSCs have mainly been used for replacing lost tissue but also to mediate trophic support to the injured- and regenerating spinal cord. MSCs have been established from adipose tissue, bone marrow, umbilical cord and evaluated for treatment of SCI due to their abundance and the fact that they are easy to access, harvest and culture and could potentially be used in autologous transplantation, even in humans. OECs are thought to be able to enhance axonal regeneration in the injured spinal cord considering that they constantly replace olfactory axons. The OECs can easily be harvested by biopsy from the nasal cavity, which could also enable autologous transplantation in humans. Schwann cells are transplanted with the intention of aiding the axonal regeneration and myelination. Activating intrinsic macrophages using pro-inflammatory agents or depleting them completely have also been evaluated repeatedly as a method for aiding regeneration and functional recovery. ESCs have mainly been transplanted following *in vitro* differentiation into neurons or oligodendrocytes. More tempting are the induced pluripotent stem cells (iPSCs) which in addition to being pluripotent enables autologous transplantation of neural- and glial cells [1].

Other approaches are focusing on exoskeletons, electrical stimulation, temperature and/or tissue engineering to enhance recovery following SCI [72, 73]. Hypothermia slows down the basic enzymatic activity resulting in reduced energy consumption and maintained ATP levels intracellularly with potentially neuroprotective effects. Systemic hypothermia (30-34°C) have therefore been evaluated as a treatment for SCI [74]. Tissue engineering in the form of three-dimensional devices composed of hydrogels, sponges or other synthetic materials shaped as guidance tubes for nerves and axons are also being evaluated [75]. The main idea of such guiding devices is that the devices can serve as a vehicle for cells or substances and/or to bridge gaps or replace damaged tissue in the spinal cord [1]. Numerous studies are also investigating the synergistic effects between various therapies mentioned above.

Partial recovery is often observed following injury, but so are complications

The severity and the level of the injury mainly determine recovery following SCI. Most of the recovery is experienced during the first 6 months following SCI, although recovery have been observed several years following the primary injury [76, 77]. Unfortunately, there are several complications which can occur in patients with SCI. Syringomyelia is an uncommon complication which can best be described as an extended cystic cavity filled with CSF and/or interstitial fluid. These patients might need surgery in which the fluid is shunted away [78]. Charcot arthropathy is a complication characterized by pain below the level of the SCI in combination with deformity of the vertebrae. Complex cases might be treated using surgery [79]. Spasticity is a common complication caused by injury to upper motor neurons resulting in a

velocity-induced increase in muscular tone. Spasticity can have significant negative impact on the mobility and reduce ventilation. Spasticity can be treated using physiotherapy, botulinum toxin injections, surgery or GABA-ergic drugs [80]. Loss of sympathetic innervation below the injury can result in orthostatic hypotension. This is observed in more than half of the patients. Common approaches for treatment of lost sympathetic innervation include compression socks, vasoconstrictive drugs or augmentation of volume [81, 82]. Autonomic dysreflexia is defined by acute and dangerous hypertension caused by stimulus below the injury level (bladder, bowel) mediating overstimulation of spinal sympathetic neurons [83]. The condition is best treated by resolving the cause for the stimulus and in more severe cases using drugs. Autonomic dysreflexia is a potential danger even for patients with chronic SCI and these patients should avoid and counteract all types of such stimuli [2]. Reduced or lost innervation to abdominal- or intercostal muscles can result in reduced ventilation and inefficient cough leading to atelectasis and pneumonia [84]. Lower quality of ventilation can have significant negative impact on the rehabilitation efforts and is a leading cause of mortality among patients with SCI [85, 86]. Immune paralysis is a phenomenon in which the immune cells become dysfunctional due to lost sympathetic innervation in combination with splenic atrophy. This might increase the rate of various infections (pneumonia, urinary tract infections) [87]. Depending on the level of the injury the detrusor and sphincter muscles might become dysfunctional [88]. The urinary dysfunction can however be managed by frequent catheterization or pharmacological therapies [89]. Patients might also suffer from various sexual dysfunctions such as reduced reflexive arousal depending on the injury type [90]. Obstipation as a result of reduced parasympathetic innervation of the intestine in combination with reduced control of the anal sphincter can significantly reduce the quality of life for individuals with SCI [91]. Pressure scores are common on the lower limbs if preventive measures are not taken [92]. Neurogenic heterotopic ossification is the formation of bone in the large joints of the lower- and upper limbs and is an ectopic formation of bone from connective tissue adjacent to the joints. The condition usually presents clinically with increased spasticity and signs of inflammation [93]. These bone formations can be managed using drugs, physical therapy, surgery or radiation [94]. Neuropathic pain is present in almost half of the patients and is thought to be a consequence of lost inhibitory signals in combination with enhanced pain reception. The pain can be managed using pharmacology (opioids) or using surgery [95]. Rehabilitation efforts in general consist of a multidisciplinary team aiming at regaining function and preventing complications. The rehabilitation can be aimed at enhancing regeneration using weight-supported locomotor training or functional electrical stimulation in skeletal muscles [2].

Mortality is increased and life expectancy is reduced

A SCI implies increased mortality for the troubled individual. During the first year following the injury about 4 % of the patients perish [96]. In particular, life expectancy is greatly reduced and the mortality increased for patients suffering a *cervical* SCI. The mortality increases with high age, presence of comorbidities and/or additional injuries acquired in relation to the SCI [97].

Traumatic spinal cord injury is commonly simulated and studied in rodents

A suitable model of SCI is one that can generate results that can be translated to or be interpreted in the context of clinical therapy for humans. Thus, the animal model should have significant similarities to humans in terms of anatomy and pathophysiology. Reproducibility and consistency of results is also a key feature of a useful animal model of SCI [2]. Importantly, the animal type, location and type of SCI should be adapted to the hypothesis stated [98]. Although the path from rodent research to human therapy is long and translation is difficult, rodent models of SCI (rat, mouse) are commonly used. Rats constitute the most common type of research animal used in SCI (72 %) followed by mice (16 %) [99]. These models are preferable since the tissue response is similar to that in humans and the accessibility, in comparison to primate models, is high [100]. Mice, in comparison to rats, have become increasingly popular due to the availability of transgenic animals which allow for detailed analysis of selected genes and/or pathways [1]. Other much less common models include rabbit, dog, cat, pig, non-human primate and guinea pig [99]. However, promising results detected in one animal type should be evaluated in several animal types, including primates, prior to translation into clinical research as a measure of safety [2].

Mechanical injuries are more commonly studied as compared to non-mechanical injuries. Even though cervical injuries are more common than thoracic injuries in humans [2] thoracic injuries are more commonly studied in animals (~80 %) as compared to cervical injuries (~12 %) [99]. Mechanical traumatic SCI can be induced using a contusion (rapid one-hit injury), compression (prolonged exposure to force) or transection model (partial- or complete cut) [2]. The most common model of mechanical SCI in animals is the contusion injury model (~43 %) followed by transection (34 %) and compression injury (20 %) [99]. The contusion model is the most realistic model and can be induced using a pneumatic impactor or by weight drop. The New York University impactor [101] or the commercially available Infinite Horizon Impactor allows for induction of a precise and reproducible contusion SCI [1]. The weight drop model is the most common approach (38 %) followed by the New York University Impactor (27 %) and the Infinite Horizon Impactor (21 %) [99]. The impactors also record the exact force and displacement as well as the dynamics of the injury [1]. Compression injury involves applying a specific amount of force for a specific amount of time using an aneurysm clip, a balloon or similar device [102]. For compression type SCI an aneurysm clip is most commonly used followed by balloon compression [99]. The compression model is particularly suitable for investigation of the effects of treatment in relation to the injury severity [103]. Transection injury involves cutting the spinal cord using a sharp instrument. The cut can be total or aim for specific parts/tracts of the spinal cord. Approximately the same number of hemi/partial and complete transection injuries are conducted [99]. The transection model is best suited for the study of axonal regeneration and/or implantation of devices. The transection model however does not properly represent the SCIs observed in the clinical setting [1]. Although non-mechanical injuries are not within the scope of this thesis it is worth mentioning that the most common type of non-mechanical injury is ischemic injury [99].

The evaluation post SCI should naturally be adapted to the hypothesis. Motor function can be evaluated using the Basso, Beattie and Bresnahan (BBB) score in rats, the Basso Mouse Scale (BMS) in mice, runway walking and recording of dynamical and static aspects of movement, ladder- or grid test for evaluation of coordination. The BBB score is one of the most commonly used evaluations and was designed for thoracic SCI and is considered by many to be reproducible, reliable and produces a clinically relevant measure [104]. The development of gait

analysis using video recordings has allowed for more accurate measurement of locomotion (e.g. stride width, stride length, stepping pattern, velocity) [105, 106]. This type of analysis allows for a detailed measurement of locomotor function and collection of data for several evaluations at the same time. However, data collection and analysis is time consuming and interpretation of the measurements is far from easy. Therefore, in recent years efforts have been made to automate the translation of video recordings to data sets [107]. The horizontal ladder walking test assesses the animals ability to place its limbs on irregularly placed bars but it requires training and cannot be used in animals with paraplegia [108]. The strength in the hind limbs can be measured using an inclined plane test. Sensory outcome is equally important. Temperature sensation can be evaluated using the tail-flick test, allodynia can be evaluated using the von Frey Filament test, Hargreaves test or the hot plate test. The hot plate test evaluates the NMDA-receptors by evaluating sensitivity to temperature [109]. The von Frey Filament test involves stimulating the plantar surface of the paws using a filament and observing the retraction of the limb due to allodynia. However, sensory evaluations are difficult to interpret since all of them require a motor reaction, which can itself be compromised due to the SCI [1]. Evaluation of the tissue and cells is commonly performed using immunohistochemistry. Anterograde (biotinylated dextran amine, cholera toxin B subunit) or retrograde (Fluoro-Gold, Fast Blue) tracing can be used for studying axonal regeneration [1]. Repair and regeneration of the corticospinal tract can also be measured using motor-evoked potentials (MEPs) by stimulating the sensorimotor cortex and conducting electromyography in hind limb muscles below the injury level. Somatosensory-evoked potentials (SEPs) are measured by stimulating peripheral nerves (tibia nerve, sciatic nerve) and recording the activity in the sensory cortex. SEPs measure the conductance along the dorsal column [110].

The immune system

The immune system is composed of a structural barrier as well as cell-mediated and antibody-mediated immune response. The structural barrier is a mechanical barrier but also a biochemical barrier inhibiting foreign and potentially harmful bacteria, viruses and microbes to enter. Immune cells and chemical processes make up the cell- and antibody mediated response. The innate immune system responds first and do so without memory or antibodies. The adaptive immune system is activated afterwards and acts using antibodies directed towards certain and specific antigens [111, 112].

The innate immune system is fast but has no memory

Innate immunity is a non-specific no-memory rapid action defense mechanism composed of four parts: anatomical- and mechanical defense (skin, mucosa), physiological response (temperature, pH), phagocytosis and inflammation. The innate immune system relies on pattern recognition receptors (PRR). These receptors are present on immune cells and detect pathogen associated molecular patterns (PAMPs, e.g. LPS in bacteria or RNA in viruses). Following detection of pathogens the innate immune system responds by releasing cytokines and chemokines (e.g. TNF-α, IL-1, IL-6) which recruits immune cells and mediates inflammation in the affected tissue. The cytokines and the immune cells alter the pain sensitivity and vascular permeability in the tissue. The innate immune system is also capable of identifying and labelling pathogens or cells for phagocytosis, thereby clearing cells, debris and antibody-complexes that might be present in the tissue, blood or the lymphatic system. The innate immune response involve cell types such as dendritic cells (DCs), macrophages, granulocytes (neutrophils, eosinophils, basophils), mast cells, natural killer cells (NK-cells), monocytes and innate lymphoid cells (ILCs). Dendritic cells (DCs) bind peptide fragments of antigens by means of major histocompatibility complex receptors (MHC). Thereafter, the cells migrate to regional lymph nodes in which they present the peptide fragments to naive T-cells. Consequently, the naive T-cells undergo proliferation and differentiation into helper T-cells and cytotoxic T-cells [111-113]. Both macrophages and neutrophils have the ability to phagocytose debris, dead cells or pathogens. The macrophages but also the DCs can recruit T-cells by acting as antigen presenting cells (APCs). Fibroblasts and B-cells can also act as APCs. Macrophages are found in many forms and are termed microglia if resident in the CNS. Mast cells are present in tissues surrounding blood vessels and initiate an acute inflammatory response by secretion of cytokines. Eosinophils mediate the destruction of pathogens, which are too large to be phagocytized. NK-cells are important for destruction of cells infected with viruses as well as for rejection of tumor cells. These cells also release perforins and granzymes, which induces apoptosis in target cells. Moreover, NK-cells are important for the activation of APCs and do so by releasing IFN-γ. The ILCs produce cytokines adapted to the pathogen that is to be eliminated or acted against. Inflammation is defined as the process during which immune cells release pro-inflammatory cytokines and chemokines (e.g. TNF-α, IL-1, IL-6) which recruits inflammatory cells (neutrophils, monocytes) [111-113].

The adaptive immune system acts by direct targeting and antibodies

The adaptive immune system consists of T- and B-cells. T-cells arise from the bone marrow, mature in the thymus and carry T-cells receptors (TCRs). Each T-cell carry only one type of TCR. APCs are required to activate the T-cells and express major histocompatibility complex (MHC) proteins on their cell surface. MHC-I is expressed by all nucleated cells in the body while MHC-II is expressed only by B-cells, DCs and macrophages. MHC-I presents molecules from within cells affected by pathogens or cells that have been phagocytized. MHC-II presents molecules not originating from the APC itself. T-cells are circulating in the blood and lymph. The APCs and T-cells aggregate in lymph nodes in which the APCs present antigens to T-cells and thereby activates the T-cells. Upon ligation between the MHC-complex of the APC and the T-cell, the T-cells secrete cytokines and differentiate into $CD8^+$ (cytotoxic) or $CD4^+$ (helper) T-cells. The $CD8^+$ T-cells mediate their action by interaction between their TCR and the MHC-I of target cells and can for example induce apoptosis in the target cells. The majority of the effector cells are phagocytized when they have mediated their desired effect, but a few remain as memory T-cells. These memory T-cells can rapidly differentiate into effector cells given that the same antigen is presented once again. The helper T-cells are activated when their TCR binds to MHC-II which carries antigen. Helper T-cells mediate their action by releasing cytokines into the surrounding tissue, which activate B-cells, macrophages and promotes inflammation. The most common types of helper T-cells are Th1, Th2 and Th17 although many other types exist. The Th1 cells release IFN-γ, which mainly activates macrophages and results in elimination of intracellular pathogens. The Th1 cells also enhance the differentiation of B-cells into plasma cells and thereby contribute to antibody production and efficient phagocytosis. The helper T-cells perish following the acute immune response and leave a small amount of helper T-cells that develop into memory helper T-cells. T-regulatory cells are a subtype of $CD4^+$ T-cells and aid in the suppression of the immune response and especially immune response to self-antigens.

B-cells originate from hematopoietic stem cells and maturate in the bone marrow. B-cells carry antibodies on their cell surface and can recognize pathogens by themselves without the need for APCs. Upon ligation of these surface antibodies, the B-cells differentiate into plasma cells (effector cells) and produce antibodies (IgD, IgM, IgG, IgE and IgA). At the same time the B-cells also differentiate into memory B-cells that are similar to memory T-cells in the sense that they can rapidly act against similar pathogens should they encounter them again. Cytokines (e.g. IL-6) secreted by helper T-cells enhance proliferation and differentiation of B-cells into plasma cells but also aid them in developing the correct type of antibodies. The antibodies produced by the plasma cells adhere to the pathogens and label them for elimination through either phagocytosis or complement activation. Plasma cells undergo apoptosis when they have mediated their action [111, 112, 114].

Microglia are the resident macrophages of the central nervous system

Microglia were first described and defined by Del Rio-Hortega P (1932). He defined nine criteria that a macrophage has to fulfill in order to be called microglia. Microglia are cells with mesodermal origin (I), which enter the CNS during early development (II) and migrate along blood vessels and tracts in the white matter by which they spread in the entire CNS (III) and distribute themselves evenly (IV). These cells acquire a ramified morphology during maturation, which they maintain in homeostasis (V). Each microglia have a defined compartment in which they rest (VI). Following injury or insult to the CNS microglia undergo activation (VII) and

gain an amoeboid morphology (VIII). Following activation microglia proliferate, migrate to the site of injury and phagocytose debris and cells undergoing necrosis (IX) [115].

Microglia are derived either from monocytes which are progenitors originating from the bone marrow [116-119], or from fetal macrophages with an amoeboid morphology which enter the CNS during the first- and second trimester [117-119]. In adults, the population of resting microglia is stable and is not undergoing any exchange [120, 121]. Resting microglia are said to have a ramified morphology and are characterized by having a small soma with delicate processes [122-132]. The mechanism controlling the transformation from amoeboid into a ramified morphology is not fully understood, but seems to be mediated by factors released from astrocytes (e.g. TGF-β, M-CSF, GM-CSF) and/or the existence of Cl^- channels on microglia [130-132]. The processes are utilized to scan the surroundings and enable rapid response to injury or pathology in the CNS resulting in activation [133, 134]. However, the housekeeping activity of microglia is mainly unknown [115]. Microglia are activated upon trauma, infection, ischemia, degenerative disorder or other alterations in the homeostasis of the CNS resulting in altered neuronal activity [122-129]. During activation microglia regain their amoeboid morphology which is characterized by a retraction of the processes into the soma and thereby a simplification of the morphology [135-137]. The transformation from a ramified morphology to an amoeboid morphology during activation is distinct [136] but the exact morphology of activated microglia can be of various types. Reports have indicated spindle- or rod like shapes or even bi- or tripolar shapes [138, 139]. Additionally, microglia alters their expression of surface markers and secretion of immunomodulatory compounds. Their phagocytic activity and production of neurotrophic factors is also elevated [123, 124, 126]. The activation however is heterogeneous in the sense that microglia can take many different functional states [126, 140]. This results in microglia having regional differences in morphology, proliferation rate, expression of surface markers and secretion of factors [137, 141-145]. The functional properties and expression profile of microglia seem to differ with localization in the CNS (white- or gray matter). The level of myelin and/or oligodendrocytes seems to determine their functional properties [146]. The functional state does not necessarily correlate with the morphology of microglia [147-149]. Microglia can be detected with markers such as IBA1 [150, 151], CD16, CD32, CD45, CD11B, CD169, CD206 and F4/80 [115]. However, these markers also label macrophages [141]. CD45 can be used to separate microglia from macrophages in the sense that microglia express low- or intermediate levels while macrophages express higher levels of CD45 [152] while both are positive for CD11B [115].

Mesenchymal stem cells can activate microglia alternatively

Microglia stimulated with lipopolysaccharide (LPS) are alternatively activated after exposure to mesenchymal stem cells (MSCs) [153-156]. The LPS induced round morphology of microglia can be shifted to a ramified morphology by colony-stimulating factor-1 (present in MSC-conditioned medium) [157]. Alternative activation of microglia has also been reported following exposure of stimulated microglia to MSC-conditioned cell culture medium [157, 158]. The alternative activation has been correlated to the suppression of pro-inflammatory cytokines and chemokines (e.g. TNF-α, IL-1β, IL-6, iNOS, NO) [153, 155-160]. The alternative activation has been suggested to be mediated by suppression of the phosphorylation of MAPK, ERK1/2 and JNKs [153]. The alternative activation caused by MSC-conditioned medium is due to TGF-β mediated inhibition of the NF-κβ pathway [158]. The alternative activation induced by MSC-conditioned medium has also been proposed to be a consequence of activation of the PI3Ks

pathway as well as *Rac1* and *Cdc42* [157]. Moreover, MSCs can increase the phagocytic activity [154, 156, 158], reduce the rate of proliferation [160-162] and elevate the expression of neurotrophic factors (IGF-1, BDNF) from stimulated microglia [155]. Secretion of CX3CL1 from MSCs in an inflammatory environment can shift the phenotype of microglia from detrimental to neuroprotective [154]. Following SCI, transplanted MSCs can decrease the recruitment of microglia to the injury epicenter [163-165] and reduce the number of reactive microglia in total [166-169]. MSCs transplanted into SCI alternatively activate microglia and thereby reduce the phagocytic activity of microglia [170, 171]. This has been correlated to the reduction in secretion of pro-inflammatory cytokines and chemokines from microglia [171].

Stem cells

The common denominator for stem cells is that they can proliferate (self-renew) and differentiate (transform into specific somatic cells) [172]. Stem cells are divided into five major categories based on their differentiation potential: totipotent/omnipotent, pluripotent, multipotent, oligopotent- and unipotent cells. Totipotent cells can differentiate into embryonal- or extra-embryonal tissue and thereby form a new viable organism. Pluripotent stem cells can differentiate into embryonal cells and thereby form all/any of the three germ layers. Multipotent cells can differentiate into cells of one specific germ layer. Oligopotent cells are similar to multipotent stem cells but with an even more limited range of differentiation while unipotent cells can form only one type of somatic cells. Stem cells can also be categorized based on the tissue from which they are established: embryonic, fetal, perinatal, adult- and induced pluripotent stem cells [172].

Embryonic- and fetal stem cells

The product of an oocyte merging with a spermatozoon is a zygote and is totipotent. Totipotent cells can give rise to both extra-embryonal- (embryonic membranes and placenta) and embryonal cells. The zygote undergoes 32-64 cell divisions forming a morula and eventually a blastocyst at about 5 days following fertilization. The blastocyst consists of a trophoblast which encapsulates the inner cell mass. The trophoblast forms the embryonic membranes and placenta while the inner cells mass develops into the fetus. The inner cell mass is pluripotent and can thus form cells belonging to any of the three germ layers (endoderm, ectoderm and mesoderm) [173]. Therefore, embryonic stem cells (ESCs) are established from the inner cell mass of the blastocyst [174]. ESCs are characterized in terms of surface markers ALP, CD24, CD9 and in terms of gene expression of *Sox2*, *Oct4*, *Nanog*, *Thy1* and *Rex1* [175]. The benefit of using ESCs is an unlimited supply of cells as well as the potential to create and replace any type of cell in the adult. The limitation comes from the ethical issue of harvesting human embryos, risk of teratoma formation and need for allogeneic transplantation [173]. Fetal stem cells can be harvested from embryos of aborted fetuses, but are not as potent as ESCs [172].

Perinatal stem cells

These cells can be harvested from three areas: amniotic fluid, umbilical cord (cord blood or Wharton's jelly) or placenta (villi, blood and amnion). The cells in the amniotic fluid are mainly fetal epithelial cells originating from the fetal skin and seem to be multipotent. Cells established from the placenta have many similarities with mesenchymal- and hematopoietic stem cells. The mesenchymal component (fibroblasts) of Wharton's jelly in the umbilical cord are thought to mediate the stem cell effect of this tissue [172].

Adult/somatic stem cells

Adult stem cells can self-renew but have a more specific differentiation potential. These stem cells aim at replacing cells lost due to pathology or in physiological processes in the adult [173]. The differentiation potential and properties of the stem cell is controlled by their niche, which is defined as the extracellular matrix, surrounding cells and signal substances. The stem cell characteristics are maintained only when the adult stem cells are associated with the niche. Adult stem cells undergo asymmetric cell division through which the stem cells are maintained but also eventually form a committed precursor/progenitor cell which is underway to becoming a somatic/differentiated cell (**Figure 1**) [176]. Adult stem cells associated with neural, epithelial, hematopoietic- and muscular tissue have been characterized most extensively [177].

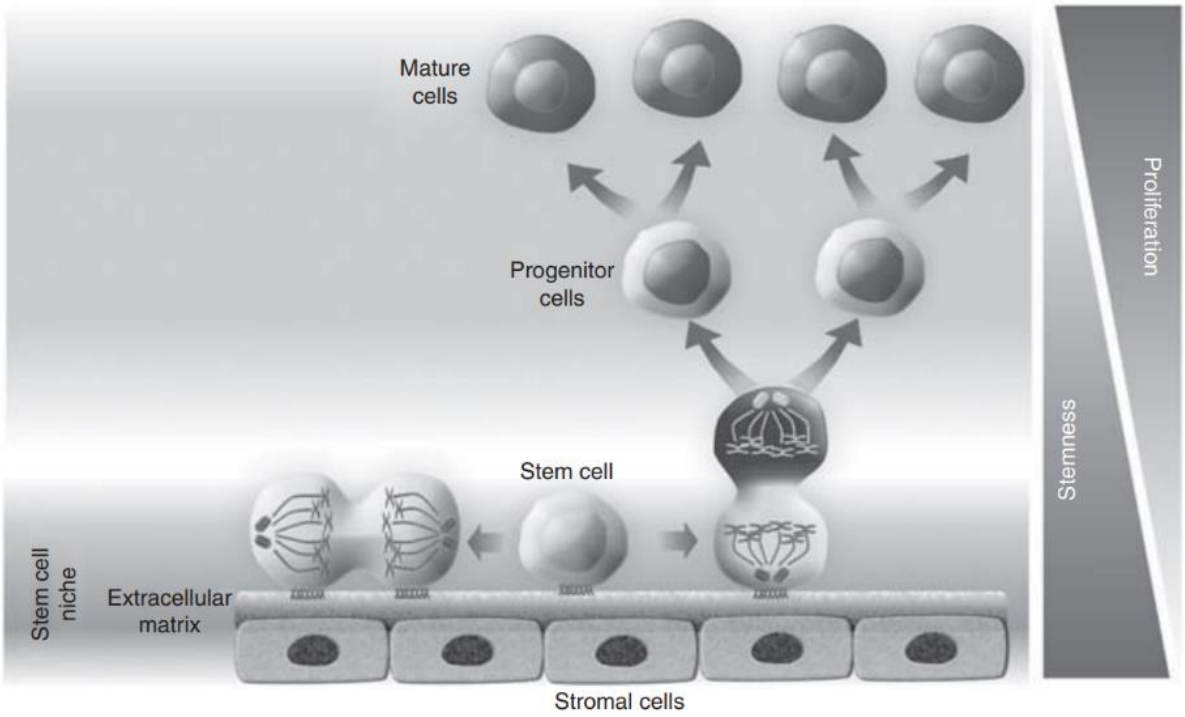

Figure 1. The adult stem cell niche. The adult stem cell niche is a microenvironment consisting of signal substances, extracellular matrix and stromal cells. The microenvironment in combination with the intrinsic characteristics of the cells determines their differentiation potential. The stem cells in the niche maintain their population by cell division and at the same time give rise to progenitor/precursor cells, which eventually differentiate and form mature cells.

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Induced pluripotent stem cells

Somatic cells can be re-programmed into becoming pluripotent stem cells (induced pluripotent stem cells, iPSCs) by using transcriptions factors (*Klf4*, *Myc*, *Oct4*, *Sox2*) [178, 179]. The iPSCs are similar to ESCs in terms of growth dynamics, gene- and protein expression as well as differentiation potential. The iPSCs offer a method for producing pluripotent cells that can be used for autologous cell transplantation. iPSCs might solve several ethical- and immunological issues related to the usage of human-derived ESCs in clinical therapy and trials [173].

Neural stem cells

The neural stem cell niche

Neural stem cells (NSCs) are located in the ventricular-subventricular zone (V-SVZ) in the lateral wall of the fourth ventricle or in the subgranular zone (SGZ) of the hippocampus [180-183]. NSCs can differentiate into neurons in the olfactory bulb and in the hippocampus and adopt morphological- and electrophysiological characteristics similar to existing neurons [184-188]. The NSCs located in the SGZ form excitatory neurons mediating pattern recognition, learning and memory [180-183] while the NSCs in the V-SVZ form interneurons in the olfactory bulb [189]. NSCs were initially identified as a subtype of astrocytes and are therefore in some contexts termed radial astrocytes, radial cells, neural/type-1 progenitors or radial glia-like cells [190, 191]. In the developing brain, the NSCs take the form of radial glia [192, 193], which is the precursor cell for most of the neurons, and glia in the embryo. In the adult brain the NSCs take the form of B1-cells [194] but still share many characteristics with radial glia [195, 196]. The NSCs reside in a niche [176] which is defined by immature- and mature NSCs as well as by ependymal- and vascular cells [197]. The V-SVZ has three domains, each with different cell composition: I) the ependymal cell layer and the apical process of B-cells, II) the A-, B-, and C-cells and III) the basal processes of the B-cells with associated vasculature (**Figure 2**).

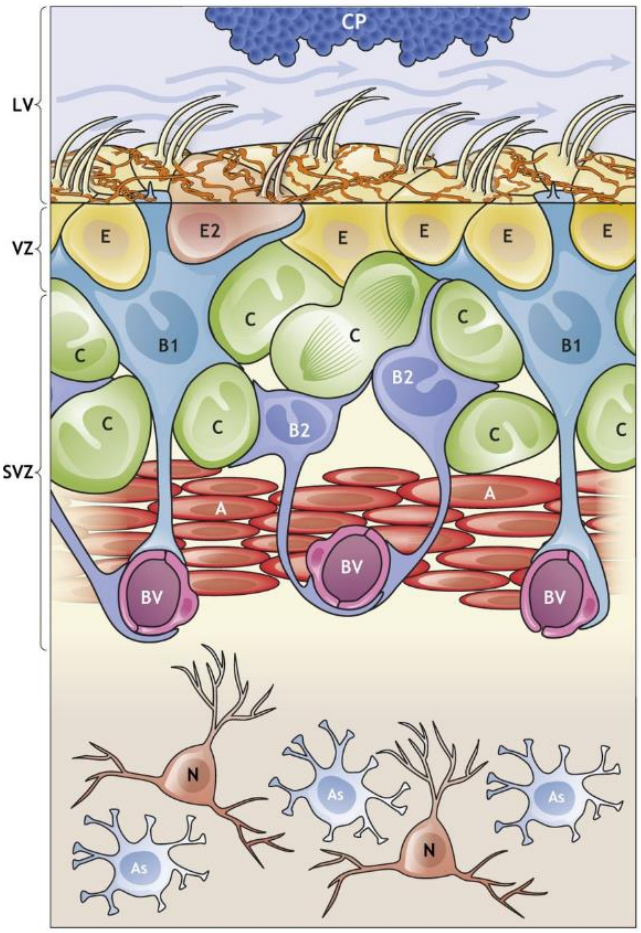

Figure 2. The ventricular-subventricular zone (V-SVZ) in the lateral wall of the fourth ventricle in the adult brain is the neural stem cell (NSC) niche. B1-cells: NSCs; B2-cells: derived from B1-cells; C-cells: transient-amplifying cells; A-cells: migratory neuroblasts; CP: choroid plexus; E/E2-cells: ependymal cells; BV: blood vessel; As: astrocyte; N: neuron. Supraependymal axons are indicated with orange color.

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B1-cells give rise to interneurons in the olfactory bulb

The B1-cells generate transient amplifying intermediate progenitors (C-cells) [194] which undergo proliferation and eventually form migratory neuroblasts (A-cells) which migrate towards the olfactory bulb [198]. The A-cells migrate by chain migration along the rostral migratory stream (RMS), which is a network of interconnecting paths [199], to the olfactory bulb [199-201]. The neuroblasts divide 1-2 times during their migration along the RMS [198]. In the

olfactory bulb, the A-cells differentiate into interneurons, integrate into the circuitry [202, 203] and contribute to odor-reward association and fine odor discrimination [180, 204-207]. Six subtypes of interneurons in the olfactory bulb have so far been described: $CalB^+$ periglomerular cells ($CalB^+$PGCs), $CalR^+$PGCs, deep granule cells (GCs), $CalR^+$GCs, superficial GCs and TH^+PGCs. The interneurons are derived from other regions in addition to the lateral ventricle such as the pallium [208] as well as from the RMS and the septum [208, 209]. The formation of A-cells, migration and differentiation into interneurons takes about 2 weeks. This indicates that the signals controlling the proliferation of NSCs in the V-SVZ must have come well in advance in relation to the need for new interneurons in the olfactory bulb. The number of NSCs decrease during aging, which results in a reduced level of neurogenesis. The reduced ability of neurogenesis is likely a consequence of protein aggregates forming due to lower lysosomal activity [210, 211]. Migrating neurons in infants can be found in the RMS but also in the frontal lobe [212, 213]. However, in the adult the migrating neurons are found in the lateral walls of the ventricles but not in the RMS or the frontal lobes [212, 214]. The existence of neurogenesis in the adult brain however is debated and while some authors report a distinct neurogenesis [215, 216] others report the opposite [217, 218]. The main function of NSCs seems to be a source of plasticity [182].

B1-cells are a heterogeneous population of cells

B1-cells can give rise to B2-cells [189, 219] which, in comparison to the B1-cells, have processes reaching blood vessels but not the ventricle [220]. The B2-cells are proliferative but their function is not yet fully understood [221]. The number of B1-cells decrease with age but the supply of A-cells and interneurons to the olfactory bulb is essentially unchanged indicating a transformation of B1-cells into B2-cells [189]. In addition to forming A-cells, the B1-cells can also differentiate into oligodendrocytes [222, 223]. These oligodendrocytes migrate to and myelinate axons in the corpus callosum [224]. The myelinating abilities of these oligodendrocytes increase following injury to the CNS [225]. However, uncertainty remains concerning the *in vivo* differentiation potential of NSCs in the V-SVZ. *In vivo* studies have indicated that only neuronal differentiation is possible [226] but *in vitro* studies have indicated that NSCs can differentiate into both neurons and oligodendrocytes [227]. There is heterogeneity within the B1-cell population. One subset of radial glia diverts during development (mid-embryonic phase) and starts to express VCAM1 which is essential for maintaining stem cell characteristics [228, 229]. Another subset of radial glia starts to express GFAP during development and form pre-B1-cells, which do not proliferate until activated by the need for A-cells [230, 231]. The type of interneuron which the B1-cells actually give rise to depends on their location on the ventricle wall along the ventral/dorsal and anterior/posterior direction [232-234]. The region itself does not determine the fate of the B1-cells but this is intrinsic to the cells [232]. NSCs in the ventral part of the V-SVZ form deep granule cells and periglomerular cells expressing calbindin while NSCs in the dorsal part of the V-SVZ differentiate into superficial granule- and periglomerular cells expressing tyrosine-hydroxylase [232, 235].

B1-cells are regulated by a complex microenvironment of cells, substances and nerves

The differentiation and renewal of the B1-cells in the V-SVZ is affected by signaling molecules from neighboring cells in the CNS, the CSF as well as blood- and epithelial cells [236].

Furthermore, NSCs in the adult brain release factors and act in an autocrine and paracrine fashion [237]. NSCs communicate with each other using gap junctions and with other cell types using cell-cell-interactions [238]. NSCs release substances (GABA, Dll1, Notch-ligand) to maintain the NSCs in the quiescent state [239]. Release of GABA from A-cells decreases the rate of proliferation of B1-cells [240] while diazepam-binding inhibitor protein released from B1- and/or C-cells accelerate proliferation of B1-cells [237]. The B1-cells are actively communicating with each other by exchanging signals and this might control the rate of proliferation [236]. The B1-cells also interact and exchange information with cells in their surrounding by extending and retracting processes [189].

NSCs are affected by signaling molecules transported to the V-SVZ through the blood stream and detected by the basal processes but also by signaling molecules secreted by the endothelial cells in the blood vessels [241]. The endothelial cells in general seem to have an inhibiting effect on the proliferation (G0/G1-arrest) of the cells in the V-SVZ [242-244]. On the other hand, betacellulin (EGF-like growth factor) released by the endothelial cells can increase proliferation of B1- and C-cells resulting in increased neurogenesis [245]. Betacellulin can also promote proliferation and production of migratory neuroblasts [245]. Moreover, the vasculature of the V-SVZ contributes to the migration of A-cells along the RMS [246, 247]. The endothelial cells also release VEGF and NT-3 which result in NSC self-renewal and maintenance of NSCs in a quiescent state [241]. The interaction between B1-cells and blood vessels seems to be dynamic in the sense that the processes extending from B1-cells to the blood vessels can move along the vessels [189]. In the NSC-niche the blood brain barrier is less dense in comparison to other areas of the brain which results in NSCs having a high access to substances transported by the blood stream [248].

The apical process of B1-cells extends through the ependymal cell layer and is in touch with the ventricle. This single apical cilium is not motile, which is in contrast to the cilia of the ependymal cells [194], but is instead similar to the cilia of radial glial cells [207]. Ependymal cells can also produce regulating substances such as SDF-1 and PEDF [243, 249]. The ependymal cells adjacent to the B1-cells release Noggin which acts as an antagonist to BMP-4 which results in increased proliferation of B1-cells enhancing neurogenesis [242, 250]. BMP-signaling in the V-SVZ can also promote differentiation of the NSCs into glial cells instead of undergoing neurogenesis [250-252]. The CSF carries signaling molecules such as BMP, WNT, RA, FGF, LIF and SHH [253] which regulate the cells in the V-SVZ [254]. WNT can maintain NSCs in their stem cell condition [255] which is also true for SHH but the levels thereof has to be tightly regulated in order to avoid depletion of the NSCs following a rapid expansion [256]. The B1-cells sense these molecules using their processes, which are in contact with the ventricles [257]. The presence of IL-1β in the CSF results in reduced proliferation of NSCs and elevated expression of VCAM1 on NSCs [229]. The choroid plexus secretome is however altered during aging [258] and the concentration of IGF2, which enhances proliferation of NSCs, is reduced in the CSF with aging [254]. Moreover, the flow of CSF guide the migration of the A-cells along the RMS [259].

Supraependymal axons originating in the dorsal raphe nucleus form a dense network in the apical surface of the V-SVZ. Serotonin brought by these serotonergic axons to the V-SVZ through this pathway can enhance the proliferation of B1-cells [260, 261]. The 5-HT axons form a plexus in the V-SVZ, are in touch with B1- and ependymal cells and regulate neurogenesis and proliferation [260-262]. Neurons originating from the ventral tegmental area and the substantia nigra also project axons to the V-SVZ and mediate proliferation of NSCs and thereby neurogenesis [263, 264]. GABA-A released from neurons which project into the V-SVZ

can promote the proliferation of NSCs [265, 266]. Glutamate released from striatal neurons can enhance the proliferation of A-cells by activating AMPA receptors [267]. C-cells express dopaminergic receptors, receive dopaminergic innervation from the midbrain and regulate- and enhance neurogenesis and seems to be mediated by EGF [268, 269].

The extracellular matrix (ECM) is in close connection with the V-SVZ and interacts using adhesion molecules (VCAM1) [229]. Fractones in the ECM can collect circulating growth factors and present these to the NSCs [270]. The ECM can thereby interact with the NSCs and control their behavior [271]. Fractones can also release FGF2 that promotes proliferation of B1-cells. The B1-cells themselves can control the release of FGF2 from fractones [270, 272]. The presence of microglia in the V-SVZ increases the rate of neurogenesis and oligodendrogenesis and at the same time suppresses expression of cytokines. In the postnatal phase, the presence of microglia is higher in the brain, which correlates with the higher level of neurogenesis and oligodendrogenesis [273-275]. The microglia in the V-SVZ in the uninjured adult have, in comparison to the remaining CNS parenchyma, an activated phenotype [142, 276]. The microglia in the V-SVZ seem to enhance and contribute to the migration of A-cells along the RMS [277]. Astrocytes can affect the differentiation of NSCs by expressing ephrinB2 [238] similar to epithelial cells which can promote a quiescent state of NSCs by expressing ephrinB2 but also by expressing Jagged1 [244]. Moreover, astrocytes can promote differentiation of NSCs by secreting cytokines (IL-1β, IL-6) [278].

Quiescent and active form of neural stem cells

Two subtypes of NSCs have been defined in the V-SVZ: quiescent- (qNSCs) and active NSCs (aNSCs) (**Figure 3**). In addition, several subtypes of qNSCs and aNSCs have been described [279, 280]. NSCs in the adult are mainly in the quiescent state [194]. Both these subtypes express GFAP, BLBP, GLAST and prominin but only aNSCs express the EGFR and Nestin [281, 282]. qNSCs have a lower rate of protein synthesis but have larger lysosomes as compared to aNSCs which enables them to efficiently eliminate protein aggregates [283]. *In vitro* propagation of qNSCs result in far less neurospheres as compared to aNSCs. Both qNSCs and aNSCs have processes which are in touch with the ventricle but only qNSCs express sphingosine-1-phosphate and prostaglandin-D2 which can respond to substances present in the CSF [281]. The aNSCs express genes related to cell-cycle regulation and repair of DNA while qNSCs express genes related to cell-cell-adhesion and signaling [284]. GABA can maintain NSCs in a quiescent state by inhibiting cell-cycle progression [285] and is regulated through a feedback-loop [240]. NSCs have been shown to be able to transition from qNSCs into aNSCs and to differentiate selectively [286]. Activation of qNSCs results in up-regulation of Nestin and EGFR in the qNSCs [281]. When the qNSCs transition into aNSCs the cells down-regulate genes related to glycolysis and up-regulate genes related to oxidative phosphorylation [279]. When qNSCs are exposed to EGF and FGF the cells increase their lysosomal activity which results in activation and transformation into aNSCs [283].

Transcriptional plasticity and epigenetic control of neural stem cells

Transcriptional plasticity regulates the lineage specification of stem cells and is mediated by changes in the chromatin state which promotes or suppresses transcription [287]. This transcriptional regulation mediated by chromatin controls the neurogenesis mediated by the B1-cells in the V-SVZ [288]. ATP mediated hydrolysis altering the chromatin structure promotes

transcriptional programs mediating neurogenesis (*Pax6* and *Brg1* interaction) [289]. Histone acetylation mediated by transcription factors (*Bmi1*, *Ezh2*) are also important for neurogenesis, proliferation and self-renewal [290-292]. Nitric oxide (NO) is a key mediator of the innate immune response and direct NSCs in culture conditions to undergo glial differentiation using the *NRSF/ REST* transcription factor [293]. Moreover, DNA methylation is also important for neurogenesis [294]. Methyl-CpG binding proteins can bind to methylated regions and regulate cell division, neurogenesis as well as differentiation by regulating micro RNAs (miRNAs) [295-297]. Non-coding RNA (miRNA, long non-coding RNA) are also important for regulation of neurogenesis of NSCs in the adult [298]. *mi-137* can for example enhance proliferation of NSCs and prevent their differentiation by modifying *Ezh2* [297]. Long non-coding RNAs such as *Six30* and *Dlx1* can enhance and direct the differentiation of NSCs towards astrocytes [299]. The regional differences in the types of interneurons in the olfactory bulb is likely regulated by transcription factors (e.g. *Emx1, Nkx6.2, Pax6, Sp8*) [235, 300] which are partially controlled by miRNAs (*mi-7a, mi-124*) [297, 301].

The transcriptome of NSCs is regulated by transcription factors [302]. qNSCs express transcription factors such as *ID2, ID3* and *Sox9* as well as the Notch2-receptor [279]. aNSCs express the transcription factor *Ascl1* that regulates several pathways in aNSCs including neuronal differentiation [303, 304]. Some of the B1-cells also express *Ascl1* which can promote differentiation into neural- and glial cells [305]. *Ascl1* can also activate NSC cell-cycle regulation [304]. A subpopulation of B- and C-cells express *Olig2*, which promotes differentiation into oligodendrocytes as compared to neuroblasts [306-308]. C-cells in the V-SVZ also express *Ascl1* and use a Notch-feedback loop to regulate the quiescent state of NSCs [309]. Hence, notch-receptor activation results in increased proliferation and neurogenesis by repressing genes inhibiting neural differentiation [310]. In this way, Notch-signaling helps to maintain the pool of undifferentiated qNSCs in the V-SVZ. Thus, notch-signaling preserves NSCs in their stem cell niche and the lack of Notch-signaling results in NSCs entering a more committed progenitor stage [311]. The qNSCs also express *Notch3* which is essential for maintaining the cells in an undifferentiated state [312]. On the other hand, aNSCs and C-cells express *Notch1* and *Ascl1*, which are important for maintaining proliferation of NSCs [313]. *Sox2* is a key transcription factor and is expressed by all cell types in the V-SVZ [314]. *Sox2* interacts with transcription factors such as *Prx1, Ars2* and *Tlx* that result in control of self-renewal and proliferation of B1-cells in the V-SVZ [315, 316]. *Sox2* is essential for maintaining the NSC population and the expression of *Sox2* is regulated by Notch-signaling [302].

Symmetric- and asymmetric cell division

The type of cell division that the NSCs undergo depends on the current developmental stage of the individual. In the early embryonic stage the NSCs undergo symmetric self-renewal [207] while radial glia undergo asymmetric cell division. Asymmetric cell division results in self-renewal as well as fully differentiated neurons [317, 318]. Moreover, NSCs can self-renew for an extended period of time in the V-SVZ by undergoing symmetrical cell division [189]. GLAST$^+$NSCs cannot self-renew for an extended period of time since the cells after a few cycles of symmetric cell division end up in asymmetric cell division [226]. GFAP$^+$NSCs undergo asymmetric cell division, which results in self-renewal or differentiation. With time, these cells undergo more consuming divisions reducing the number of B1-cells [189, 319].

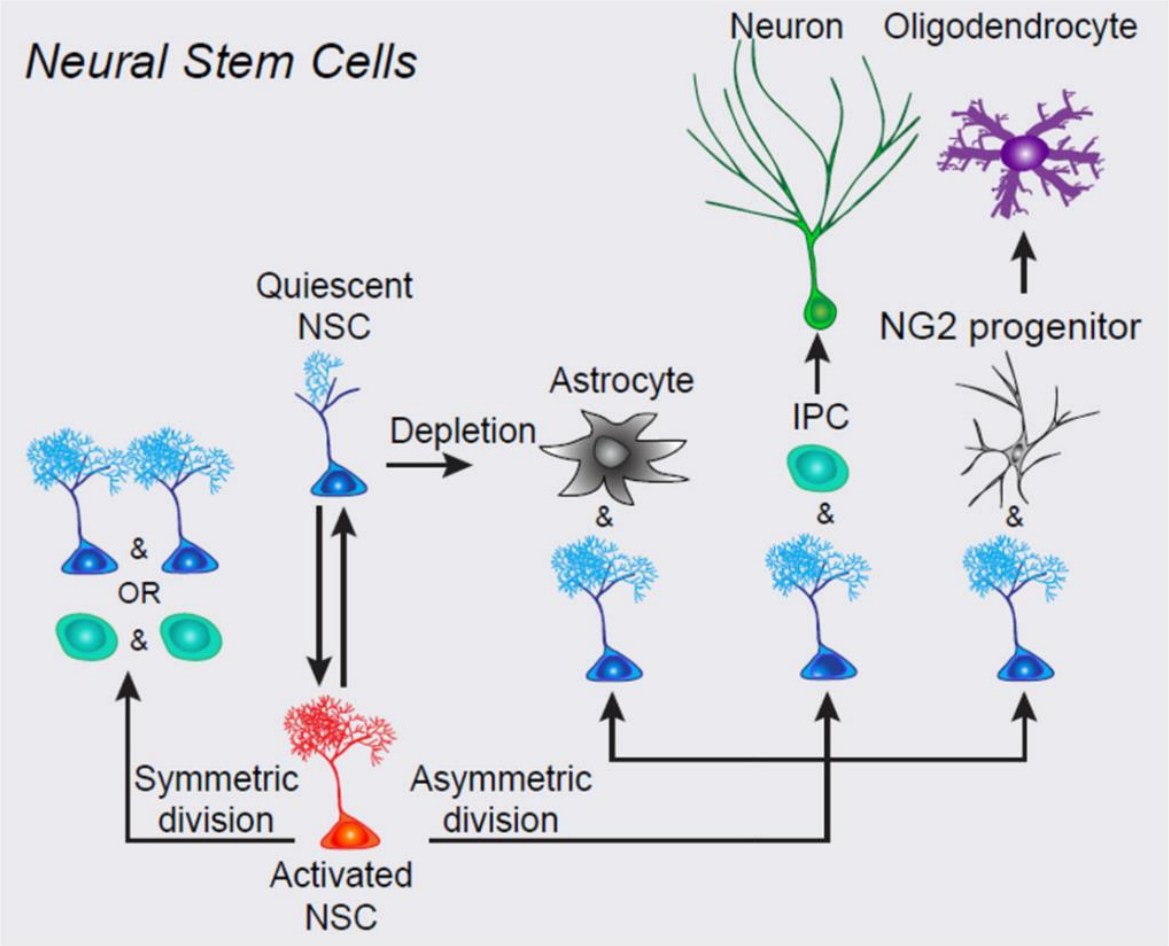

Figure 3. The life cycle of a neural stem cell (NSC). NSCs transform between being in a quiescent- and an active state. Symmetric cell division of activated NSCs maintains the cell population while asymmetric division results in progenitor- or glial cells.

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Approaches for establishing neural stem cells

NSCs can be harvested from the spinal cord tissue and cultured with EGF and bFGF which induce proliferation, self-renewal and expansion in culture conditions [320]. Moreover, NSCs can established from the filum terminale, which is a structure that extends from the conus medullaris located in the caudal most part of the spinal cord [321]. Harvested and dissociated NSCs grow in neurospheres [322, 323]. Currently NSCs are established from tissue harvested from the lateral walls of the fourth ventricle or the olfactory bulb [324, 325]. Pluripotent cells (iPSCs, ESCs) can be differentiated into NSCs by culturing the cells in serum-free media [326, 327]. Generation of NSCs using trans-differentiation is achieved by using specific transcription factors (*Sox2, Zfp521*) in combination with chemical compounds (valproic acid, TGF-β, CHIR99021). Fibroblasts have been used for induction of trans-differentiation but other cell types such as lymphocytes, astrocytes or liver cells might also make good candidates for induction of trans-differentiation. Experiments in which the culture environment (three- dimensional cultures) and/or the addition of growth factors (LIF or heparin in addition to EGF and bFGF) have been investigated as methods to promote trans-differentiation of pluripotent cells into NSCs [328].

Mesenchymal stem cells

Colony forming unit fibroblasts

Multipotent stromal precursor cells harvested from guinea pig with spindle-shaped morphology growing in monolayer cultures capable of supporting hematopoiesis as well as osteogenesis were first described by Friedenstein in 1970 [329]. He termed these cells colony-forming unit fibroblasts (CFUs). CFUs can differentiate into cells of mesodermal origin (adipocytes, chondrocytes, osteocytes) in culture conditions but also following transplantation into a recipient [330, 331]. These cells also adhere to plastic and grow in colonies with a fibroblast-like morphology [332]. Caplan reported in 1991 that these stromal progenitor cells could proliferate and differentiate, which fulfills the definition of a stem cell, and he therefore termed these cells mesenchymal stem cells (MSCs) [333]. The stem- and stromal cell properties of the MSCs were confirmed a few years later when MSCs from humans transplanted into immunodeficient mice formed bone and supported niches capable of hematopoiesis [334]. Therefore, stromal progenitors harvested from the bone marrow proliferated in culture conditions are now commonly termed MSCs [332]. MSCs can be isolated from most connective tissues including bone marrow, adipose tissue, kidney, umbilical cord, dental tissue, liver and lung [335]. However, bone marrow is most commonly used as donor tissue [336]. The immunomodulatory capacity of MSCs vary depending on the tissue from which they are harvested [337] and even MSCs established from the same tissue type seem to be a heterogeneous population of cells [338]. Adipose tissue derived MSCs, for example, express higher levels of TGF-β1 and IL-6 in comparison to MSCs established from bone marrow [337].

MSCs regulate proliferation and differentiation of hematopoietic stem cells

The population of stromal cells in the bone marrow is heterogeneous and contains progenitors in different stages of mesodermal differentiation. The fraction of proliferating multipotent stem cells is however very low [339] and only about 0.01-0.001% of the bone marrow cells are considered as stromal progenitors [330]. MSCs are part of a highly specific niche in the bone marrow, in which the MSCs regulate hematopoietic stem cells (HSCs) (**Figure 4**) [336, 340]. MSCs and HSCs differ by their differentiation potential; MSCs differentiate into cells with mesodermal origin while HSCs differentiate into immune- and blood cells [341]. More precisely, there are two niches in the bone marrow: the endosteal- and the vascular niche. The endosteal niche is made up of osteoblasts along the trabecular bone while the vascular niche is made up of endothelial- and subendothelial stromal cells ($CD146^+$) surrounding the bone marrow sinusoids. The MSCs are present in both niches and in these they are responsible for maintaining HSCs in their quiescent state (G0-phase) meaning that the HSCs proliferate but do not differentiate or undergo apoptosis. The niche is responsible for the survival, proliferation, differentiation and release of the HSCs into the vasculature when necessary [339]. Hence, the MSCs protect and maintain the population of HSCs and control the differentiation and release of these cells [342, 343].

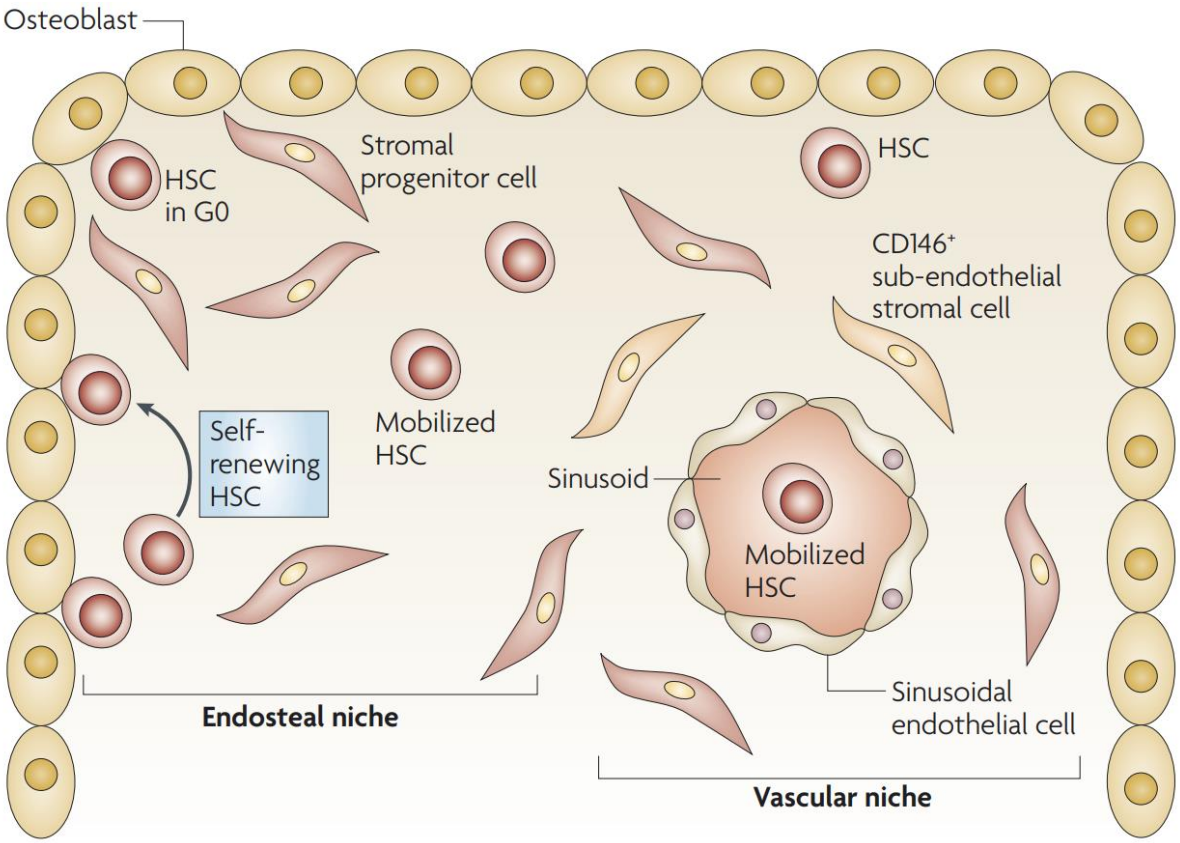

Figure 4. Stromal progenitor cells in combination with the trabecular bones form the hematopoietic stem cell (HSC) niche in the bone marrow. Stromal progenitor cells together with osteoblasts form the endosteal niche and keep HSCs in a quiescent state. In the vascular niche, the endothelial and sub-endothelial cells regulate differentiation and proliferation of HSCs as well as recruitment of HSCs into the vascular niche.

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Phenotypic profile of MSCs

Human MSCs lack expression of surface markers CD11B, CD14, CD34, CD19, CD79a, CD45, CD31 and HLA-DR but do express surface markers CD13, CD44, CD54, CD73, CD90, CD105, CD166 and Stro-1 [344]. Murine MSCs (mMSCs) from bone marrow do not express CD34 or CD45 but do express CD105, CD44, CD90 [345], CD29 and SCA1. Furthermore, human MSCs (hMSCs) do express MHC-I on the cell surface and MHC-II intracellularly [346-348]. In the presence of IFN-γ, expression of MHC-II molecules are significantly up-regulated on the cell surface [347, 348]. This up-regulation seems to be valid only for undifferentiated cells [346]. Exposure to IFN-γ also significantly- and rapidly increases the expression of MHC-I on the cell surface. The increase in MHC-I and MHC-II expression implies that MSCs can act as APCs in addition to acting as immune modulating cells, in the presence of IFN-γ [347-350].

MSCs act by secreting signal substances and extracellular vesicles

MSCs secrete proteins with anti-apoptotic, neuroprotective- and immunomodulatory capabilities [351-353] such as trophic factors, growth factors and cytokines [354, 355] but also miRNA and mRNA [355]. More precisely, MSCs secrete extracellular vesicles (EVs) (MSC-EVs) from their endosomal compartments [356, 357] which are able to impact the microenvironment of surrounding cells [358]. The MSC-EVs transport proteins [359, 360], lipids, organic compounds [361], mRNA [362], tRNA [363], miRNA [362] which modify target cells. Specifically, MSCs have been demonstrated to secrete factors such as IL-6, IL-10, IL-1α, PGE2, HGF, IDO, NO, TGF-β1, VEGF, PDGF, GDNF, BDNF, IGF-1, EGF, EPO, PDGFR-β, RANTES and COX2 [364-367]. The secretome of MSCs varies with the origin of the MSCs, the batch as well as with the mix of functionally distinct subtypes of cells in the culture or transplant [364-367]. The miRNA content in the EVs, for example, varies with the origin and species of the MSCs [368, 369]. Moreover, the secretome of the MSCs can be modified by pre-conditioning the cells [370, 371]. MSC-EVs are thought to mediate cell-to-cell communication and thereby the immune-modulatory effect of MSCs [153, 354, 356, 359, 360, 363, 368, 372-414]. Inflammation triggers MSCs to produce and release growth factors which mediate remodeling of extracellular matrix, angiogenesis as well as differentiation of adult stem cells which enables MSCs to contribute to tissue repair and regeneration [415]. These cytokines and growth factors have been demonstrated to suppress the immune system, decrease apoptosis and scar formation and contribute to angiogenesis following SCI [416]. However, the immunomodulatory action mediated by the MSCs seem to vary depending on the type and intensity of inflammation [415]. Taken together, MSCs seem to mediate the majority of their immunosuppressive- and immune modulatory effects following transplantation by secretion of soluble factors using EVs [345, 417, 418].

MSCs migrate to injured tissue using chemokine receptors

Systemically administrated MSCs migrate to injured tissue in the periphery [419]. Chemokine receptors (e.g. CXCR4) expressed by MSCs facilitate their movement to the injured area [420-422]. MSCs can extravasate from capillaries using their surface adhesion molecules and move between endothelial cells by shifting from an adhesion- into a rolling state using VCAM1 [423, 424]. The ability of MSCs to cross the basement membrane is controlled by metalloproteases which the MSCs secrete when affected by inflammatory cytokines such as TNF-α, TGF-β1 and IL-1β [425].

Neural stem cells transplanted into spinal cord injury

NSCs transplanted into SCI in rodents survive in the recipient [426-436]. Although NSCs transplanted in the chronic phase of SCI experience good survival [437], transplantation in the subacute phase seem to result in better graft survival [438, 439]. Moreover, Parr et al. (2007) found that transplantation in the subacute phase resulted in superior graft survival as compared to transplantation in the acute phase [440]. NSCs transplanted into intact parenchyma seem to experience better survival as compared to NSCs transplanted into the SCI epicenter [441].

NSCs transplanted into SCI can differentiate into all neural lineages [429, 442, 443]. However, some authors found that NSCs only differentiated into astrocytes [432, 434, 435, 444, 445]. Ricci-Vitiani et al. (2006) suggested that the astrocytic differentiation is controlled by pro-inflammatory cytokines such as TNF-α, IL-1β and IFN-γ [445]. On the other hand, some authors documented that NSCs differentiated into oligodendrocytes which myelinated axons in the recipient [438, 439, 446]. Vroemen et al. (2003) among others noted that the transplanted NSCs differentiated into glial cells but not into neurons [430, 431, 436, 440]. Reports also indicate that NSCs differentiate into neurons and oligodendrocytes but not astrocytes in the recipient [426, 427, 447]. Yan et al. (2007) suggested that the microenvironment into which the NPCs are transplanted determines if and how the cells differentiate. The authors found that NSCs placed close to the injury epicenter differentiated into neurons to a higher extent while NSCs placed just under the pia mater more often differentiated into astrocytes. The NSCs, which differentiated into neurons, also formed synapses with resident neurons in the recipient [448]. Akesson et al. (2007) confirmed that the NSCs mainly differentiated into neurons and astrocytes and rarely into oligodendrocytes [428]. Thus, great controversy exist concerning the differentiation potential of NSCs transplanted into SCI. However, NSCs transplanted following SCI integrate well in the spinal cord of the recipient [427, 429, 436]. Moreover, neurons derived from transplanted NSCs form functional synapses with neurons in the recipient [438, 443, 449-452]. Thus, the grafted NSCs act as neuronal/electrophysiological relays by extending active axons across the injured spinal cord [438, 443, 450-452]. However, neither Pallini et al. (2005) nor Gu et al. (2012) could detect any synaptic formation between the grafted NSCs and the axons and/or neurons in the recipient [432, 453]. However, Lu et al. (2003) observed that the grafted NSCs gave rise to significant growth of axons, which could be further enhanced by administration of NT-3 [454]. Grafted NSCs have also been reported to enhance axonal regeneration following SCI [430, 431]. NSCs show a great ability to migrate following engraftment into the spinal cord [426, 427, 438, 455]. Chen et al. (2016) observed migration towards the site of the injury while Pallini et al. (2005) observed migration away from the site of the injury [432, 456]. It has also been documented that grafted NSCs migrate to and integrate with the white matter to a higher extent than gray matter [438, 444].

Transplanted NSCs can suppress the inflammatory response following SCI [443]. Cusimano et al. (2012) suggested that this was due to the suppression of classical activation of macrophages, which was confirmed by Cheng et al. (2016) [456, 457]. Alternative activation of macrophages was enhanced following NSC transplantation in the subacute phase of SCI but not when NSCs were transplanted in the chronic phase [455]. Grafted NSCs have been reported to modulate activation and proliferation of microglia through secretion of VEGF [276]. In addition, NSCs transplanted following SCI have been observed to suppress apoptosis [443, 453]. Moreover, NSCs transplanted following SCI contribute significantly to recovery of hind limb

function [426, 427, 429, 432, 438, 443, 446, 447, 451-453, 455-460]. However, Jin et al. (2016) among others have documented that NSCs transplanted in the chronic phase of SCI do not contribute to functional improvement [455, 457, 461]. Some authors could not detect any improvement of hind limb function following transplantation of NSCs into SCI regardless of time point of transplantation [434, 440]. Enhanced angiogenesis through VEGF secretion [458] and neurotrophic support have been proposed as explanations for the enhancement in recovery of hind limb function [453, 459]. Interactive synaptic reorganization between grafted NSCs and neurons in the recipient has also been proposed as an explanatory model for the improvement in hind limb function [462]. NSCs transplanted post SCI improve motor recovery but also results in aberrant axonal sprouting resulting in hypersensitivity [460].

Transplantation of NSCs has been combined with other treatments aiming at enhancing the effect of NSCs. Wang and Zhang (2015) combined transplantation of NSCs with hypothermia and observed that the combination enhanced recovery of hind limb function and axonal regeneration as compared to NSC transplantation alone [463]. This was confirmed by Tashiro et al. (2016) who also observed increased neuronal differentiation [464]. Hwang et al. (2014) combined transplantation of NSCs with treadmill locomotor training and observed enhanced recovery of hind limb function, graft survival, re-myelination and axonal regeneration [465]. Administration of valproic acid in combination with transplantation of NSCs enhance recovery of hind limb function, neuronal differentiation and the formation of synapses between the grafted NSCs and resident neurons [466]. Su et al. (2007) observed that differentiation of NSCs into neurons could be enhanced by administration of clinically relevant doses of lithium [467]. Yuan et al. (2014) transplanted NSCs in double-layer collagen membranes and observed an enhanced differentiation of NSCs into neurons and significant improvement of hind limb function [468]. Devices consisting of fibrin matrix have also been utilized for transplantation of NSCs and resulted in enhanced neuronal differentiation [469].

Immunomodulatory effect of mesenchymal stem cells

Innate immunity

Dendritic cells

hMSCs can by means of IL-4 and GM-CSF signaling [470-472] inhibit monocytes from differentiating into DCs (**Figure 5**) [470, 471, 473, 474]. hMSCs achieve this by inhibiting monocytes from entering the G1-phase of the cell cycle (G0/G1 arrest) [474] and is most likely regulated by secretion of prostaglandin-E2 (PGE2) from hMSCs [472]. Adipose tissue-derived hMSCs (hAMSCs) are more capable of suppressing the differentiation of monocytes into DCs as compared to hBMSCs [475]. Moreover, hAMSCs promote secretion of IL-10 from mature DCs [475]. In addition to preventing differentiation of monocytes into DCs, hMSCs can also inhibit secretion of IL-12 from mature DCs, which results in inhibition of T-cell activation- and proliferation [471, 472]. Furthermore, hMSCs can polarize mature DCs to adopt an immature phenotype ($CD83^{low}$) [471].

mMSCs can impair TLR4-mediated activation of DCs *in vivo*. This reduces the ability of DCs to migrate, secrete cytokines and present antigens to $CD4^+$- and $CD8^+$ T-cells *in vivo* [476]. Furthermore, mMSCs can polarize DCs towards an immature phenotype ($CD11b^{high}$) [477].

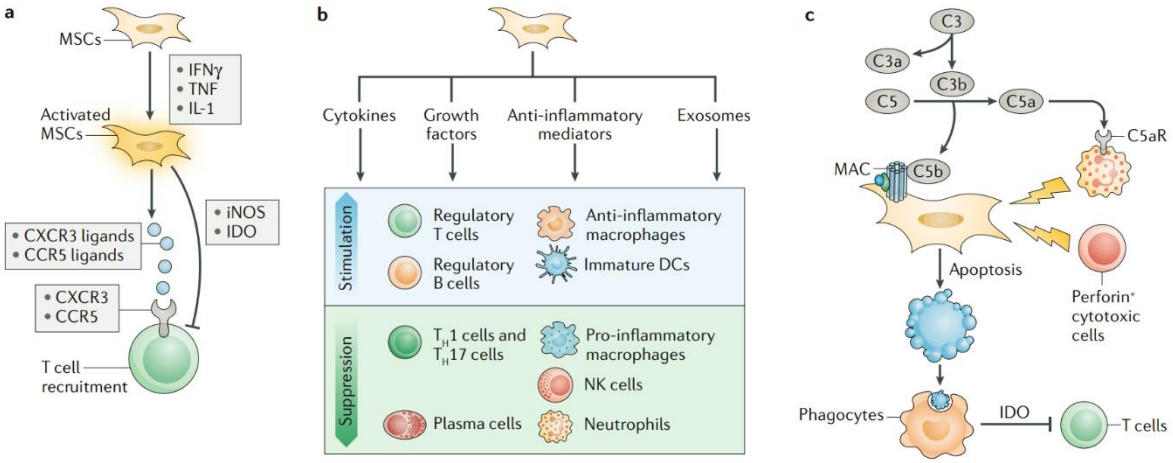

Figure 5. Effect of mesenchymal stem cells (MSCs) on the innate- and adaptive immune system. a) MSCs activated by IFN-γ together with TNF-α or IL-1 produce chemokine receptor ligands, which results in activation of T-cells. MSCs can suppress the activation of T-cells by secreting iNOS (in murine) or IDO (in humans). b) MSCs suppress proliferation and effector functions of $CD4^+$ T-cells, macrophages, NK-cells, granulocytes and B-cells by secreting cytokines (TGF-α, IL-6), growth factors (HGF, LIF), anti-inflammatory mediators (PGE2, TSG6, HO1, exosomes) and enhance the number of regulatory T- and B-cells and alternatively activate macrophages. c) The complement system as well as cytotoxic cells can promote MSCs to undergo apoptosis. Phagocytic cells clearing apoptotic MSCs secrete IDO, which results in reduced proliferation of T-cells.

Natural killer cells

hMSCs can inhibit IL-2 activated proliferation of resting- and activated NK-cells [418, 478]. However, NK-cells activated by IL-2 can lyse both autologous- and allogeneic hMSCs [478, 479]. The NK-cell mediated lysis of hMSCs is accompanied by an elevation in secretion of cytokines from NK-cells. hMSCs primed by TLR ligation are less sensitive to death by IL-2 activated NK-cells. In the presence of IFN-γ, expression of MHC-I on the cell surface of hMSCs is up-regulated which protects hMSCs from NK-cell mediated lysis [478]. Hence, hMSCs in an inflammatory environment are more protected from NK-cells [480]. However, contradictory evidence has been reported suggesting that MHC-I on hMSCs do not protect them from NK-cell mediated lysis [479]. Moreover, hMSCs can inhibit effector functions (cytokine secretion and cytotoxic activity) of NK-cells by down-regulation of NKp30, NKp44 and NKG2D. Furthermore, PGE2 and indoleamine 2,3-dioxygenase (IDO) secreted by hMSCs also have an inhibitory effect on effector functions of NK-cells [481]. Additionally, by altering the phenotype of NK-cells the hMSCs can reduce the cytotoxicity, cytokine secretion and proliferative capacity of NK-cells when the ratio of NK-cells to hMSCs is low. These effects seem to be mediated by PGE2 but also by TGF-β1 [482]. NK-cells secrete TNF-α and IFN-γ as a response to cell-to-cell interaction between hMSCs. This cellular interaction and response is dependent on ICAM1 expression on hMSCs and expression of LFA1 on NK-cells [479].

Adaptive immunity

T-cells

In an inflammatory environment hMSCs can inhibit proliferation of activated T-cells [348, 418, 483-493] which is mediated by inhibiting activated T-cells from entering the G1-phase of the cell cycle (G0/G1 arrest) [483, 485]. This arrest is mediated by secretion of soluble factors (TGF-β1, HGF) from hMSCs primed in an inflammatory environment [484]. Additionally, IFN-γ secreted by T- and NK-cells enhances secretion of IDO from hMSCs, which further suppresses proliferation of T-cells [418, 494]. However, contradictory results do exist suggesting that neither TGF-β1, HGF, PGE2 nor IL-10 are responsible for inhibiting the proliferation of T-cells [348]. hMSCs secrete IL-6 and VEGF which induce phenotypic changes in DCs (reduction of MHC-II, CD40, CD86 expression) which in turn results in the inhibition of T-cells [495]. Some contradictory evidence suggest that hMSCs can inhibit the proliferation of activated T-cells by secretion of IDO following exposure to IFN-γ which results in apoptosis of T-cells [490]. Others suggest that the inhibition of proliferation is not mediated by apoptosis of T-cells [418, 484]. Moreover, hMSCs can inhibit the proliferation of both $CD4^+$- and $CD8^+$ T-cells [418, 484] and is true for both autologous- and allogeneic hMSCs [484]. The inhibition of T-cell proliferation seems to depend on the dose of hMSCs and rate of contact between T-cells and hMSCs. In low concentrations of hMSCs the cells support proliferation of T-cells by cell-to-cell interaction and secretion of IL-6. In higher concentrations of hMSCs the cells instead inhibit the proliferation of T-cells [486]. Furthermore, hMSCs can indirectly inhibit the activation of T-cells by interacting with APCs which results in secretion of large quantities of IL-10 [496]. Stimulation of T-cells results in secretion of TNF-α which in turn activates the NF-κβ pathway in hMSCs resulting in inhibition of the proliferation of T-cells [487]. The TNF-α and IFN-γ induced inhibition of T-cell proliferation mediated by hMSCs seems to be related to an up-

regulation in IDO [488]. hMSCs activated by pro-inflammatory agents (IFN-γ, TNF-α) secrete Galectin-9 [492, 493] which can inhibit the proliferation of T-cells [492]. In addition, hMSCs secrete OH-1 and iNOS, which also contribute to inhibition of proliferation of T-cells. This inhibition only occurs when both OH-1 and iNOS are present [489]. Moreover, hMSCs promote survival of unstimulated T-cells by down-regulating Fas ligand- and receptor on the surface of the T-cells. Hence, MSCs can inhibit proliferation of activated T-cells and promote the survival of non-activated T-cells [491]. IL-1β secreted from monocytes can induce secretion of TGF-β1 from hMSCs which inhibits activation of both $CD4^+$- and $CD8^+$ T-cells [497]. Another method by which hMSCs can modulate the immune response is by generating T-regulatory cells. PGE2 and TGF-β1 secreted following cell-to-cell interaction between hMSCs and $CD4^+$ T-cells enhance generation of T-regulatory cells [498, 499]. In addition, hMSCs can increase the generation of T-regulatory lymphocytes indirectly by alternatively activating macrophages expressing CCL18. These alternatively activated macrophages also express high levels of IL-10 [500].

mMSCs stimulated with IFN-γ and/or pro-inflammatory cytokines (IL-1β, IL-1α, TNF-α) secrete iNOS which is further converted into nitric oxide (NO) which reduces the responsiveness of T-cells [501]. mMSCs can express NO in the presence of both $CD4^+$- and $CD8^+$ T-cells. Moreover, NO suppresses Stat5 phosphorylation in T-cells, which leads to inhibition of proliferation of T-cells [502]. Guanylate cyclase activation is a key step in the NO mediated inhibition of T-cell proliferation [503]. TGF-β can however reduce the secretion of iNOS from mMSCs [504]. The proliferation of T-cells can also be inhibited by matrix metalloproteinases (MMP), especially MMP-2 and MMP-9. These MMPs down-regulate the expression of CD25 on the surface of activated T-cells which results in a reduced proliferation [505]. The inhibition of T-cell proliferation can partially be explained by cell-to-cell interaction between mMSCs and T-cells, which activates the inhibitory molecule programmed death 1 pathway. Inhibition of proliferation of $CD4^+$ T-cells seems to be mediated primarily by cell-to-cell interaction between mMSCs and T-cells [506]. The T-cells respond to the interaction by modulating their expression of cytokine receptors [507]. mMSCs pre-treated with adenosine triphosphate (ATP) increase their expression of IL-2, IFN-γ and IL-12 and decrease their expression of IL-10 *in vivo*. This results in a reduced ability of mMSCs to inhibit the proliferation of T-cells [508]. Following systemic infusion of mMSCs, T-cells undergo apoptosis through the FAS ligand dependent pathway. The apoptosis results in increased expression of TGF-β from macrophages involved in the apoptosis. The elevated expression of TGF-β results in immune tolerance by up-regulating levels of T-regulatory cells [509].

B-cells

hMSCs can inhibit proliferation of B-cells [510-512]. Inhibition of proliferation of B-cells is mediated by cell-to-cell interaction between hMSCs and $CD4^+$- and $CD8^+$ T-cells and is not dependent on interaction between B-cells and hMSCs [345, 510]. The inhibition of proliferation results from G0/G1 arrest [511] and is thought to be mediated by secreted soluble factors in addition to cell-to-cell interaction [511]. The inhibition of proliferation is however not due to apoptosis [511]. Furthermore, by inhibiting the differentiation of B-cells the hMSCs indirectly inhibit the production of antibodies [510, 511, 513]. The antibody secretion from B-cells is hMSC dose dependent. Thus the antibody secretion can be enhanced or reduced depending on the ratio between hMSCs and B-cells [514]. The inhibition of proliferation and differentiation

is dependent on cell-to-cell interaction and presence of IFN-γ and is probably mediated through the programmed death 1 pathway [512]. Additionally, the chemotactic properties of B-cells are significantly down-regulated by hMSCs [511]. However, following ligation of TLR9 hMSCs can strongly enhance the proliferation and differentiation of B-cells into Ig-secreting cells [515].

General immunosuppressive properties

hMSC can alternatively activate macrophages to express high levels of IL-10 and IL-6 and low levels of IL-12 and TNF-α. Additionally, hMSCs improve the phagocytic activity of these alternatively polarized macrophages [516]. Monocytes co-cultured with placental derived hMSCs differentiate to a higher extent into alternatively activated macrophages rather than classically activated macrophages. These alternatively activated macrophages have a high expression of IL-10 and a low expression of IL-1β, IL-12 and MIP-1α as well as enhanced phagocytic capability. The shift in activation mediated by hMSCs is most likely mediated by soluble factors, which bind to progesterone- and glucocorticoid receptors [517]. The differentiation of monocytes towards IL-10 secreting macrophages is thought to be partially mediated by IDO secreted from hMSCs. These macrophages can inhibit proliferation of T-cells through secretion of IL-10 [488]. Moreover, hMSCs can induce DCs to reduce their expression of TNF-α and instead increase their expression of IL-10. In the presence of hMSCs effector T-cells can increase their expression of IL-4 and reduce their expression of IFN-γ. The effect of hMSCs on T-, DCs and NK-cells is thought to be mediated mainly by secretion of PGE2 from hMSCs [518]. COX2 mediates the production of PGE2 in hMSCs and might be an important enzyme to consider therapeutically [519]. hBMSCs can either have an immunosuppressive effect or act as APCs following pre-treatment with IFN-γ. Ligation of TLR3- and 4 on hMSCs enhances their expression of IL-1β, IL-6, IL-8 and CCL5. In the presence of IFN-γ the hMSCs elevate their expression of iNOS but not IL-12 and TNF-α following ligation of TLR3- and 4 [520]. Taken together, hMSCs secrete the following immunosuppressive factors: IDO, NO, PGE2, HGF, COX1, COX2, VEGF, IL-10 and TGF-β [417, 521, 522].

mMSCs can reduce expression of IL-2, IL-12, IFN-γ, TNF-α, IL-4, IL-5, IL-1β and IL-10 but not TGF-β and IDO [506]. mMSCs pre-treated with IFN-γ *in vitro* up-regulate surface expression of MHC-I but have their anti-inflammatory profile preserved (TGF-β and IL-10 expression). *In vivo* this up-regulation results in hypersensitivity reactions (delayed-type) in allogeneic recipients [346]. The kynurenine pathway (including IDO1 and IDO2) is active in both hMSCs and mMSCs. This pathway is important for the proliferation and differentiation of MSCs and is regulated by IFN-γ. In the presence of IFN-γ the proliferation- and differentiation of MSCs is inhibited [523]. Cell culture medium conditioned by mBMSCs contains insulin-like growth factor 1 (IGF-1), HGF, VEGF and TGF-β1 which contribute to neuronal survival and neurite outgrowth when mBMSCs and neurons are co-cultured [524].

Mesenchymal stem cells transplanted into spinal cord injury

Transplanted BMSCs [525-532], AMSCs [527, 533, 534], umbilical cord BMSCs (UCBMSCs) [535] and UCMSCs [169] do survive in the recipient. The reported survival is varying but is in the range of weeks and in some cases months. In contrast, some authors have not detected any survival of transplanted MSCs [536, 537]. The time point of transplantation, time point of evaluation, number of cells transplanted and route of transplantation are important parameters to take into consideration when concluding if transplanted cells survive or not. Both allogeneic- and autologous mBMSCs survive following intrathecal transplantation in the subacute phase of SCI. Although both autologous- and allogeneic mBMSCs could be detected up to 4 weeks post transplantation, the survival of autologous mBMSCs was superior to the survival of allogeneic mBMSCs. However, the transplanted mBMSCs, regardless of histocompatibility, did not trans-differentiate into neural cells. Although both autologous- and allogeneic mBMSCs did contribute significantly to recovery of hind limb motor function the autologous mBMSCs contributed significantly more [525]. Administration of immunosuppression in combination with transplantation of mBMSCs has synergistic effects and can reduce the cystic cavity size and enhance the regeneration of axons following SCI. Thus, Torres-Espín et al. (2015) suggested that a large amount of mBMSCs transplanted shortly after SCI combined with administration of immunosuppression is the most efficient approach. The authors also emphasized that survival of transplanted mBMSCs is important for functional improvement [538].

Reports indicate that MSCs can trans-differentiate and form neural tissue in general [529, 530, 534, 535, 539-544] and specifically astrocytes [530, 539, 540, 542], oligodendrocytes [529, 534] and neurons [530, 534, 535, 540, 541]. In contrast, many authors could not detect any trans-differentiation of MSCs transplanted into SCI [164, 169, 525, 527, 528, 545-547]. Even though differentiation of MSCs might occur in some cases and might contribute to repair of the injured tissue, many authors have suggested that the paracrine effects mediated by MSCs explains the majority of their beneficial effect [164, 525, 527-529, 537, 545]. Thus, transplanted MSCs can increase expression of neurotrophic- and growth factors such as BDNF [164, 527, 528, 539, 548, 549], GDNF [528], VEGF [164, 169, 525, 544], NGF [537, 539, 542, 548, 549], CNTF [534, 537], bFGF [169], TGF-β [525], SDF-1 [525] and NT-3 [169, 549] following transplantation into SCI. Cantinieaux et al. (2013) elaborated with this theory and evaluted if transplantation of MSC-conditioned cell culture medium into the injury epicenter could improve the hind limb locomotor function. The authors found that the conditioned medium could reduce the size of the cystic cavity, protect neurons from apoptosis and promote angiogenesis, which correlated with enhanced functional recovery [550]. Although transplanted MSCs can elevate levels of neurotrophic- and growth factors it is not certain if these factors are secreted by MSCs or if the secretion stems from endogenous sources up-regulated by the transplanted MSCs. Furthermore, it is reasonable to argue that the secretome of transplanted *in vivo* primed MSCs might be different from the secretome of unprimed MSCs *in vitro*, especially considering the complexity and dynamics of the *in vivo* environment during SCI.

Various routes for transplantation have been utilized: intra-lesion [164, 169, 526-528, 530-536, 538, 539, 542-546, 548, 549, 551-556], intravenous [529, 537, 539, 541, 542, 551, 557-561] and intrathecal [525, 526, 540, 547, 551, 556, 562, 563]. The ability of MSCs to migrate from venous blood [529, 541, 557, 559] and subdural space [526, 540, 563] to the lesion area has been demonstrated repeatedly. This suggests that less invasive transplantation approaches (intrathecal and intravenous) might be more appealing than intra-lesion

transplantation. Some groups have combined several transplantation routes [551]. Kim et al. (1976) compared intra-lesion to intravenous transplantation of MSCs and found that intra-lesion transplantation results in superior engraftment of MSCs and elevated expression of BDNF and NGF as compared to intravenous transplantation. However, the authors could not detect any difference in functional outcome between the two transplantation routes [539]. On the other hand, Kang et al. (2012) did describe a better functional outcome (BBB score) following intravenous transplantation as compared to intra-lesion transplantation. The authors suggested that this supports the fact that MSCs can migrate across the blood spinal cord barrier but also that the intra-lesion approach contributes to the injury. Moreover, the authors observed that the number of cells engrafted following intravenous transplantation was lower and that the cells differentiated mainly towards astrocytes as compared to intra-lesion transplanted MSCs which mainly differentiated into neurons [542]. Shin et al. (2013) compared intra-lesion, intrathecal- and intravenous transplantation of MSCs. They found that intrathecal transplantation results in a more even distribution of MSCs as compared to intra-lesion transplantation, which instead produces a mass-effect on the uninjured tissue. Superior functional recovery was observed in animals which received intrathecal transplantation. Although many more cells were transplanted using the intravenous route as compared to the intra-lesion route, the intravenous approach resulted in very poor engraftment in the spinal cord [551]. Furthermore, the improvement in functional recovery likely dependents on the number of engrafted MSCs [526]. In conclusion, the route for transplantation seems to have a significant effect on the efficiency of the MSC therapy for SCI and should be taken into consideration in the experimental planning and during evaluation.

Transplanted MSCs have proven the ability to favorably modulate the microenvironment in the injured spinal cord. Specifically, MSCs have proven the ability to reduce glial scar formation [169, 533, 554, 562], cystic cavity size [525, 547, 550, 558], lesion size [533, 553] and improve angiogenesis [164, 536, 550, 557] and tissue sparing [528, 532, 534, 536-538, 545, 550, 555, 562]. Additionally, MSCs can also improve axonal regeneration [164, 528, 532, 533, 544, 554, 557, 562] and re-myelination [529, 557]. Furthermore, transplanted MSCs can reduce the apoptotic activity in the recipient [164, 534, 543, 550, 552] and alternatively activate macrophages [553, 562]. Moreover, transplanted MSCs can also reduce expression of pro-inflammatory cytokines (TNF-α, IL-6, IL-12, IL-4, IL-2, IL-1β, IL-12, COX2) [541, 553, 559, 560, 562] and up-regulate expression of anti-inflammatory cytokines (IL-10, IL-4, IL-13) [537, 553]. Thus, MSCs contribute to neuroprotection and reduce the degree of the injury but also contribute to regeneration in the injured spinal cord.

The functional outcome (motor, sensory and autonomic) is perhaps the most relevant measurement of efficiency for a patient. MSCs have in several studies proven the ability to significantly improve the hind limb motor function [164, 169, 525, 529-538, 541, 543, 546-550, 552-555, 557, 558, 561-563]. This significant enhancement of hind limb motor function is suggested to be a consequence of secreted soluble factors (e.g. neurotrophic- and growth factors) resulting in neuroprotection with or without association to axonal regeneration and re-myelination [164, 527, 529, 537, 547-549, 552, 558]. In contrast, fewer studies claim that the trans-differentiation of MSCs explain the functional improvements [530, 535]. Taken together, transplanted MSCs survive, trans-differentiate under some circumstances, elevate levels of neurotrophic- and growth factors, favorably modulate the microenvironment and enhance functional recovery in the recipient. Moreover, MSCs seem to improve the functional outcome regardless of survival or trans-differentiation, which supports the theory that elevated expression of neurotrophic- and growth factors is the main therapeutic effect that the MSCs contribute

with. Therefore, in contrast to other cell transplantation approaches, MSCs are most often not transplanted with the intention to replace damaged cells in the recipient but to contribute temporarily with immunomodulation.

Genetic modification

In addition to enhancing the survival and engraftment of MSCs by seeding them onto scaffolds prior to transplantation, modification of their transcriptome using plasmids or viral vectors have been investigated. Transplantation of BDNF-hBMSCs intra-lesion in the acute phase of SCI results in significant improvement of hind limb motor function and increased sprouting of corticospinal- and serotonergic axons. Thus, the enhanced axonal regeneration mediated by BDNF most likely explains the improvement of hind limb motor function [564]. Similarly BDNF-hMSCs, transplanted in three-dimensional rather than in two-dimensional conformation, contribute to recovery of hind limb motor function. The seeded three-dimensional devices significantly reduce the expression of pro-inflammatory cytokines (TNF-α, IL-1β) and the apoptotic activity in the injured spinal cord [565]. Functional improvement has also been described following transplantation of BDNF-mBMSCs in combination with chABC. This functional improvement has been associated with observations of improved axonal regeneration and increased expression of NGF and BDNF [566]. Enhancement of axonal regeneration and re-myelination has also been achieved by transplantation of mBMSCs over-expressing CDNF, which correlate with improved hind limb motor function. These CDNF-mBMSCs have also managed to reduce the level of pro-inflammatory cytokines and thereby further contribute to a favorable microenvironment for regeneration [567]. Neurotrophic factors can be administrated either by means of MSCs over-expressing them or directly into the spinal cord. Transplantation of mBMSCs over-expressing GDNF seems to be a feasible alternative to direct administration of GDNF [568]. Wei et al. (2017) achieved over-expression of GDNF in hBMSCs by suppressing *mi-383*. The GDNF secreted by the hBMSCs resulted in reduced cystic cavity size and improved hind limb motor function [569]. Gene silencing has also been utilized for the Nogo-66 receptor gene. This has resulted in improved neuronal survival and neurite outgrowth as well as hind limb motor function [570]. Moreover, over-expression of NT-3 by transplanted mBMSCs do enhance the survival of mBMSCs following transplantation into SCI. In addition, the NT-3-mBMSCs reduce apoptosis of motor neurons [571]. Additionally, Wang et al. (2014) reported that NT-3-mBMSCs could significantly improve hind limb motor function, reduce the cystic cavity size and increase expression of BDNF and VEGF. The NT-3-mBMSCs can also reduce astrogliosis and enhance axonal regeneration [572]. hUCMSCs over-expressing NT-3 have also been transplanted into SCI and managed to improve the hind limb locomotor function and reduce the cystic cavity size [573]. mBMSCs over-expressing bFGF have also enhanced axonal outgrowth which did result in significant hind limb motor function improvement, although the expression was only significant during the first week post transplantation [574].

Transplantation of mBMSCs over-expressing the anti-inflammatory cytokine IL-13 enhances the recovery of hind limb motor function but also reduce lesion volume, number of resident microglia and increase the number of alternatively activated macrophages as well as the macrophage-axonal contact. This seems to be a consequence of IL-13-induced apoptosis of microglia [575]. Favorable modification of the inflammatory environment has also been achieved by transplantation of heme-oxygenase-1 (OH-1)-MSCs, which can reduce the level of pro-

inflammatory cytokines (TNF-α, IL-6, COX2), as well as the microglial infiltration. Consequently, the OH-1-MSCs significantly improve the functional outcome following SCI [576]. Promotion of axonal regeneration (elevated expression of GAP43 and MAP2) has been observed post transplantation of Wnt3a-hUCBMSCs which has been associated with improvement of hind limb motor function (BBB score) [577]. Transplanted mAMSCs over-expressing Neurogenin-2 can differentiate into neurons ($NEUN^+$- and/or $TUJ1^+$ cells) and elevate the levels of BDNF and VEGF in a recipient. This is associated with improved functional outcome [578]. Taken together, neurotrophic factors (BDNF, CDNF, NT-3) but also growth factors (bFGF) secreted by genetically modified MSCs can enhance the axonal regeneration and re-myelination, which correlates with, enhanced hind limb motor function following SCI.

Seeded scaffolds

In order to enhance engraftment of transplanted MSCs in the lesion area several projects have investigated the effects of transplanting scaffolds seeded with MSCs. Transplantation of mBMSCs on acellular spinal cord scaffolds results in improved survival of the transplanted MSCs as well as enhanced trans-differentiation of the MSCs into glial cells. Additionally, this treatment approach has managed to reduce apoptosis (lower expression of CASPASE-3) but also significantly improve the hind limb motor function [579]. Acellular spinal cord scaffolds have also been seeded with hUCMSCs prior to transplantation and been able to support proliferation and differentiation of resident oligodendrocytes resulting in enhanced re-myelination and hind limb locomotor function. However, the hUCMSCs did not survive for more than 2 weeks in the recipient [580]. hBMSCs transplanted on polymer scaffolds have proven the ability to differentiate into oligodendrocytes, astrocytes and neurons and enhanced the motor- and sensory functions post SCI [581]. On the other hand, mBMSCs transplanted on polymer scaffolds survive at least 8 weeks in the recipient and enhance axonal regeneration and recovery of hind limb function (BBB score and motor evoked-potentials, MEPs) [582]. Collagen scaffolds seeded with mBMSCs can reduce astrogliosis, apoptosis and infiltration as well as enhance alternative activation of macrophages, axonal regeneration and functional recovery following SCI [583]. A three-dimensional, rather than two-dimensional, structure of collagen scaffold seeded with mBMSCs seem to be more beneficial in improving hind limb motor function and axonal regeneration and in reducing expression of pro-inflammatory cytokines (IL-6, IL-1β, TNF-α) [584]. Biodegradable chitin conduits seeded with mBMSCs can reduce glial scar formation and muscular atrophy following SCI. mBMSCs seeded on these scaffolds survive, proliferate and differentiate into Schwann cell-like cells which promote axonal regeneration [585]. Transplantation of mBMSCs on gelatin sponge scaffolds promote angiogenesis, reduce expression of pro-inflammatory cytokines (TNF-α, IL-1β) as well as the cystic cavity size. Gelatin sponge scaffolds also enhance survival and proliferation of transplanted mBMSCs [586]. Scaffolds seeded with genetically modified MSCs have also been investigated. Jiao et al. (2017) found that hUCMSCs over-expressing GDNF loaded onto composite scaffolds (silk fibroin/alginates) significantly improve the hind limb motor function and host neuronal survival following SCI [587]. Furthermore, BDNF-mBMSCs seeded onto alginate-based anisotropic capillary hydrogels survive up to 4 weeks in the recipient, promote axonal regeneration and aid the guidance of axons through the lesion area [588]. Additionally, scaffolds seeded with BMSCs in combination with activated Schwann cells survive up to 8 weeks, differentiate into neuron-like cells in the recipient and improve axonal regeneration and recovery of hind limb motor

function (BBB score & electrophysiological response) [589]. Taken together, scaffolds seem to further enhance the positive effects on rehabilitation executed by MSCs. The impression is that scaffolds improve survival and tend to promote trans-differentiation to a higher extent. Partial- or complete transection SCI, as compared to contusion injuries, might be better suited for scaffold transplantation.

Co-transplantation

In order to further enhance the therapeutic effects of transplanted MSCs some researchers have assessed the potential benefit of co-transplanting MSCs with other cell types. Co-transplantation of olfactory ensheathing cells (OECs) and mBMSCs intra-lesion results in reduced loss of white matter, reduced levels of apoptosis (reduction of CASPASE-3, CASPASE-9 and Bax/Bcl2 ratio) and improved axonal regeneration which has been correlated with enhanced functional improvement [590]. In contrast, intra-lesion co-transplantation of murine olfactory ensheathing glia (mOEG) and mBMSCs results in poor graft survival which correlate with a lack of enhancement of functional recovery [591]. Co-transplantation of mBMSCs and chABC reduce the necrotic area, the astrocytic scar and expression of GFAP and CSPGs in the recipient. The axonal regeneration is promoted which correlates with significant improvement of hind limb motor function [592]. Lee et al. (2016) further refined this approach by transplanting BDNF-AMSCs together with chABC followed by two additional equivalent transplantations at 1- and 2 weeks following the first transplantation. Already the first injection resulted in significant improvement of hind limb motor function while the two additional transplantations increased the recovery of hind limb motor function further but also managed to reduce the fibrotic scar formation and expression of TNF-α, COX2 and IL-6. The therapy elevated expression of BDNF in the recipient [593]. In contrast, co-transplantation of AMSCs together with chABC results in increased levels of pro-inflammatory cytokines (COX2, TNF-α) but can on the other hand reduce levels of CSPGs which results in significant improvement of hind limb motor function [594]. Pre-treatment of MSCs prior to transplantation is another approach that has been investigated. Chen et al. (2014) pre-treated mBMSCs with valproic acid prior to intravenous transplantation. The authors documented improved survival of the transplanted mBMSCs and improved ability for the cells to migrate to the injury epicenter by means of chemokine receptors. Additionally, the authors documented improved functional outcome in animals which received these pre-treated mBMSCs [595]. NT-3-mBMSCs pre-treated with retinoic acid contribute to enhanced survival of resident neuronal cells and reduce cystic cavity size. These pre-treated cells also enhance the axonal regeneration, which is associated with significant improvement of hind limb motor function (BBB score) [596].

Another approach that has been investigated is transplantation of MSCs in combination with growth factors. Intrathecal transplantation of hBMSCs in combination with bFGF results in trans-differentiation into neural cells and significantly enhances functional recovery [597]. mBMSCs transplanted in combination with NGF differentiate into astrocytes ($GFAP^+$ cells) and neurons ($NEUN^+$ cells). Furthermore, axonal regeneration is improved and is correlated with improved hind limb motor function [598]. Intra-lesion transplantation of mBMSCs followed by administration of minocycline (tetracycline antibiotic) during the first 2 weeks post transplantation reduces the infiltration of neutrophils and increases the expression of BDNF and VEGF. Moreover, this therapy reduces the level of apoptosis and enhances recovery of hind limb motor function [599]. Transplantation of mBMSCs in combination with

intravenous administration of propofol improves the recovery of hind limb motor function and axonal regeneration [600]. Hyperbaric oxygen treatment post mBMSCs transplantation do not only reduce the size of the lesion but also reduces the expression of pro-inflammatory cytokines (TNF-α, IL-6, IL-1β and IFN-γ) and enhances recovery of hind limb motor function [601]. In conclusion, several combinatory therapies have been investigated and the majority have indicated promising results. It is however difficult to conclude which one of these therapies that is the most efficient one.

Clinical trials involving mesenchymal stem cells as a treatment for SCI

Several aspects are important to take into consideration in clinical studies evaluating MSCs (**Figure 6**). First, the immune status of the recipient has to be evaluated. If allogeneic cells are to be used the age and gender of the donor is important to take into account. Allogeneic MSCs can be used of the shelf and enables transplantation at any time point during the disease progression but raises questions concerning graft rejection. MSC cultures rely on basal medium containing xenogeneic serum. Since this might be associated with risk, serum-free culture conditions are preferred when establishing cells which are to be administrated to humans. Moreover, the timing, dose and administration route are key elements that probably have to be adapted to the disease and condition which is to be treated. The dose has to be sufficient and might be difficult to know beforehand. The administration route has to ensure proper migration of cells to the injury epicenter or mediate a systemic immunomodulatory effect [338].

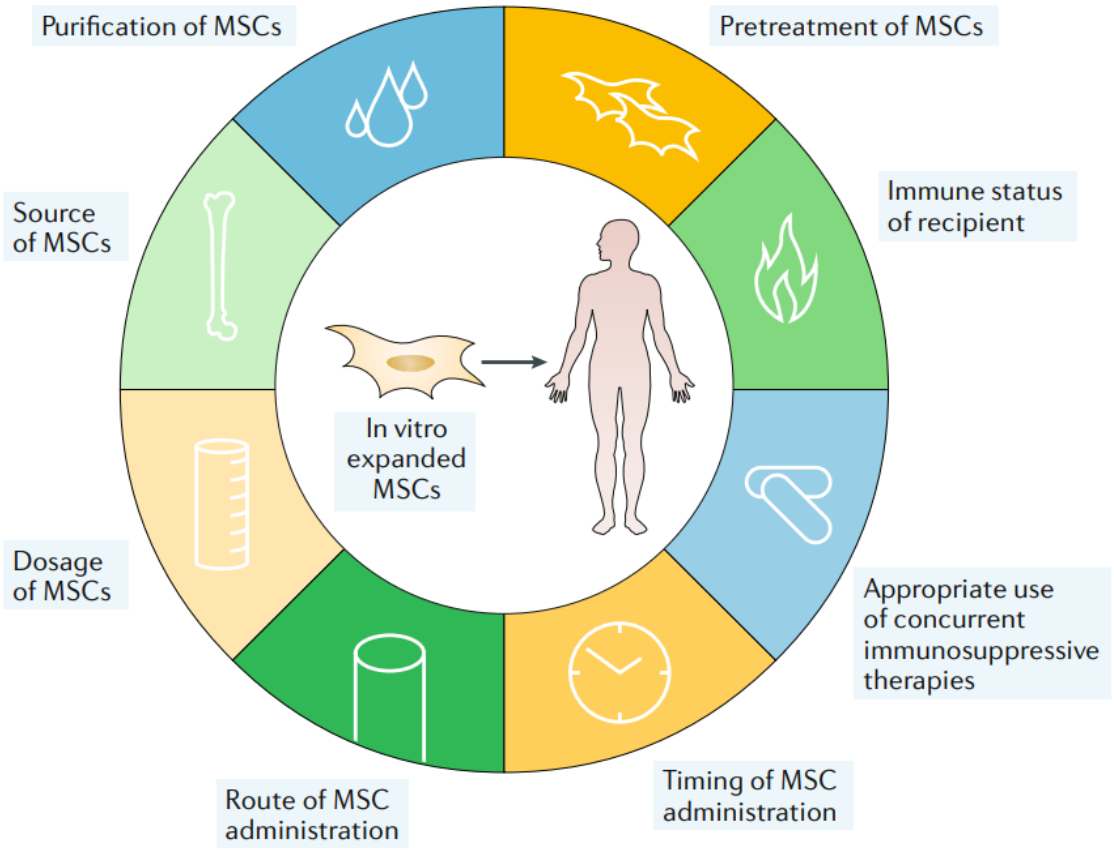

Figure 6. Key aspects to consider in clinical studies and therapies using or evaluating mesenchymal stem cells (MSCs).

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Transplantation of hAMSCs [602], hBMSCs [603-616] or hUCMSCs [617] to patients in clinical studies is safe. However, adverse events and side effects such as head ache [608, 609, 615, 617], fever [608, 609, 615, 616], dizziness [608], lower back pain [617], neuropathic pain [618] and spasticity [618] have been reported. On the other hand, several clinical trials have reported a complete lack of complications following transplantation [602-607, 610-612, 614]. In those cases in which side effects did occur, these usually subsided within 2-3 days post transplantation [608, 609, 615, 617]. A systematic review of 36 studies including 1012 patients conducted by Lalu et al. (2012) including intravenous and intra-arterial transplantation confirms that transplantation of hMSCs in prospective clinical trials is safe and is not associated with death, malignancy or organ system complications. However, the authors did detect an association between transplantation and fever [616]. Although not all clinical trials investigated tumor formation, the ones that did could not detect any such formation [602, 608, 611]. Transplantations in these studies were autologous [602-615, 618] except when hUCMSCs were used [617]. However, establishing and verifying MSCs takes months, which implies that transplantation of autologous MSCs in the acute- and subacute phase of SCI is not possible. Common transplantation routes such as intra-lesion [603, 606-608, 611, 612], intrathecal [603-606, 609, 610, 613-615, 617, 618] and intravenous [602, 611, 614] transplantation have all been used for transplantation of hMSCs to patients. Intrathecal transplantation seems to be the most commonly used route. In some cases, 2 or 3 routes have been combined [603, 606, 611, 612, 614]. hMSCs have been transplanted in the chronic [602, 604, 605, 607, 608, 613] or subacute phase [604, 605, 614] of SCI. Transplantation has resulted in improvement in motor [602, 603, 607-610, 612-615, 617], sensory [607-609, 612, 614, 615, 617], autonomic [609] and urological functions [607, 608, 614, 617]. Furthermore, cystic cavity size has been reported to decrease as a result of transplantation of MSCs [603]. However, some authors have not been able to observe any functional improvement [618]. In conclusion, the functional improvement following MSC transplantation is modest which might be a consequence of transplantation in the subacute- or chronic phase.

Disease-associated microglia

Microglia, the tissue resident myeloid-like cell in the CNS, have traditionally been defined as $CD11B^{+}CD45^{low}$ cells. Recently, single-cell RNA sequencing (scRNAseq) has become a popular method for characterizing heterogeneity of cell populations by determination of marker genes and regulatory factors in subpopulations of cells over time, conditions and/or development in various tissues and species [619-623].

Disease-associated microglia are detected in various pathological conditions in the CNS

In recent years, several disease models of CNS pathology in rodents have been used as a platform for investigation of disease-associated transformations of microglia. O'Koren et al. (2019) reported that degeneration of retina resulted in a down-regulation of genes associated with homeostasis of microglia (*P2ry12, Tmem119, Selplg, Cst3*) and up-regulation of genes defining disease-associated microglia (DAM) (*Lgals3, Spp1, Lpl, Cd68*) (**Figure 7**). In addition, DAM relocated within the retina and contributed to the protection of the retinal epithelium [624]. Mathys et al. (2017) reported that microglia in Alzheimer's disease transformed over time and formed specific sets of subpopulations with a transcriptional profile different from microglia in homeostasis. Early on in the disease microglia were characterized by expression of *Ctsb, Ctsz, Cst7, Mif, Ccl12, Ccl3, Ccl4, Lilrb4* while microglia later on in the disease (i.e. DAM) were characterized by expression of *Apoe, Lgals3bp, Axl* and *H2-D1* [625]. Keren-Shaul et al. (2017) made similar observations and reported that DAM in Alzheimer's disease indeed down-regulate expression of genes associated with homeostatic microglia (*P2ry12, P2ry13, Tmem119, Cx3cr1*) and instead up-regulate genes associated with phagocytosis and lipid metabolism (*Apoe, Ctsd, Lpl, Tyrobp, Trem2, Cst7*). Furthermore, they observed that DAM co-localized with Aβ-plaques [626]. Masuda et al. (2019) used a model of demyelination (cuprizone) and confirmed that microglia in these circumstances up-regulate expression of genes associated with DAM (*Apoe, Mafb, Apoc1, Gpnmb, Anxa2, Lglas1, Spp1*, *Lpl*) while suppressing expression of genes associated with homeostatic microglia (*Tmem119*, *P2ry12*). Moreover, they found that the expression profile of DAM in rodents and humans was similar [627]. In addition, DAM has also been detected and described in experimental models of multiple sclerosis (EAE, experimental autoimmune encephalomyelitis). While these microglia also down-regulate genes associated with homeostatic microglia (*P2ry12, Tmem119, Siglech, Selplg, Gpr34, P2ry12, Maf, Slc2a5, Sal1, Serpine2, Bhlhe41*) they specifically up-regulate genes associated with chemokines and proliferation (*Mki67, Ly86, Ccl2* and *Cxcl10*) [628]. However, in EAE the down-regulation of genes associated with homeostatic microglia seem to be temporary and the cells regain their original expression after the acute phase of the disease [629]. Krasemann et al. (2017) injected apoptotic neurons into the CNS that were phagocytized but also mediated the transformation of homeostatic microglia into DAM. The authors concluded that the formation of DAM was a result of suppression of TGF-β-signaling and enhancement of *Trem2*-regulated *Apoe*-signaling. The *Apoe*-signaling mediated suppression of transcription factors (*Mafb, Mef2a, Smad3*) associated with homeostatic microglia and up-regulation of transcription factors (*Atf3, Tfec, Bhlhe40*) associated with inflammatory programs [629]. Facial nerve axotomy used as a model of transient neurodegeneration has also been associated with elevated expression levels of *Apoe, Axl, Ccl12, H2-K1, Ctss, H2-D1* and suppression of *Cst3, Maf, Gpr34, P2ry12*, *Sparc* implying formation and existence of DAM [630]. Activation of microglia using LPS, which simulates

acute inflammation, also induces a transformation of microglia into DAM characterized by suppression of homeostatic microglia genes (*Siglech, Tmem119, P2ry12, Mef2c, Fcrls, Olfml3*), genes related to anti-inflammation (*Mrc1, Arg1*) and phagocytosis (*Tyrobp, Trem2*). LPS stimulation however up-regulate expression of genes related to pro-inflammation (*Il1b, Ccl2, Tnf, Nfkbia, Gpr84, Spp1, Tlr2*) in microglia [631]. Moreover, myeloid cells in the CSF of patients infected with human immunodeficiency virus (HIV) have an up-regulated expression of genes associated with DAM (*Apoe, Axl, Trem2, Apoc1, Ctsb, C1qa, C1qb, C1qc*) [632]. Taken together, DAM seem to be present in various types of CNS pathology indicating that microglia respond in a homogenous fashion to injury and disease.

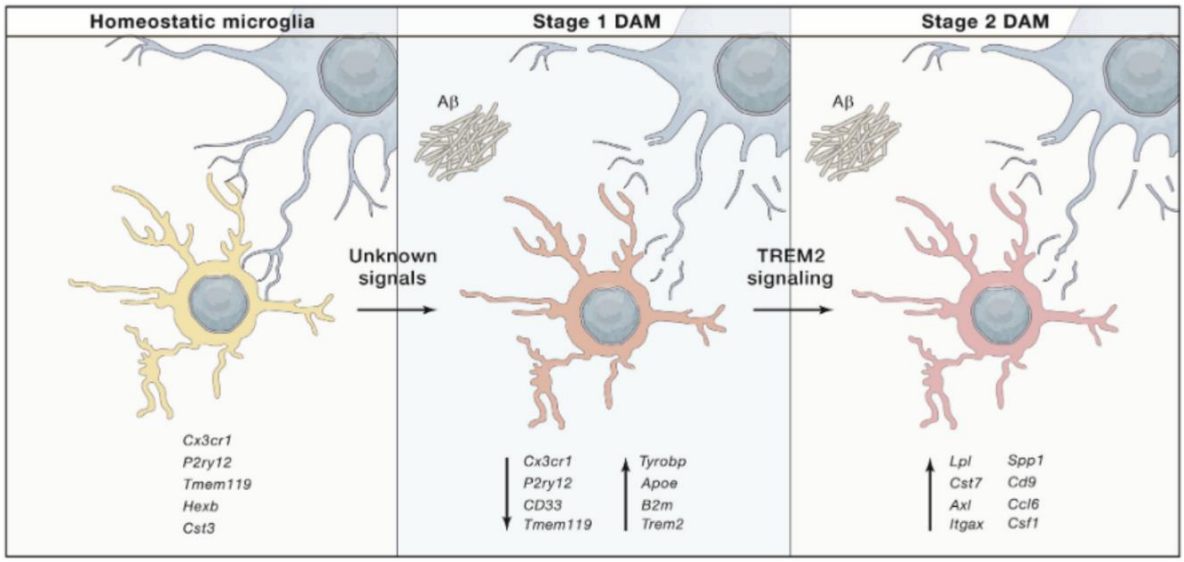

Figure 7. *Trem2*-independent and *Trem2*-dependent transformation of homeostatic microglia into disease-associated microglia (DAM) type one and two.

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Disease-associated microglia in the developing brain

Microglia with a transcriptional signature of DAM have been detected in brains of healthy developing mice, in addition to in CNS pathology. Li et al. (2019) reported that microglia in the developing brain, especially in the white matter, express low levels of genes associated with microglia in homeostasis (*P2ry12, Tmem119, Tgfbr1*) and instead express high levels of genes characteristic for DAM (*Igf1, Lpl, Clec7a, Fabp5, Cd63, Spp1, Itgax, Gpnmb, Apoe, Tyrobp*). These cells were especially characterized by expression of *Ccl3* and *Ccl4*, were able to phagocytize oligodendrocytes and astrocytes, and were therefore termed *postnatal proliferative-region-associated microglia* (pPAM) or *white matter-associated microglia* (WAM). In contrast to DAM in CNS pathology, the formation of WAM was not regulated by the *Trem2-Apoe* signaling-pathway [633]. Moreover, Masuda et al. (2019) reported that postnatal microglia have an expression profile similar to microglia in homeostasis (*Tmem119, Selplg, Slc2a5*) but also over-express *Cst3, Sparc* and *Iba1* while embryonic microglia instead are characterized by elevated expression of genes associated with lysosomal activity (*Ctsb, Ctsd, Lamp1*) but also by *Apoe* and *Iba1* [627].

Formation and origin of disease-associated microglia is not yet fully understood

Currently, the transformation of homeostatic microglia into DAM is thought to be mediated mainly by a *Trem2*-dependent pathway. More precisely, the initial transformation of homeostatic microglia into DAM1 is not mediated by *Trem2* but is then followed by a *Trem2*-dependent transformation from DAM1 into DAM2 (**Figure 7**) [626, 634]. It is in the first transformation into DAM1 in which microglia down-regulate expression of *Tmem119* and *P2ry12* and up-regulate expression of *Tyrbop* and *Apoe*. During the second transformation from DAM1 into DAM2 these cells instead up-regulate expression of *Cst7, Lpl* and *Axl*. Interestingly, the *Trem2*-mediated activation of microglia seems to enhance the survival and proliferation of DAM [635]. Moreover, in the absence of *Trem2,* microglia do not seem to transform from DAM1 into DAM2 but they do acquire a phagocytic phenotype [629, 636]. The *Trem2*-signaling seems to be partially activated by phospholipids in Aβ-plaques in disease models of Alzheimer's disease [637]. Moreover, *Trem2* can bind to lipoproteins on Aβ-plaques [638] which results in microglia phagocytosing Aβ-plaques at a higher rate [639].

Although *Trem2*-signaling seems to mediate the transformation of DAM1 into DAM2, the initial activation of homeostatic microglia is not yet fully understood. One hypothesis is that microglia respond to specific molecular patterns associated with CNS pathology which they detect using sensory mechanisms. Extracellular protein aggregates (e.g. Aβ-plaques), ADP, myelin debris and apoptotic bodies have been suggested and to some extent demonstrated to be able to act as such activating molecular patterns [635]. *P2ry12* for example, a gene which defines microglia in homeostasis, is a receptor for ADP which is a danger signal in the CNS [626]. Moreover, down-regulation of regulators such as *Cx3cr1* and CD200 which are associated with homeostatic condition of microglia is possibly inducing the transformation [626]. Activation of microglia mediated by other immune cells responding to CNS pathology is another likely mechanism which might contribute to initiating the transformation [640]. Moreover, the ability of microglia to transform seems to be reduced with increased age and is currently explained by a dysregulation of transcription factors and chromatin modifiers in microglia [641]. In conclusion, DAM down-regulate expression of homeostatic markers for microglia (*Tmem119, Cx3cr1, P2ry12, P2ry13*) [642] and up-regulate genes related to lipid metabolism, phagocytosis and lysosomal activity (*Trem2, Apoe, Tyrobp, Ctsd, Lpl*) [643]. Several questions concerning DAM are still unanswered. The relation between DAM, disease progression and outcome in CNS pathology has not been properly evaluated. The mechanisms, which mediates transformation of homeostatic microglia into DAM, is not fully understood and neither is the mechanism that triggers and initiates this transformation.

AIM

The cellular- and immune response following SCI is complex and has implications for regeneration and recovery. The immune cell response and the microglial response in particular, following SCI has not yet been investigated at single-cell resolution. Furthermore, several questions remain concerning the importance of histocompatibility of transplanted stem cells, their cellular response and their causal contribution to functional recovery following SCI. Therefore, this thesis aimed at investigating stem cell transplantation as a therapeutic approach for SCI as well as disease-specific transformations of immune cells in SCI.

The general aim was divided into four specific aims:

1. Investigate neural progenitor cells transplanted into SCI in terms of their differentiation, transcriptional changes, effect on neuroinflammation and causal contribution to functional recovery (**Paper I**).

2. Evaluate differences in therapeutic potential between syngeneic- and allogeneic mesenchymal stem cells transplanted into SCI (**Paper II**).

3. Investigate the cellular response of mesenchymal stem cells transplanted into SCI (**Paper III**).

4. Investigate disease-specific transformations of immune cells over time in SCI at single-cell resolution (**Paper IV**).

MATERIAL AND METHODS

Ethical considerations

All animal experiments were conducted according to ethical permits granted by the ethical committee in Stockholm (Sweden) (**Table 1**). In addition, specific regulations and guidelines issued by veterinarians at Karolinska Institutet were implemented at all times. Human spinal cord injury (SCI) samples were retrieved from an ongoing clinical trial (SC0806-A101, NCT02490501) approved by the Swedish Medical Products Agency Study Protocol. All study participants gave informed written consent for participation.

Table 1. Ethical permits.

Permit	Principal investigator	Date (approval)	Date (valid)	Purpose
N38/16	Lou Brundin	2016-01-28	2021-02-28	Cell harvesting
N196/15	Lou Brundin, Mikael Svensson	2015-11-12	2020-11-12	SCI, cell transplantation
N12317/2017	Lou Brundin, Mikael Svensson	2017-09-25	2020-11-12	SCI, cell transplantation
N124/15	Mikael Svensson	2015-08-20	2020-08-20	SCI
N138/14	Robert Harris, Tomas Olsson, Maja Jagodic	2014-07-08	2020-07-08	Breeding
2013/2257-31/1 2015/1436-31 2019-03671	Hans Basun, Mikael Svensson	2013-01-29 2015-09-10 2019-07-31	No time limit (*maximum 45 patients*)	Resection of glial scar in human SCI and replacement with a FGF1 soaked device

Abbreviations: SCI: Spinal Cord Injury; FGF1: Fibroblast Growth Factor 1.

Experimental animals

Each researcher involved in experiments involving laboratory animals was educated and certified at FELASA B equivalent level. Experimental animals were kept at an approved and regularly controlled facility at Karolinska Institutet. Animals were kept in room temperature with constant access to food and water. The room was lit twelve out of twelve hours per day. Cages were enriched and no more than five animals were kept in each cage. Veterinarians were always informed of ongoing experiments and were available for secondary opinion on questions concerning animal health. Animals were acclimatized to the facility during at least 7 days prior to surgical intervention and/or functional evaluation. Animals in ongoing experiments experiencing deterioration in health were scored using a template developed by the veterinarians at Karolinska Institutet (*Utökad bedömning av djurhälsa för smågnagare och kanin postoperativt eller annars när det är påkallat*) and treated accordingly.

Mus musculus

Inbred mice were purchased through Scanbur (Stockholm, Sweden). SCI was induced in C57BL/6J (The Jackson Laboratory, 000664) female mice. Female mice have shorter urinary tracts, which makes assisted bladder compression significantly easier, which is the reason for only inducing SCI in female mice. Cells were harvested from C57BL/6J or BALB/cJ (The Jackson

Laboratory, 000651) mice. Male Cx3cr1^{GFP} (The Jackson Laboratory, 005582) were crossed with female C57BL/6J mice in order to obtain C57BL/6J-Cx3cr1^{GFP} mice. Microglia/macrophage- and monocyte populations were isolated from the spinal cords of healthy and injured C57BL/6J-Cx3cr1^{GFP} mice and the cells were subjected to bulk RNA sequencing. Cx3cr1^{CreER} (The Jackson Laboratory, 021160) were crossed with Rosa26^{DTA} (The Jackson Laboratory, 010527) to obtain Cx3cr1^{CreER+}Rosa26^{DTA+} mice. All *Cx3cr1*$^{+}$ cells (~microglia) in the central nervous system (CNS) of the Cx3cr1^{CreER+}Rosa26^{DTA+} mice were labelled with yellow fluorescent protein (YFP). Cx3cr1^{CreER+}Rosa26^{DTA+} mice induced with tamoxifen resulted in a depletion of *Cx3cr1*$^{+}$ cells in the entire CNS of the animal. All inbred mice were genotyped prior to use (below). Mice used in experiments were between 8-10 weeks old with a weight between 18-20g, except BALB/cJ mice that were all male and between 4-6 weeks old.

Rattus norvegicus

The ventricular-subventricular zone (V-SVZ) from adult Lewis rats (LEW-Tg(EGFP)455Rrrc) heterozygous for the enhanced green fluorescent protein (eGFP) under the ubiquitin C promotor on chromosome 5 were harvested from which neural progenitor cells (NPCs) were established. These GFP^{+}NPCs were transplanted into wild-type littermates. All rats were genotyped prior to use (below).

Genotyping

Ear biopsies from C57BL/6J-Cx3cr1^{GFP} mice were incubated in 100 µl of ddH_2O with 1:200 5 M NaOH (in-house) and 1:2500 0.5 M EDTA (Thermo Fisher, AM9260G) for 1 hour at 95°C. 100 µl ddH_2O with 1:25 Tris-HCl (Sigma, 10812846001) was added to the suspension and samples were kept on ice. 16 µl of PCR master mix (62.5 % REDExtract-N-Amp PCR (Sigma, R4775), 28 % RNase-free H_2O, 3 % mutant primer, 3 % wild-type primer and 3 % common primer) was combined with 4 µl of the suspension in each well of a 96-well PCR plate (Life technologies, N8010560). Primer stock concentration was 20 µM (Eurofins Genomics) (**Table 2**). Thermal cycling was performed at 94°C for 2 min, 10 cycles of: 94°C for 20 s; 65°C for 15 s (decreased 0.5°C/cycle) and 68°C for 10 s followed by 28 cycles of: 94°C for 15 s; 60°C for 15 s and 72°C for 10 s followed by 2 min at 72°C. The samples were added onto an agarose gel SYBR™ Safe DNA Gel stain (Invitrogen™, G521802) in combination with 20 µl of DNA ladder (Invitrogen™, SM0241) and connected to a power-supply (Bio-Rad, Power-Pac 100) delivering 90 V during ~15 min. Gels were then analyzed (Bio-Rad, ChemiDoc™ XRS^{+}).

Ear biopsies from Cx3cr1^{CreER+}Rosa26^{DTA+} mice were first denatured for 5 min at 94°C and then subjected to 35 cycles of: 94°C for 30 s, 60°C for 60 s and 72°C for 60 s followed by incubation at 72°C for 2 min using a PCR apparatus (Applied Biosystems, 2720) (**Table 2**). DNA fragments were analyzed on gels (4 % agarose) and visualized using GelRed fluorescent nucleic acid gel stain (Bio-Rad, ChemiDoc™ XRS^{+}).

Genotyping was performed on ear biopsies using standard protocols (Rat Research Resource center RRRC, Columbia). REDExtract-N-AMP™ Tissue PCR Kit was used for obtaining total DNA from each biopsy using manufacturer's protocol. The animals were inspected before use by illuminating the eyes using a flashlight with an attached dichroic filter (excitation at 470/40 nm) enhancing the GFP fluorescence which was inspected by placing an emission filter (515/30 nm) above the eye of the rat.

Table 2. Primers for genotyping.

Strain	Primer	Sequence 5'→3'
C57BL/6J-Cx3cr1^{GFP}	moIMR0003	GGGCCAGCTCATTCCTCCCACTCAT
	oIMR4281	CCACAGGATTTCAGCCTGAACTTTG
	oIMR4282	CGTGCACTATGCTCAGATATCTGTC
Cx3cr1^{CreER+}Rosa26^{DTA+}	Rosa26 Mutant Forward	CGACCTGCAGGTCCTCG
	Rosa26 Mutant Reverse	CTCGAGTTTGTCCAATTATGTCAC
	Rosa26 WT Forward	CGTGATCTGCAACTCCAGTC
	Rosa26 WT Reverse	GGAGCGGGAGAAATGGATATG

Experimental spinal cord injury

Intraperitoneal anesthesia was used in order to ensure a reproducible and stable anesthesia that allowed one to easily move the animal during surgery. Medetomidin (0.5 mg/kg, Domitor® vet. 1 mg/ml) and ketamine (75 mg/kg, Ketador vet. 100 mg/ml) were given i.p. to mice. Rats were anesthetized using 5 % isoflurane (Baxter) with 30 % oxygen, which was reduced to 2.5 % isoflurane during surgery. At the time of induction buprenorfin (0.05 mg/kg, Temgesic® 0.3 mg/ml) and karprofen (5 mg/kg, Rimadyl® vet. 50 mg/ml) were given s.c. to counteract pain. Dehydration was antagonized by s.c. injection of normal saline (Baxter) prior to surgery. Weight was documented and animals were marked using ear biopsy. Eyes were protected using cerate (Oculentum simplex) and the fur on the back on the place for the upcoming incision was shaved (Aesculap, ISIS 273278). Animals were placed on a heating pad, a small skin incision was made and the muscles were gently set aside and the spinal column stabilized by using a stereotaxic frame (Model 900 & 900-c, Kopf®). The dorsal part of the vertebrae above Th10/11 was removed using a drill (Anspach®, EMAX® 2). The dura mater was cut open and a severe contusion SCI (75 kdyn in mice, 200 kdyn in rats) was induced using a IH-0400 impactor (Infinite Horizon). A SCI was called successful if the measurement displayed by the apparatus showed a distinct impact peak and both hind limbs extended at the moment of impact and retracted and ended up flaccid. Bupivakain (Marcain®, 2.5 mg/ml) was administrated into the muscles surrounding the site of incision prior to suturing the skin. The skin was closed using a running suture (Ethicon, Vicryl 4.0) supplemented with two or three single sutures in order to properly secure the closure. Animals were allowed to recover on a heating pad and observed for a while prior to a second administration of analgesics. Analgesics were administrated twice daily during the first 3 days following surgery. Assisted bladder emptying was applied until reflexive bladder emptying returned. Mice were weighed at the day of surgery and weekly thereafter. Animals were sacrificed given a weight loss exceeding 25 % of their initial weight.

Mesenchymal stem cells

Bone marrow for establishment of mesenchymal stem cells (MSCs) was harvested from mice (C57BL/6J, BALB/cJ). Animals were given a lethal dose of pentobarbitalnatrium i.p. (Apotek Produktion & Laboratorier, 60 mg/ml) and underwent cervical dislocation prior to initiation of harvest. Following rinse in 70 % ethanol (Solveco, 1000) the skin was cut open in a circular fashion around the abdomen and the skin was removed by pulling in a caudal direction. The tibia and femur were removed, isolated and kept on ice in HBSS (Gibco®, 14170112) containing 1:50 Penicillin-Streptomycin (Pen-Strep, Gibco® 15140122). Muscles and connective tissue were removed from the bone and the epiphysis was cut open on both sides in

a ventilated cell culture hood. The bone marrow was removed from the bone by flushing with pre-warmed basal medium (89 % α-MEM (Gibco®, 22561054), 1 % Pen-Strep, 10 % fetal bovine serum (FBS, Gibco® 10082147) through the bone using a 27 gauge needle connected to a 10 ml syringe. The suspension was passaged several times through a thick needle (18 gauge) attached to a 10 ml syringe to properly dissociate the cells. The suspension was run through a 70 μm cell strainer (Corning®, 352350) prior to counting (BLAUBRAND®, Bürker 718920). The cells were centrifuged at 300xg for 10 min and re-suspended in basal medium and plated ($1.45x10^6$ cells/cm^2) in 100 mm cell culture dishes with tissue culture treated surfaces (Nunc™, 150350) and kept at 37°C and 5 % CO_2. Three days following initial plating the basal medium was replaced.

Immunodepletion

The MSCs were harvested for immunodepletion 8-10 days following initial plating. Plates were washed with 1xDPBS (Gibco®, 14190250) and incubated for 5 min at 37°C in 4 ml of 0.5 % Trypsin-EDTA (Gibco®, 15400054). Non-lifted cells were mechanically removed using a cell scraper (Nunc™, 179693). Trypsin was inactivated with 0.5 ml FBS, cells collected and stored on ice. Cells were washed twice in 20 ml α-MEM (500xg, 15 min, 4°C). Cells were immunodepleted using biotinylated antibodies against CD11B, CD45 and CD34 (**Table 3**) using Dynabeads™ M-280 Streptavidin (Gibco®, 11206D) and a magnetic particle concentrator (DYNAL MPC®-1, 120.01) as described by Boregowda et al. (2016). Five Dynabeads™ per cell and 10 μg antibody per target was used. The immunodepleted MSCs were plated in pre-warmed basal medium at $1x10^6$ cells/cm^2 in T75 flasks (Nunc™, 156499) and kept at 37°C and 5 % CO_2. Basal medium was replaced 2-3 times per week [644].

Tri-lineage differentiation potential

MSCs were cultured 5-7 days post immunodepletion prior to evaluation of tri-lineage differentiation potential. Adipogenic differentiation was induced by plating MSCs at 12.000 cells/cm^2 in 35 mm tissue culture treated dishes (Falcon®, 353001) in basal medium. One day post plating the basal medium was replaced with adipogenic induction medium (α-MEM, 1 % Pen-Strep, 10^{-8} M Dexamethasone (Sigma, D4902), 20 μM 5, 8, 11, 14 – Eicosatetraynoic acid (Sigma, E1768), 25 μg/ml bovine insulin (Sigma, I0516), 10 % rabbit serum (Sigma, R4505)) and replaced 2-3 times/week. After 2-3 weeks of culture, the cells were fixed with 4 % paraformaldehyde (PFA) (Solveco, 1198) for 30 min. After wash in dH_2O, cells were stained in a working solution (60 %, 0.5 % stock in isopropanol diluted in dH_2O) of Oil Red O Staining (Sigma, O1391) for 20 min, washed in tap water and then analyzed under a microscope (Zeiss, Axiovert 200). Gene expression for *Cfd* and *Lpl* (Eurofins Genomics) was estimated using RTqPCR (**Table 4**) [644]. Osteogenic differentiation was induced by plating 8.000 cells/cm^2 in 35 mm tissue culture treated culture dishes in basal medium. One day post plating the basal medium was replaced with osteogenic induction medium (high glucose DMEM (Sigma, D5796), 1 % Pen-Strep, 10 % FBS, 10 mM β-glycerol phosphate (Sigma, G9422), 50 μg/ml ascorbic acid-2-phosphate (AA2P) (Sigma, A8960), 10^{-8} M Dexamethasone (Sigma, D4902)). The induction medium was changed 2-3 times/week for a total of 3 weeks. After 3 weeks the cells were washed in 1xPBS and fixed in 4 % paraformaldehyde (PFA) 20-30 min at RT and finally stained in 2 % Alizarin Red S (Sigma, A5533) for 5 min, washed in tap water, and then

visualized under a microscope. Gene expression for *Ibsp*, *Spp1*, *Ctsk*, *Runx2* and *Sparc* (Eurofins Genomics) (**Table 4**) was estimated using RTqPCR [644]. Chondrogenic differentiation was induced in a three-dimensional culture [645, 646]. 2.5x10^6 MSCs (500.000 cells/ml) were re-suspended in chondrogenic induction medium (high glucose DMEM, 1 % Pen-Strep, 10 ng/ml TGF-β3 (Sigma, 80330), 100 nM Dexamethasone, 50 µg/ml ascorbic acid-2-phosphate (AA2P), 100 nM sodium pyruvate (Sigma, P5280), 40 µg/ml proline (Sigma, P5607), 6.25 µg/ml bovine insulin (Sigma, I0516), 6.25 µg/ml transferrin (Sigma, T8158), 6.25 µg/ml selenous acid (Sigma, 211176), 5.33 µg/ml linoleic acid (Sigma, L1012), 1.25 mg/ml bovine serum albumin (Sigma, A7906) and centrifuged at 500xg for 10 min at 4°C to pellet the cells. Induction medium was changed 2-3 times per week without disturbing the pellet. Gene expression of *Sox9* (Eurofins Genomics) (**Table 4**) was estimated using RTqPCR [644].

Phenotypic characterization

Undifferentiated MSCs (P3, P12) were washed using 1xDPBS and harvested by incubation in 0.5 % Trypsin-EDTA at 37°C for 5 min. Cells were washed twice in α-MEM (500xg, 15 min, 4°C). CD105, CD29 and SCA1 were used as positive markers and CD45 as a negative marker for MSCs using a multi-color flow kit (R&D Systems, FMC003). Non-specific background signal caused by primary antibodies was measured using cell marker specific isotype controls. Fc receptor-mediated binding by antibodies was reduced by incubating samples in α-CD16/CD32 antibody at a concentration of 10 µl per 100 µl cell suspension for 5 min on ice prior to staining (**Table 3**). Cells were analyzed using a BD LSRFortessa™ cell analyzer. Phenotypic characterization of MSCs was also performed following transfection (below).

Traditional colony-forming unit assay

The growth potential of MSCs was assessed using traditional colony forming unit assay (tCFU-assay). MSCs (P10) were plated in a six-well plate (Nunc™, 140675) at a density of 100 cells/cm^2 in basal medium and incubated at 37°C and 5 % CO_2. Basal medium was replaced at 3- and 6 days post plating [647, 648]. After 7 days in culture the cells were washed (1xDPBS) and fixed with 4 % PFA for 20-30 min and stained with 3 % crystal violet (Sigma, C3886) in methanol at RT for 20 min [649] followed by wash in dH_2O. An inverted microscope was used for scoring and counting the colonies. A colony was defined as 5 or more cells with fibroblast-like morphology [648, 650].

Population growth dynamics and doubling time

MSCs (P10) were plated in two six-well plates at ~220 cells/cm^2 in basal medium and kept at 37°C and 5 % CO_2 for a total of 12 days. Every other day two six-wells were harvested using 0.5 % Trypsin-EDTA in 1xDPBS. Cells were counted using a counting chamber and the population doubling time (PDT) was estimated. The PDT takes into account the number of cells (N) at plating (0) and at a later time point (t) [648].

$$PDT = \frac{t \times \log(2)}{\log(N_t/N_0)}$$

Table 3. Antibodies.

Type	Host	Target	Fluorochrome	Manufacturer	ID
Primary	Goat	IBA1	NA	Abcam	ab5076
	Rabbit	IBA1	NA	Wako	019-19741
	Goat	IBA1	NA	Abcam	ab48004
	Mouse	NEUN	NA	Merck Millipore	MAB377
	Mouse	TUJ1	NA	Merck Millipore	MAB1637
	Rabbit	GFAP	NA	Dako	Z0334
	Rat	MHC-II	NA	Abcam	ab25333
	Rabbit	CASPASE-3	NA	Abcam	ab13847
	Mouse	NESTIN	NA	Merck Millipore	MAB353
	Mouse	CC1	NA	Merck Millipore	OP80
	Mouse	CNPase	NA	Abcam	ab6319
	Rabbit	SOX2	NA	Merck Millipore	AB5603
	Rabbit	OLIG2	NA	Abcam	ab109186
	Rabbit	GFP	NA	Merck Millipore	AB3080
	Rat	MBP	NA	Abcam	ab7349
	Mouse	NF	NA	Dako	GA607
	Rabbit	CST7	NA	Nordic Biosite	12073-1-AP
	Rabbit	TMEM119	NA	Abcam	ab209064
	Mouse	APOE	NA	Abcam	ab1907
	Rabbit	IGF1	NA	Abcam	ab40657
Secondary	NA	GOAT	Alexa Fluor® 488	Invitrogen	A11055
	NA	MOUSE	Alexa Fluor® 488	Invitrogen	A11001
	NA	RABBIT	Alexa Fluor® 488	Invitrogen	A11008
	NA	MOUSE	Alexa Fluor® 594	Invitrogen	A11005
	NA	RABBIT	Alexa Fluor® 594	Invitrogen	A21207
	NA	GOAT	Alexa Fluor® 594	Invitrogen	A27016
	NA	RAT	Alexa Fluor® 568	Invitrogen	A11077
	NA	MOUSE	Alexa Fluor® 647	Invitrogen	A28181
	NA	RABBIT	Alexa Fluor® 647	Invitrogen	A27040
	NA	GOAT	Alexa Fluor® 647	Invitrogen	A27018
Pre-conjugated	Rat	CD45	PE/Cy7	BD Biosciences	552848
	Rat	CD45	PE/Cy7	Biolegend	103113
	Mouse	CD45	v450	BD Biosciences	560697
	Rat	CD45	PE	BD Biosciences	553081
	Rat	CD11B	PE	Biolegend	101208
	Rat	CD11B	PerCP-Cy5.5	Biolegend	101227
	Rat	CD11B	APC	BD Biosciences	553312
	Rat	CD11B	BV421	BD Biosciences	562605
	Mouse	CD64	PerCP-Cy5.5	Biolegend	139308
	Rat	LY6G	v450	BD Biosciences	560603
	Rat	LY6G	BV421	BD Biosciences	562737
	Rat	LY6C	PE	Biolegend	128007
	Hamster	CD29	APC	eBioscience	17-0291-80
	Rat	SCA1	FITC	BD Biosciences	561077
	Rat	MHC-I	FITC	Biolegend	125508
	Rat	MHC-II	FITC	Biolegend	107606
	Hamster	CD3E	BV421	BD Biosciences	562600
	Rat	CD19	BV421	BD Biosciences	562701
	Rat	SIGLEC-F	BV421	BD Biosciences	562681
	Mouse	NK1.1	BV421	Biolegend	108741
	Rat	CD14	FITC	Biolegend	123307
	Rat	CD11B	Biotin	BD Biosciences	553309
	Rat	CD45	Biotin	BD Biosciences	553078
	Rat	CD34	Biotin	eBioscience	13-0341-85
Fc block, Counterstain, Live/dead marker	Rat	CD16/32	NA	BD Biosciences	553141
	NA	dsDNA	Hoechest 33258	Abcam	ab228550
	NA	dsDNA	Hoechest 33342	Thermo Fisher	62249
	NA	dsDNA	Propidium iodide	Sigma	P4170
	NA	Free amines	Near-IR	Invitrogen	L34959

Expression construct and transfection

HBEGF/DTR linked to mCherry was inserted into the CAG promotor of a modified episomal expression vector (EEV, System Biosciences EEV600A-1) (Eurofins Genomics) [651, 652]. Prior to use in culture the EEV plasmid was made endotoxin-free using a kit and manufacturer's instructions (Qiagen, 12362). 10.000 MSCs were plated per cm^2, in basal medium, 4 days prior to transplantation. MSCs were transfected using a kit (Lipofectamine™ 3000, Invitrogen) 2 days following plating in combination with replacing the medium with fresh basal medium. Cells were incubated for an additional 2 days, washed in 1xDPBS, harvested and stored in α-MEM on ice until transplantation. mCherry fluorescence of transfected MSCs was inspected and confirmed prior to harvesting and transplantation (Zeiss, Axiovert 200).

Mouse stromal vascular fraction

Adipose tissue (brown and white) was harvested from mice and kept on ice in HBSS with 1:50 Pen-Strep. Using two scalpels the tissue was first mechanically dissociated/minced followed by incubation in 2 mg/ml Collagenase P (Sigma, 11213857001) at 37°C for 1 hour. Suspension was triturated every 10 min. The suspension was passed through 100 µm (Corning®, 431752) and 40 µm (Corning®, 431750) cell strainers in order to remove debris and aggregation of cells. Following wash with basal medium (600xg, 7 min) the cells were re-suspended in 3 ml ACK lysis buffer (Gibco®, A1049201) and kept at RT for 5 min. Following wash in 1xDPBS twice (600xg, 5 min), the cells were re-suspended in α-MEM and kept on ice until transplantation [653].

Neural progenitor cells

Harvesting and culturing

The V-SVZ was harvested from adult rats expressing GFP and used for isolation of NPCs according to the modified protocol from Johansson et al. (1999) [654]. Following a lethal injection of pentobarbitalnatrium i.p., the rats were decapitated and the cerebrum dissected and kept in 1xDPBS on ice. The V-SVZ was identified using a surgical microscope (Nikon, SMZ-2T) and removed using surgical scissors. The V-SVZ was enzymatically dissociated with 10 U/ml papain (Worthington, LS003126), washed in L-15 medium (Invitrogen™, 11415064) and cultured in growth medium (DMEM/F-12 (Gibco®, 31331093), B27 (Gibco®, 17504044), Pen-Strep (Sigma, 15140122), epidermal growth factor (20 ng/ml, Sigma E4127), basic fibroblast growth factor (10 ng/ml, R&D Systems 3339-FB). Growth factors were added to the cultures every second day. Neurospheres were passaged twice, the first time 4-5 days following initial plating. Size and fluorescence of neurospheres were evaluated using a table microscope prior to transplantation (Nikon, eclipse TS100).

Multi-lineage differentiation potential

Neurospheres or single NPCs (dissociated using papain) were plated in growth medium at 10.000 cells/well in a 24-well plate (Nunc™, 142475) onto coverslips coated with poly-D-Lysine (Sigma, A-003-M). Some cells were kept undifferentiated, while cells intended for differentiation

were induced 1 day after plating and cultured for 7 days in L-15 with 1 % FBS. Cells were then fixed with PFA (2 %) and evaluated using immunocytochemistry.

Stem cell transplantation

Mesenchymal stem cells

One day following SCI surgery, the mice were anesthetized once again. The spinal cord was exposed with no additional incision of the skin or surrounding tissue. A total of $0.5x10^6$ MSCs in 3-4 µl α-MEM were transplanted using a pipette prepared from a glass capillary needle (WPI, 1B150F-6) connected to a 10 µl syringe (Hamilton®, 80330) into 4 sites adjacent to the injury epicenter. A pipette puller (HEKA, PIP5) was utilized to form the glass capillary needle into a pipette tip. The syringe was attached to a fixating arm on a stereotaxic frame (Model 900 & 900-c, Kopf®) and the cell suspension was delivered under microscopic visualization. The suspension was injected slowly, the needle was left in place for 5 minutes and then slowly retracted with intermittent pausing. The skin was closed (Ethicon, Vicryl 4.0) and analgesics administrated in the same fashion as during SCI surgery. Transplantation of MSCs established from C57BL/6J mice to C57BL/6J mice was defined as *syngeneic* transplantation while transplantation of MSCs established from BALB/cJ mice into C57BL/6J mice was defined as *allogeneic*.

Neural progenitor cells

NPCs were transplanted using a method similar to MSC transplantation. However, rats were treated with 10 mg/kg cyclosporine (Novartis, Sandimmun® 50 mg/ml) once daily during the first week following SCI. $0.5–0.6x10^6$ NPCs (neurospheres and single NPCs) diluted in 6 µl 1xDPBS were transplanted into the SCI epicenter at 8-10 days following SCI.

Locomotor and kinematical assessment of hind limb function

The hind limb function of rats and mice were objectively documented and observed when walking on a runway. The animals were recorded (Canon EOS 6D, 60 fps, 1/800 shutter speed) and the recordings analyzed offline (Click Joint, AEA solutions). In order to ensure proper visualization, the animals were placed under a brief anesthesia using 4 % isoflurane. The fur on the hind limbs was removed, and the joints of the hind limbs were labelled using a permanent marker. The following anatomical landmarks on the hind limbs were labelled: I) iliac crest, II) greater trochanter, III) lateral malleolus, IV) metatarsophalangeal joint of the fifth toe, and V) tip of the toe. Animals were given 2–3 practice runs prior to induction of SCI in order to acclimatize to the measurement environment and equipment. A 100 cm runway was used for walking analysis from which the first and last 10 cm were excluded from analysis (80 cm of efficient runway was used in analysis). The glass was illuminated with LED light, which enabled visualization of the stepping pattern of the animals. By using a small amount of water coating the surface, the paws were clearly visible when pressed onto the bottom surface. Random frames from recordings were selected. Using the software, the height of the iliac crest, stride- width and length, footsteps, number of steps on runway, velocity, and protraction- and retraction distances were measured. The iliac crest height (ICH) was defined as the distance from the foot to the iliac crest at mid-stance face. The stride width was defined as the horizontal distance between the legs,

while the stride length is the vertical distance between steps (for the same foot) along the axis of movement [106]. Protraction was defined as the forward dragging of the hind limbs, while retraction is defined as the opposite. Using the recordings the Basso, Beattie and Bresnahan (BBB) locomotor rating scale was estimated for each animal at each time point of observation [104]. For mice the Basso Mouse Scale (BMS) score was estimated instead of the BBB using an open field environment constructed using Plexiglas [655]. The locomotor rating scale and kinematical analysis were assessed prior to the SCI and up to 12 weeks post SCI.

Table 4. Primers for RTqPCR.

	Gene	Sense	Antisense
Mus musculus	*Cfd*	ATGGTATGATGTGCAGAGTGTAG	CACACATCATGTTAATGGTGAC
	Lpl	GAGGACACTTGTCATCTCATTC	CCTTCTTATTGGTCAGACTTCC
	Ibsp	CAAGCGTCACTGAAGCAGGTG	CATGCCCCTTGTAGTAGCTGTATT
	Ctsk	TGCCTTCCAATACGTGCAGCA	TGCATTTAGCTGCCTTTGCCG
	Runx2	CCGCACGACAACCGCACCAT	CGCTCCGGCCCACAAATCTC
	Sparc	AGCGCCTGGAGGCTGGAGAC	CTTGATGCCAAAGCAGCCGG
	Spp1	CAGTGATTTGCTTTTGCCTGTTTG	GGTCTCATCAGACTCATCCGAATG
	Sox9	CTCTGGAGGCTGCTGAACG	TTGTAATCGGGGTGGTCTTTCTT
	Actb	TGGAATCCTGTGGCATCCATGAAAC	TAAAACGCAGCTCAGTAACAGTCCG
	Gapdh	AAGGGCTCATGACCACAGTC	CAGGGATGATGTTCTGGGCA
	Ccl2	GCTCAGCCAGATGCAGTTA	TACGGGTCAACTTCACATTC
	Ccl3	CCATGACACTCTGCAACCAA	CGTGGAATCTTCCGGCTGTA
	Ccl4	TGCTCGTGGCTGCCTTCT	CAGGAAGTGGGAGGGTCAGA
	Ccl5	GACAGCACATGCATCTCCCA	CCTTCGAGTGACAAACACGACT
	Ccl11	GGCTGACCTCAAACTCACAGAAA	ACATTCTGGCTTGGCATGGT
	Cxcl1	CTGGGATTCACCTCAAGAACATC	CAGGGTCAAGGCAAGCCTC
	Csf2	CTGTCACGTTGAATGAAGAGGT	GGCTTCCTCATTTTTGGCCT
	Ifng	AGCTGATCCTTTGGACCCTC	GTCACCATCCTTTTGCCAGTT
	Il1b	CTGTGTCTTTCCCGTGGACC	CAGCTCATATGGGTCCGACA
	Il1a	TGCGCTGCCAGGGGTCTT	ATTCCACGGTTCCACGGTTAGG
	Il5	GACAAGCAATGAGACGATGA	GAACTCTTGCAGGTAATCCA
	Il6	TAGTCCTTCCTACCCCAATTTCC	TTGGTCCTTAGCCACTCCTTC
	Il10	ACCTGGTAGAAGTGATGCCC	ACAGGGGAGAAATCGATGACA
	Il12a	CCAAACCAGCACATTGAAGA	AGCTCCCTCTTGTTGTGGAA
	Il12b	GGGACATCATCAAACCAGACCC	GCCTTTGCATTGGACTTCGG
	Tnfa	AAGCCTGTAGCCCACGTCGTA	GGCACCACTAGTTGGTTGTCTTTG
	Fcgr3a	TTTGGACACCCAGATGTTTCAG	GTCTTCCTTGAGCACCTGGATC
	Fcgr2a	AATCCTGCCGTTCCTACTGATC	GTGTCACCGTGTCTTCCTTGAG
	Mrc1	TCTTTGCCTTTCCCAGTCTCC	TGACACCCAGCGGAATTTC
	Arg1	TTGGGTGGATGCTCACACTG	TTGCCCATGCAGATTCCC
Rattus norvegicus	*Actb*	AGCTGAGAGGGAAATCGTGC	ACCAGACAGCACTGTGTTGG
	Gapdh	ATGGGAGTTGCTGTTGAAGTCA	CCGAGGGCCCACTAAAGG
	Il1a	GGCTAAGTTTCAATCAGCCCTTT	AGGTGCTGATCTGGGTTGGAT
	Il1b	TCCAGTCAGGCTTCCTTGTG	GGTCATTCTCCTCACTGTCGAA
	Il12a	TGTCAATCACGCTACCTCCTC	AAGACACTTGGCAGGTCCAG
	Csf2	TGCTCTGGAGAACGAAAAGAACG	ATTGAGTTTGGTGAGGTTGCCCC
	Ccl3	TCCTATGGACGGCAAATTCCACGA	AGATCTGCCGGTTTCTCTTGGTCA
	Tnfa	GGTGATCGGTCCCAACAAGGA	CACGCTGGCTCAGCCACTC

Active elimination of engrafted neural progenitor cells

The same expression construct used for transfection of the MSCs (above) was used for transfecting NPCs enabling them to be actively eliminated following transplantation. Hence, in this case the mCherry expression and the DTR was both of interest. Approximately 0.5–3 μg DNA was added to $0.2x10^6$ NPCs in buffer T. The transfection was conducted using electroporation (Neon® Transfection System) using settings: 990 V, 40 ms, 1 pulse. Cells were plated in growth medium following transfection and kept in culture for 2 days prior to transplantation. The transplanted NPCs were actively eliminated immediately post transplantation by injecting the rats with 100 mg/kg diphtheria toxin i.p. per day on 3 consecutive days.

Induction of Cre recombinase

Tamoxifen was used for induction of the Cre recombinase in the $Cx3cr1^{CreER+}Rosa26^{DTA+}$ mice. The tamoxifen (Sigma, T5648) was suspended in corn oil (Sigma, C8267) and incubated at 75°C for at least 1 hour. Five mg (200 μl) tamoxifen was administrated to each animal s.c., once daily for a total of 3 consecutive days.

Animal sacrifice and tissue harvesting

Animals were sacrificed by administrating a lethal dose of pentobarbitalnatrium. Following loss of conscience the animals were mounted in a stereotaxic frame, the skin above the cervical region incised and the musculature separated. Cerebrospinal fluid (CSF) from mice was collected from the cisterna magna using a pipette prepared from a glass capillary needle and connected to a 10 μl syringe (Hamilton®). CSF from rats was also collected from the cisterna magna but using a safety needle (Venofix® Safety, 4056501-01) attached to a 1 ml syringe. The CSF was collected into Eppendorf® tubes and snap-frozen in liquid nitrogen. The animals then underwent cervical dislocation and the SCI epicenter +/- 3 mm was dissected and snap-frozen in Eppendorf® tubes using liquid nitrogen. CSF and spinal cord samples were stored at -80°C. Animals (spinal cords) subjected to evaluation using fluorescence activated cell sorting (FACS) or flow cytometry did not undergo cervical dislocation but were instead transcardially perfused with 1xDPBS (Watson Marlow, 120S) and the spinal cords were kept in 1xDPBS on ice until dissociation. Animals evaluated using immunohistochemistry were first transcardially perfused with 1xDPBS and then with PFA (4 %). The spinal cords were fixed in PFA (4 %) at 4°C O/N.

Human spinal cord samples

The injured human spinal cord segment was removed surgically according to the study protocol in the ongoing clinical trial (above) and the rostral 1/3 of the sample was immediately fixed in PFA (4 %) at 4°C O/N and then subjected to histological evaluation.

Fluorescence activated cell sorting

Bulk cell samples

Spinal cords containing transplanted MSCs or NPCs underwent enzymatic dissociation using 10 U/ml papain (Worthington, L5003126) with a combination of trituration and incubation at 37°C. The same protocol was used for isolating microglia/macrophages, microglia-like macrophages and monocyte-like cells from spinal cords of C57BL/6J-Cx3cr1^{GFP} and C57BL/6J mice respectively. DNAse 200 U/ml (Sigma, D7291) was added to the cell suspension during dissociation. 1 % BSA (Gibco®, 15260-037) in 1xDPBS was added to the cell suspension to inactivate the papain. Cells were collected and 10 ml of 30 % Percoll (Sigma, P1644) diluted in 1xDPBS was added. The myelin was separated from the cells by centrifugation at 750xg at 4°C for 10 min with brake set at 2. The pellet was aspirated and filtered through pre-wet cell strainers (100 µm, 40 µm). Cells were washed in 2 ml fluorescence activated cell sorting (FACS)-buffer (1 % BSA, 2 mM EDTA (Gibco®, 15575-038), 25 mM HEPES (Sigma, H0887)). Fc receptor-mediated binding by antibodies was reduced by incubating cells in 1 µg Mouse Fc Block (CD16/CD32) per 1x10^6 cells in 100 µl FACS-buffer for 5 min on ice (**Table 3**). Pre-conjugated antibodies were added at a concentration of 1µg/10^6 cells to the cell suspension and incubated for 30 min on ice (**Table 3**). For isolation of MSCs and NPCs from the dissociated spinal cords their mCherry and GFP fluorescence, respectively, were utilized and no additional antibodies were added in order to distinguish them. Microglia/macrophages were defined as CX3CR1$^+$CD19$^-$Ly6G$^-$CD3$^-$NK1.1$^-$SIGLECF$^-$CD11b$^+$CD45$^{high/low}$ cells while microglia-like macrophages were defined as CD11b$^+$CD45^{low}Ly6G$^-$CD64$^+$ and monocyte-like cells were defined as CD11b$^+$CD45^{high}Ly6G$^-$CD64$^-$ cells. Cells were washed twice, re-suspended in 300 µl FACS-buffer, and sorted using a BD Influx™ cell sorter. Cells were collected in FACS-buffer, and re-suspended in 1 ml Trizol (Thermo Fisher, 15596026) following centrifugation at 300xg for 5 min and eventually stored at -80°C until downstream processing.

Single cells

SCI epicenters from C57BL/6J and tamoxifen induced Cx3cr1^{CreER+}Rosa26^{DTA+} mice were dissected and dissociated using enzymatic dissociation (above). Single cell suspensions from 3-5 spinal cords per condition were pooled and stained with antibodies against CD45, CD11B, CD64 and CD14 using the procedure described above (**Table 3**). CD45$^+$ cells were isolated from the aggregated suspension of cells using FACS (Sony SH800S). A mixture of unstained cells from healthy- and injured spinal cords was used as negative control when setting the voltages. Cells were sorted into wells containing 2 µl lysis buffer (**Table 5**) in Hard-Shell® 384-well PCR plates (Bio-Rad, hsp3805). Plates were covered with a seal, centrifuged at 4000 rpm (Hettich, Universal 320R) then snap-frozen on dry ice and stored at -80°C. A compensation matrix was estimated using UltraComp eBeads (Thermo Fisher, 01-2222-41) stained with 1 µg antibody per fluorophore. ArC™ Amine Reactive Compensation Bead Kit (Invitrogen, A10628) was used when setting up the compensation matrix for the live/dead marker. When using transgenic animals (Cx3cr1^{CreER+}Rosa26^{DTA+}), unstained spinal cord tissue (containing YFP$^+$ cells) was used for compensation setup for the YFP-fluorescence.

Flow cytometry

Spinal cords were dissociated as described above. Phenotypic characterization of spinal cords containing transplanted MSCs was performed using a cell analyzer (BD Biosciences, LSRFortessa™). A compensation matrix was calculated based on single stains. Data was analyzed in Kaluza Analysis Software (Beckman Coulter). During analysis, dead cells and non-singlets were excluded. Phenotypic characterization and quantification of number of microglia ($CD11b^{+}Cx3cr1^{+}$ and $CD11b^{+}CD45^{low}$ cells) in $Cx3cr1^{CreER+}Rosa26^{DTA+}$ mice prior to and following depletion of microglia was conducted using another cell analyzer (Beckman Coulter, Gallios flow cytometer) but analyzed using the same analysis software.

Table 5. Library preparation using the Smart-Seq2 protocol.

Type	Ingredient	Ratio	Manufacturer	Identifier
Lysis buffer	H_2O	1:53	NA	
	Recombinant RNase Inhibitor	1:40	Takara	2313A
	ERCC 1:600000	1:40	In-house	
	10 % Triton™ X-100	1:50	Sigma	93443
	10 mM dNTP	1:4	In-house	
	100 µM dT	1:40	In-house	
Reverse transcription mix	SMARTScribe	1:6.32	Takara	639538
	Recombinant RNase Inhibitor	1:24	Takara	2313A
	5x First Strand Buffer	1:3	Takara	639538
	100 mM DTT	1:12	Promega	P117A
	5 M Betaine	1:3	Sigma	B0300
	1 M $MgCl_2$	1:100	Sigma	M1028
	100 µM TSO	1:60	In-house	
	H_2O	1:43	NA	
Pre-amplification mix	H_2O	1:7	NA	
	Kapa HiFi HotStart ReadyMix (2x)	1:1.2	Kapa Biosystems	KK2601
	10 µM ISPCR primer	1:60	In-house	
	Lambda exonuclease	1:133	New England Biolabs	M0262S
Tn5 mix	H_2O	1:1.7	NA	
	TAPS-PEG	1:3.6	In-house	
	Tn5 stock	1.7.2	In-house	
PCR/barcode mix	H_2O	1:1.47	NA	
	5x buffer	1:3.9	KAPA	KB2500
	dNTPs	1:26	KAPA	KN1009
	Hifi	1:39	KAPA	KE2004

Proliferation assay

Proliferation of MSCs in culture was investigated by incubating the cells in 1:1000 EdU (Thermo Fisher, A10044) for 24 hours prior to analysis. Proliferation rate of transplanted MSCs was investigated by administrating EdU (0.75 mg/ml) in drinking water supplemented with 1 % sucrose (Sigma, S0389) available from induction of SCI until sacrifice. Proliferation was assessed using flow cytometry (cells in culture) and immunohistochemistry (transplanted cells) using the Click-iT™ Plus EdU Alexa Fluor™ 488/555 Imaging Kit (Thermo Fisher, C10637). Manufacturer's instructions were used throughout. Proliferation of $CD45^{+}$ immune cells in SCI was assessed by incubating dissociated cells with Hoechest 33342 (**Table 3**) according to manufacturer's instructions. The S-phase of the cell cycle was defined as the right-most peak in a histogram of 33342 on a log-scale and used as the definition of proliferating cells.

RNA isolation

Spinal cords and differentiated mesenchymal stem cells

RNA isolation and clean up was performed using the RNeasy mini kit (Qiagen, 74104) or RNeasy micro kit (Qiagen, 74004). The tissue was mechanically dissociated (IKA®, T8.01) in lysis buffer containing β-mercaptoethanol (1 %). Genomic DNA was digested using DNase I (Qiagen, 79254). The RNA was re-suspended in nuclease-free H_2O and stored at -70°C.

Bulk cell samples

Isolated and Trizol treated samples of NPCs, MSCs and microglia/macrophages, microglia-like macrophages and monocyte-like cells were thawed and incubated at RT for 5 min. Following incubation, the samples were vortexed and 0.2 ml chloroform (Merck Millipore, 102445) was added. Following vigorous shaking samples were incubated on bench-top for 5 min and then centrifuged at 12000xg for 15 min at 4°C. The aqueous phase was removed and placed in a separate Eppendorf® tube containing 10 µg glycogen (Invitrogen™, 10814-010) in 0.5 ml 100 % isopropanol (Solveco, 1131). Samples were incubated at -20°C O/N. The next day, samples were centrifuged at 12000xg for 10 min at 4°C. After removing the supernatant 1 ml of 75 % ethanol (Solveco, 1000) was added followed by centrifugation at 7500xg at 4°C for 5 min. The supernatant was aspirated, the RNA dried at RT and then diluted in nuclease-free water. Genomic DNA was digested using DNase I (Qiagen, 79254). RNA was purified using the RNeasy micro kit (Qiagen, 74004), diluted in 14 µl nuclease-free H_2O and stored at -70°C.

Reverse transcription and real-time quantitative polymerase chain reaction

Spinal cords and differentiated mesenchymal stem cells

RNA (500 ng) was converted into cDNA using the iScript™ cDNA Synthesis Kit (Bio-Rad, 1708891) on a block heater (Eppendorf® ThermoStat Plus, 022670204). The cDNA was diluted 1:5 in RNase-free water (Sigma, W4502). The cDNA was then used in a 10 µl PCR reaction using the iQ™ SYBR® Green Supermix (Bio-Rad, 170-8887) in combination with 25 mM forward- and reverse primers (Eurofins Genomics) (**Table 4**) in a 384-well PCR plate. RTqPCR was performed at 95°C for 3 min followed by 40 cycles of: 95°C for 10 s; 60°C for 30 s and 72°C for 30 s with a CFX384 Touch™ Real-Time PCR detection System (Bio-Rad, 1855485). The protocol was finalized with a melt curve (65°C to 95°C, increment 0.5°C every 5 s). The ΔΔCt method was used for data analysis. *Gapdh* and/or *Actb* were used as housekeeping genes. Plate setup and normalization of expression was performed in Bio-Rad CFX manager (Bio-Rad Laboratories Inc., v3.1).

Isolated microglia/macrophages and monocytes

RNA was converted to cDNA as described above. The cDNA was pre-amplified prior to RTqPCR. Briefly, 500 µl pre-amplification assay pool was prepared using 5 µl of each TaqMan Assay (20x) and nuclease-free H_2O. Pre-amplification reaction mix was prepared (1 for each sample) using 25 µl of the pre-amplification assay pool, 12.5 µl SsoAdvanced™ PreAmp

Supermix (Bio-Rad, 172-5160) and 12.5 µl of template. Following thorough vortex, the reaction mix was dispensed into a 96-well PCR plate (Life technologies, N8010560). The PCR plate was then loaded onto a PCR instrument and kept at 95°C for 3 min followed by 15 cycles of: 95 °C for 15 s; 58 °C for 4 min and finally diluted 1:5 in TE buffer (Invitrogen™, 12090015). Master mix for a 10 µl RTqPCR reaction was prepared with 5 µl SsoAdvanced™ Universal Probes Supermix (Bio-Rad, 172-5280), 1 µl TaqMan assay (20x), 2 µl template and 2 µl nuclease-free H_2O. Master mix and template were dispensed into a 384-well PCR plate (Bio-Rad, Hard-Shell®) and RTqPCR was conducted at 95°C for 30 s followed by 40 cycles of: 95°C for 10 s; 60°C for 20 s. Expression was normalized to *Actb*. The ΔΔCt was used for analysis in Bio-Rad CFX manager (Bio-Rad Laboratories Inc., v3.1).

RNA sequencing

Libraries for sequencing were prepared from RNA isolated from isolated cells (microglia/macrophages, MSCs, NPCs) using the SMARTer Stranded Total RNA-Seq Kit - Pico Input Mammalian (Clontech) due to low amounts of RNA obtained from each sample. Sequencing libraries for RNA isolated from spinal cord tissue were prepared using TruSeq stranded mRNA library preparation kit implementing polyA selection (Illumina Inc.). Libraries were sequenced 2x125bp using HiSeq2500 (Illumina Inc.) on high output mode. For each sample, a minimum of $15x10^6$ read-pairs were used. The read-pairs were aligned to a reference genome (GRCm38: mouse; Rnor_6.0: rat) using STAR [656] followed by summarization of read counts over genes using featureCounts [657] and Ensembl annotation (release 81) (**Table 6**). Differential gene expression analysis was performed in R (v3.3.2-3.6.0) [658] using a limma [659] and edgeR [660] approach.

Enrichment- and network topology-based analysis

Genes were determined to be significantly differentially expressed in any given contrast if they had a FDR<0.01 and (logFC>1 OR logFC<-1). Up- and down-regulated genes from each contrast were analyzed using over-representation enrichment analysis (ORA) and network topology-based analysis (NTA). The WEB-based Gene SeT AnaLysis Toolkit (WebGestalt) was implemented through the R package WebGestaltR [661]. All three subcategories of Gene Ontology (GO) terms were investigated: biological process (BP), molecular function (MF) and cellular component (CC). Pathway analysis was conducted using the Kyoto Encyclopedia of Genes and Genomes (KEGG) terms. Network retrieval & prioritization (NRP) and network expansion (NE) algorithms were used for network construction in the NTA. All GO-terms (and associated genes) significant at FDR<0.01 were exported. Terms were grouped into general categories and the median FDR was estimated for each contrast and category. All unique genes within each category and contrast were subjected to competitive gene set testing (accounting for inter-gene correlation). Given that the median FDR for the terms and the FDR for the competitive gene set test were both <0.05, the category for that contrast was deemed significant. The Molecular Signatures Database (MSigDB, v6.2) was used for performing gene set enrichment analysis (GSEA). Hallmark gene sets, curated gene sets (C2) an immunologic gene sets (C7) were used.

Single-cell RNA sequencing

Library preparation

RNA was converted to cDNA and further into sequencing libraries using the Smart-Seq2 protocol [662]. Briefly, primers were annealed at 72°C for 3 min (Bio-Rad, 1000C Touch™ Thermal Cycler) followed by immediate reverse transcription and template switching by adding 3 µl of reverse transcription mix (**Table 5**) to each well (Eppendorf, epMotion 5073) and kept at 42°C for 90 min and 70°C for 5 min. DNA was pre-amplified by adding 7.5 µl of pre-amplification mix into each well and kept at 37°C for 30 min, 95°C for 3 min, 22 cycles of: 98°C for 20 s, 67°C for 15 s; 72°C for 4 min followed by 72°C for 5 min. DNA was purified by adding solid phase reversible immobilization (SPRI) beads at a ratio of 0.75:1, incubated for 5 min at RT, washed with 30 µl 80 % EtOH twice, air dried for 15 min and eluted in 15 µl EB buffer (Qiagen, 19086). A custom 3D-printed magnetic plate was used for bead separation during processing. DNA concentration was measured in 16 random wells per 384-well plate using a step-wise pattern with QUBIT™ dsDNA HS Assay Kit (Q33231). 3 µl sample was added to 97 µl buffer in a 96-well plate (Invitrogen™, M33089) and concentrations were measured and compared to standard (Tecan spark 10M, ex/em 485/528nm). DNA was then diluted to a concentration of 0.15 µg/µl using RNase-free H_2O. Tagmentation was performed by adding 1.8 µl Tn5 mix to each well in a 384-well plate. 0.7 µl diluted cDNA (0.15 ng/µl) was then added into each well containing the Tn5 mix. The plate was vortexed (Thermo Fisher, 88880017TS), centrifuged and kept at 55°C for 10 min. Tn5 was inactivated by adding 1 µl 0.1 % sodium dodecyl sulfate (SDS, Sigma L3771) to each well. Plate was vortexed and incubated at RT for 7 min. Libraries were barcoded by adding 19.5 µl PCR/barcode mix into each well now containing 3.5 µl sample. 2 µl primer (3.75 µM/primer) was finally added to each well. PCR/barcoding was then conducted at 72°C for 3 min, 95°C for 3 min, 12 cycles of: 95°C for 15 s; 55°C for 30 s; 72°C for 45 s. DNA was pooled (5 µl from each well) into an Eppendorf® tube. Clean up, as described above, was repeated twice and DNA was eventually eluted in 30 µl EB buffer. Concentration was again measured using QUBIT™ followed by 37 bp paired-end sequencing typically with dual index 8 bp using 2.6 pm on a 309 bp peak on Illumina NextSeq.

Data analysis

Demultiplexing and alignment

Reads were aligned to the GRCm38 reference genome following trimming and removal of adaptor sequences using STAR [656]. Following removal of duplicate reads [663] HT-seq was used for obtaining raw transcript counts [664]. Raw count (Rc) for each gene (i) and cell (j) were log2-transformed into log2-counts per million (CPM) using the total number of raw counts for each cell (TRc) and adding pseudo count (1) to handle zero counts. This value is denoted in text and figures as *log2(CPM)*:

$$\log2\left(\frac{Rc_{ij}*10^6}{TRc_j}+1\right) = \log2(CPM+1) \approx \log2(CPM).$$

Data pre-processing and quality control

The count matrix was cleared from wells intentionally containing 0 (empty) or 5 (bulk) cells as well as non-genes, ERCC and genes not expressed in any cell. Cells with low *Actb* expression (<0.01 quantile) were removed by filtering. The cutoff for the minimum number of counts per cell was determined by visualizing all cells in a log2-log2 plot of number of features *vs* number of counts per cell. The cutoff was eventually determined at 2^{16} counts per cell. Following processing the data set used for analysis had 606.166 reads/cell (IQR: 643.913) and 2191 genes/cell (IQR: 1228). 4734 cells were sequenced out of which 3687 cells and 20469 genes with a mean of 307 cells/condition remained for downstream analysis.

Clustering and dimensionality reduction

The downstream data analysis was conducted solely in R (v3.6.0) [658] using the Seurat package (v3.0.0) [665]. The NormalizeData() function using scale.factor=10^4 was used for data normalization. Highly variable genes given to the FindVariableFeatures() function had a log2(cpm)>0.3 and a normalized dispersion>0.5. Following scaling of the data using the ScaleData() function the first 50 principal components (PCs) were determined using RunPCA(). The first 17 PCs were determined as highly variable by plotting the st.dev for each PC. A Shared Nearest Neighbor (SNN) graph was constructed using the first 17 PCs in the FindNeighbors() function. Clusters in the SNN graph were identified using the FindClusters() function in which the Louvain algorithm with resolution 1.3 was selected. The resolution was selected by varying it in a range between 0.5 and 4 and carefully evaluating the relevance of the clustering. The t-distributed stochastic neighbor embedding (tSNE) algorithm was used for dimensionality reduction. Using the RunTSNE() function the first 15 variables were estimated using seed 132 and perplexity 70. When cells isolated and sequenced from spinal cords of mice depleted of *Cx3cr1*$^+$ cells prior to SCI were analyzed key settings were adjusted to: FindNeighbors(dims=1:20); FindClusters(resolution=0.75); RunTSNE(dims=1:16, seed.use=246).

Pseudotime ordering of microglial cells

A pseudotime axis was constructed by combining the cell clustering and the actual time series. Firstly, a subset of all microglial cell clusters was created. Secondly, for each condition (time point) a centroid, defined as the position of the mean tSNE-1 and tSNE-2 value for all cells in that condition, was estimated. Thirdly, each cell was assigned to the closest centroid measured using the Euclidian distance in two-dimensional space. Within each newly formed cluster, the cells were arranged based on their tSNE-1 value following a 0-1 normalization. The clusters were arranged in chronological order and the gene expression for each cell expressed as log2(cpm) was plotted over pseudotime.

Cluster marker genes and differential gene expression analysis

The FindAllMarkers() function was used for determining marker genes for each cell cluster. The threshold for logFC (logfc.threshold) was set at log(2). Differential gene expression analysis between clusters was conducted using the edgeR [660] and limma [659] packages in R [658] implementing the trimmed mean of M-values (TMM) function for normalization of data.

Integration with peer data sets

The current data was integrated with three published data sets using the Seurat package (v3.0.0) [665]. As described above, NormalizeData() was used for normalizing the data and FindVariableFeatures() used for the identification of variable genes. For the latter function selection.method=vst and nfeatures=2000 was used. Anchors for the integrated data were identified using the FindIntegrationAnchors() function and the data sets were eventually integrated using the IntegrateData() function. Scaling of the data and determination of top PCs was conducted as described above. The SNN graph was constructed using the first 10 PCs in the FindNeighbors() function. Clusters were identified using resolution 1 and the Louvain algorithm in the FindClusters() function. The first 10 dimensions for the tSNE was estimated using seed 246 and perplexity 70 in RunTSNE(). Data published by Li et al. (2019) [633] was integrated with the current data in the first part of this analysis. Secondly, this data set was expanded with data published by Mathys et al. (2017) [625] and Masuda et al. (2019) [627] using the settings in Seurat described in this section (**Table 6**).

Correlogram of transcription factors

The FindAllMarkers() function was used for determining transcription factors for each microglial cell cluster. Key settings in the function were: min.diff.pct=0.1; logfc.threshold=0.4; min.pct=0.3. The transcription factors were ranked in terms of logFC and top transcription factors from each cell cluster were used in the construction of a correlogram.

Index sorting and FACS trace-back

The surface expression of CD11B, CD45, CD64 and CD14 (**Table 3**) was recorded, normalized to voltage settings and plotted for each cell in order to correlate cell clusters to phenotypic profile (index sorting). Moreover, the isolated, sequenced and QC-checked cells were identified among all analyzed and/or sorted cells and labelled using color in a reconstructed FACS plot reporting CD45 *vs* BSC-A. This was termed a FACS trace-back plot and was reported for each plate separately due to variations in voltage settings between sorting occasions.

Table 6. RNA sequencing data sets.

Study	Title	GEO ID	PMID
I	Adult neural progenitor cells transplanted into spinal cord injury differentiate into oligodendrocytes, enhance myelination, and contribute to recovery.	GSE125134	31031190
II	Syngeneic, in contrast to allogeneic, mesenchymal stem cells have superior therapeutic potential following spinal cord injury.	GSE139227	30551037
III	Mesenchymal stem cells transplanted into spinal cord injury adopt immune cell-like characteristics.	GSE125176	30944028
IV	Permanently re-programmed microglia in spinal cord injury contribute to functional recovery.	*In progress*	*In progress*
Li et al. 2019	Developmental heterogeneity of microglia and brain myeloid cells revealed by deep single-cell RNA sequencing.	GSE123022, GSE123024	30606613
Masuda et al. 2019	Spatial and temporal heterogeneity of mouse and human microglia at single-cell resolution.	GSE120744, GSE120745	30760929
Mathys et al. 2017	Temporal tracking of microglia activation in neurodegeneration at single-cell resolution.	GSE103334	29020624

Immunoassay

Expression of cytokines/chemokines in the CSF of mice was investigated using a Bio-Plex Pro™ Mouse Cytokine 23-plex Assay (Bio-Rad). 10 µl CSF from each mouse was used. Expression of cytokines/chemokines in CSF of rats was analyzed using the Bio-Plex Pro Rat Cytokine 24-plex Assay (Bio-Rad, 10014905). 25 µl CSF from each rat was used. A Bio-Plex® MAGPIX (Bio-Rad Laboratories, Hercules, CA, USA) was used for detection. Manufacturer's protocols were used throughout.

Immunohistochemistry

Thin tissue

Post-fixed spinal cords were cryo-preserved in 15 % sucrose (Sigma, S9378) O/N followed by 30 % sucrose O/N diluted in 1xPBS. A 25x20x5 mm cryomold (Tissue-Tek® Cryomold®, 420572) was filled with compound (Tissue-Tek® O.C.T.™), the spinal cord placed in the compound and frozen to -60°C. A cryostat (Leica, CM1850 or CryoStar™ NX70 Cryostat) was used for producing 14-20 µm transversal- or coronal sections. The sections were mounted on adhesion slides (VWR, SuperFrost® Plus 48311-703) and stored at -20°C until staining. Prior to staining the sections were thawed ~10 min at RT and excessive cryomold was peeled of and each section encircled with a PAP pen. Sections were rehydrated ~20 min in 1xPBS at RT. Blocking solution (0.3 % Triton X-100 (Sigma, 93443), 5 % normal donkey serum (Millipore, S30-100), 1xPBS and 0.01 % NaN_3 (Sigma, S-2002)) was added and the sections were incubated for 1 hour at RT. Excess blocking buffer was removed and primary antibody diluted in blocking buffer was added onto the sections and incubated at 4°C for 24 hours (**Table 3**). Sections were rinsed in 1xPBS three times and incubated in secondary antibody diluted in 1xPBS for 1 hour at RT (**Table 3**). Following wash in 1xPBS three times the cell nuclei were labelled by adding nucleic acid stain onto the sections. The sections were incubated for ~20 min at RT. Following wash three times in 1xPBS Mowiol® (Sigma, 81381) was added onto each slide followed by a 22x50 mm cover slip (Marienfeld, 010243). Sections were imaged using a confocal microscope (below).

Thick tissue

The dura mater was removed from the fixed spinal cords using forceps under a table microscope. The spinal cords were blocked (20 % donkey serum (Merck, S30), 0.3 % Triton X-100 (Sigma, 93443) and 0.01 % NaN_3, 1xPBS) for 24 hours at 37°C. After 24 hours, the tissue pieces were rinsed twice in washing solution (1xPBS, 0.3 % Triton X-100) and incubated in primary antibody (**Table 3**) diluted in blocking solution at 37°C during 4 days. The sections were then rinsed twice in washing buffer and incubated in secondary antibody for 3 days at 37°C (**Table 3**). The tissue was rinsed twice in washing solution. Sca/eCUBIC-1 (25 % urea (Sigma, 208884), 25 % *N,N,N',N'*-tetrakis (2-hydroxypropyl) ethylenediamine (Tokyo Chemical Industry CO., T0781), and 15 % polyethylene glycol mono-*p*-isooctylphenyl ether/Triton X-100 (Nacalai Tesque Inc., 25987-85), 35 % dH_2O) was used for tissue clearing. Propidium iodide (1 mg/ml stock) 1:20000 was added to the clearing solution (**Table 3**). The spinal cord sections were incubated in the suspension at 50°C during 3 days [666].

Immunocytochemistry

MSCs intended for immunocytochemistry were plated at 20.000 cells/cm^2 in tissue culture-coated slides (Nunc™ Lab-Tek® Chamber Slide™, 177402) in 0.3 ml basal medium and incubated for 3 days. Cells were then fixed (2 % PFA) during 30 min and stained using same procedures described for fixed thin spinal cord sections. The same procedure for fixation and staining was implemented for evaluation of both differentiated- and undifferentiated NPCs. Cells were imaged using a confocal microscope (below).

Confocal microscopy

A confocal microscope (Zeiss, LSM 880 Airyscan) was used for imaging thin spinal cord sections as well as cell cultures following fixation and staining. Images were exported using Zeiss Zen Blue software as tagged image file format (tiff) files.

Light sheet fluorescence microscopy

Thick transparent tissue was imaged using a light sheet fluorescence microscope (LSFM). The microscope was placed on a metric optical breadboard (Technical Manufacturing Corporation, 78-25555-01). An argon laser (JDS Uniphase, 2211-10SLHP) with a wavelength of 488 nm was used for illuminating the tissue. The laser was directed towards a 10x beam expander (Rodenstock). Secondly, the laser was reflected on a Galvo Scanner (8660*231, Cambridge Technology). Thirdly, the laser traveled through a theta lens (Rodenstock) to focus the light into a single point. The light sheet was formed by letting the Galvo scanner oscillate. For imaging, a 10x objective (Olympus), a fluorescence filter and a tube lens 135 mm f/2.8 (Yashica) was placed in front of a camera (Canon, EOS 6D) equipped with Magic Lantern. Recordings were exported and rendered in Da Vinci Resolve (v14).

Luxol fast blue staining

Spinal cord sections were thawed, re-hydrated for 15 min in 1xPBS at RT followed by hydration in 95 % EtOH at RT for 15 min. Sections were stained at 56°C O/N in 0.1 % luxol fast blue solution (0.1 mg luxol fast blue (Waldeck, 1B-389), 100 ml 95 % EtOH and 0.5 ml glacial acetic acid (Millipore)). Following incubation, the sections were first washed in 95 % EtOH and then rinsed in dH_2O. Slides were incubated for 30 s in 0.05 % lithium carbohydrate solution (0.05 mg lithium carbonate (Karolinska apoteket, 941122), 100 ml dH_2O) followed by incubation in 70 % EtOH for 30 s and finally rinsed thoroughly in dH_2O. Sections were mounted (VWR, Xylene) and imaged using a microscope (Leica reichert polyvar 2).

Automatic cell quantification

Cells of interest in confocal images were quantified using Image Processing and Analysis in Java (ImageJ, 64-bit Java 1.6.0_24). Individual images were imported as an image sequence and saved. A custom-built macro for automatic cell quantification was developed. Briefly, each image in the image sequence was split into its three channels (red, green, blue). The channels of interest were kept and the remaining channels discarded. The remaining images were then multiplied in

order to enhance the overlap between the selected stainings/channels. The product image was then converted into binary format followed by water shedding to enhance separation between structures. Remaining- and separated structures were counted allowing the user to set a range of interest for the cell size- and circularity. The counted regions were laid on top of the original image sequence for verification purposes.

Data- and statistical analysis

Data for each biological replicate was reported in combination with mean (95 % confidence interval) or median (25^{th}– and 75^{th} percentile). P-values <0.05 were considered significant. Assumptions for statistical tests were evaluated and used as a basis for test selection. Assumption of normality was evaluated using Shapiro Wilk's test. Assumption of homogeneity of variances between groups was evaluated using Bartlett's test for normally distributed data and using the Fligner-Killeen test for non-normally distributed data. A mixed ANOVA followed by one-way ANOVA of significant main effects followed by Tukey's post hoc test was implemented for determination of the interaction between dependent- and independent continuous variables.

In general, continuous variables for two or more categorical groups were evaluated using one-way ANOVA and Tukey's post hoc test given normality and sphericity of data. Repeated measures ANOVA was implemented in the case of dependent groups. The Kruskal-Wallis H test followed by pair-wise Mann-Whitney U-tests using Holm-Bonferroni correction was implemented given that the assumptions for ANOVA were not fulfilled. Student's t-test was used for investigation of difference between two normally distributed continuous variables. Consideration was taken to if samples were dependent of independent as well as the homogeneity of variances between the samples. The Wilcoxon rank sum test was used as the non-parametrical equivalent to the Student's t-test for independent samples while the Wilcoxon signed-rank test was used as the non-parametrical equivalent to Student's t-test for dependent samples. Sensitivity of statistics was evaluated by resampling using the ordinary bootstrap approach. Correlation between two continuous, normally distributed, variables was estimated using Pearson's product moment correlation given a linear relationship and homoscedastic data. Correlation between two continuous variables with a monotonic but non-linear relationship was investigated using Spearman's rank-order and/or Kendall's Tau-b correlation. Data, statistical- and sensitivity analysis was conducted in R (v3.3.2-3.6.0) [658].

RESULTS

Paper I: Neural progenitor cells as a treatment for spinal cord injury

NPCs transplanted into SCI form myelinating oligodendrocytes

Conflicting results have been reported concerning the differentiation of neural progenitor cells (NPCs) transplanted into SCI. In this study, the NPCs transplanted into SCI were found in the SCI epicenter in which they filled the cystic cavity. In the chronic phase of SCI, the transplanted NPCs had almost no expression of SOX2 or NESTIN suggesting a loss of their stem cell potential. This was also true for TUJ1, which was expressed to a low extent during the chronic phase of SCI, suggesting that the NPCs do not differentiate into neurons. On the other hand, 25 % of the NPCs expressed GFAP at this time point indicating that transplanted NPCs differentiated into astrocytes. Moreover, more than half of the NPCs expressed CC1 in the recipient and many of which expressed OLIG2 and CNPase. Thus, the NPCs differentiated mainly into oligodendrocytes following transplantation. In proximity to the engrafted NPCs, a high density of MBP was detected. The differentiation of NPCs into oligodendrocytes and the enhanced myelination adjacent to the NPCs was confirmed by global transcriptional profiling and luxol fast blue staining respectively. Moreover, most of the engrafted NPCs were localized in the white matter. In addition, no co-localization between CASPASE-3 and the NPCs could be detected, indicating that the NPCs did not undergo apoptosis. In conclusion, NPCs transplanted into SCI migrate into the white matter, differentiate mainly into oligodendrocytes, and enhance myelination of axons close to the transplantation site.

NPCs transplanted into SCI up-regulate expression of genes related to oligodendrocytes and myelination

The precise response of transplanted NPCs in the subacute phase of SCI has never been properly investigated. Therefore, at 3- and 4 weeks following transplantation the NPCs were isolated using FACS and subjected to global transcriptional profiling. We found that transplanted NPCs were significantly different from NPCs in culture in terms of gene expression. We also documented a significant difference in gene expression between NPCs transplanted into injured and those transplanted into uninjured spinal cord. The injury environment induced NPCs to up-regulate expression of genes related to proliferation, metabolism and signaling while the same cells down-regulated expression of genes associated with migration, inflammation and immune response at 3 weeks post SCI. Four weeks post SCI the injury environment still mediated up-regulation of genes related to synaptic signaling but caused down-regulation of genes associated with response to stimuli and cell structure. The up-regulation of genes associated with signaling seemed to be related to inhibitory signaling mediated by GABA. Moreover, the global transcriptional profiling of NPCs exposed to SCI revealed that they up-regulated expression of genes associated with oligodendrocytes (*Ascl1, Nkx2-2, Hes5, Tcf7l2*) and myelination (*Atp1a3, Cntn1, Dnm1, Erbb2, Ina, Uchl1*) at both 3- and 4 weeks post SCI. In conclusion, the SCI environment mediates transcriptional changes in the NPCs up-regulating expression of genes associated with metabolism and signaling and in particular genes related to oligodendrocytes and myelination during the subacute phase of SCI.

Transplantation of NPCs into SCI results in suppression of neuroinflammation

The effect of NPC transplantation on neuroinflammation during SCI was investigated by measuring the levels of pro-inflammatory cytokines/chemokines in the cerebrospinal fluid (CSF). Transplantation of NPCs mediated lower levels of pro-inflammatory cytokines/chemokines (IL-1α, IL-1β, IL-2, TNF-α, GRO/KC, MCP-1 and MIP-1α), as compared to saline injection, in the CSF at 3 weeks post SCI. However, the NPCs, in contrast to saline, could not suppress inflammation at later time points (6 & 12 weeks) following SCI. The suppressive effect of the NPCs on expression of inflammatory cytokines/chemokines in the CSF was also detected in the global transcriptional profiling of NPCs that revealed down-regulation of genes related to pro-inflammatory cytokines/chemokines (*Il1a, Il1b, Il6*). In conclusion, transplanted NPCs can suppress pro-inflammation in the subacute phase of SCI.

Enhancement of hind limb locomotor function recovery is observed following transplantation of NPCs into SCI

The implications of the beneficial modulations mediated by NPCs in SCI were investigated by evaluating the hind limb function during 12 weeks following SCI. Animals treated with NPCs recovered significantly in terms of Basso, Beattie and Bresnahan (BBB) locomotor rating score over time. NPC-transplanted animals reached a BBB score of about 15 (0-21 point scale), while saline treated animals had an average score of about 8.5. In addition, animals treated with NPCs recovered their iliac crest height (ICH) to a level comparable to uninjured rats, while this was not the case for saline treated animals. Moreover, the number of steps needed to cover a specific distance was fewer for the NPC treated animals. These animals also had longer protraction- and shorter retraction distances as compared to the saline treated animals at 12 weeks post SCI. The stride width was shorter and the stride length longer for NPC treated animals. The stepping pattern for the NPC treated animals normalized to the level of uninjured rats at 12 weeks post SCI. In conclusion, NPC-transplantation is positively correlated with significant recovery of hind limb motor function following SCI.

Causality do exist between transplantation of NPCs and functional recovery following SCI

In order to determine if the significant recovery of hind limb motor function had a causal connection to the transplantation of NPCs we eliminated the NPCs following transplantation and observed the hind limb function during 6 weeks. We found that the BBB score and the ICH were significantly higher at 6 weeks following SCI in animals that received transplantation of NPCs as compared to animals in which the transplanted NPCs were ablated. Moreover, we found that the ablated NPCs were phagocytosed by $IBA1^{+}$ cells. In conclusion, NPCs do contribute causally to recovery of hind limb motor function following SCI.

Paper II: Importance of histocompatibility of mesenchymal stem cells transplanted into spinal cord injury

Transplanted syngeneic MSCs suppress immune response, microglial activation and neuroinflammation in SCI

The global transcriptional profile of spinal cords of animals subjected to transplantation of mesenchymal stem cells (MSCs) with different histocompatibility was investigated in the subacute phase (20 days post SCI). The expression pattern of spinal cords subjected to syngeneic MSC transplantation was more similar to uninjured spinal cord tissue than to spinal cords subjected to injury but no treatment. The opposite was true for spinal cords subjected to transplantation with allogeneic MSCs. We found that 62 genes were significantly down-regulated in spinal cords of animals given syngeneic MSCs in comparison to those treated with allogeneic MSCs. Using enrichment analysis for this subset of genes we identified gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) terms related to both innate- and adaptive immune response. The global transcriptional analysis also revealed that animals given syngeneic MSCs had a lower expression of genes associated with pro-inflammatory cytokines/chemokines in the spinal cord (*Ccl2, Ccl3, Ccl4, Ccl5, Cxcl1, Il1b, Tnfa*). Furthermore, the syngeneic MSCs also suppressed the expression of genes related to homeostatic microglia (*Tmem119, P2ry12, Hexb, Fcrls, Sall1, Olfml3*). In conclusion, syngeneic but not allogeneic MSCs suppress pro-inflammation, the innate- and adaptive immune response and genes associated with homeostatic microglia in the injured spinal cord.

Transplanted syngeneic MSCs reduce levels of pro-inflammatory cytokines/chemokines in the acute- and subacute phase of SCI

The global transcriptional analysis was followed by an investigation focusing on the protein expression of pro-inflammatory cytokines/chemokines in the CSF during the acute- and subacute phase of SCI. Transplantation of allogeneic- or syngeneic MSCs resulted in elevated levels of pro-inflammatory cytokines/chemokines in the CSF at 3- and 10 days following SCI as compared to injury- and transplantation control. In the subacute phase (20 days post SCI) animals subjected to transplantation of syngeneic MSCs had lower expression levels of pro-inflammatory cytokines/chemokines (CCL2, CCL3, CCL4, CCL5, GM-CSF, IFN-γ, IL-1β, IL-6, CXCL1 and TNF-α) in the CSF as compared to animals subjected to allogeneic MSC transplantation as well as compared to injury control. Gene expression data obtained in the global transcriptional profiling, and using RTqPCR, of spinal cord tissue was in line with the protein expression of inflammatory cytokines/chemokines detected in the CSF. In conclusion, syngeneic MSCs suppress the pro-inflammatory response following SCI, which is not the case for allogeneic MSCs.

Transplanted syngeneic MSCs activate macrophages alternatively in SCI

Differences in activation of macrophages was investigated as a potential and partial explanation for the observed differences in the immune system- and inflammatory response. In the subacute phase of SCI, transplantation of allogeneic MSCs mediated classical activation of macrophages (M1) while syngeneic MSCs resulted in alternative activation of macrophages (M2) in the spinal

cord. The activation profile (M1/M2 ratio) of the macrophages demonstrated a positive correlation with the inflammatory response. Moreover, the activation profile of microglia/macrophages ($CD11B^{+}CD45^{Low}Ly6G^{-}CD64^{+}$) and monocytes ($CD11B^{+}CD45^{High}Ly6G^{-}CD64^{-}$) were investigated specifically. The alternative activation of macrophages observed in the spinal cord was confirmed to be true in isolated microglia/macrophages but not in isolated monocytes. In addition, transplantation of syngeneic MSCs resulted in a lower proliferation rate of $IBA1^{+}$ cells in the spinal cord, as compared to transplantation of allogeneic MSCs. In conclusion, transplantation of syngeneic MSCs results in an alternative activation of microglia/macrophages, which correlates with lower levels of pro-inflammatory cytokines/chemokines in the CSF and in the spinal cord as well as with a general suppression of the immune response.

Transplanted syngeneic MSCs enhance neuronal survival and recovery of hind limb function in SCI

The implications of the immune system alterations mediated by the syngeneic- and allogeneic MSCs on the functional recovery was investigated. Animals transplanted with syngeneic- or allogeneic MSCs both recovered in hind limb function (iliac crest height index, ICHI) over time, which was not the case for injury- and transplantation control. However, transplantation of syngeneic MSCs, as compared to allogeneic MSCs, resulted in a higher rate of recovery in ICHI over time. Animals treated with syngeneic MSCs had a higher ICHI at steady state (>7 weeks post SCI) in comparison to animals subjected to allogeneic MSCs as well as compared to injury- and transplantation control. We could not detect any difference in the trochanter major height index at steady state when comparing animals given syngeneic- or allogeneic MSCs. The density of $NEUN^{+}$ cells in the spinal cord of animals treated with syngeneic MSCs was higher at sacrifice (10 weeks post SCI), in relation to spinal cords subjected to allogeneic MSC transplantation. In conclusion, transplantation of syngeneic MSCs contribute to enhanced neuronal survival and hind limb recovery, as compared to allogeneic MSCs, following SCI.

Transplanted syngeneic MSCs have superior survival in SCI

The survival of transplanted syngeneic- and allogeneic MSCs was estimated as a measure of the importance of histocompatibility. Both syngeneic- and allogeneic MSCs survived for 3 days in the recipients injured spinal cord. Most allogeneic MSCs had perished at 10 days post SCI while syngeneic MSCs at 10 days post SCI had a rate of survival similar to the one at 3 days following SCI. Twenty days following SCI almost no transplanted MSCs could be detected in the spinal cord, regardless of their histocompatibility. Taken together, although the survival of transplanted MSCs is poor in SCI the histocompatibility is of importance for MSC graft survival.

Paper III: Cellular response of mesenchymal stem cells transplanted into spinal cord injury

Transplanted MSCs up-regulate expression of genes related to phagocytosis, inflammation and immune response in SCI

The global transcriptional profile of MSCs was investigated 7 days following transplantation into SCI. MSCs transplanted into healthy spinal cord and MSCs in culture were used as control. We detected that transplanted MSCs had a distinctly different gene expression profile in comparison to MSCs in culture. A slight difference was detected between MSCs transplanted into SCI and MSCs transplanted into healthy spinal cord. Gene expression analysis indicated that genes related to proliferation and DNA processes and repair of DNA were down-regulated in transplanted MSCs in comparison to MSCs in culture. On the other hand, genes related to functions mediated by immune cells were up-regulated including genes related to cytokine production, cytokine signaling, phagocytosis, stress response and apoptosis in transplanted MSCs. The genes related to the immune system response were associated with both innate- and adaptive immunity and especially activation of macrophages. With regards to cytokines, predominately genes related to pro-inflammatory cytokines (*I1a, I1b, Ifng, Il12a, Il8, Il10, Il2*) were up-regulated in MSCs following transplantation. In conclusion, the transcriptional profile of MSCs transplanted into SCI suggest that the *in vivo* primed MSCs have similarities with classically activated macrophages.

Transplanted MSCs up-regulate surface expression of CD45 in SCI

Phenotypic characterization of transplanted and non-transplanted MSCs was conducted. Seven days post transplantation the MSCs had a preserved surface expression of MSC markers CD29 and SCA1 in injured- and uninjured spinal cord. This was confirmed by gene expression analysis. MSCs should by definition not express CD45 on the cell surface, which was also true for the MSCs used in this study, prior to transplantation. Following transplantation however the MSCs up-regulated both gene- and surface expression of CD45. Moreover, we observed that *Cx3r1*$^{+}$ cells surrounded but did not infiltrate the graft at evaluation. In conclusion, MSCs maintain expression of MSC specific markers while they up-regulate expression of CD45, which is a common marker for immune cells, on their cell surface following transplantation into SCI.

Transplanted MSCs up-regulate expression of MHC-I and MHC-II in SCI

MSCs express MHC-I but not MHC-II on the cell surface. MSCs exposed to IFN-γ in culture conditions up-regulate expression of MHC-II on the cell surface. We investigated whether this was the case for MSCs transplanted into SCI. MSCs did not express MHC-II on the cell surface prior to transplantation but did express MHC-I. Seven days following transplantation into SCI the MSCs maintained their expression of MHC-I on the cell surface but up-regulated their surface expression of MHC-II. We confirmed this significant up-regulation with gene expression data extracted from the global transcriptional profiling of MSCs as well as by using immunohistochemistry. In conclusion, MSCs up-regulate surface expression of MHC-II following transplantation into SCI which raises questions concerning their immune-privilege.

Transplanted MSCs proliferate but do not undergo trans-differentiation in SCI

Investigations concerning the fate of MSCs following transplantation into SCI has produced conflicting results. In this study, we found that transplanted MSCs survived for 7, but not 14 days, following transplantation into SCI. The survival rate was not different in injured as compared to non-injured spinal cord. MSCs in culture conditions proliferated to a high extent while only a small portion of the MSCs engrafted in the recipients spinal cord proliferated. Transplanted MSCs did not seem to undergo apoptosis due to undetectable co-expression between MSCs and CASPASE-3. Using histological examination, we could not detect any $TUJ1^{+}$-, $NEUN^{+}$- or $GFAP^{+}$MSCs suggesting that no trans-differentiation occurred following transplantation. This was confirmed with gene expression analysis. In conclusion, MSCs transplanted into SCI proliferate to a low extent during the first week but do not undergo trans-differentiation. No MSCs could be detected at 2 weeks following transplantation but it did not seem to be a consequence of apoptosis.

Paper IV: Defining spinal cord injury associated microglia

Disease-associated microglia are detected in SCI

We characterized the transcriptional profile of 3069 CD45$^+$ immune cells isolated from the injury epicenter of traumatic SCI in mice over time. Healthy spinal cord tissue was used as reference. SCI induced a distinct, rapid and permanent transformation of the immune cells in the spinal cord. The changes were evident already at 0.5 hour post SCI and stabilized at 21 days post SCI. Homeostatic microglia (hMicroglia) expressing *P2ry12, Tmem119* and *Siglech* had transformed already at 0.5 hour following SCI. The hMicroglia transformed into activated microglia (aMicroglia) expressing *Il1a* and *Cd83.* At 2 hours post SCI the aMicroglia had transformed into a homogenous cell population expressing *Hbegf* and *Edn1* (proliferation-mediating microglia, pmMicroglia). This population further transformed into a population expressing *Mt2* and *Msr1* which persisted between 6- and 36 hours post SCI (monocyte-activating microglia, maMicroglia). At 3 days post SCI a cell population defined by expression of *Cst7, Igf1, Apoe*, *Ctsd, Clec7a, Lilrb4a, Lpl* and *Axl* was evident. This population was stable from 21 days post SCI onwards and was identical at 90 days post SCI. This population of immune cells had an expression profile similar to disease-associated microglia (DAM) detected in experimental models of Alzheimer's disease, neurodegeneration, demyelination and inflammation. Therefore, this population was deemed DAM in SCI. This was confirmed using protein expression of CST7, IGF1 and APOE on thin sections from SCI in mouse and humans. Using FACS index sorting and by constructing trace back plots (indicating sorted, sequenced and annotated cells in a plot of CD45 *vs* BSC-A) we concluded that surface expression of CD45 cannot be used for distinguishing hMicroglia from DAM in SCI. We also documented, using flow cytometry, that the proliferation of DAM was virtually non-existent. The gene expression of DAM in SCI and hMicroglia detected using scRNAseq were confirmed in bulk RNA sequenced CD11B$^+$CD45$^{low/high}$ cells at 7- and 21 days post SCI. In conclusion, hMicroglia are permanently transformed into a cell type characterized by a low rate of proliferation and up-regulated expression of genes associated with lipoprotein- and cholesterol metabolism, lysosomal proteolysis as well as cell- and organelle adhesion.

Disease-associated microglia in SCI originate from homeostatic microglia and enhance recovery of hind limb locomotor function

The data suggested that DAM originated from hMicroglia. In order to confirm this hypothesis we created a pseudotime axis by re-clustering the microglial cells. We estimated centroids in the two-dimensional tSNE for each condition and then assigned cells to the closest centroid. Secondly, the cells were normalized according to the tSNE-1 value for each cell within each condition. Thirdly, all conditions were arranged in chronological order. Expression of genes associated with hMicroglia were down-regulated and expression of genes associated with DAM in SCI were up-regulated when moving along the pseudotime axis. This was confirmed using protein expression of CST7, IGF1 and APOE on thin sections from SCI in mouse. Moreover, unique transcription factors could be identified for each microglial subtype. The gene expression pattern in combination with the unique transcription factors for each microglial subtype supported the hypothesis that DAM are indeed a product derived from hMicroglia. In order to fully confirm this hypothesis we implemented a mouse model of microglial depletion. At full depletion of *Cx3cr1*$^+$ cells (~microglia) SCI was induced. Isolation and scRNAseq of CD45$^+$

immune cells at 7- and 21 days post SCI in the *Cx3cr1*$^{+}$ cell depleted mice revealed no trace of DAM. Thus, DAM could not be formed when there was a lack of hMicroglia at the moment of SCI, which confirms that DAM are indeed re-programmed hMicroglia. Mice, which did not have DAM during SCI, had a significantly lower recovery of hind limb locomotor function measured using the Basso Mouse Scale during the first 90 days post SCI. In conclusion, DAM in SCI are re-programmed homeostatic microglia, which do enhance the recovery of hind limb locomotor function.

Disease-associated microglia have the same transcriptional profile regardless of CNS pathology

DAM in SCI had a transcriptional profile similar to DAM detected in disease models of neurodegeneration and demyelination. Therefore, we integrated published data from these studies with the data in the current study. First, data published by Li et al. (2019) who sequenced microglia- and myeloid cells from mice undergoing development was integrated. The authors documented a subpopulation of cells in the white matter of developing mice, which was metabolically active and phagocytized newly formed oligodendrocytes and termed this population *postnatal proliferative-region-associated microglia* (pPAM). pPAM co-localized with DAM in SCI in the integrated data set indicating a distinct similarity between the two cells types. Moreover, aMicroglia detected in SCI was found adjacent to immediate early genes (IEG)+Microglia in the aggregated tSNE. Microglial cells in SCI that up-regulated expression of *C1qa-c* were detected adjacent to embryonic microglia (eMicroglia) in the aggregated tSNE and we therefore termed these cells embryonic-like microglia (elMicroglia). We then added data published by Masuda et al. (2019) and data published by Mathys et al. (2017). Masuda et al. investigated microglia in models of demyelination (cuprizone) and neurodegeneration (n. VII axotomy) while Mathys et al. investigated microglia in the hippocampus of mice in an experimental model of Alzheimer's disease (CKp25 inducible mice). We found that microglia in demyelination co-localized with DAM in SCI while this was not the case for microglia in neurodegeneration. Unfortunately, we could not detect any distinct co-localization between the disease-associated cells in Alzheimer's disease with any of the cell clusters in our data set. In conclusion, the transcriptional profile of DAM do not seem to be affected by the type of pathology affecting the CNS. In addition, DAM seem to have similarities with microglia in development that supports the beneficial rather than detrimental function of these cells.

DISCUSSION

The end of correlation

In Paper I, we investigated neural progenitor cells (NPCs) as a therapy for spinal cord injury (SCI). Transplantation of NPCs into SCI has been associated with enhanced recovery of hind limb function [426, 427, 438, 443, 446, 451, 452, 456, 458, 459]. Moreover, results concerning differentiation of transplanted NPCs has been conflicting [426, 427, 429-432, 434-436, 438-440, 442-447]. Therefore, this project aimed at understanding the fate of transplanted NPCs as well the causal contribution of NPCs to recovery of hind limb function. The importance of the differentiation stems from NPCs having similarities with astrocytes (B1-cells) in the ventricular-subventricular zone (V-SVZ) [299]. Some suggest that NPCs differentiating into astrocytes following transplantation into SCI might contribute and add to the glial scar. Scar formation is one of the main obstacles for regenerating axons and thus for functional recovery. However, consistent reports indicate a correlation between transplantation of NPCs and recovery of hind limb motor function following SCI [426, 427, 438, 443, 446, 451, 452, 456, 458, 459], which is not in line with this theory. Moreover, NPCs preferably differentiate into oligodendrocytes, which have the ability to myelinate CNS axons and especially following injury to the CNS [222-225]. Therefore, in Paper I the main aim was to investigate the causality between transplantation of NPCs and the recovery of hind limb motor function. By transplanting NPCs and actively eliminating them and measuring the hind limb function we found that the NPCs did indeed contribute in a causal fashion to hind limb locomotor function recovery. Moreover, using histology we found that NPCs seem to enhance the recovery by differentiating into myelinating oligodendrocytes. Interestingly, ~25 % of the NPCs differentiated into $GFAP^+$ astrocytes but their relation to the glial scar was never investigated. In this project, we showed that the NPCs have a differentiation potential and pattern similar to what has been observed in the V-SVZ in the sense that the NPCs favorably differentiate into oligodendrocytes. The increased myelination observed in relation to the NPCs is a likely explanation for the enhanced recovery of hind limb function.

The transplanted NPCs were actively eliminated immediately following transplantation in order to avoid any loss of the diphtheria-toxin receptor. This setup is inferior to active elimination in the chronic phase of the injury since such a setup has the potential to more clearly reveal the long-term effects of the cells and how they relate to function. Stem cell transplantation studies suffer from difficulties in judging the success of the transplantations when evaluating the hind limb function with or without active elimination. The only way to find out is by evaluation of the tissue after sacrifice, which consumes a lot of time and effort, and results in a vague conclusion after all. However, this is a step in the right direction, a direction towards proving causality and not only correlation between therapeutic efforts and functional recovery. Far too many publications report correlation and not causation. In order to develop therapies for SCI which are efficient enough to restore normal or near-normal function first the causation and second the mechanism has to be shown. Proving causation allows one to distinguish the potential while the mechanism reveals potential targets to be modulated.

Moreover, NPCs are harvested from adult V-SVZ. Thus, the experimental setup used in this study (transplantation at 8-10 days post SCI) would not be possible in a clinical setting considering that harvesting and establishing NPC cultures takes a minimum of 2 weeks. Transplantation in the subacute phase would be the only option. Secondly, harvesting the

V-SVZ implies that the affected patient has to undergo complex surgery and risk potential functional loss by the removal of tissue from the V-SVZ. Harvesting V-SVZ from a donor is also highly unlikely to be a feasible option considering that few would risk such complex surgery but also to lose valuable tissue from their brain. Moreover, the usage of NPCs has to be placed in relation to the expected beneficial effects of the NPCs. Thirdly, the timing of transplantation has to be carefully evaluated prior to the initiation of clinical trials in order to evaluate if harvesting the V-SVZ and establishing NPCs is even worthwhile considering that they cannot be transplanted until the subacute phase. The NPCs could perhaps be established from embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs). iPSCs first have to be established and then differentiated into NPCs, which is vastly time consuming. Hence, autologous transplantation might be possible but the question of the timing of transplantation again becomes an issue. Both ESCs and iPSCs would most likely produce populations with a low fraction of NPCs, which implies the need for selection and intense evaluation prior to transplantation in order to eliminate the risk of teratoma formation. Moreover, the usage of NPCs should be placed in relation to other therapeutic options, not limited to stem cells, and their beneficial effects as well as the risk and complexity of the treatment. In this project, we transplanted the NPCs at 8-10 days following severe SCI and evaluated during 12 weeks following SCI. As for Paper II-IV, the results in this project are valid only for the experimental setup implemented. The results might be different for a less severe injury, for a different time point of transplantation and different time points of evaluation.

Implications of histocompatibility

In Paper II we investigated the importance of histocompatibility of mesenchymal stem cells (MSCs) transplanted into SCI. The importance of this study stems from the rapid and varying cellular- and molecular response following SCI as well as from reports indicating that MSCs are able to beneficially modulate the immune response following SCI [541, 553, 559, 560, 562]. We hypothesized that MSCs had to be transplanted in the acute phase of SCI for them to be able to modulate the immune response considering that the immune response is most prominent during the acute- and subacute phase of SCI (0-3 weeks post SCI). This raises questions concerning MSC availability and histocompatibility as well as the timing and route of transplantation. MSCs take weeks to establish and validate, which eliminates the possibility for transplantation of autologous MSCs during the acute- and subacute phase of SCI. Therefore, established, verified and stored allogeneic MSCs are the only MSCs that can be offered to patients during this period. Technological advances are not likely to overcome this issue since this limitation is a consequence of the time required for culturing and validating the MSCs. Although syngeneic MSCs were superior to allogeneic MSCs it is not possible to transplant autologous MSCs in the acute phase of SCI in the clinical setting as in this experimental design. We found that allogeneic MSCs activated macrophages classically and thereby contributed to enhancing pro-inflammation and did not enhance recovery of hind limb function. Hence, the usage of allogeneic MSCs might have a negative rather than a beneficial effect for the patient. Moreover, MSCs have been evaluated as a therapy for several diseases, not only in the CNS. The results concerning the immune-privilege of MSCs and the usage of autologous *vs* allogeneic MSCs could be of importance for other disease models as well.

However, several aspects of the experimental design in this paper are important to take into account when interpreting the results: the SCI was severe, the MSCs were harvested from the bone marrow and transplanted during the acute phase of SCI into the injury epicenter.

Moreover, variation in surgical technique, variation in SCI induction and MSC transplantation accuracy are also important sources of error to take into consideration. The transplantation accuracy is by far the most crucial aspect to keep in mind. All transplantations were conducted with caution and patience in order to minimize additional injury and to ensure accurate placement of the cells. Moreover, the SCI was induced using a commercially available standardized contusion apparatus (IH-0400) and the SCI was induced with the spinal column fixated and stabilized in a stereotaxic frame. This minimized variations in injury severity (tissue damage and impact on the corticospinal tract). This experimental design also implied transplantation of MSCs into the injury epicenter, which creates an additional injury to the spinal cord since a needle has to be inserted and a cavity created into which the MSCs are placed. The beneficial effect of the MSCs therefore has to exceed the additional injury caused by the transplantation. Thus, the experimental procedures are complex and several pitfalls and difficulties have to be taken into consideration when interpreting the results. It is possible that these results will vary given a different experimental setup (time of transplantation, time of evaluation, number of transplanted cells, injury severity, strain), evaluation of different parameters or when including more biological replicates.

This project aimed at investigating features and characteristics of MSCs focusing on their immune-privilege and immunomodulatory effect. Although immune system modulation certainly is of importance for regeneration and recovery, reduction of scar formation and enhancement of myelination are perhaps even more important aspects to focus on. In conclusion, although the histocompatibility of MSCs is of importance and MSCs can beneficially modify the microenvironment and contribute to recovery of hind limb function [164, 166, 533, 534, 536, 538, 541, 550, 552, 554, 555, 557, 558] there might be better therapeutic options for SCI to explore and to invest in.

Replacing stem cells

In Paper III, we developed the ideas and findings from Paper II. The main goal was to understand and map the mechanisms of action of MSCs and we therefore sought to investigate the cellular response of MSCs following transplantation into SCI. Understanding the cellular response is the first step in understanding how MSCs are actually mediating their beneficial function and effect in the recipient. Until this day MSCs have been evaluated using methods investigating specific pre-determined aspects of the cellular response of MSCs including secretion of neurotrophic factors and differentiation [164, 527, 530, 535, 549]. Considering that transplantation of MSCs can enhance the recovery of hind limb function following SCI there is certainly a mechanism, but it has not yet been described. The majority of studies investigating the cellular response and secretome of MSCs have been conducted in culture conditions. We hypothesized that replication of the SCI conditions cannot be done accurately in culture conditions considering the complex cellular- and molecular response in combination with effects such as edema, ischemia and instability of the spinal column. Therefore, we sought to investigate the cellular response of MSCs following exposure to a SCI environment. We found that MSCs exposed to a SCI environment for 7 days adopt immune-cell like characteristics in terms of their gene- and surface marker expression. The MSCs seem to adopt characteristics of macrophages but did not seem to be immune-privileged or trans-differentiate as reported in the literature. It is likely that the MSCs mediate their beneficial modulations and effect in the SCI microenvironment by adopting macrophage-like characteristics and phagocytizing cells undergoing apoptosis and contributing to modulation of the immune response by secretion of cytokines/chemokines. It is likely that the

adoption of immune-cell like characteristics has implications for other disease models in the CNS and outside the CNS in which MSCs are being transplanted. The similarity between MSCs transplanted into SCI and the MSCs transplanted into uninjured spinal cord is most likely a result of a non-significant injury caused by the transplantation. The additional injury is a major limitation in the cell transplantation approach for treatment of SCI. Furthermore, important to keep in mind when interpreting these results are the facts that these results might vary with the source from which the MSCs were harvested, the time point of transplantation as well as the time point of evaluation, strain, age, sex as well as the type and severity of the SCI. However, MSCs do not survive for too long in the recipient and evaluation at a later time point might have resulted in too few cells to evaluate.

More importantly, this project aimed at identifying the cellular response of MSCs with the intention of finding a way to replace the need for MSC transplantation. If MSCs could be replaced the discussion on the histocompatibility and route of transplantation would be eliminated, while the question concerning the timing would still be present. In addition to the adoption of immune-cell like characteristics, the secretome (proteins, trophic factors, cytokines, mRNA, miRNA) of MSCs is thought to mediate a significant part of the beneficial effects of MSCs. These substances are thought to be secreted in extracellular vesicles (MSC-EVs) [351-353, 355, 359, 362, 369, 376, 391, 667]. Although these MSCs-EVs have been quite extensively studied in culture conditions, the important thing going forward is to characterize the MSC-EVs secreted from MSCs in a SCI environment. In order to characterize these MSC-EVs one would have to develop a method in which the EVs secreted from MSCs can be isolated and distinguished from EVs released by other cells in the spinal cord. Given that the content of the MSC-EVs could be identified, the MSC-EVs could be replicated and administrated as a synthetic drug. This drug could then be evaluated in clinical trials in which the safety, dosage and time point of administration could be assessed. Such a drug in combination with decompressive- and stabilizing surgery could perhaps help to minimize the extent of the secondary injury. Even though the secretome of MSCs is beneficial and could perhaps be replicated and amplified, it might not be the entire solution to reducing or eliminating the secondary injury. In conclusion, understanding the basic science of SCI in combination with understanding and replicating the beneficial modulations mediated by (mesenchymal) stem cells and other therapeutic approaches could perhaps, in the distant future, be combined into a simple and efficient treatment/drug which minimizes the extent of the secondary injury.

Disease-associated non-immune cells in spinal cord injury

In Paper IV, we investigated the immune cell response following SCI focusing on microglia. We utilized scRNAseq and characterized the heterogeneity of the cell population over time and identified marker genes and subpopulations. We found that homeostatic microglia (hMicroglia) in SCI undergo transcriptional re-programming and eventually adopt a gene expression profile similar disease-associated microglia (DAM) described in various disease- and injury models of the CNS [625, 630, 634, 668, 669].

Although we did observe DAM in SCI, we did not investigate the mechanisms that initiates the transformation of hMicroglia nor the mechanisms regulating the transformation of microglia into DAM. Transcriptional programs have been suggested as mediators and regulators of the transformation. Using a pseudotime approach, we were able to identify unique transcription factors for each subtype of microglia. However, the transcription factors we identified were not in line with those previously published [629, 634]. This might indicate

differences between disease- and injury conditions or indicate the need for more detailed studies on this issue. *Trem2* has been reported to be an important regulator and mediator of transformation from hMicroglia into DAM [626, 629, 634, 636]. In this study, however we did not investigate the importance of *Trem2* due to the simple lack of access to such knock out animals. However, we have no reason to doubt that *Trem2* is most likely a key regulator for DAM formation in SCI as well. Although the transcriptional profile of DAM in SCI was similar to DAM detected in other disease and injury models of the CNS the origin of these cells in SCI was not certain. Moreover, the distinction between tissue resident immune cells and infiltrating immune cells is a recurring question if the field of SCI research. Using a microglia (*Cx3cr1*$^{+}$ cell) depletion model we were able to determine that a lack of hMicroglia at the moment of SCI results in a lack of DAM at 21 days post SCI. Thus, DAM are with high probability re-programmed hMicroglia, which is also in line with the literature. In addition, this gave us an indication of the importance and function of DAM in SCI by measuring and recording the hind limb locomotor function over time. We found that DAM enhance recovery of hind limb function and that this effect is mainly mediated during the first week following SCI. There are however a few limitations to keep in mind concerning the microglia depletion model. Firstly, the depletion is not specific for microglia and includes all *Cx3cr1*$^{+}$ cells. Immune cells such as NK-cells, DCs and monocytes are also *Cx3cr1*$^{+}$. Hence, the lower rate of functional recovery observed in the *Cx3cr1*$^{+}$ cell depleted animals might be a consequence of depletion of other immune cells and not due to the lack of DAM. Secondly, the depletion is not specific for the spinal cord nor the injury epicenter and instead depletes all *Cx3cr1*$^{+}$ cells in the entire CNS. Thus, there is a potential risk of a systemic response in the CNS to the local and delimited injury in the spinal cord. This might have implications for the functional recovery. A model that depletes microglia only in the injury epicenter could eliminate such a potential bias. Thirdly, the depleted microglia were re-placed by infiltrating monocytes that eventually formed macrophages. We observed this re-population to be complete at 28 days post the last day of tamoxifen injection in non-injured animals. In mice which received an SCI we also detected monocyte-derived macrophages implicating re-population following injury as well. The hind limb function of animals depleted of microglia and which did not receive a SCI did not deteriorate in hind limb locomotor function. However, it is difficult to determine if the inferior functional recovery is due to the lack of DAM or the presence and actions of monocyte-derived macrophages, both or neither of these two effects. Moreover, depleting immediately at injury or 3-4 weeks post injury could help explain additional aspects concerning the role and importance of DAM. The co-localization between *postnatal proliferative-region-associated microglia* (pPAM) in the developing brain and DAM in SCI as revealed by the integrated data analysis is in line with the beneficial effect mediated by the DAM in SCI. When interpreting the results limitations such as: no individual biological replicates were used, only adult females were used and a small number of animals were used in the functional assessment, are important to keep in mind. Thus, alterations in the current experimental design might yield different results and conclusions than those presented in this thesis.

Microglia is an important cell type to investigate and consider in SCI with implications on inflammatory- and immune response. Although the mechanism that activates and regulates the transformation of hMicroglia into DAM remains to be elucidated other cell types such as astrocytes, oligodendrocytes and pericytes are more directly related to the mechanical- and chemical barriers of re-generation in the spinal cord. The mechanical barrier mainly consists of the glial scar formed by reactive astrocytes surrounding the cystic cavity. Characterization of the heterogeneity and temporal changes of astrocytes in the glial scar as well

as the mechanisms mediating the formation of the various subpopulations could potentially unfold novel targets for therapy and approaches for treatment. The biochemical barrier to regeneration consists mainly of demyelination and the lack of re-myelination of spared- or newly formed axons in the spinal cord. Characterization of oligodendrocytes over time following SCI might contribute to the identification of subpopulations whose survival could be enhanced resulting in better myelination. Individually sequencing a larger number of cells from an unsorted population of cells from spinal cord (injury) could result in a data set in which the above-mentioned and several other cell types could be identified and studied. Later on, using knock out models and/or cell depletion models the importance and function of these cell populations could be determined. This would result in a better understanding for the pathology of SCI at single-cell resolution but hopefully also result in new clues on how to construct new treatment approaches for SCI. In conclusion, the efforts in this study can and should be broadened to include additional cell types of the CNS and thereby dig deeper in the function of these cell (sub)populations during SCI, how they are formed as well as their origin.

Ethical considerations

The single most important potential ethical issue in this doctoral project is the implementation of research animals. Each project defined in this thesis is centralized around experimental SCI. Several perspectives of SCI in each project have to be covered which results in usage of a large number of research animals. The core ethical issue is actually an ethical dilemma in the sense that usage of research animals is not self-evident in all cultures, countries or for all individuals. The chances are also that the course of time will alter the attitude towards use of research animals. Therefore, it is of substantial importance to actively discuss the potential benefits of using research animals for studying SCI in relation to the obvious harm and suffering brought upon the mice.

The 3Rs (Replace, Reduce, Refine) defined by the Bologna declaration and accepted in 1999 is generally accepted as the basis for discussion for the usage of research animals. Replacement alternatives are no longer available. Stem cells have been studied extensively *in vitro*. The environment following a SCI *in vivo* is utterly complex and is not fully understood or mapped. Due to the complexity and the lack of full understanding, the *in vivo* environment is impossible to replicate *in vitro*. In order to study and evaluate the effect and the modulation of stem cells and immune cells they have to be studied *in vivo*. Furthermore, *in vivo* studies are required to evaluate the therapeutic efficiency of stem cells for treatment of SCI. Reduction alternatives were actively discussed in the project design process. Each project was designed to provide substantial material and data in order to accurately reject or accept the null hypothesis using as few research animals as possible. One strategy used for reducing the number of animals was to describe the studied phenomena from several different perspectives using different methodologies. The proof became more rigorous and the number of animals used was minimized. Additionally, well-established and reproducible methods were used in order to minimize the risk for having to exclude animals due to technical errors or include more due to mishaps. Refinement alternatives refers to the selection of species and why the animal model is the most refined one and how any potential suffering of the animals will be minimized. Rodents have an anatomy which resembles humans and makes SCI in mice a realistic model. This ensures that any potential insights gained in mice can be transferred into treating humans. Rodents have been used for a long time within several branches of medical research. Hence, the knowledge about mice and rats as research animals is vast and several research methods and techniques have

been developed. This has enabled researchers to pursue their projects and study their hypothesis accurately. Additionally, this ensures that the wellbeing and living conditions for the animals can be optimized. Moreover, the pre-, intra- and post-operative treatment and care of the animals is similar to the clinically accepted practice used in humans.

From an utilitaristic perspective, this doctoral project is difficult to advocate for. Humans do not suffer any negative consequences but do have a large potential gain. However, the research animals do not have any potential gain at all, only suffering. The doctoral project is also difficult to motivate from a deontological perspective. Far from every human/individual would classify this type of animal research as law or generally accepted practice. The contrary is more probably true. The opposition towards use of animals in research has always been present and these individuals and groups frequently express their opinion not only in words but also in action. Furthermore, the animals are not free and cannot express their opinion and thereby not execute their autonomy, which makes this approach unfair. Hence, this project have both positive and negative consequences and fulfills three out of four criteria in the doctrine of double effect. The nature of the act is to do well, the intentions are right and the potential positive effects are in proportion to the negative side effects (suffering of the mice). However, the positive effect is achieved through a deliberate negative action toward the mice. This implies that the doctrine is not fulfilled.

Currently a vast number of SCI studies are conducted in research animals, mainly rodents of various types. These animal models however are highly standardized which certainly is not the case in the clinical setting. The problem of translation from basic science to clinical application has proven to be difficult and could be explained by the heterogeneity of injuries in the clinical setting to which the treatment developed in rodents might not be sufficiently applicable. Thus, the robustness of the results should be properly evaluated by altering the experimental design and parameters investigating the sensitivity of both negative and positive effects of the treatment. Therefore, results obtained in animal models should be validated thoroughly in other animal models prior to trials involving humans. It is however common that far too many general conclusions are made and clinical trials initiated following experiments conducted only in one animal model. Unfortunately, in this thesis only one animal model of SCI has been implemented, which is usually the case in SCI research and a consequence of limited resources. Although the results are promising they should be evaluated in other animal models while also extending the number of biological replicates prior to even considering initiation of a clinical trial.

CONCLUSION

This thesis investigated stem cell transplantation as a therapeutic approach for SCI as well as disease-specific transformations of immune cells, focusing on microglia, following SCI. We found that stem cell therapy for SCI shows a great deal of potential but is, at the moment, not sufficient or efficient enough to restore function to a near-normal level. Following SCI, microglia undergo disease-associated transformations, which persists in the chronic phase of the injury, and contribute to functional recovery.

Specifically:

1. Immunocompatible NPCs transplanted into SCI contribute causally to recovery of hind limb function. The NPCs seem to mediate this effect by differentiating into oligodendrocytes through which they enhance myelination of CNS axons (**Paper I**).

2. The histocompatibility of transplanted MSCs is of importance for their therapeutic potential in SCI. Syngeneic MSCs have a higher therapeutic potential, as compared to allogeneic MSCs, by alternatively activating macrophages and by enhancing neuronal survival, which results in suppression of inflammation and enhancement of hind limb functional recovery (**Paper II**).

3. MSCs transplanted into SCI adopt immune-cell like characteristics. The MSCs adopt this profile by up-regulating expression of genes and surface markers associated with immune cells, production of cytokines/chemokines as well as phagocytosis and endocytosis (**Paper III**).

4. Following CNS pathology homeostatic microglia undergo a distinct temporal transformation resulting in a disease-associated subtype of microglia. These disease-associated microglia persist during the chronic phase of SCI in murine and humans and seem to enhance the recovery of hind limb motor function (**Paper IV**).

OUTLOOK

The field of spinal cord injury (SCI) research has for an extended period of time been investigating a large variety of different approaches and treatments. However, at the moment, no therapy can restore lost functions to a normal or near-normal level.

The extent- and occurrence of the primary injury could be reduced by means of technical development and increased emphasis on safety in everyday life, sports, traffic and work places. Non-traumatic injuries could be counteracted by early identification and treatment of the underlying diseases. In the end, injuries to the spinal cord can perhaps never be avoided. However, the extent of the secondary injury could in the best of scenarios be significantly reduced or eliminated. A large number of research projects have aimed at enhancing regeneration and restoring function using stem cells, grafts, devices, bioengineering, drugs, hypothermia, stimulation and many other approaches. All of these methods have shown some potential to enhance regeneration and functional recovery. However, the spinal cord is a vastly complex structure and simulating the innervation of skeletal muscles, organs and smooth muscle is a highly complex task. This perhaps explains the limited success of many of the treatment approaches. Furthermore, the complexity of the spinal cord tissue makes it very difficult to replace. If the tissue cannot be adequately repaired using current treatment approaches and not replaced either, efforts should perhaps be directed towards characterizing the cellular- and molecular response following SCI in greater detail prior to evaluating any treatments. Hence, SCI is not yet fully understood which limits our ability to design a therapy that can reduce and hopefully eliminate the extent of the secondary injury. The simple lack of success in SCI research supports the idea that something has to change. One of the critical areas to focus on is scar formation. The glial scar creates a mechanical barrier that inhibits regenerating axons from growing through the injured area. The prevention of scar formation in combination with enhanced myelination of regenerating axons is likely to significantly enhance recovery. Utilizing modern techniques such as single-cell RNA sequencing has the potential to uncover subtypes of cells, their characteristics and dynamics over time. Identifying the scar-forming cells and the pathways that mediate the formation of these cells is the first step. In the second step drugs that inhibit these pathways and thus the formation of these cells has the potential to minimize the scar formation and thus the mechanical barrier. Moreover, a better understanding for oligodendrocytes and their myelinating capabilities has the potential to uncover ways of enhancing myelination of regeneration axons. In the best of scenarios, a medicament could be developed which modulates the cellular response and minimizes the scar formation and maximizes the myelination of regenerating axons. Thus, the spinal cord tissue is immensely difficult to replicate and replace which is the motivation for the development of a therapy that minimizes or reduces the negative effects of the secondary injury. Given that the scar formation can be eliminated and regenerating axons are successfully re-innervating the skeletal muscles emphasis could be shifted towards enhancing neuronal survival by reducing or eliminating excitotoxicity. In this case, the patient would only loose the cells which were damaged in the primary injury which might give some (but hopefully less) functional reduction. This functional reduction could in best case be managed by the plasticity of the CNS and in combination with rehabilitation efforts be reduced to almost none. Thus, one vision would perhaps be to develop the “SCI drug” which could be administrated to the patient when a SCI is suspected. Decompressive surgery should be preserved and is likely necessary for decompression itself but also to stabilize and reconstruct the spinal column and manage any fractures to the vertebrae.

Taken together, an increased focus on basic science focusing on mechanisms in SCI should perhaps, at the moment, be prioritized in favor of projects blindly evaluating various therapeutic approaches.

Alongside increased efforts in basic science, intense efforts should be made to exploit technical innovation to replace lost functions and/or to manage them in the best possible manner. Highly sophisticated exoskeletons in combination with artificial intelligence could perhaps be able to restore several somatic functions. Utilizing sensors on the motor cortex recording the activation of regions associated with walking connected to mechanical devices attached to the lower limbs is one potential solution for voluntary control of walking. Thus, some extent of voluntary control in combination with artificial intelligence might be a feasible option. Devices implanted into the muscles and/or nerves regulating these functions and attached to the motor- and sensory cortex in a similar fashion as the artificial legs could perhaps control urogenital- and anal functions. Sensory functions could perhaps be replaced using a lower body suit that has sensors detecting touch and relays this information to the correct region of the sensory cortex. If connection between the mechanical devices and the motor- and sensory cortex is difficult to establish this could be replaced using mobile applications, through which the individual could activate functions at any given time. Hence, technical development could provide patients with more instant relief and recovery until research in basic science has identified mechanisms which have a distinct and important causal connection to functional outcome and which can be distinctly, precisely and efficiently targeted and/or modulated.

Taken together, an increased emphasis on basic science focusing on characterizing and understanding the mechanisms of SCI *in combination* with application of technical innovation and artificial intelligence has a high probability of assisting and aiding these patients in the short- and long run.

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REFERENCES

1. Silva NA, Sousa N, Reis RL, Salgado AJ: From basics to clinical: a comprehensive review on spinal cord injury. Prog Neurobiol 2014, 114:25-57.
2. Ahuja CS, Wilson JR, Nori S, Kotter MRN, Druschel C, Curt A, Fehlings MG: Traumatic spinal cord injury. Nat Rev Dis Primers 2017, 3:17018.
3. Dobkin BH, Havton LA: Basic advances and new avenues in therapy of spinal cord injury. Annu Rev Med 2004, 55:255-282.
4. Singh A, Tetreault L, Kalsi-Ryan S, Nouri A, Fehlings MG: Global prevalence and incidence of traumatic spinal cord injury. Clin Epidemiol 2014, 6:309-331.
5. Cripps RA, Lee BB, Wing P, Weerts E, Mackay J, Brown D: A global map for traumatic spinal cord injury epidemiology: towards a living data repository for injury prevention. Spinal Cord 2011, 49(4):493-501.
6. Chen Y, He Y, DeVivo MJ: Changing Demographics and Injury Profile of New Traumatic Spinal Cord Injuries in the United States, 1972-2014. Arch Phys Med Rehabil 2016, 97(10):1610-1619.
7. van den Berg ME, Castellote JM, Mahillo-Fernandez I, de Pedro-Cuesta J: Incidence of spinal cord injury worldwide: a systematic review. Neuroepidemiology 2010, 34(3):184-192; discussion 192.
8. Pineau I, Lacroix S: Proinflammatory cytokine synthesis in the injured mouse spinal cord: multiphasic expression pattern and identification of the cell types involved. J Comp Neurol 2007, 500(2):267-285.
9. LaPlaca MC, Simon CM, Prado GR, Cullen DK: CNS injury biomechanics and experimental models. Prog Brain Res 2007, 161:13-26.
10. Tator CH: Review of experimental spinal cord injury with emphasis on the local and systemic circulatory effects. Neurochirurgie 1991, 37(5):291-302.
11. Tator CH, Fehlings MG: Review of the secondary injury theory of acute spinal cord trauma with emphasis on vascular mechanisms. J Neurosurg 1991, 75(1):15-26.
12. Farooque M, Hillered L, Holtz A, Olsson Y: Changes of extracellular levels of amino acids after graded compression trauma to the spinal cord: an experimental study in the rat using microdialysis. J Neurotrauma 1996, 13(9):537-548.
13. Schanne FA, Kane AB, Young EE, Farber JL: Calcium dependence of toxic cell death: a final common pathway. Science 1979, 206(4419):700-702.
14. Liu M, Wu W, Li H, Li S, Huang LT, Yang YQ, Sun Q, Wang CX, Yu Z, Hang CH: Necroptosis, a novel type of programmed cell death, contributes to early neural cells damage after spinal cord injury in adult mice. J Spinal Cord Med 2015, 38(6):745-753.
15. Li S, Mealing GA, Morley P, Stys PK: Novel injury mechanism in anoxia and trauma of spinal cord white matter: glutamate release via reverse Na+-dependent glutamate transport. J Neurosci 1999, 19(14):RC16.
16. Li S, Stys PK: Mechanisms of ionotropic glutamate receptor-mediated excitotoxicity in isolated spinal cord white matter. J Neurosci 2000, 20(3):1190-1198.
17. Liu D, Xu GY, Pan E, McAdoo DJ: Neurotoxicity of glutamate at the concentration released upon spinal cord injury. Neuroscience 1999, 93(4):1383-1389.
18. Hausmann ON: Post-traumatic inflammation following spinal cord injury. Spinal Cord 2003, 41(7):369-378.
19. Dizdaroglu M, Jaruga P, Birincioglu M, Rodriguez H: Free radical-induced damage to DNA: mechanisms and measurement. Free Radic Biol Med 2002, 32(11):1102-1115.
20. Beattie MS, Farooqui AA, Bresnahan JC: Review of current evidence for apoptosis after spinal cord injury. J Neurotrauma 2000, 17(10):915-925.
21. Norenberg MD, Smith J, Marcillo A: The pathology of human spinal cord injury: defining the problems. J Neurotrauma 2004, 21(4):429-440.
22. McKeon RJ, Schreiber RC, Rudge JS, Silver J: 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 1991, 11(11):3398-3411.
23. Gallo V, Bertolotto A, Levi G: The proteoglycan chondroitin sulfate is present in a subpopulation of cultured astrocytes and in their precursors. Dev Biol 1987, 123(1):282-285.
24. Tator CH: Update on the pathophysiology and pathology of acute spinal cord injury. Brain Pathol 1995, 5(4):407-413.
25. Silver J, Miller JH: Regeneration beyond the glial scar. Nat Rev Neurosci 2004, 5(2):146-156.
26. Wanner IB, Anderson MA, Song B, Levine J, Fernandez A, Gray-Thompson Z, Ao Y, Sofroniew MV: Glial scar borders are formed by newly proliferated, elongated astrocytes that interact to corral inflammatory and fibrotic cells via STAT3-dependent mechanisms after spinal cord injury. J Neurosci 2013, 33(31):12870-12886.
27. Anderson MA, Burda JE, Ren Y, Ao Y, O'Shea TM, Kawaguchi R, Coppola G, Khakh BS, Deming TJ, Sofroniew MV: Astrocyte scar formation aids central nervous system axon regeneration. Nature 2016, 532(7598):195-200.
28. Domeniconi M, Cao Z, Spencer T, Sivasankaran R, Wang K, Nikulina E, Kimura N, Cai H, Deng K, Gao Y et al: Myelin-associated glycoprotein interacts with the Nogo66 receptor to inhibit neurite outgrowth. Neuron 2002, 35(2):283-290.
29. GrandPré T, Nakamura F, Vartanian T, Strittmatter SM: Identification of the Nogo inhibitor of axon regeneration as a Reticulon protein. Nature 2000, 403(6768):439-444.

30. Li M, Shibata A, Li C, Braun PE, McKerracher L, Roder J, Kater SB, David S: Myelin-associated glycoprotein inhibits neurite/axon growth and causes growth cone collapse. J Neurosci Res 1996, 46(4):404-414.
31. Fawcett JW, Asher RA: The glial scar and central nervous system repair. Brain Res Bull 1999, 49(6):377-391.
32. Kotter MR, Li WW, Zhao C, Franklin RJ: Myelin impairs CNS remyelination by inhibiting oligodendrocyte precursor cell differentiation. J Neurosci 2006, 26(1):328-332.
33. Meletis K, Barnabé-Heider F, Carlén M, Evergren E, Tomilin N, Shupliakov O, Frisén J: Spinal cord injury reveals multilineage differentiation of ependymal cells. PLoS Biol 2008, 6(7):e182.
34. Barnabé-Heider F, Göritz C, Sabelström H, Takebayashi H, Pfrieger FW, Meletis K, Frisén J: Origin of new glial cells in intact and injured adult spinal cord. Cell Stem Cell 2010, 7(4):470-482.
35. de Leon RD, Hodgson JA, Roy RR, Edgerton VR: Locomotor capacity attributable to step training versus spontaneous recovery after spinalization in adult cats. J Neurophysiol 1998, 79(3):1329-1340.
36. Ballermann M, Fouad K: Spontaneous locomotor recovery in spinal cord injured rats is accompanied by anatomical plasticity of reticulospinal fibers. Eur J Neurosci 2006, 23(8):1988-1996.
37. Courtine G, Song B, Roy RR, Zhong H, Herrmann JE, Ao Y, Qi J, Edgerton VR, Sofroniew MV: Recovery of supraspinal control of stepping via indirect propriospinal relay connections after spinal cord injury. Nat Med 2008, 14(1):69-74.
38. Christensen MD, Hulsebosch CE: Chronic central pain after spinal cord injury. J Neurotrauma 1997, 14(8):517-537.
39. Rabchevsky AG, Kitzman PH: Latest approaches for the treatment of spasticity and autonomic dysreflexia in chronic spinal cord injury. Neurotherapeutics 2011, 8(2):274-282.
40. Krenz NR, Weaver LC: Sprouting of primary afferent fibers after spinal cord transection in the rat. Neuroscience 1998, 85(2):443-458.
41. Qi HX, Stepniewska I, Kaas JH: Reorganization of primary motor cortex in adult macaque monkeys with long-standing amputations. J Neurophysiol 2000, 84(4):2133-2147.
42. Levy WJ, Amassian VE, Traad M, Cadwell J: Focal magnetic coil stimulation reveals motor cortical system reorganized in humans after traumatic quadriplegia. Brain Res 1990, 510(1):130-134.
43. Topka H, Cohen LG, Cole RA, Hallett M: Reorganization of corticospinal pathways following spinal cord injury. Neurology 1991, 41(8):1276-1283.
44. Jones EG: GABAergic neurons and their role in cortical plasticity in primates. Cereb Cortex 1993, 3(5):361-372.
45. Roy FD, Zewdie ET, Gorassini MA: Short-interval intracortical inhibition with incomplete spinal cord injury. Clin Neurophysiol 2011, 122(7):1387-1395.
46. Acheson MB, Livingston RR, Richardson ML, Stimac GK: High-resolution CT scanning in the evaluation of cervical spine fractures: comparison with plain film examinations. AJR Am J Roentgenol 1987, 148(6):1179-1185.
47. Lammertse D, Dungan D, Dreisbach J, Falci S, Flanders A, Marino R, Schwartz E, Rehabilitation NIoDa: Neuroimaging in traumatic spinal cord injury: an evidence-based review for clinical practice and research. J Spinal Cord Med 2007, 30(3):205-214.
48. Kirshblum SC, Waring W, Biering-Sorensen F, Burns SP, Johansen M, Schmidt-Read M, Donovan W, Graves D, Jha A, Jones L et al: Reference for the 2011 revision of the International Standards for Neurological Classification of Spinal Cord Injury. J Spinal Cord Med 2011, 34(6):547-554.
49. SCHNEIDER RC, CHERRY G, PANTEK H: The syndrome of acute central cervical spinal cord injury; with special reference to the mechanisms involved in hyperextension injuries of cervical spine. J Neurosurg 1954, 11(6):546-577.
50. McKinley W, Santos K, Meade M, Brooke K: Incidence and outcomes of spinal cord injury clinical syndromes. J Spinal Cord Med 2007, 30(3):215-224.
51. Ko HY, Ditunno JF, Graziani V, Little JW: The pattern of reflex recovery during spinal shock. Spinal Cord 1999, 37(6):402-409.
52. Lehmann KG, Lane JG, Piepmeier JM, Batsford WP: Cardiovascular abnormalities accompanying acute spinal cord injury in humans: incidence, time course and severity. J Am Coll Cardiol 1987, 10(1):46-52.
53. Ryken TC, Hurlbert RJ, Hadley MN, Aarabi B, Dhall SS, Gelb DE, Rozzelle CJ, Theodore N, Walters BC: The acute cardiopulmonary management of patients with cervical spinal cord injuries. Neurosurgery 2013, 72 Suppl 2:84-92.
54. Hall ED, Braughler JM: Acute effects of intravenous glucocorticoid pretreatment on the in vitro peroxidation of cat spinal cord tissue. Exp Neurol 1981, 73(1):321-324.
55. Carlson GD, Minato Y, Okada A, Gorden CD, Warden KE, Barbeau JM, Biro CL, Bahnuik E, Bohlman HH, Lamanna JC: Early time-dependent decompression for spinal cord injury: vascular mechanisms of recovery. J Neurotrauma 1997, 14(12):951-962.
56. Dolan EJ, Tator CH, Endrenyi L: The value of decompression for acute experimental spinal cord compression injury. J Neurosurg 1980, 53(6):749-755.
57. Fehlings MG, Perrin RG: The role and timing of early decompression for cervical spinal cord injury: update with a review of recent clinical evidence. Injury 2005, 36 Suppl 2:B13-26.
58. Ahuja CS, Martin AR, Fehlings M: Recent advances in managing a spinal cord injury secondary to trauma. F1000Res 2016, 5.
59. Bethea JR, Nagashima H, Acosta MC, Briceno C, Gomez F, Marcillo AE, Loor K, Green J, Dietrich WD: Systemically administered interleukin-10 reduces tumor necrosis factor-alpha production and significantly improves functional recovery following

traumatic spinal cord injury in rats. J Neurotrauma 1999, 16(10):851-863.
60. Stirling DP, Koochesfahani KM, Steeves JD, Tetzlaff W: Minocycline as a neuroprotective agent. Neuroscientist 2005, 11(4):308-322.
61. Lee SM, Yune TY, Kim SJ, Park DW, Lee YK, Kim YC, Oh YJ, Markelonis GJ, Oh TH: Minocycline reduces cell death and improves functional recovery after traumatic spinal cord injury in the rat. J Neurotrauma 2003, 20(10):1017-1027.
62. Wilson JR, Fehlings MG: Riluzole for acute traumatic spinal cord injury: a promising neuroprotective treatment strategy. World Neurosurg 2014, 81(5-6):825-829.
63. Konishi Y, Chui DH, Hirose H, Kunishita T, Tabira T: Trophic effect of erythropoietin and other hematopoietic factors on central cholinergic neurons in vitro and in vivo. Brain Res 1993, 609(1-2):29-35.
64. Freund P, Wannier T, Schmidlin E, Bloch J, Mir A, Schwab ME, Rouiller EM: Anti-Nogo-A antibody treatment enhances sprouting of corticospinal axons rostral to a unilateral cervical spinal cord lesion in adult macaque monkey. J Comp Neurol 2007, 502(4):644-659.
65. Caroni P, Schwab ME: Two membrane protein fractions from rat central myelin with inhibitory properties for neurite growth and fibroblast spreading. J Cell Biol 1988, 106(4):1281-1288.
66. Zuo J, Neubauer D, Dyess K, Ferguson TA, Muir D: Degradation of chondroitin sulfate proteoglycan enhances the neurite-promoting potential of spinal cord tissue. Exp Neurol 1998, 154(2):654-662.
67. Fouad K, Schnell L, Bunge MB, Schwab ME, Liebscher T, Pearse DD: Combining Schwann cell bridges and olfactory-ensheathing glia grafts with chondroitinase promotes locomotor recovery after complete transection of the spinal cord. J Neurosci 2005, 25(5):1169-1178.
68. David S, Lacroix S: Molecular approaches to spinal cord repair. Annu Rev Neurosci 2003, 26:411-440.
69. Maisonpierre PC, Belluscio L, Friedman B, Alderson RF, Wiegand SJ, Furth ME, Lindsay RM, Yancopoulos GD: NT-3, BDNF, and NGF in the developing rat nervous system: parallel as well as reciprocal patterns of expression. Neuron 1990, 5(4):501-509.
70. Rabchevsky AG, Fugaccia I, Turner AF, Blades DA, Mattson MP, Scheff SW: Basic fibroblast growth factor (bFGF) enhances functional recovery following severe spinal cord injury to the rat. Exp Neurol 2000, 164(2):280-291.
71. Tetzlaff W, Okon EB, Karimi-Abdolrezaee S, Hill CE, Sparling JS, Plemel JR, Plunet WT, Tsai EC, Baptiste D, Smithson LJ et al: A systematic review of cellular transplantation therapies for spinal cord injury. J Neurotrauma 2011, 28(8):1611-1682.
72. Bachmann LC, Matis A, Lindau NT, Felder P, Gullo M, Schwab ME: Deep brain stimulation of the midbrain locomotor region improves paretic hindlimb function after spinal cord injury in rats. Sci Transl Med 2013, 5(208):208ra146.
73. Miller LE, Zimmermann AK, Herbert WG: Clinical effectiveness and safety of powered exoskeleton-assisted walking in patients with spinal cord injury: systematic review with meta-analysis. Med Devices (Auckl) 2016, 9:455-466.
74. Erecinska M, Thoresen M, Silver IA: Effects of hypothermia on energy metabolism in Mammalian central nervous system. J Cereb Blood Flow Metab 2003, 23(5):513-530.
75. Wang M, Zhai P, Chen X, Schreyer DJ, Sun X, Cui F: Bioengineered scaffolds for spinal cord repair. Tissue Eng Part B Rev 2011, 17(3):177-194.
76. Burns AS, Ditunno JF: Establishing prognosis and maximizing functional outcomes after spinal cord injury: a review of current and future directions in rehabilitation management. Spine (Phila Pa 1976) 2001, 26(24 Suppl):S137-145.
77. Marino RJ, Ditunno JF, Donovan WH, Maynard F: Neurologic recovery after traumatic spinal cord injury: data from the Model Spinal Cord Injury Systems. Arch Phys Med Rehabil 1999, 80(11):1391-1396.
78. Brodbelt AR, Stoodley MA: Post-traumatic syringomyelia: a review. J Clin Neurosci 2003, 10(4):401-408.
79. Aebli N, Pötzel T, Krebs J: Characteristics and surgical management of neuropathic (Charcot) spinal arthropathy after spinal cord injury. Spine J 2014, 14(6):884-891.
80. Adams MM, Hicks AL: Spasticity after spinal cord injury. Spinal Cord 2005, 43(10):577-586.
81. Claydon VE, Steeves JD, Krassioukov A: Orthostatic hypotension following spinal cord injury: understanding clinical pathophysiology. Spinal Cord 2006, 44(6):341-351.
82. Krassioukov A, Eng JJ, Warburton DE, Teasell R, Team SCIRER: A systematic review of the management of orthostatic hypotension after spinal cord injury. Arch Phys Med Rehabil 2009, 90(5):876-885.
83. Krassioukov A, Warburton DE, Teasell R, Eng JJ, Team SCIRER: A systematic review of the management of autonomic dysreflexia after spinal cord injury. Arch Phys Med Rehabil 2009, 90(4):682-695.
84. Linn WS, Adkins RH, Gong H, Waters RL: Pulmonary function in chronic spinal cord injury: a cross-sectional survey of 222 southern California adult outpatients. Arch Phys Med Rehabil 2000, 81(6):757-763.
85. Brown R, DiMarco AF, Hoit JD, Garshick E: Respiratory dysfunction and management in spinal cord injury. Respir Care 2006, 51(8):853-868;discussion 869-870.
86. DeVivo MJ, Black KJ, Stover SL: Causes of death during the first 12 years after spinal cord injury. Arch Phys Med Rehabil 1993, 74(3):248-254.
87. Brommer B, Engel O, Kopp MA, Watzlawick R, Müller S, Prüss H, Chen Y, DeVivo MJ, Finkenstaedt FW, Dirnagl U et al: Spinal cord injury-induced immune deficiency syndrome enhances infection susceptibility dependent on lesion level. Brain 2016, 139(Pt 3):692-707.

88. Taweel WA, Seyam R: Neurogenic bladder in spinal cord injury patients. Res Rep Urol 2015, 7:85-99.
89. Sezer N, Akkuş S, Uğurlu FG: Chronic complications of spinal cord injury. World J Orthop 2015, 6(1):24-33.
90. Hess MJ, Hough S: Impact of spinal cord injury on sexuality: broad-based clinical practice intervention and practical application. J Spinal Cord Med 2012, 35(4):211-218.
91. Coggrave MJ, Norton C: The need for manual evacuation and oral laxatives in the management of neurogenic bowel dysfunction after spinal cord injury: a randomized controlled trial of a stepwise protocol. Spinal Cord 2010, 48(6):504-510.
92. Guidelines CfSCMCP: Pressure ulcer prevention and treatment following spinal cord injury: a clinical practice guideline for health-care professionals. J Spinal Cord Med 2001, 24 Suppl 1:S40-101.
93. van Kuijk AA, Geurts AC, van Kuppevelt HJ: Neurogenic heterotopic ossification in spinal cord injury. Spinal Cord 2002, 40(7):313-326.
94. Rush PJ: The rheumatic manifestations of traumatic spinal cord injury. Semin Arthritis Rheum 1989, 19(2):77-89.
95. Cardenas DD, Felix ER: Pain after spinal cord injury: a review of classification, treatment approaches, and treatment assessment. PM R 2009, 1(12):1077-1090.
96. Krause JS, Sternberg M, Lottes S, Maides J: Mortality after spinal cord injury: an 11-year prospective study. Arch Phys Med Rehabil 1997, 78(8):815-821.
97. Center NSCI: Spinal Cord Injury Facts and Figures at a Glance. J Spinal Cord Med 2014, 37(3):355-356.
98. Akhtar AZ, Pippin JJ, Sandusky CB: Animal models in spinal cord injury: a review. Rev Neurosci 2008, 19(1):47-60.
99. Sharif-Alhoseini M, Khormali M, Rezaei M, Safdarian M, Hajighadery A, Khalatbari MM, Meknatkhah S, Rezvan M, Chalangari M, Derakhshan P et al: Animal models of spinal cord injury: a systematic review. Spinal Cord 2017, 55(8):714-721.
100. Zhang N, Fang M, Chen H, Gou F, Ding M: Evaluation of spinal cord injury animal models. Neural Regen Res 2014, 9(22):2008-2012.
101. Gruner JA: A monitored contusion model of spinal cord injury in the rat. J Neurotrauma 1992, 9(2):123-126; discussion 126-128.
102. Rivlin AS, Tator CH: Effect of duration of acute spinal cord compression in a new acute cord injury model in the rat. Surg Neurol 1978, 10(1):38-43.
103. Fehlings MG, Tator CH, Linden RD: The relationships among the severity of spinal cord injury, motor and somatosensory evoked potentials and spinal cord blood flow. Electroencephalogr Clin Neurophysiol 1989, 74(4):241-259.
104. Basso DM, Beattie MS, Bresnahan JC: A sensitive and reliable locomotor rating scale for open field testing in rats. J Neurotrauma 1995, 12(1):1-21.
105. Hamers FP, Koopmans GC, Joosten EA: CatWalk-assisted gait analysis in the assessment of spinal cord injury. J Neurotrauma 2006, 23(3-4):537-548.
106. Zorner B, Filli L, Starkey ML, Gonzenbach R, Kasper H, Rothlisberger M, Bolliger M, Schwab ME: Profiling locomotor recovery: comprehensive quantification of impairments after CNS damage in rodents. Nat Methods 2010, 7(9):701-708.
107. Beare JE, Morehouse JR, DeVries WH, Enzmann GU, Burke DA, Magnuson DS, Whittemore SR: Gait analysis in normal and spinal contused mice using the TreadScan system. J Neurotrauma 2009, 26(11):2045-2056.
108. Metz GA, Whishaw IQ: Cortical and subcortical lesions impair skilled walking in the ladder rung walking test: a new task to evaluate fore- and hindlimb stepping, placing, and co-ordination. J Neurosci Methods 2002, 115(2):169-179.
109. Woolf CJ, Thompson SW: The induction and maintenance of central sensitization is dependent on N-methyl-D-aspartic acid receptor activation; implications for the treatment of post-injury pain hypersensitivity states. Pain 1991, 44(3):293-299.
110. Zileli M, Schramm J: Motor versus somatosensory evoked potential changes after acute experimental spinal cord injury in rats. Acta Neurochir (Wien) 1991, 108(3-4):140-147.
111. Marshall JS, Warrington R, Watson W, Kim HL: An introduction to immunology and immunopathology. Allergy Asthma Clin Immunol 2018, 14(Suppl 2):49.
112. Abbas AK, Lichtman AH, Pillai S, Tyndale-Biscoe P: Basic immunology
functions and disorders of the immune system. In. Johanneshov: MTM,; 2017.
113. Turvey SE, Broide DH: Innate immunity. J Allergy Clin Immunol 2010, 125(2 Suppl 2):S24-32.
114. Bonilla FA, Oettgen HC: Adaptive immunity. J Allergy Clin Immunol 2010, 125(2 Suppl 2):S33-40.
115. Kettenmann H, Hanisch UK, Noda M, Verkhratsky A: Physiology of microglia. Physiol Rev 2011, 91(2):461-553.
116. Chan WY, Kohsaka S, Rezaie P: The origin and cell lineage of microglia: new concepts. Brain Res Rev 2007, 53(2):344-354.
117. Rezaie P, Dean A, Male D, Ulfig N: Microglia in the cerebral wall of the human telencephalon at second trimester. Cereb Cortex 2005, 15(7):938-949.
118. Brockhaus J, Ilschner S, Banati RB, Kettenmann H: Membrane properties of ameboid microglial cells in the corpus callosum slice from early postnatal mice. J Neurosci 1993, 13(10):4412-4421.
119. Haas S, Brockhaus J, Verkhratsky A, Kettenmann H: ATP-induced membrane currents in ameboid microglia acutely isolated from mouse brain slices. Neuroscience 1996, 75(1):257-261.
120. Ajami B, Bennett JL, Krieger C, Tetzlaff W, Rossi FM: Local self-renewal can sustain CNS microglia maintenance and function throughout adult life. Nat Neurosci 2007, 10(12):1538-1543.

121. Mildner A, Schmidt H, Nitsche M, Merkler D, Hanisch UK, Mack M, Heikenwalder M, Brück W, Priller J, Prinz M: Microglia in the adult brain arise from Ly-6ChiCCR2+ monocytes only under defined host conditions. Nat Neurosci 2007, 10(12):1544-1553.
122. Block ML, Zecca L, Hong JS: Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. Nat Rev Neurosci 2007, 8(1):57-69.
123. Colton C, Wilcock DM: Assessing activation states in microglia. CNS Neurol Disord Drug Targets 2010, 9(2):174-191.
124. Davoust N, Vuaillat C, Androdias G, Nataf S: From bone marrow to microglia: barriers and avenues. Trends Immunol 2008, 29(5):227-234.
125. Graeber MB, Streit WJ: Microglia: biology and pathology. Acta Neuropathol 2010, 119(1):89-105.
126. Hanisch UK, Kettenmann H: Microglia: active sensor and versatile effector cells in the normal and pathologic brain. Nat Neurosci 2007, 10(11):1387-1394.
127. Kreutzberg GW: Microglia: a sensor for pathological events in the CNS. Trends Neurosci 1996, 19(8):312-318.
128. Streit WJ, Conde JR, Fendrick SE, Flanary BE, Mariani CL: Role of microglia in the central nervous system's immune response. Neurol Res 2005, 27(7):685-691.
129. van Rossum D, Hanisch UK: Microglia. Metab Brain Dis 2004, 19(3-4):393-411.
130. Sievers J, Parwaresch R, Wottge HU: Blood monocytes and spleen macrophages differentiate into microglia-like cells on monolayers of astrocytes: morphology. Glia 1994, 12(4):245-258.
131. Schilling T, Nitsch R, Heinemann U, Haas D, Eder C: Astrocyte-released cytokines induce ramification and outward K+ channel expression in microglia via distinct signalling pathways. Eur J Neurosci 2001, 14(3):463-473.
132. Eder C, Klee R, Heinemann U: Involvement of stretch-activated Cl- channels in ramification of murine microglia. J Neurosci 1998, 18(18):7127-7137.
133. Davalos D, Grutzendler J, Yang G, Kim JV, Zuo Y, Jung S, Littman DR, Dustin ML, Gan WB: ATP mediates rapid microglial response to local brain injury in vivo. Nat Neurosci 2005, 8(6):752-758.
134. Nimmerjahn A, Kirchhoff F, Helmchen F: Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science 2005, 308(5726):1314-1318.
135. Lynch MA: The multifaceted profile of activated microglia. Mol Neurobiol 2009, 40(2):139-156.
136. Stence N, Waite M, Dailey ME: Dynamics of microglial activation: a confocal time-lapse analysis in hippocampal slices. Glia 2001, 33(3):256-266.
137. Streit WJ, Walter SA, Pennell NA: Reactive microgliosis. Prog Neurobiol 1999, 57(6):563-581.
138. Nelson PT, Soma LA, Lavi E: Microglia in diseases of the central nervous system. Ann Med 2002, 34(7-8):491-500.
139. Rezaie P, Trillo-Pazos G, Greenwood J, Everall IP, Male DK: Motility and ramification of human fetal microglia in culture: an investigation using time-lapse video microscopy and image analysis. Exp Cell Res 2002, 274(1):68-82.
140. Colton CA: Heterogeneity of microglial activation in the innate immune response in the brain. J Neuroimmune Pharmacol 2009, 4(4):399-418.
141. Carson MJ, Bilousova TV, Puntambekar SS, Melchior B, Doose JM, Ethell IM: A rose by any other name? The potential consequences of microglial heterogeneity during CNS health and disease. Neurotherapeutics 2007, 4(4):571-579.
142. Goings GE, Kozlowski DA, Szele FG: Differential activation of microglia in neurogenic versus non-neurogenic regions of the forebrain. Glia 2006, 54(4):329-342.
143. Kraft AD, McPherson CA, Harry GJ: Heterogeneity of microglia and TNF signaling as determinants for neuronal death or survival. Neurotoxicology 2009, 30(5):785-793.
144. Kuwabara Y, Yokoyama A, Yang L, Toku K, Mori K, Takeda I, Shigekawa T, Zhang B, Maeda N, Sakanaka M et al: Two populations of microglial cells isolated from rat primary mixed glial cultures. J Neurosci Res 2003, 73(1):22-30.
145. Ladeby R, Wirenfeldt M, Garcia-Ovejero D, Fenger C, Dissing-Olesen L, Dalmau I, Finsen B: Microglial cell population dynamics in the injured adult central nervous system. Brain Res Brain Res Rev 2005, 48(2):196-206.
146. Hristova M, Cuthill D, Zbarsky V, Acosta-Saltos A, Wallace A, Blight K, Buckley SM, Peebles D, Heuer H, Waddington SN et al: Activation and deactivation of periventricular white matter phagocytes during postnatal mouse development. Glia 2010, 58(1):11-28.
147. Ilschner S, Brandt R: The transition of microglia to a ramified phenotype is associated with the formation of stable acetylated and detyrosinated microtubules. Glia 1996, 18(2):129-140.
148. Suzumura A, Marunouchi T, Yamamoto H: Morphological transformation of microglia in vitro. Brain Res 1991, 545(1-2):301-306.
149. Markovic DS, Vinnakota K, Chirasani S, Synowitz M, Raguet H, Stock K, Sliwa M, Lehmann S, Kälin R, van Rooijen N et al: Gliomas induce and exploit microglial MT1-MMP expression for tumor expansion. Proc Natl Acad Sci U S A 2009, 106(30):12530-12535.
150. Imai Y, Ibata I, Ito D, Ohsawa K, Kohsaka S: A novel gene iba1 in the major histocompatibility complex class III region encoding an EF hand protein expressed in a monocytic lineage. Biochem Biophys Res Commun 1996, 224(3):855-862.
151. Imai Y, Kohsaka S: Intracellular signaling in M-CSF-induced microglia activation: role of Iba1. Glia 2002, 40(2):164-174.
152. Zhang GX, Li J, Ventura E, Rostami A: Parenchymal microglia of naïve adult C57BL/6J mice

express high levels of B7.1, B7.2, and MHC class II. Exp Mol Pathol 2002, 73(1):35-45.
153. Jaimes Y, Naaldijk Y, Wenk K, Leovsky C, Emmrich F: Mesenchymal Stem Cell-Derived Microvesicles Modulate Lipopolysaccharides-Induced Inflammatory Responses to Microglia Cells. Stem Cells 2017, 35(3):812-823.
154. Giunti D, Parodi B, Usai C, Vergani L, Casazza S, Bruzzone S, Mancardi G, Uccelli A: Mesenchymal stem cells shape microglia effector functions through the release of CX3CL1. Stem Cells 2012, 30(9):2044-2053.
155. Wang F, Zhang KH, Hu HM, Liu XB, Bai HR, Jiang F, Wang XD, An YH: Alternatively activated microglia co-cultured with BMSCS offers a new strategy in the treatment of CNS-associated disease. Cell Biol Int 2015, 39(3):341-349.
156. Hegyi B, Környei Z, Ferenczi S, Fekete R, Kudlik G, Kovács KJ, Madarász E, Uher F: Regulation of mouse microglia activation and effector functions by bone marrow-derived mesenchymal stem cells. Stem Cells Dev 2014, 23(21):2600-2612.
157. Neubrand VE, Pedreño M, Caro M, Forte-Lago I, Delgado M, Gonzalez-Rey E: Mesenchymal stem cells induce the ramification of microglia via the small RhoGTPases Cdc42 and Rac1. Glia 2014, 62(12):1932-1942.
158. Noh MY, Lim SM, Oh KW, Cho KA, Park J, Kim KS, Lee SJ, Kwon MS, Kim SH: Mesenchymal Stem Cells Modulate the Functional Properties of Microglia via TGF-β Secretion. Stem Cells Transl Med 2016, 5(11):1538-1549.
159. Ooi YY, Dheen ST, Tay SS: Paracrine effects of mesenchymal stem cells-conditioned medium on microglial cytokines expression and nitric oxide production. Neuroimmunomodulation 2015, 22(4):233-242.
160. Yan K, Zhang R, Sun C, Chen L, Li P, Liu Y, Peng L, Sun H, Qin K, Chen F et al: Bone marrow-derived mesenchymal stem cells maintain the resting phenotype of microglia and inhibit microglial activation. PLoS One 2013, 8(12):e84116.
161. Jose S, Tan SW, Ooi YY, Ramasamy R, Vidyadaran S: Mesenchymal stem cells exert anti-proliferative effect on lipopolysaccharide-stimulated BV2 microglia by reducing tumour necrosis factor-α levels. J Neuroinflammation 2014, 11:149.
162. Ooi YY, Ramasamy R, Rahmat Z, Subramaiam H, Tan SW, Abdullah M, Israf DA, Vidyadaran S: Bone marrow-derived mesenchymal stem cells modulate BV2 microglia responses to lipopolysaccharide. Int Immunopharmacol 2010, 10(12):1532-1540.
163. Wang YH, Chen J, Zhou J, Nong F, Lv JH, Liu J: Reduced inflammatory cell recruitment and tissue damage in spinal cord injury by acellular spinal cord scaffold seeded with mesenchymal stem cells. Exp Ther Med 2017, 13(1):203-207.
164. Zhou HL, Zhang XJ, Zhang MY, Yan ZJ, Xu ZM, Xu RX: Transplantation of Human Amniotic Mesenchymal Stem Cells Promotes Functional Recovery in a Rat Model of Traumatic Spinal Cord Injury. Neurochem Res 2016, 41(10):2708-2718.
165. Zhang R, Liu Y, Yan K, Chen L, Chen XR, Li P, Chen FF, Jiang XD: Anti-inflammatory and immunomodulatory mechanisms of mesenchymal stem cell transplantation in experimental traumatic brain injury. J Neuroinflammation 2013, 10:106.
166. Ryu HH, Kang BJ, Park SS, Kim Y, Sung GJ, Woo HM, Kim WH, Kweon OK: Comparison of mesenchymal stem cells derived from fat, bone marrow, Wharton's jelly, and umbilical cord blood for treating spinal cord injuries in dogs. J Vet Med Sci 2012, 74(12):1617-1630.
167. Seo JH, Jang IK, Kim H, Yang MS, Lee JE, Kim HE, Eom YW, Lee DH, Yu JH, Kim JY et al: Early Immunomodulation by Intravenously Transplanted Mesenchymal Stem Cells Promotes Functional Recovery in Spinal Cord Injured Rats. Cell Med 2011, 2(2):55-67.
168. Abrams MB, Dominguez C, Pernold K, Reger R, Wiesenfeld-Hallin Z, Olson L, Prockop D: Multipotent mesenchymal stromal cells attenuate chronic inflammation and injury-induced sensitivity to mechanical stimuli in experimental spinal cord injury. Restor Neurol Neurosci 2009, 27(4):307-321.
169. Yang CC, Shih YH, Ko MH, Hsu SY, Cheng H, Fu YS: Transplantation of human umbilical mesenchymal stem cells from Wharton's jelly after complete transection of the rat spinal cord. PLoS One 2008, 3(10):e3336.
170. Zanier ER, Pischiutta F, Riganti L, Marchesi F, Turola E, Fumagalli S, Perego C, Parotto E, Vinci P, Veglianese P et al: Bone marrow mesenchymal stromal cells drive protective M2 microglia polarization after brain trauma. Neurotherapeutics 2014, 11(3):679-695.
171. Xu C, Fu F, Li X, Zhang S: Mesenchymal stem cells maintain the microenvironment of central nervous system by regulating the polarization of macrophages/microglia after traumatic brain injury. Int J Neurosci 2017:1-12.
172. Ilic D, Polak JM: Stem cells in regenerative medicine: introduction. Br Med Bull 2011, 98:117-126.
173. Chagastelles PC, Nardi NB: Biology of stem cells: an overview. Kidney Int Suppl (2011) 2011, 1(3):63-67.
174. Evans MJ, Kaufman MH: Establishment in culture of pluripotential cells from mouse embryos. Nature 1981, 292(5819):154-156.
175. Rippon HJ, Bishop AE: Embryonic stem cells. Cell Prolif 2004, 37(1):23-34.
176. Fuchs E, Tumbar T, Guasch G: Socializing with the neighbors: stem cells and their niche. Cell 2004, 116(6):769-778.
177. Alison MR, Islam S: Attributes of adult stem cells. J Pathol 2009, 217(2):144-160.
178. Maherali N, Hochedlinger K: Guidelines and techniques for the generation of induced pluripotent stem cells. Cell Stem Cell 2008, 3(6):595-605.
179. Takahashi K, Yamanaka S: Induction of pluripotent stem cells from mouse embryonic and adult

fibroblast cultures by defined factors. Cell 2006, 126(4):663-676.
180. Li WL, Chu MW, Wu A, Suzuki Y, Imayoshi I, Komiyama T: Adult-born neurons facilitate olfactory bulb pattern separation during task engagement. Elife 2018, 7.
181. Ming GL, Song H: Adult neurogenesis in the mammalian brain: significant answers and significant questions. Neuron 2011, 70(4):687-702.
182. Christian KM, Song H, Ming GL: Functions and dysfunctions of adult hippocampal neurogenesis. Annu Rev Neurosci 2014, 37:243-262.
183. Deng W, Aimone JB, Gage FH: New neurons and new memories: how does adult hippocampal neurogenesis affect learning and memory? Nat Rev Neurosci 2010, 11(5):339-350.
184. Reynolds BA, Weiss S: Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. Science 1992, 255(5052):1707-1710.
185. Richards LJ, Kilpatrick TJ, Bartlett PF: De novo generation of neuronal cells from the adult mouse brain. Proc Natl Acad Sci U S A 1992, 89(18):8591-8595.
186. Gage FH, Coates PW, Palmer TD, Kuhn HG, Fisher LJ, Suhonen JO, Peterson DA, Suhr ST, Ray J: Survival and differentiation of adult neuronal progenitor cells transplanted to the adult brain. Proc Natl Acad Sci U S A 1995, 92(25):11879-11883.
187. Lim DA, Alvarez-Buylla A: The Adult Ventricular-Subventricular Zone (V-SVZ) and Olfactory Bulb (OB) Neurogenesis. Cold Spring Harb Perspect Biol 2016, 8(5).
188. Paton JA, Nottebohm FN: Neurons generated in the adult brain are recruited into functional circuits. Science 1984, 225(4666):1046-1048.
189. Obernier K, Cebrian-Silla A, Thomson M, Parraguez JI, Anderson R, Guinto C, Rodas Rodriguez J, Garcia-Verdugo JM, Alvarez-Buylla A: Adult Neurogenesis Is Sustained by Symmetric Self-Renewal and Differentiation. Cell Stem Cell 2018, 22(2):221-234.e228.
190. Eckenhoff MF, Rakic P: Radial organization of the hippocampal dentate gyrus: a Golgi, ultrastructural, and immunocytochemical analysis in the developing rhesus monkey. J Comp Neurol 1984, 223(1):1-21.
191. Seri B, García-Verdugo JM, McEwen BS, Alvarez-Buylla A: Astrocytes give rise to new neurons in the adult mammalian hippocampus. J Neurosci 2001, 21(18):7153-7160.
192. Anthony TE, Klein C, Fishell G, Heintz N: Radial glia serve as neuronal progenitors in all regions of the central nervous system. Neuron 2004, 41(6):881-890.
193. Noctor SC, Flint AC, Weissman TA, Dammerman RS, Kriegstein AR: Neurons derived from radial glial cells establish radial units in neocortex. Nature 2001, 409(6821):714-720.
194. Doetsch F, Caillé I, Lim DA, García-Verdugo JM, Alvarez-Buylla A: Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. Cell 1999, 97(6):703-716.
195. Mirzadeh Z, Merkle FT, Soriano-Navarro M, Garcia-Verdugo JM, Alvarez-Buylla A: Neural stem cells confer unique pinwheel architecture to the ventricular surface in neurogenic regions of the adult brain. Cell Stem Cell 2008, 3(3):265-278.
196. Fuentealba LC, Obernier K, Alvarez-Buylla A: Adult neural stem cells bridge their niche. Cell Stem Cell 2012, 10(6):698-708.
197. Ma DK, Ming GL, Song H: Glial influences on neural stem cell development: cellular niches for adult neurogenesis. Curr Opin Neurobiol 2005, 15(5):514-520.
198. Ponti G, Obernier K, Guinto C, Jose L, Bonfanti L, Alvarez-Buylla A: Cell cycle and lineage progression of neural progenitors in the ventricular-subventricular zones of adult mice. Proc Natl Acad Sci U S A 2013, 110(11):E1045-1054.
199. Doetsch F, Alvarez-Buylla A: Network of tangential pathways for neuronal migration in adult mammalian brain. Proc Natl Acad Sci U S A 1996, 93(25):14895-14900.
200. Lois C, Alvarez-Buylla A: Long-distance neuronal migration in the adult mammalian brain. Science 1994, 264(5162):1145-1148.
201. Lois C, García-Verdugo JM, Alvarez-Buylla A: Chain migration of neuronal precursors. Science 1996, 271(5251):978-981.
202. Imayoshi I, Sakamoto M, Ohtsuka T, Takao K, Miyakawa T, Yamaguchi M, Mori K, Ikeda T, Itohara S, Kageyama R: Roles of continuous neurogenesis in the structural and functional integrity of the adult forebrain. Nat Neurosci 2008, 11(10):1153-1161.
203. Petreanu L, Alvarez-Buylla A: Maturation and death of adult-born olfactory bulb granule neurons: role of olfaction. J Neurosci 2002, 22(14):6106-6113.
204. Grelat A, Benoit L, Wagner S, Moigneu C, Lledo PM, Alonso M: Adult-born neurons boost odor-reward association. Proc Natl Acad Sci U S A 2018, 115(10):2514-2519.
205. Lledo PM, Saghatelyan A: Integrating new neurons into the adult olfactory bulb: joining the network, life-death decisions, and the effects of sensory experience. Trends Neurosci 2005, 28(5):248-254.
206. Alvarez-Buylla A, Theelen M, Nottebohm F: Proliferation "hot spots" in adult avian ventricular zone reveal radial cell division. Neuron 1990, 5(1):101-109.
207. Kriegstein A, Alvarez-Buylla A: The glial nature of embryonic and adult neural stem cells. Annu Rev Neurosci 2009, 32:149-184.
208. Ventura RE, Goldman JE: Dorsal radial glia generate olfactory bulb interneurons in the postnatal murine brain. J Neurosci 2007, 27(16):4297-4302.
209. Alonso M, Ortega-Pérez I, Grubb MS, Bourgeois JP, Charneau P, Lledo PM: Turning astrocytes from the rostral migratory stream into neurons: a role for the olfactory sensory organ. J Neurosci 2008, 28(43):11089-11102.
210. Enwere E, Shingo T, Gregg C, Fujikawa H, Ohta S, Weiss S: Aging results in reduced epidermal growth factor receptor signaling, diminished olfactory

neurogenesis, and deficits in fine olfactory discrimination. J Neurosci 2004, 24(38):8354-8365.
211. Capilla-Gonzalez V, Cebrian-Silla A, Guerrero-Cazares H, Garcia-Verdugo JM, Quiñones-Hinojosa A: Age-related changes in astrocytic and ependymal cells of the subventricular zone. Glia 2014, 62(5):790-803.
212. Sanai N, Nguyen T, Ihrie RA, Mirzadeh Z, Tsai HH, Wong M, Gupta N, Berger MS, Huang E, Garcia-Verdugo JM et al: Corridors of migrating neurons in the human brain and their decline during infancy. Nature 2011, 478(7369):382-386.
213. Paredes MF, James D, Gil-Perotin S, Kim H, Cotter JA, Ng C, Sandoval K, Rowitch DH, Xu D, McQuillen PS et al: Extensive migration of young neurons into the infant human frontal lobe. Science 2016, 354(6308).
214. Wang C, Liu F, Liu YY, Zhao CH, You Y, Wang L, Zhang J, Wei B, Ma T, Zhang Q et al: Identification and characterization of neuroblasts in the subventricular zone and rostral migratory stream of the adult human brain. Cell Res 2011, 21(11):1534-1550.
215. Spalding KL, Bergmann O, Alkass K, Bernard S, Salehpour M, Huttner HB, Boström E, Westerlund I, Vial C, Buchholz BA et al: Dynamics of hippocampal neurogenesis in adult humans. Cell 2013, 153(6):1219-1227.
216. Boldrini M, Fulmore CA, Tartt AN, Simeon LR, Pavlova I, Poposka V, Rosoklija GB, Stankov A, Arango V, Dwork AJ et al: Human Hippocampal Neurogenesis Persists throughout Aging. Cell Stem Cell 2018, 22(4):589-599.e585.
217. Knoth R, Singec I, Ditter M, Pantazis G, Capetian P, Meyer RP, Horvat V, Volk B, Kempermann G: Murine features of neurogenesis in the human hippocampus across the lifespan from 0 to 100 years. PLoS One 2010, 5(1):e8809.
218. Dennis CV, Suh LS, Rodriguez ML, Kril JJ, Sutherland GT: Human adult neurogenesis across the ages: An immunohistochemical study. Neuropathol Appl Neurobiol 2016, 42(7):621-638.
219. Lacar B, Young SZ, Platel JC, Bordey A: Gap junction-mediated calcium waves define communication networks among murine postnatal neural progenitor cells. Eur J Neurosci 2011, 34(12):1895-1905.
220. Hansen DV, Lui JH, Parker PR, Kriegstein AR: Neurogenic radial glia in the outer subventricular zone of human neocortex. Nature 2010, 464(7288):554-561.
221. Doetsch F, García-Verdugo JM, Alvarez-Buylla A: Cellular composition and three-dimensional organization of the subventricular germinal zone in the adult mammalian brain. J Neurosci 1997, 17(13):5046-5061.
222. Fogarty M, Richardson WD, Kessaris N: A subset of oligodendrocytes generated from radial glia in the dorsal spinal cord. Development 2005, 132(8):1951-1959.
223. Casper KB, McCarthy KD: GFAP-positive progenitor cells produce neurons and oligodendrocytes throughout the CNS. Mol Cell Neurosci 2006, 31(4):676-684.
224. Xing YL, Röth PT, Stratton JA, Chuang BH, Danne J, Ellis SL, Ng SW, Kilpatrick TJ, Merson TD: Adult neural precursor cells from the subventricular zone contribute significantly to oligodendrocyte regeneration and remyelination. J Neurosci 2014, 34(42):14128-14146.
225. Bond AM, Ming GL, Song H: Adult Mammalian Neural Stem Cells and Neurogenesis: Five Decades Later. Cell Stem Cell 2015, 17(4):385-395.
226. Calzolari F, Michel J, Baumgart EV, Theis F, Götz M, Ninkovic J: Fast clonal expansion and limited neural stem cell self-renewal in the adult subependymal zone. Nat Neurosci 2015, 18(4):490-492.
227. Ortega F, Gascón S, Masserdotti G, Deshpande A, Simon C, Fischer J, Dimou L, Chichung Lie D, Schroeder T, Berninger B: Oligodendrogliogenic and neurogenic adult subependymal zone neural stem cells constitute distinct lineages and exhibit differential responsiveness to Wnt signalling. Nat Cell Biol 2013, 15(6):602-613.
228. Hu XL, Chen G, Zhang S, Zheng J, Wu J, Bai QR, Wang Y, Li J, Wang H, Feng H et al: Persistent Expression of VCAM1 in Radial Glial Cells Is Required for the Embryonic Origin of Postnatal Neural Stem Cells. Neuron 2017, 95(2):309-325.e306.
229. Kokovay E, Wang Y, Kusek G, Wurster R, Lederman P, Lowry N, Shen Q, Temple S: VCAM1 is essential to maintain the structure of the SVZ niche and acts as an environmental sensor to regulate SVZ lineage progression. Cell Stem Cell 2012, 11(2):220-230.
230. Yuzwa SA, Borrett MJ, Innes BT, Voronova A, Ketela T, Kaplan DR, Bader GD, Miller FD: Developmental Emergence of Adult Neural Stem Cells as Revealed by Single-Cell Transcriptional Profiling. Cell Rep 2017, 21(13):3970-3986.
231. Furutachi S, Miya H, Watanabe T, Kawai H, Yamasaki N, Harada Y, Imayoshi I, Nelson M, Nakayama KI, Hirabayashi Y et al: Slowly dividing neural progenitors are an embryonic origin of adult neural stem cells. Nat Neurosci 2015, 18(5):657-665.
232. Merkle FT, Mirzadeh Z, Alvarez-Buylla A: Mosaic organization of neural stem cells in the adult brain. Science 2007, 317(5836):381-384.
233. Young KM, Fogarty M, Kessaris N, Richardson WD: Subventricular zone stem cells are heterogeneous with respect to their embryonic origins and neurogenic fates in the adult olfactory bulb. J Neurosci 2007, 27(31):8286-8296.
234. Alvarez-Buylla A, Kohwi M, Nguyen TM, Merkle FT: The heterogeneity of adult neural stem cells and the emerging complexity of their niche. Cold Spring Harb Symp Quant Biol 2008, 73:357-365.
235. Lim DA, Alvarez-Buylla A: Adult neural stem cells stake their ground. Trends Neurosci 2014, 37(10):563-571.
236. Obernier K, Alvarez-Buylla A: Neural stem cells: origin, heterogeneity and regulation in the adult mammalian brain. Development 2019, 146(4).
237. Alfonso J, Le Magueresse C, Zuccotti A, Khodosevich K, Monyer H: Diazepam binding inhibitor promotes progenitor proliferation in the postnatal SVZ by reducing GABA signaling. Cell Stem Cell 2012, 10(1):76-87.

238. Ashton RS, Conway A, Pangarkar C, Bergen J, Lim KI, Shah P, Bissell M, Schaffer DV: Astrocytes regulate adult hippocampal neurogenesis through ephrin-B signaling. Nat Neurosci 2012, 15(10):1399-1406.
239. Kawaguchi D, Furutachi S, Kawai H, Hozumi K, Gotoh Y: Dll1 maintains quiescence of adult neural stem cells and segregates asymmetrically during mitosis. Nat Commun 2013, 4:1880.
240. Liu X, Wang Q, Haydar TF, Bordey A: Nonsynaptic GABA signaling in postnatal subventricular zone controls proliferation of GFAP-expressing progenitors. Nat Neurosci 2005, 8(9):1179-1187.
241. Delgado AC, Ferrón SR, Vicente D, Porlan E, Perez-Villalba A, Trujillo CM, D'Ocón P, Fariñas I: Endothelial NT-3 delivered by vasculature and CSF promotes quiescence of subependymal neural stem cells through nitric oxide induction. Neuron 2014, 83(3):572-585.
242. Shen Q, Wang Y, Kokovay E, Lin G, Chuang SM, Goderie SK, Roysam B, Temple S: Adult SVZ stem cells lie in a vascular niche: a quantitative analysis of niche cell-cell interactions. Cell Stem Cell 2008, 3(3):289-300.
243. Kokovay E, Goderie S, Wang Y, Lotz S, Lin G, Sun Y, Roysam B, Shen Q, Temple S: Adult SVZ lineage cells home to and leave the vascular niche via differential responses to SDF1/CXCR4 signaling. Cell Stem Cell 2010, 7(2):163-173.
244. Ottone C, Krusche B, Whitby A, Clements M, Quadrato G, Pitulescu ME, Adams RH, Parrinello S: Direct cell-cell contact with the vascular niche maintains quiescent neural stem cells. Nat Cell Biol 2014, 16(11):1045-1056.
245. Gómez-Gaviro MV, Scott CE, Sesay AK, Matheu A, Booth S, Galichet C, Lovell-Badge R: Betacellulin promotes cell proliferation in the neural stem cell niche and stimulates neurogenesis. Proc Natl Acad Sci U S A 2012, 109(4):1317-1322.
246. Snapyan M, Lemasson M, Brill MS, Blais M, Massouh M, Ninkovic J, Gravel C, Berthod F, Götz M, Barker PA et al: Vasculature guides migrating neuronal precursors in the adult mammalian forebrain via brain-derived neurotrophic factor signaling. J Neurosci 2009, 29(13):4172-4188.
247. Bovetti S, Hsieh YC, Bovolin P, Perroteau I, Kazunori T, Puche AC: Blood vessels form a scaffold for neuroblast migration in the adult olfactory bulb. J Neurosci 2007, 27(22):5976-5980.
248. Tavazoie M, Van der Veken L, Silva-Vargas V, Louissaint M, Colonna L, Zaidi B, Garcia-Verdugo JM, Doetsch F: A specialized vascular niche for adult neural stem cells. Cell Stem Cell 2008, 3(3):279-288.
249. Ramírez-Castillejo C, Sánchez-Sánchez F, Andreu-Agulló C, Ferrón SR, Aroca-Aguilar JD, Sánchez P, Mira H, Escribano J, Fariñas I: Pigment epithelium-derived factor is a niche signal for neural stem cell renewal. Nat Neurosci 2006, 9(3):331-339.
250. Lim DA, Tramontin AD, Trevejo JM, Herrera DG, García-Verdugo JM, Alvarez-Buylla A: Noggin antagonizes BMP signaling to create a niche for adult neurogenesis. Neuron 2000, 28(3):713-726.
251. Gajera CR, Emich H, Lioubinski O, Christ A, Beckervordersandforth-Bonk R, Yoshikawa K, Bachmann S, Christensen EI, Götz M, Kempermann G et al: LRP2 in ependymal cells regulates BMP signaling in the adult neurogenic niche. J Cell Sci 2010, 123(Pt 11):1922-1930.
252. Mira H, Andreu Z, Suh H, Lie DC, Jessberger S, Consiglio A, San Emeterio J, Hortigüela R, Marqués-Torrejón MA, Nakashima K et al: Signaling through BMPR-IA regulates quiescence and long-term activity of neural stem cells in the adult hippocampus. Cell Stem Cell 2010, 7(1):78-89.
253. Zappaterra MW, Lehtinen MK: The cerebrospinal fluid: regulator of neurogenesis, behavior, and beyond. Cell Mol Life Sci 2012, 69(17):2863-2878.
254. Lehtinen MK, Zappaterra MW, Chen X, Yang YJ, Hill AD, Lun M, Maynard T, Gonzalez D, Kim S, Ye P et al: The cerebrospinal fluid provides a proliferative niche for neural progenitor cells. Neuron 2011, 69(5):893-905.
255. Seib DR, Corsini NS, Ellwanger K, Plaas C, Mateos A, Pitzer C, Niehrs C, Celikel T, Martin-Villalba A: Loss of Dickkopf-1 restores neurogenesis in old age and counteracts cognitive decline. Cell Stem Cell 2013, 12(2):204-214.
256. Ferent J, Cochard L, Faure H, Taddei M, Hahn H, Ruat M, Traiffort E: Genetic activation of Hedgehog signaling unbalances the rate of neural stem cell renewal by increasing symmetric divisions. Stem Cell Reports 2014, 3(2):312-323.
257. Tong CK, Han YG, Shah JK, Obernier K, Guinto CD, Alvarez-Buylla A: Primary cilia are required in a unique subpopulation of neural progenitors. Proc Natl Acad Sci U S A 2014, 111(34):12438-12443.
258. Silva-Vargas V, Maldonado-Soto AR, Mizrak D, Codega P, Doetsch F: Age-Dependent Niche Signals from the Choroid Plexus Regulate Adult Neural Stem Cells. Cell Stem Cell 2016, 19(5):643-652.
259. Sawamoto K, Wichterle H, Gonzalez-Perez O, Cholfin JA, Yamada M, Spassky N, Murcia NS, Garcia-Verdugo JM, Marin O, Rubenstein JL et al: New neurons follow the flow of cerebrospinal fluid in the adult brain. Science 2006, 311(5761):629-632.
260. Tong CK, Chen J, Cebrián-Silla A, Mirzadeh Z, Obernier K, Guinto CD, Tecott LH, García-Verdugo JM, Kriegstein A, Alvarez-Buylla A: Axonal control of the adult neural stem cell niche. Cell Stem Cell 2014, 14(4):500-511.
261. Brezun JM, Daszuta A: Depletion in serotonin decreases neurogenesis in the dentate gyrus and the subventricular zone of adult rats. Neuroscience 1999, 89(4):999-1002.
262. Banasr M, Hery M, Printemps R, Daszuta A: Serotonin-induced increases in adult cell proliferation and neurogenesis are mediated through different and common 5-HT receptor subtypes in the dentate gyrus and the subventricular zone. Neuropsychopharmacology 2004, 29(3):450-460.
263. Baker SA, Baker KA, Hagg T: Dopaminergic nigrostriatal projections regulate neural precursor proliferation in the adult mouse subventricular zone. Eur J Neurosci 2004, 20(2):575-579.

264. Lennington JB, Pope S, Goodheart AE, Drozdowicz L, Daniels SB, Salamone JD, Conover JC: Midbrain dopamine neurons associated with reward processing innervate the neurogenic subventricular zone. J Neurosci 2011, 31(37):13078-13087.
265. Young SZ, Taylor MM, Wu S, Ikeda-Matsuo Y, Kubera C, Bordey A: NKCC1 knockdown decreases neuron production through GABA(A)-regulated neural progenitor proliferation and delays dendrite development. J Neurosci 2012, 32(39):13630-13638.
266. Young SZ, Lafourcade CA, Platel JC, Lin TV, Bordey A: GABAergic striatal neurons project dendrites and axons into the postnatal subventricular zone leading to calcium activity. Front Cell Neurosci 2014, 8:10.
267. Song M, Yu SP, Mohamad O, Cao W, Wei ZZ, Gu X, Jiang MQ, Wei L: Optogenetic stimulation of glutamatergic neuronal activity in the striatum enhances neurogenesis in the subventricular zone of normal and stroke mice. Neurobiol Dis 2017, 98:9-24.
268. Kim Y, Wang WZ, Comte I, Pastrana E, Tran PB, Brown J, Miller RJ, Doetsch F, Molnár Z, Szele FG: Dopamine stimulation of postnatal murine subventricular zone neurogenesis via the D3 receptor. J Neurochem 2010, 114(3):750-760.
269. O'Keeffe GC, Tyers P, Aarsland D, Dalley JW, Barker RA, Caldwell MA: Dopamine-induced proliferation of adult neural precursor cells in the mammalian subventricular zone is mediated through EGF. Proc Natl Acad Sci U S A 2009, 106(21):8754-8759.
270. Kerever A, Schnack J, Vellinga D, Ichikawa N, Moon C, Arikawa-Hirasawa E, Efird JT, Mercier F: Novel extracellular matrix structures in the neural stem cell niche capture the neurogenic factor fibroblast growth factor 2 from the extracellular milieu. Stem Cells 2007, 25(9):2146-2157.
271. Porcheri C, Suter U, Jessberger S: Dissecting integrin-dependent regulation of neural stem cell proliferation in the adult brain. J Neurosci 2014, 34(15):5222-5232.
272. Douet V, Kerever A, Arikawa-Hirasawa E, Mercier F: Fractone-heparan sulphates mediate FGF-2 stimulation of cell proliferation in the adult subventricular zone. Cell Prolif 2013, 46(2):137-145.
273. Shigemoto-Mogami Y, Hoshikawa K, Goldman JE, Sekino Y, Sato K: Microglia enhance neurogenesis and oligodendrogenesis in the early postnatal subventricular zone. J Neurosci 2014, 34(6):2231-2243.
274. Tong CK, Fuentealba LC, Shah JK, Lindquist RA, Ihrie RA, Guinto CD, Rodas-Rodriguez JL, Alvarez-Buylla A: A Dorsal SHH-Dependent Domain in the V-SVZ Produces Large Numbers of Oligodendroglial Lineage Cells in the Postnatal Brain. Stem Cell Reports 2015, 5(4):461-470.
275. Batista-Brito R, Close J, Machold R, Fishell G: The distinct temporal origins of olfactory bulb interneuron subtypes. J Neurosci 2008, 28(15):3966-3975.
276. Mosher KI, Andres RH, Fukuhara T, Bieri G, Hasegawa-Moriyama M, He Y, Guzman R, Wyss-Coray T: Neural progenitor cells regulate microglia functions and activity. Nat Neurosci 2012, 15(11):1485-1487.
277. Ribeiro Xavier AL, Kress BT, Goldman SA, Lacerda de Menezes JR, Nedergaard M: A Distinct Population of Microglia Supports Adult Neurogenesis in the Subventricular Zone. J Neurosci 2015, 35(34):11848-11861.
278. Barkho BZ, Song H, Aimone JB, Smrt RD, Kuwabara T, Nakashima K, Gage FH, Zhao X: Identification of astrocyte-expressed factors that modulate neural stem/progenitor cell differentiation. Stem Cells Dev 2006, 15(3):407-421.
279. Llorens-Bobadilla E, Zhao S, Baser A, Saiz-Castro G, Zwadlo K, Martin-Villalba A: Single-Cell Transcriptomics Reveals a Population of Dormant Neural Stem Cells that Become Activated upon Brain Injury. Cell Stem Cell 2015, 17(3):329-340.
280. Dulken BW, Leeman DS, Boutet SC, Hebestreit K, Brunet A: Single-Cell Transcriptomic Analysis Defines Heterogeneity and Transcriptional Dynamics in the Adult Neural Stem Cell Lineage. Cell Rep 2017, 18(3):777-790.
281. Codega P, Silva-Vargas V, Paul A, Maldonado-Soto AR, Deleo AM, Pastrana E, Doetsch F: Prospective identification and purification of quiescent adult neural stem cells from their in vivo niche. Neuron 2014, 82(3):545-559.
282. Mich JK, Signer RA, Nakada D, Pineda A, Burgess RJ, Vue TY, Johnson JE, Morrison SJ: Prospective identification of functionally distinct stem cells and neurosphere-initiating cells in adult mouse forebrain. Elife 2014, 3:e02669.
283. Knobloch M, Braun SM, Zurkirchen L, von Schoultz C, Zamboni N, Araúzo-Bravo MJ, Kovacs WJ, Karalay O, Suter U, Machado RA et al: Metabolic control of adult neural stem cell activity by Fasn-dependent lipogenesis. Nature 2013, 493(7431):226-230.
284. Cebrián-Silla A, Alfaro-Cervelló C, Herranz-Pérez V, Kaneko N, Park DH, Sawamoto K, Alvarez-Buylla A, Lim DA, García-Verdugo JM: Unique Organization of the Nuclear Envelope in the Post-natal Quiescent Neural Stem Cells. Stem Cell Reports 2017, 9(1):203-216.
285. Fernando RN, Eleuteri B, Abdelhady S, Nussenzweig A, Andäng M, Ernfors P: Cell cycle restriction by histone H2AX limits proliferation of adult neural stem cells. Proc Natl Acad Sci U S A 2011, 108(14):5837-5842.
286. Bonaguidi MA, Wheeler MA, Shapiro JS, Stadel RP, Sun GJ, Ming GL, Song H: In vivo clonal analysis reveals self-renewing and multipotent adult neural stem cell characteristics. Cell 2011, 145(7):1142-1155.
287. Kouzarides T: Chromatin modifications and their function. Cell 2007, 128(4):693-705.
288. Gonzales-Roybal G, Lim DA: Chromatin-based epigenetics of adult subventricular zone neural stem cells. Front Genet 2013, 4:194.
289. Ninkovic J, Steiner-Mezzadri A, Jawerka M, Akinci U, Masserdotti G, Petricca S, Fischer J, von Holst A, Beckers J, Lie CD et al: The BAF complex interacts with Pax6 in adult neural progenitors to establish

a neurogenic cross-regulatory transcriptional network. Cell Stem Cell 2013, 13(4):403-418.
290. Davie JR, Hendzel MJ: Multiple functions of dynamic histone acetylation. J Cell Biochem 1994, 55(1):98-105.
291. Molofsky AV, Pardal R, Iwashita T, Park IK, Clarke MF, Morrison SJ: Bmi-1 dependence distinguishes neural stem cell self-renewal from progenitor proliferation. Nature 2003, 425(6961):962-967.
292. Zhang J, Ji F, Liu Y, Lei X, Li H, Ji G, Yuan Z, Jiao J: Ezh2 regulates adult hippocampal neurogenesis and memory. J Neurosci 2014, 34(15):5184-5199.
293. Bergsland M, Covacu R, Perez Estrada C, Svensson M, Brundin L: Nitric oxide-induced neuronal to glial lineage fate-change depends on NRSF/REST function in neural progenitor cells. Stem Cells 2014, 32(9):2539-2549.
294. Wu H, Coskun V, Tao J, Xie W, Ge W, Yoshikawa K, Li E, Zhang Y, Sun YE: Dnmt3a-dependent nonpromoter DNA methylation facilitates transcription of neurogenic genes. Science 2010, 329(5990):444-448.
295. Ma DK, Marchetto MC, Guo JU, Ming GL, Gage FH, Song H: Epigenetic choreographers of neurogenesis in the adult mammalian brain. Nat Neurosci 2010, 13(11):1338-1344.
296. Liu C, Teng ZQ, Santistevan NJ, Szulwach KE, Guo W, Jin P, Zhao X: Epigenetic regulation of miR-184 by MBD1 governs neural stem cell proliferation and differentiation. Cell Stem Cell 2010, 6(5):433-444.
297. Szulwach KE, Li X, Smrt RD, Li Y, Luo Y, Lin L, Santistevan NJ, Li W, Zhao X, Jin P: Cross talk between microRNA and epigenetic regulation in adult neurogenesis. J Cell Biol 2010, 189(1):127-141.
298. Li X, Jin P: Roles of small regulatory RNAs in determining neuronal identity. Nat Rev Neurosci 2010, 11(5):329-338.
299. Ramos AD, Diaz A, Nellore A, Delgado RN, Park KY, Gonzales-Roybal G, Oldham MC, Song JS, Lim DA: Integration of genome-wide approaches identifies lncRNAs of adult neural stem cells and their progeny in vivo. Cell Stem Cell 2013, 12(5):616-628.
300. Merkle FT, Fuentealba LC, Sanders TA, Magno L, Kessaris N, Alvarez-Buylla A: Adult neural stem cells in distinct microdomains generate previously unknown interneuron types. Nat Neurosci 2014, 17(2):207-214.
301. de Chevigny A, Coré N, Follert P, Gaudin M, Barbry P, Béclin C, Cremer H: miR-7a regulation of Pax6 controls spatial origin of forebrain dopaminergic neurons. Nat Neurosci 2012, 15(8):1120-1126.
302. Hsieh J: Orchestrating transcriptional control of adult neurogenesis. Genes Dev 2012, 26(10):1010-1021.
303. Kopan R, Ilagan MX: The canonical Notch signaling pathway: unfolding the activation mechanism. Cell 2009, 137(2):216-233.
304. Andersen J, Urbán N, Achimastou A, Ito A, Simic M, Ullom K, Martynoga B, Lebel M, Göritz C, Frisén J et al: A transcriptional mechanism integrating inputs from extracellular signals to activate hippocampal stem cells. Neuron 2014, 83(5):1085-1097.
305. Kohwi M, Osumi N, Rubenstein JL, Alvarez-Buylla A: Pax6 is required for making specific subpopulations of granule and periglomerular neurons in the olfactory bulb. J Neurosci 2005, 25(30):6997-7003.
306. Covacu R, Perez Estrada C, Arvidsson L, Svensson M, Brundin L: Change of fate commitment in adult neural progenitor cells subjected to chronic inflammation. J Neurosci 2014, 34(35):11571-11582.
307. Hack MA, Saghatelyan A, de Chevigny A, Pfeifer A, Ashery-Padan R, Lledo PM, Götz M: Neuronal fate determinants of adult olfactory bulb neurogenesis. Nat Neurosci 2005, 8(7):865-872.
308. Marshall CA, Novitch BG, Goldman JE: Olig2 directs astrocyte and oligodendrocyte formation in postnatal subventricular zone cells. J Neurosci 2005, 25(32):7289-7298.
309. Aguirre A, Rubio ME, Gallo V: Notch and EGFR pathway interaction regulates neural stem cell number and self-renewal. Nature 2010, 467(7313):323-327.
310. Imayoshi I, Sakamoto M, Yamaguchi M, Mori K, Kageyama R: Essential roles of Notch signaling in maintenance of neural stem cells in developing and adult brains. J Neurosci 2010, 30(9):3489-3498.
311. Breunig JJ, Silbereis J, Vaccarino FM, Sestan N, Rakic P: Notch regulates cell fate and dendrite morphology of newborn neurons in the postnatal dentate gyrus. Proc Natl Acad Sci U S A 2007, 104(51):20558-20563.
312. Kawai H, Kawaguchi D, Kuebrich BD, Kitamoto T, Yamaguchi M, Gotoh Y, Furutachi S: Area-Specific Regulation of Quiescent Neural Stem Cells by Notch3 in the Adult Mouse Subependymal Zone. J Neurosci 2017, 37(49):11867-11880.
313. Basak O, Giachino C, Fiorini E, Macdonald HR, Taylor V: Neurogenic subventricular zone stem/progenitor cells are Notch1-dependent in their active but not quiescent state. J Neurosci 2012, 32(16):5654-5666.
314. Ellis P, Fagan BM, Magness ST, Hutton S, Taranova O, Hayashi S, McMahon A, Rao M, Pevny L: SOX2, a persistent marker for multipotential neural stem cells derived from embryonic stem cells, the embryo or the adult. Dev Neurosci 2004, 26(2-4):148-165.
315. Shimozaki K, Clemenson GD, Gage FH: Paired related homeobox protein 1 is a regulator of stemness in adult neural stem/progenitor cells. J Neurosci 2013, 33(9):4066-4075.
316. Shimozaki K, Zhang CL, Suh H, Denli AM, Evans RM, Gage FH: SRY-box-containing gene 2 regulation of nuclear receptor tailless (Tlx) transcription in adult neural stem cells. J Biol Chem 2012, 287(8):5969-5978.
317. Haubensak W, Attardo A, Denk W, Huttner WB: Neurons arise in the basal neuroepithelium of the early mammalian telencephalon: a major site of neurogenesis. Proc Natl Acad Sci U S A 2004, 101(9):3196-3201.
318. Noctor SC, Martínez-Cerdeño V, Ivic L, Kriegstein AR: Cortical neurons arise in symmetric and

asymmetric division zones and migrate through specific phases. Nat Neurosci 2004, 7(2):136-144.
319. Basak O, Krieger TG, Muraro MJ, Wiebrands K, Stange DE, Frias-Aldeguer J, Rivron NC, van de Wetering M, van Es JH, van Oudenaarden A et al: Troy+ brain stem cells cycle through quiescence and regulate their number by sensing niche occupancy. Proc Natl Acad Sci U S A 2018, 115(4):E610-E619.
320. Weiss S, Dunne C, Hewson J, Wohl C, Wheatley M, Peterson AC, Reynolds BA: Multipotent CNS stem cells are present in the adult mammalian spinal cord and ventricular neuroaxis. J Neurosci 1996, 16(23):7599-7609.
321. Arvidsson L, Fagerlund M, Jaff N, Ossoinak A, Jansson K, Hägerstrand A, Johansson CB, Brundin L, Svensson M: Distribution and characterization of progenitor cells within the human filum terminale. PLoS One 2011, 6(11):e27393.
322. Ferrari D, Binda E, De Filippis L, Vescovi AL: Isolation of neural stem cells from neural tissues using the neurosphere technique. Curr Protoc Stem Cell Biol 2010, Chapter 2:Unit2D.6.
323. Azari H, Rahman M, Sharififar S, Reynolds BA: Isolation and expansion of the adult mouse neural stem cells using the neurosphere assay. J Vis Exp 2010(45).
324. Mothe A, Tator CH: Isolation of Neural Stem/Progenitor Cells from the Periventricular Region of the Adult Rat and Human Spinal Cord. J Vis Exp 2015(99):e52732.
325. Gritti A, Bonfanti L, Doetsch F, Caille I, Alvarez-Buylla A, Lim DA, Galli R, Verdugo JM, Herrera DG, Vescovi AL: Multipotent neural stem cells reside into the rostral extension and olfactory bulb of adult rodents. J Neurosci 2002, 22(2):437-445.
326. Zhang SC, Wernig M, Duncan ID, Brüstle O, Thomson JA: In vitro differentiation of transplantable neural precursors from human embryonic stem cells. Nat Biotechnol 2001, 19(12):1129-1133.
327. Meneghini V, Frati G, Sala D, De Cicco S, Luciani M, Cavazzin C, Paulis M, Mentzen W, Morena F, Giannelli S et al: Generation of Human Induced Pluripotent Stem Cell-Derived Bona Fide Neural Stem Cells for Ex Vivo Gene Therapy of Metachromatic Leukodystrophy. Stem Cells Transl Med 2017, 6(2):352-368.
328. Tang Y, Yu P, Cheng L: Current progress in the derivation and therapeutic application of neural stem cells. Cell Death Dis 2017, 8(10):e3108.
329. Friedenstein AJ, Chailakhjan RK, Lalykina KS: The development of fibroblast colonies in monolayer cultures of guinea-pig bone marrow and spleen cells. Cell Tissue Kinet 1970, 3(4):393-403.
330. Friedenstein AJ, Chailakhyan RK, Latsinik NV, Panasyuk AF, Keiliss-Borok IV: Stromal cells responsible for transferring the microenvironment of the hemopoietic tissues. Cloning in vitro and retransplantation in vivo. Transplantation 1974, 17(4):331-340.
331. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR: Multilineage potential of adult human mesenchymal stem cells. Science 1999, 284(5411):143-147.
332. Horwitz EM, Le Blanc K, Dominici M, Mueller I, Slaper-Cortenbach I, Marini FC, Deans RJ, Krause DS, Keating A, Therapy ISfC: Clarification of the nomenclature for MSC: The International Society for Cellular Therapy position statement. Cytotherapy 2005, 7(5):393-395.
333. Caplan AI: Mesenchymal stem cells. J Orthop Res 1991, 9(5):641-650.
334. Kuznetsov SA, Krebsbach PH, Satomura K, Kerr J, Riminucci M, Benayahu D, Robey PG: Single-colony derived strains of human marrow stromal fibroblasts form bone after transplantation in vivo. J Bone Miner Res 1997, 12(9):1335-1347.
335. da Silva Meirelles L, Chagastelles PC, Nardi NB: Mesenchymal stem cells reside in virtually all post-natal organs and tissues. J Cell Sci 2006, 119(Pt 11):2204-2213.
336. Méndez-Ferrer S, Michurina TV, Ferraro F, Mazloom AR, Macarthur BD, Lira SA, Scadden DT, Ma'ayan A, Enikolopov GN, Frenette PS: Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. Nature 2010, 466(7308):829-834.
337. Melief SM, Zwaginga JJ, Fibbe WE, Roelofs H: Adipose tissue-derived multipotent stromal cells have a higher immunomodulatory capacity than their bone marrow-derived counterparts. Stem Cells Transl Med 2013, 2(6):455-463.
338. Shi Y, Wang Y, Li Q, Liu K, Hou J, Shao C: Immunoregulatory mechanisms of mesenchymal stem and stromal cells in inflammatory diseases. Nat Rev Nephrol 2018, 14(8):493-507.
339. Sacchetti B, Funari A, Michienzi S, Di Cesare S, Piersanti S, Saggio I, Tagliafico E, Ferrari S, Robey PG, Riminucci M et al: Self-renewing osteoprogenitors in bone marrow sinusoids can organize a hematopoietic microenvironment. Cell 2007, 131(2):324-336.
340. Bertrand FE, Eckfeldt CE, Fink JR, Lysholm AS, Pribyl JA, Shah N, LeBien TW: Microenvironmental influences on human B-cell development. Immunol Rev 2000, 175:175-186.
341. Reyes M, Dudek A, Jahagirdar B, Koodie L, Marker PH, Verfaillie CM: Correction: Origin of endothelial progenitors in human postnatal bone marrow. J Clin Invest 2008, 118(11):3813.
342. Wilson A, Trumpp A: Bone-marrow haematopoietic-stem-cell niches. Nat Rev Immunol 2006, 6(2):93-106.
343. Kiel MJ, Morrison SJ: Uncertainty in the niches that maintain haematopoietic stem cells. Nat Rev Immunol 2008, 8(4):290-301.
344. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop D, Horwitz E: Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006, 8(4):315-317.
345. Uccelli A, Moretta L, Pistoia V: Mesenchymal stem cells in health and disease. Nat Rev Immunol 2008, 8(9):726-736.

346. Liu H, Lu K, MacAry PA, Wong KL, Heng A, Cao T, Kemeny DM: Soluble molecules are key in maintaining the immunomodulatory activity of murine mesenchymal stromal cells. J Cell Sci 2012, 125(Pt 1):200-208.
347. Le Blanc K, Tammik C, Rosendahl K, Zetterberg E, Ringdén O: HLA expression and immunologic properties of differentiated and undifferentiated mesenchymal stem cells. Exp Hematol 2003, 31(10):890-896.
348. Tse WT, Pendleton JD, Beyer WM, Egalka MC, Guinan EC: Suppression of allogeneic T-cell proliferation by human marrow stromal cells: implications in transplantation. Transplantation 2003, 75(3):389-397.
349. Chan WK, Lau AS, Li JC, Law HK, Lau YL, Chan GC: MHC expression kinetics and immunogenicity of mesenchymal stromal cells after short-term IFN-gamma challenge. Exp Hematol 2008, 36(11):1545-1555.
350. Potian JA, Aviv H, Ponzio NM, Harrison JS, Rameshwar P: Veto-like activity of mesenchymal stem cells: functional discrimination between cellular responses to alloantigens and recall antigens. J Immunol 2003, 171(7):3426-3434.
351. Yu S, Zhao Y, Ma Y, Ge L: Profiling the Secretome of Human Stem Cells from Dental Apical Papilla. Stem Cells Dev 2016, 25(6):499-508.
352. Johnson TV, DeKorver NW, Levasseur VA, Osborne A, Tassoni A, Lorber B, Heller JP, Villasmil R, Bull ND, Martin KR et al: Identification of retinal ganglion cell neuroprotection conferred by platelet-derived growth factor through analysis of the mesenchymal stem cell secretome. Brain 2014, 137(Pt 2):503-519.
353. Szekiova E, Slovinska L, Blasko J, Plsikova J, Cizkova D: The neuroprotective effect of rat adipose tissue-derived mesenchymal stem cell-conditioned medium on cortical neurons using an in vitro model of SCI inflammation. Neurol Res 2018, 40(4):258-267.
354. Baglio SR, Pegtel DM, Baldini N: Mesenchymal stem cell secreted vesicles provide novel opportunities in (stem) cell-free therapy. Front Physiol 2012, 3:359.
355. Li Y, Gao X, Wang J: Human adipose-derived mesenchymal stem cell-conditioned media suppresses inflammatory bone loss in a lipopolysaccharide-induced murine model. Exp Ther Med 2018, 15(2):1839-1846.
356. Akyurekli C, Le Y, Richardson RB, Fergusson D, Tay J, Allan DS: A systematic review of preclinical studies on the therapeutic potential of mesenchymal stromal cell-derived microvesicles. Stem Cell Rev 2015, 11(1):150-160.
357. Monsel A, Zhu YG, Gennai S, Hao Q, Hu S, Rouby JJ, Rosenzwajg M, Matthay MA, Lee JW: Therapeutic Effects of Human Mesenchymal Stem Cell-derived Microvesicles in Severe Pneumonia in Mice. Am J Respir Crit Care Med 2015, 192(3):324-336.
358. Kusuma GD, Carthew J, Lim R, Frith JE: Effect of the Microenvironment on Mesenchymal Stem Cell Paracrine Signaling: Opportunities to Engineer the Therapeutic Effect. Stem Cells Dev 2017, 26(9):617-631.
359. Ahn SY, Park WS, Kim YE, Sung DK, Sung SI, Ahn JY, Chang YS: Vascular endothelial growth factor mediates the therapeutic efficacy of mesenchymal stem cell-derived extracellular vesicles against neonatal hyperoxic lung injury. Exp Mol Med 2018, 50(4):26.
360. Anderson JD, Johansson HJ, Graham CS, Vesterlund M, Pham MT, Bramlett CS, Montgomery EN, Mellema MS, Bardini RL, Contreras Z et al: Comprehensive Proteomic Analysis of Mesenchymal Stem Cell Exosomes Reveals Modulation of Angiogenesis via Nuclear Factor-KappaB Signaling. Stem Cells 2016, 34(3):601-613.
361. Vallabhaneni KC, Penfornis P, Dhule S, Guillonneau F, Adams KV, Mo YY, Xu R, Liu Y, Watabe K, Vemuri MC et al: Extracellular vesicles from bone marrow mesenchymal stem/stromal cells transport tumor regulatory microRNA, proteins, and metabolites. Oncotarget 2015, 6(7):4953-4967.
362. Collino F, Deregibus MC, Bruno S, Sterpone L, Aghemo G, Viltono L, Tetta C, Camussi G: Microvesicles derived from adult human bone marrow and tissue specific mesenchymal stem cells shuttle selected pattern of miRNAs. PLoS One 2010, 5(7):e11803.
363. Baglio SR, Rooijers K, Koppers-Lalic D, Verweij FJ, Pérez Lanzón M, Zini N, Naaijkens B, Perut F, Niessen HW, Baldini N et al: Human bone marrow- and adipose-mesenchymal stem cells secrete exosomes enriched in distinctive miRNA and tRNA species. Stem Cell Res Ther 2015, 6:127.
364. Kalinina N, Kharlampieva D, Loguinova M, Butenko I, Pobeguts O, Efimenko A, Ageeva L, Sharonov G, Ischenko D, Alekseev D et al: Characterization of secretomes provides evidence for adipose-derived mesenchymal stromal cells subtypes. Stem Cell Res Ther 2015, 6:221.
365. Assoni A, Coatti G, Valadares MC, Beccari M, Gomes J, Pelatti M, Mitne-Neto M, Carvalho VM, Zatz M: Different Donors Mesenchymal Stromal Cells Secretomes Reveal Heterogeneous Profile of Relevance for Therapeutic Use. Stem Cells Dev 2017, 26(3):206-214.
366. Dabrowski FA, Burdzinska A, Kulesza A, Sladowska A, Zolocinska A, Gala K, Paczek L, Wielgos M: Comparison of the paracrine activity of mesenchymal stem cells derived from human umbilical cord, amniotic membrane and adipose tissue. J Obstet Gynaecol Res 2017, 43(11):1758-1768.
367. Park CW, Kim KS, Bae S, Son HK, Myung PK, Hong HJ, Kim H: Cytokine secretion profiling of human mesenchymal stem cells by antibody array. Int J Stem Cells 2009, 2(1):59-68.
368. Clark EA, Kalomoiris S, Nolta JA, Fierro FA: Concise review: MicroRNA function in multipotent mesenchymal stromal cells. Stem Cells 2014, 32(5):1074-1082.
369. Eirin A, Zhu XY, Puranik AS, Woollard JR, Tang H, Dasari S, Lerman A, van Wijnen AJ, Lerman LO: Integrated transcriptomic and proteomic analysis of the molecular cargo of extracellular vesicles derived from porcine adipose tissue-derived mesenchymal stem cells. PLoS One 2017, 12(3):e0174303.

370. Waszak P, Alphonse R, Vadivel A, Ionescu L, Eaton F, Thébaud B: Preconditioning enhances the paracrine effect of mesenchymal stem cells in preventing oxygen-induced neonatal lung injury in rats. Stem Cells Dev 2012, 21(15):2789-2797.
371. Parsha K, Mir O, Satani N, Yang B, Guerrero W, Mei Z, Cai C, Chen PR, Gee A, Hanley PJ et al: Mesenchymal stromal cell secretomes are modulated by suspension time, delivery vehicle, passage through catheter, and exposure to adjuvants. Cytotherapy 2017, 19(1):36-46.
372. Bruno S, Deregibus MC, Camussi G: The secretome of mesenchymal stromal cells: Role of extracellular vesicles in immunomodulation. Immunol Lett 2015, 168(2):154-158.
373. Crivelli B, Chlapanidas T, Perteghella S, Lucarelli E, Pascucci L, Brini AT, Ferrero I, Marazzi M, Pessina A, Torre ML et al: Mesenchymal stem/stromal cell extracellular vesicles: From active principle to next generation drug delivery system. J Control Release 2017, 262:104-117.
374. Willis GR, Fernandez-Gonzalez A, Anastas J, Vitali SH, Liu X, Ericsson M, Kwong A, Mitsialis SA, Kourembanas S: Mesenchymal Stromal Cell Exosomes Ameliorate Experimental Bronchopulmonary Dysplasia and Restore Lung Function through Macrophage Immunomodulation. Am J Respir Crit Care Med 2018, 197(1):104-116.
375. Otero-Ortega L, Gómez de Frutos MC, Laso-García F, Rodríguez-Frutos B, Medina-Gutiérrez E, López JA, Vázquez J, Díez-Tejedor E, Gutiérrez-Fernández M: Exosomes promote restoration after an experimental animal model of intracerebral hemorrhage. J Cereb Blood Flow Metab 2018, 38(5):767-779.
376. Marote A, Teixeira FG, Mendes-Pinheiro B, Salgado AJ: MSCs-Derived Exosomes: Cell-Secreted Nanovesicles with Regenerative Potential. Front Pharmacol 2016, 7:231.
377. Kourembanas S: Exosomes: vehicles of intercellular signaling, biomarkers, and vectors of cell therapy. Annu Rev Physiol 2015, 77:13-27.
378. Nguyen DC, Lewis HC, Joyner C, Warren V, Xiao H, Kissick HT, Wu R, Galipeau J, Lee FE: Extracellular vesicles from bone marrow-derived mesenchymal stromal cells support. J Extracell Vesicles 2018, 7(1):1463778.
379. Farinazzo A, Angiari S, Turano E, Bistaffa E, Dusi S, Ruggieri S, Bonafede R, Mariotti R, Constantin G, Bonetti B: Nanovesicles from adipose-derived mesenchymal stem cells inhibit T lymphocyte trafficking and ameliorate chronic experimental autoimmune encephalomyelitis. Sci Rep 2018, 8(1):7473.
380. Seo M, Kim JC, Kim HK, Choi EW, Jeong S, Nam KC, Jang M: A Novel Secretory Vesicle from Deer Antlerogenic Mesenchymal Stem Cell-Conditioned Media (DaMSC-CM) Promotes Tissue Regeneration. Stem Cells Int 2018, 2018:3891404.
381. Mayourian J, Ceholski DK, Gorski PA, Mathiyalagan P, Murphy JF, Salazar SI, Stillitano F, Hare JM, Sahoo S, Hajjar RJ et al: Exosomal microRNA-21-5p Mediates Mesenchymal Stem Cell Paracrine Effects on Human Cardiac Tissue Contractility. Circ Res 2018, 122(7):933-944.
382. Lankford KL, Arroyo EJ, Nazimek K, Bryniarski K, Askenase PW, Kocsis JD: Intravenously delivered mesenchymal stem cell-derived exosomes target M2-type macrophages in the injured spinal cord. PLoS One 2018, 13(1):e0190358.
383. Lo Sicco C, Reverberi D, Balbi C, Ulivi V, Principi E, Pascucci L, Becherini P, Bosco MC, Varesio L, Franzin C et al: Mesenchymal Stem Cell-Derived Extracellular Vesicles as Mediators of Anti-Inflammatory Effects: Endorsement of Macrophage Polarization. Stem Cells Transl Med 2017, 6(3):1018-1028.
384. Furuta T, Miyaki S, Ishitobi H, Ogura T, Kato Y, Kamei N, Miyado K, Higashi Y, Ochi M: Mesenchymal Stem Cell-Derived Exosomes Promote Fracture Healing in a Mouse Model. Stem Cells Transl Med 2016, 5(12):1620-1630.
385. Lopez-Verrilli MA, Caviedes A, Cabrera A, Sandoval S, Wyneken U, Khoury M: Mesenchymal stem cell-derived exosomes from different sources selectively promote neuritic outgrowth. Neuroscience 2016, 320:129-139.
386. Tooi M, Komaki M, Morioka C, Honda I, Iwasaki K, Yokoyama N, Ayame H, Izumi Y, Morita I: Placenta Mesenchymal Stem Cell Derived Exosomes Confer Plasticity on Fibroblasts. J Cell Biochem 2016, 117(7):1658-1670.
387. Tan CY, Lai RC, Wong W, Dan YY, Lim SK, Ho HK: Mesenchymal stem cell-derived exosomes promote hepatic regeneration in drug-induced liver injury models. Stem Cell Res Ther 2014, 5(3):76.
388. Chen TS, Lai RC, Lee MM, Choo AB, Lee CN, Lim SK: Mesenchymal stem cell secretes microparticles enriched in pre-microRNAs. Nucleic Acids Res 2010, 38(1):215-224.
389. Damania A, Jaiman D, Teotia AK, Kumar A: Mesenchymal stromal cell-derived exosome-rich fractionated secretome confers a hepatoprotective effect in liver injury. Stem Cell Res Ther 2018, 9(1):31.
390. Aghajani Nargesi A, Lerman LO, Eirin A: Mesenchymal stem cell-derived extracellular vesicles for kidney repair: current status and looming challenges. Stem Cell Res Ther 2017, 8(1):273.
391. Nargesi AA, Lerman LO, Eirin A: Mesenchymal Stem Cell-derived Extracellular Vesicles for Renal Repair. Curr Gene Ther 2017, 17(1):29-42.
392. Ragni E, Banfi F, Barilani M, Cherubini A, Parazzi V, Larghi P, Dolo V, Bollati V, Lazzari L: Extracellular Vesicle-Shuttled mRNA in Mesenchymal Stem Cell Communication. Stem Cells 2017, 35(4):1093-1105.
393. Toh WS, Lai RC, Hui JHP, Lim SK: MSC exosome as a cell-free MSC therapy for cartilage regeneration: Implications for osteoarthritis treatment. Semin Cell Dev Biol 2017, 67:56-64.
394. Luo Q, Zhang B, Kuang D, Song G: Role of Stromal-Derived Factor-1 in Mesenchymal Stem Cell Paracrine-Mediated Tissue Repair. Curr Stem Cell Res Ther 2016, 11(7):585-592.
395. Rani S, Ryan AE, Griffin MD, Ritter T: Mesenchymal Stem Cell-derived Extracellular Vesicles:

Toward Cell-free Therapeutic Applications. Mol Ther 2015, 23(5):812-823.
396. Fierabracci A, Del Fattore A, Luciano R, Muraca M, Teti A: Recent advances in mesenchymal stem cell immunomodulation: the role of microvesicles. Cell Transplant 2015, 24(2):133-149.
397. Katsuda T, Kosaka N, Takeshita F, Ochiya T: The therapeutic potential of mesenchymal stem cell-derived extracellular vesicles. Proteomics 2013, 13(10-11):1637-1653.
398. Biancone L, Bruno S, Deregibus MC, Tetta C, Camussi G: Therapeutic potential of mesenchymal stem cell-derived microvesicles. Nephrol Dial Transplant 2012, 27(8):3037-3042.
399. Ruppert KA, Nguyen TT, Prabhakara KS, Toledano Furman NE, Srivastava AK, Harting MT, Cox CS, Olson SD: Human Mesenchymal Stromal Cell-Derived Extracellular Vesicles Modify Microglial Response and Improve Clinical Outcomes in Experimental Spinal Cord Injury. Sci Rep 2018, 8(1):480.
400. Harting MT, Srivastava AK, Zhaorigetu S, Bair H, Prabhakara KS, Toledano Furman NE, Vykoukal JV, Ruppert KA, Cox CS, Olson SD: Inflammation-Stimulated Mesenchymal Stromal Cell-Derived Extracellular Vesicles Attenuate Inflammation. Stem Cells 2018, 36(1):79-90.
401. Dorronsoro A, Robbins PD: Regenerating the injured kidney with human umbilical cord mesenchymal stem cell-derived exosomes. Stem Cell Res Ther 2013, 4(2):39.
402. Arslan F, Lai RC, Smeets MB, Akeroyd L, Choo A, Aguor EN, Timmers L, van Rijen HV, Doevendans PA, Pasterkamp G et al: Mesenchymal stem cell-derived exosomes increase ATP levels, decrease oxidative stress and activate PI3K/Akt pathway to enhance myocardial viability and prevent adverse remodeling after myocardial ischemia/reperfusion injury. Stem Cell Res 2013, 10(3):301-312.
403. Roccaro AM, Sacco A, Maiso P, Azab AK, Tai YT, Reagan M, Azab F, Flores LM, Campigotto F, Weller E et al: BM mesenchymal stromal cell-derived exosomes facilitate multiple myeloma progression. J Clin Invest 2013, 123(4):1542-1555.
404. Kim HS, Choi DY, Yun SJ, Choi SM, Kang JW, Jung JW, Hwang D, Kim KP, Kim DW: Proteomic analysis of microvesicles derived from human mesenchymal stem cells. J Proteome Res 2012, 11(2):839-849.
405. Liang X, Ding Y, Zhang Y, Tse HF, Lian Q: Paracrine mechanisms of mesenchymal stem cell-based therapy: current status and perspectives. Cell Transplant 2014, 23(9):1045-1059.
406. Lee JY, Kim E, Choi SM, Kim DW, Kim KP, Lee I, Kim HS: Microvesicles from brain-extract-treated mesenchymal stem cells improve neurological functions in a rat model of ischemic stroke. Sci Rep 2016, 6:33038.
407. Eirin A, Riester SM, Zhu XY, Tang H, Evans JM, O'Brien D, van Wijnen AJ, Lerman LO: MicroRNA and mRNA cargo of extracellular vesicles from porcine adipose tissue-derived mesenchymal stem cells. Gene 2014, 551(1):55-64.
408. Wu S, Ju GQ, Du T, Zhu YJ, Liu GH: Microvesicles derived from human umbilical cord Wharton's jelly mesenchymal stem cells attenuate bladder tumor cell growth in vitro and in vivo. PLoS One 2013, 8(4):e61366.
409. Favaro E, Carpanetto A, Lamorte S, Fusco A, Caorsi C, Deregibus MC, Bruno S, Amoroso A, Giovarelli M, Porta M et al: Human mesenchymal stem cell-derived microvesicles modulate T cell response to islet antigen glutamic acid decarboxylase in patients with type 1 diabetes. Diabetologia 2014, 57(8):1664-1673.
410. Blazquez R, Sanchez-Margallo FM, de la Rosa O, Dalemans W, Alvarez V, Tarazona R, Casado JG: Immunomodulatory Potential of Human Adipose Mesenchymal Stem Cells Derived Exosomes on in vitro Stimulated T Cells. Front Immunol 2014, 5:556.
411. Fatima F, Ekstrom K, Nazarenko I, Maugeri M, Valadi H, Hill AF, Camussi G, Nawaz M: Non-coding RNAs in Mesenchymal Stem Cell-Derived Extracellular Vesicles: Deciphering Regulatory Roles in Stem Cell Potency, Inflammatory Resolve, and Tissue Regeneration. Front Genet 2017, 8:161.
412. Lee JK, Park SR, Jung BK, Jeon YK, Lee YS, Kim MK, Kim YG, Jang JY, Kim CW: Exosomes derived from mesenchymal stem cells suppress angiogenesis by down-regulating VEGF expression in breast cancer cells. PLoS One 2013, 8(12):e84256.
413. Eirin A, Zhu XY, Puranik AS, Woollard JR, Tang H, Dasari S, Lerman A, van Wijnen AJ, Lerman LO: Comparative proteomic analysis of extracellular vesicles isolated from porcine adipose tissue-derived mesenchymal stem/stromal cells. Sci Rep 2016, 6:36120.
414. Lindoso RS, Collino F, Bruno S, Araujo DS, Sant'Anna JF, Tetta C, Provero P, Quesenberry PJ, Vieyra A, Einicker-Lamas M et al: Extracellular vesicles released from mesenchymal stromal cells modulate miRNA in renal tubular cells and inhibit ATP depletion injury. Stem Cells Dev 2014, 23(15):1809-1819.
415. Wang Y, Chen X, Cao W, Shi Y: Plasticity of mesenchymal stem cells in immunomodulation: pathological and therapeutic implications. Nat Immunol 2014, 15(11):1009-1016.
416. Caplan AI, Dennis JE: Mesenchymal stem cells as trophic mediators. J Cell Biochem 2006, 98(5):1076-1084.
417. Ryan JM, Barry F, Murphy JM, Mahon BP: Interferon-gamma does not break, but promotes the immunosuppressive capacity of adult human mesenchymal stem cells. Clin Exp Immunol 2007, 149(2):353-363.
418. Krampera M, Cosmi L, Angeli R, Pasini A, Liotta F, Andreini A, Santarlasci V, Mazzinghi B, Pizzolo G, Vinante F et al: Role for interferon-gamma in the immunomodulatory activity of human bone marrow mesenchymal stem cells. Stem Cells 2006, 24(2):386-398.
419. Li Y, Chen J, Chen XG, Wang L, Gautam SC, Xu YX, Katakowski M, Zhang LJ, Lu M, Janakiraman N et al: Human marrow stromal cell therapy for stroke in rat: neurotrophins and functional recovery. Neurology 2002, 59(4):514-523.
420. Sordi V, Malosio ML, Marchesi F, Mercalli A, Melzi R, Giordano T, Belmonte N, Ferrari G, Leone BE, Bertuzzi F et al: Bone marrow mesenchymal

stem cells express a restricted set of functionally active chemokine receptors capable of promoting migration to pancreatic islets. Blood 2005, 106(2):419-427.
421. Honczarenko M, Le Y, Swierkowski M, Ghiran I, Glodek AM, Silberstein LE: Human bone marrow stromal cells express a distinct set of biologically functional chemokine receptors. Stem Cells 2006, 24(4):1030-1041.
422. Ji JF, He BP, Dheen ST, Tay SS: Interactions of chemokines and chemokine receptors mediate the migration of mesenchymal stem cells to the impaired site in the brain after hypoglossal nerve injury. Stem Cells 2004, 22(3):415-427.
423. Rüster B, Göttig S, Ludwig RJ, Bistrian R, Müller S, Seifried E, Gille J, Henschler R: Mesenchymal stem cells display coordinated rolling and adhesion behavior on endothelial cells. Blood 2006, 108(12):3938-3944.
424. Chamberlain G, Fox J, Ashton B, Middleton J: Concise review: mesenchymal stem cells: their phenotype, differentiation capacity, immunological features, and potential for homing. Stem Cells 2007, 25(11):2739-2749.
425. Ries C, Egea V, Karow M, Kolb H, Jochum M, Neth P: MMP-2, MT1-MMP, and TIMP-2 are essential for the invasive capacity of human mesenchymal stem cells: differential regulation by inflammatory cytokines. Blood 2007, 109(9):4055-4063.
426. Cummings BJ, Uchida N, Tamaki SJ, Anderson AJ: Human neural stem cell differentiation following transplantation into spinal cord injured mice: association with recovery of locomotor function. Neurol Res 2006, 28(5):474-481.
427. Salazar DL, Uchida N, Hamers FP, Cummings BJ, Anderson AJ: Human neural stem cells differentiate and promote locomotor recovery in an early chronic spinal cord injury NOD-scid mouse model. PLoS One 2010, 5(8):e12272.
428. Akesson E, Piao JH, Samuelsson EB, Holmberg L, Kjaeldgaard A, Falci S, Sundström E, Seiger A: Long-term culture and neuronal survival after intraspinal transplantation of human spinal cord-derived neurospheres. Physiol Behav 2007, 92(1-2):60-66.
429. Boido M, Garbossa D, Vercelli A: Early graft of neural precursors in spinal cord compression reduces glial cyst and improves function. J Neurosurg Spine 2011, 15(1):97-106.
430. Pfeifer K, Vroemen M, Caioni M, Aigner L, Bogdahn U, Weidner N: Autologous adult rodent neural progenitor cell transplantation represents a feasible strategy to promote structural repair in the chronically injured spinal cord. Regen Med 2006, 1(2):255-266.
431. Pfeifer K, Vroemen M, Blesch A, Weidner N: Adult neural progenitor cells provide a permissive guiding substrate for corticospinal axon growth following spinal cord injury. Eur J Neurosci 2004, 20(7):1695-1704.
432. Pallini R, Vitiani LR, Bez A, Casalbore P, Facchiano F, Di Giorgi Gerevini V, Falchetti ML, Fernandez E, Maira G, Peschle C et al: Homologous transplantation of neural stem cells to the injured spinal cord of mice. Neurosurgery 2005, 57(5):1014-1025; discussion 1014-1025.
433. Okada S, Ishii K, Yamane J, Iwanami A, Ikegami T, Katoh H, Iwamoto Y, Nakamura M, Miyoshi H, Okano HJ et al: In vivo imaging of engrafted neural stem cells: its application in evaluating the optimal timing of transplantation for spinal cord injury. FASEB J 2005, 19(13):1839-1841.
434. Macias MY, Syring MB, Pizzi MA, Crowe MJ, Alexanian AR, Kurpad SN: Pain with no gain: allodynia following neural stem cell transplantation in spinal cord injury. Exp Neurol 2006, 201(2):335-348.
435. Sandhu MS, Ross HH, Lee KZ, Ormerod BK, Reier PJ, Fuller DD: Intraspinal transplantation of subventricular zone-derived neural progenitor cells improves phrenic motor output after high cervical spinal cord injury. Exp Neurol 2017, 287(Pt 2):205-215.
436. Vroemen M, Aigner L, Winkler J, Weidner N: Adult neural progenitor cell grafts survive after acute spinal cord injury and integrate along axonal pathways. Eur J Neurosci 2003, 18(4):743-751.
437. !!! INVALID CITATION !!!
438. Karimi-Abdolrezaee S, Eftekharpour E, Wang J, Morshead CM, Fehlings MG: Delayed transplantation of adult neural precursor cells promotes remyelination and functional neurological recovery after spinal cord injury. J Neurosci 2006, 26(13):3377-3389.
439. Piltti KM, Salazar DL, Uchida N, Cummings BJ, Anderson AJ: Safety of human neural stem cell transplantation in chronic spinal cord injury. Stem Cells Transl Med 2013, 2(12):961-974.
440. Parr AM, Kulbatski I, Tator CH: Transplantation of adult rat spinal cord stem/progenitor cells for spinal cord injury. J Neurotrauma 2007, 24(5):835-845.
441. Piltti KM, Salazar DL, Uchida N, Cummings BJ, Anderson AJ: Safety of epicenter versus intact parenchyma as a transplantation site for human neural stem cells for spinal cord injury therapy. Stem Cells Transl Med 2013, 2(3):204-216.
442. Brock JH, Graham L, Staufenberg E, Im S, Tuszynski MH: Rodent Neural Progenitor Cells Support Functional Recovery After Cervical Spinal Cord Contusion. J Neurotrauma 2017.
443. Hong JY, Lee SH, Lee SC, Kim JW, Kim KP, Kim SM, Tapia N, Lim KT, Kim J, Ahn HS et al: Therapeutic potential of induced neural stem cells for spinal cord injury. J Biol Chem 2014, 289(47):32512-32525.
444. Mligiliche NL, Xu Y, Matsumoto N, Idel C: Survival of neural progenitor cells from the subventricular zone of the adult rat after transplantation into the host spinal cord of the same strain of adult rat. Anat Sci Int 2005, 80(4):229-234.
445. Ricci-Vitiani L, Casalbore P, Petrucci G, Lauretti L, Montano N, Larocca LM, Falchetti ML, Lombardi DG, Gerevini VD, Cenciarelli C et al: Influence of local environment on the differentiation of neural stem cells engrafted onto the injured spinal cord. Neurol Res 2006, 28(5):488-492.

446. Salewski RP, Mitchell RA, Shen C, Fehlings MG: Transplantation of neural stem cells clonally derived from embryonic stem cells promotes recovery after murine spinal cord injury. Stem Cells Dev 2015, 24(1):36-50.
447. Kumamaru H, Saiwai H, Kubota K, Kobayakawa K, Yokota K, Ohkawa Y, Shiba K, Iwamoto Y, Okada S: Therapeutic activities of engrafted neural stem/precursor cells are not dormant in the chronically injured spinal cord. Stem Cells 2013, 31(8):1535-1547.
448. Yan J, Xu L, Welsh AM, Hatfield G, Hazel T, Johe K, Koliatsos VE: Extensive neuronal differentiation of human neural stem cell grafts in adult rat spinal cord. PLoS Med 2007, 4(2):e39.
449. Cummings BJ, Uchida N, Tamaki SJ, Salazar DL, Hooshmand M, Summers R, Gage FH, Anderson AJ: Human neural stem cells differentiate and promote locomotor recovery in spinal cord-injured mice. Proc Natl Acad Sci U S A 2005, 102(39):14069-14074.
450. Bonner JF, Connors TM, Silverman WF, Kowalski DP, Lemay MA, Fischer I: Grafted neural progenitors integrate and restore synaptic connectivity across the injured spinal cord. J Neurosci 2011, 31(12):4675-4686.
451. Lu P, Wang Y, Graham L, McHale K, Gao M, Wu D, Brock J, Blesch A, Rosenzweig ES, Havton LA et al: Long-distance growth and connectivity of neural stem cells after severe spinal cord injury. Cell 2012, 150(6):1264-1273.
452. Ben-Hur T: Reconstructing neural circuits using transplanted neural stem cells in the injured spinal cord. J Clin Invest 2010, 120(9):3096-3098.
453. Gu YL, Yin LW, Zhang Z, Liu J, Liu SJ, Zhang LF, Wang TH: Neurotrophin expression in neural stem cells grafted acutely to transected spinal cord of adult rats linked to functional improvement. Cell Mol Neurobiol 2012, 32(7):1089-1097.
454. Lu P, Jones LL, Snyder EY, Tuszynski MH: Neural stem cells constitutively secrete neurotrophic factors and promote extensive host axonal growth after spinal cord injury. Exp Neurol 2003, 181(2):115-129.
455. Nishimura S, Yasuda A, Iwai H, Takano M, Kobayashi Y, Nori S, Tsuji O, Fujiyoshi K, Ebise H, Toyama Y et al: Time-dependent changes in the microenvironment of injured spinal cord affects the therapeutic potential of neural stem cell transplantation for spinal cord injury. Mol Brain 2013, 6:3.
456. Cheng Z, Zhu W, Cao K, Wu F, Li J, Wang G, Li H, Lu M, Ren Y, He X: Anti-Inflammatory Mechanism of Neural Stem Cell Transplantation in Spinal Cord Injury. Int J Mol Sci 2016, 17(9).
457. Cusimano M, Biziato D, Brambilla E, Donegà M, Alfaro-Cervello C, Snider S, Salani G, Pucci F, Comi G, Garcia-Verdugo JM et al: Transplanted neural stem/precursor cells instruct phagocytes and reduce secondary tissue damage in the injured spinal cord. Brain 2012, 135(Pt 2):447-460.
458. Li Z, Guo GH, Wang GS, Guan CX, Yue L: Influence of neural stem cell transplantation on angiogenesis in rats with spinal cord injury. Genet Mol Res 2014, 13(3):6083-6092.
459. Amemori T, Romanyuk N, Jendelova P, Herynek V, Turnovcova K, Prochazka P, Kapcalova M, Cocks G, Price J, Sykova E: Human conditionally immortalized neural stem cells improve locomotor function after spinal cord injury in the rat. Stem Cell Res Ther 2013, 4(3):68.
460. Hofstetter CP, Holmström NA, Lilja JA, Schweinhardt P, Hao J, Spenger C, Wiesenfeld-Hallin Z, Kurpad SN, Frisén J, Olson L: Allodynia limits the usefulness of intraspinal neural stem cell grafts; directed differentiation improves outcome. Nat Neurosci 2005, 8(3):346-353.
461. Jin Y, Bouyer J, Shumsky JS, Haas C, Fischer I: Transplantation of neural progenitor cells in chronic spinal cord injury. Neuroscience 2016, 320:69-82.
462. Yokota K, Kobayakawa K, Kubota K, Miyawaki A, Okano H, Ohkawa Y, Iwamoto Y, Okada S: Engrafted Neural Stem/Progenitor Cells Promote Functional Recovery through Synapse Reorganization with Spared Host Neurons after Spinal Cord Injury. Stem Cell Reports 2015, 5(2):264-277.
463. Wang D, Zhang J: Effects of hypothermia combined with neural stem cell transplantation on recovery of neurological function in rats with spinal cord injury. Mol Med Rep 2015, 11(3):1759-1767.
464. Tashiro S, Nishimura S, Iwai H, Sugai K, Zhang L, Shinozaki M, Iwanami A, Toyama Y, Liu M, Okano H et al: Functional Recovery from Neural Stem/Progenitor Cell Transplantation Combined with Treadmill Training in Mice with Chronic Spinal Cord Injury. Sci Rep 2016, 6:30898.
465. Hwang DH, Shin HY, Kwon MJ, Choi JY, Ryu BY, Kim BG: Survival of neural stem cell grafts in the lesioned spinal cord is enhanced by a combination of treadmill locomotor training via insulin-like growth factor-1 signaling. J Neurosci 2014, 34(38):12788-12800.
466. Abematsu M, Tsujimura K, Yamano M, Saito M, Kohno K, Kohyama J, Namihira M, Komiya S, Nakashima K: Neurons derived from transplanted neural stem cells restore disrupted neuronal circuitry in a mouse model of spinal cord injury. J Clin Invest 2010, 120(9):3255-3266.
467. Su H, Chu TH, Wu W: Lithium enhances proliferation and neuronal differentiation of neural progenitor cells in vitro and after transplantation into the adult rat spinal cord. Exp Neurol 2007, 206(2):296-307.
468. Yuan N, Tian W, Sun L, Yuan R, Tao J, Chen D: Neural stem cell transplantation in a double-layer collagen membrane with unequal pore sizes for spinal cord injury repair. Neural Regen Res 2014, 9(10):1014-1019.
469. Lu P, Graham L, Wang Y, Wu D, Tuszynski M: Promotion of survival and differentiation of neural stem cells with fibrin and growth factor cocktails after severe spinal cord injury. J Vis Exp 2014(89):e50641.
470. Tipnis S, Viswanathan C, Majumdar AS: Immunosuppressive properties of human umbilical cord-derived mesenchymal stem cells: role of B7-H1 and IDO. Immunol Cell Biol 2010, 88(8):795-806.

471. Jiang XX, Zhang Y, Liu B, Zhang SX, Wu Y, Yu XD, Mao N: Human mesenchymal stem cells inhibit differentiation and function of monocyte-derived dendritic cells. Blood 2005, 105(10):4120-4126.
472. Spaggiari GM, Abdelrazik H, Becchetti F, Moretta L: MSCs inhibit monocyte-derived DC maturation and function by selectively interfering with the generation of immature DCs: central role of MSC-derived prostaglandin E2. Blood 2009, 113(26):6576-6583.
473. Nauta AJ, Kruisselbrink AB, Lurvink E, Willemze R, Fibbe WE: Mesenchymal stem cells inhibit generation and function of both CD34+-derived and monocyte-derived dendritic cells. J Immunol 2006, 177(4):2080-2087.
474. Ramasamy R, Fazekasova H, Lam EW, Soeiro I, Lombardi G, Dazzi F: Mesenchymal stem cells inhibit dendritic cell differentiation and function by preventing entry into the cell cycle. Transplantation 2007, 83(1):71-76.
475. Ivanova-Todorova E, Bochev I, Mourdjeva M, Dimitrov R, Bukarev D, Kyurkchiev S, Tivchev P, Altunkova I, Kyurkchiev DS: Adipose tissue-derived mesenchymal stem cells are more potent suppressors of dendritic cells differentiation compared to bone marrow-derived mesenchymal stem cells. Immunol Lett 2009, 126(1-2):37-42.
476. Chiesa S, Morbelli S, Morando S, Massollo M, Marini C, Bertoni A, Frassoni F, Bartolomé ST, Sambuceti G, Traggiai E et al: Mesenchymal stem cells impair in vivo T-cell priming by dendritic cells. Proc Natl Acad Sci U S A 2011, 108(42):17384-17389.
477. Zhang B, Liu R, Shi D, Liu X, Chen Y, Dou X, Zhu X, Lu C, Liang W, Liao L et al: Mesenchymal stem cells induce mature dendritic cells into a novel Jagged-2-dependent regulatory dendritic cell population. Blood 2009, 113(1):46-57.
478. Spaggiari GM, Capobianco A, Becchetti S, Mingari MC, Moretta L: Mesenchymal stem cell-natural killer cell interactions: evidence that activated NK cells are capable of killing MSCs, whereas MSCs can inhibit IL-2-induced NK-cell proliferation. Blood 2006, 107(4):1484-1490.
479. Poggi A, Prevosto C, Massaro AM, Negrini S, Urbani S, Pierri I, Saccardi R, Gobbi M, Zocchi MR: Interaction between human NK cells and bone marrow stromal cells induces NK cell triggering: role of NKp30 and NKG2D receptors. J Immunol 2005, 175(10):6352-6360.
480. Giuliani M, Bennaceur-Griscelli A, Nanbakhsh A, Oudrhiri N, Chouaib S, Azzarone B, Durrbach A, Lataillade JJ: TLR ligands stimulation protects MSC from NK killing. Stem Cells 2014, 32(1):290-300.
481. Spaggiari GM, Capobianco A, Abdelrazik H, Becchetti F, Mingari MC, Moretta L: Mesenchymal stem cells inhibit natural killer-cell proliferation, cytotoxicity, and cytokine production: role of indoleamine 2,3-dioxygenase and prostaglandin E2. Blood 2008, 111(3):1327-1333.
482. Sotiropoulou PA, Perez SA, Gritzapis AD, Baxevanis CN, Papamichail M: Interactions between human mesenchymal stem cells and natural killer cells. Stem Cells 2006, 24(1):74-85.
483. Jones S, Horwood N, Cope A, Dazzi F: The antiproliferative effect of mesenchymal stem cells is a fundamental property shared by all stromal cells. J Immunol 2007, 179(5):2824-2831.
484. Di Nicola M, Carlo-Stella C, Magni M, Milanesi M, Longoni PD, Matteucci P, Grisanti S, Gianni AM: Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. Blood 2002, 99(10):3838-3843.
485. Glennie S, Soeiro I, Dyson PJ, Lam EW, Dazzi F: Bone marrow mesenchymal stem cells induce division arrest anergy of activated T cells. Blood 2005, 105(7):2821-2827.
486. Najar M, Rouas R, Raicevic G, Boufker HI, Lewalle P, Meuleman N, Bron D, Toungouz M, Martiat P, Lagneaux L: Mesenchymal stromal cells promote or suppress the proliferation of T lymphocytes from cord blood and peripheral blood: the importance of low cell ratio and role of interleukin-6. Cytotherapy 2009, 11(5):570-583.
487. Dorronsoro A, Ferrin I, Salcedo JM, Jakobsson E, Fernández-Rueda J, Lang V, Sepulveda P, Fechter K, Pennington D, Trigueros C: Human mesenchymal stromal cells modulate T-cell responses through TNF-α-mediated activation of NF-κB. Eur J Immunol 2014, 44(2):480-488.
488. François M, Romieu-Mourez R, Li M, Galipeau J: Human MSC suppression correlates with cytokine induction of indoleamine 2,3-dioxygenase and bystander M2 macrophage differentiation. Mol Ther 2012, 20(1):187-195.
489. Chabannes D, Hill M, Merieau E, Rossignol J, Brion R, Soulillou JP, Anegon I, Cuturi MC: A role for heme oxygenase-1 in the immunosuppressive effect of adult rat and human mesenchymal stem cells. Blood 2007, 110(10):3691-3694.
490. Plumas J, Chaperot L, Richard MJ, Molens JP, Bensa JC, Favrot MC: Mesenchymal stem cells induce apoptosis of activated T cells. Leukemia 2005, 19(9):1597-1604.
491. Benvenuto F, Ferrari S, Gerdoni E, Gualandi F, Frassoni F, Pistoia V, Mancardi G, Uccelli A: Human mesenchymal stem cells promote survival of T cells in a quiescent state. Stem Cells 2007, 25(7):1753-1760.
492. Ungerer C, Quade-Lyssy P, Radeke HH, Henschler R, Königs C, Köhl U, Seifried E, Schüttrumpf J: Galectin-9 is a suppressor of T and B cells and predicts the immune modulatory potential of mesenchymal stromal cell preparations. Stem Cells Dev 2014, 23(7):755-766.
493. Gieseke F, Kruchen A, Tzaribachev N, Bentzien F, Dominici M, Müller I: Proinflammatory stimuli induce galectin-9 in human mesenchymal stromal cells to suppress T-cell proliferation. Eur J Immunol 2013, 43(10):2741-2749.
494. Meisel R, Zibert A, Laryea M, Göbel U, Däubener W, Dilloo D: Human bone marrow stromal cells inhibit allogeneic T-cell responses by indoleamine 2,3-dioxygenase-mediated tryptophan degradation. Blood 2004, 103(12):4619-4621.

495. Djouad F, Charbonnier LM, Bouffi C, Louis-Plence P, Bony C, Apparailly F, Cantos C, Jorgensen C, Noël D: Mesenchymal stem cells inhibit the differentiation of dendritic cells through an interleukin-6-dependent mechanism. Stem Cells 2007, 25(8):2025-2032.
496. Beyth S, Borovsky Z, Mevorach D, Liebergall M, Gazit Z, Aslan H, Galun E, Rachmilewitz J: Human mesenchymal stem cells alter antigen-presenting cell maturation and induce T-cell unresponsiveness. Blood 2005, 105(5):2214-2219.
497. Groh ME, Maitra B, Szekely E, Koç ON: Human mesenchymal stem cells require monocyte-mediated activation to suppress alloreactive T cells. Exp Hematol 2005, 33(8):928-934.
498. English K, Ryan JM, Tobin L, Murphy MJ, Barry FP, Mahon BP: Cell contact, prostaglandin E(2) and transforming growth factor beta 1 play non-redundant roles in human mesenchymal stem cell induction of CD4+CD25(High) forkhead box P3+ regulatory T cells. Clin Exp Immunol 2009, 156(1):149-160.
499. Crop MJ, Baan CC, Korevaar SS, Ijzermans JN, Weimar W, Hoogduijn MJ: Human adipose tissue-derived mesenchymal stem cells induce explosive T-cell proliferation. Stem Cells Dev 2010, 19(12):1843-1853.
500. Melief SM, Schrama E, Brugman MH, Tiemessen MM, Hoogduijn MJ, Fibbe WE, Roelofs H: Multipotent stromal cells induce human regulatory T cells through a novel pathway involving skewing of monocytes toward anti-inflammatory macrophages. Stem Cells 2013, 31(9):1980-1991.
501. Ren G, Zhang L, Zhao X, Xu G, Zhang Y, Roberts AI, Zhao RC, Shi Y: Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. Cell Stem Cell 2008, 2(2):141-150.
502. Sato K, Ozaki K, Oh I, Meguro A, Hatanaka K, Nagai T, Muroi K, Ozawa K: Nitric oxide plays a critical role in suppression of T-cell proliferation by mesenchymal stem cells. Blood 2007, 109(1):228-234.
503. Bingisser RM, Tilbrook PA, Holt PG, Kees UR: Macrophage-derived nitric oxide regulates T cell activation via reversible disruption of the Jak3/STAT5 signaling pathway. J Immunol 1998, 160(12):5729-5734.
504. Xu C, Yu P, Han X, Du L, Gan J, Wang Y, Shi Y: TGF-β promotes immune responses in the presence of mesenchymal stem cells. J Immunol 2014, 192(1):103-109.
505. Ding Y, Xu D, Feng G, Bushell A, Muschel RJ, Wood KJ: Mesenchymal stem cells prevent the rejection of fully allogenic islet grafts by the immunosuppressive activity of matrix metalloproteinase-2 and -9. Diabetes 2009, 58(8):1797-1806.
506. Han KH, Ro H, Hong JH, Lee EM, Cho B, Yeom HJ, Kim MG, Oh KH, Ahn C, Yang J: Immunosuppressive mechanisms of embryonic stem cells and mesenchymal stem cells in alloimmune response. Transpl Immunol 2011, 25(1):7-15.
507. Augello A, Tasso R, Negrini SM, Amateis A, Indiveri F, Cancedda R, Pennesi G: Bone marrow mesenchymal progenitor cells inhibit lymphocyte proliferation by activation of the programmed death 1 pathway. Eur J Immunol 2005, 35(5):1482-1490.
508. Ferrari D, Gulinelli S, Salvestrini V, Lucchetti G, Zini R, Manfredini R, Caione L, Piacibello W, Ciciarello M, Rossi L et al: Purinergic stimulation of human mesenchymal stem cells potentiates their chemotactic response to CXCL12 and increases the homing capacity and production of proinflammatory cytokines. Exp Hematol 2011, 39(3):360-374, 374.e361-365.
509. Akiyama K, Chen C, Wang D, Xu X, Qu C, Yamaza T, Cai T, Chen W, Sun L, Shi S: Mesenchymal-stem-cell-induced immunoregulation involves FAS-ligand-/FAS-mediated T cell apoptosis. Cell Stem Cell 2012, 10(5):544-555.
510. Rosado MM, Bernardo ME, Scarsella M, Conforti A, Giorda E, Biagini S, Cascioli S, Rossi F, Guzzo I, Vivarelli M et al: Inhibition of B-cell proliferation and antibody production by mesenchymal stromal cells is mediated by T cells. Stem Cells Dev 2015, 24(1):93-103.
511. Corcione A, Benvenuto F, Ferretti E, Giunti D, Cappiello V, Cazzanti F, Risso M, Gualandi F, Mancardi GL, Pistoia V et al: Human mesenchymal stem cells modulate B-cell functions. Blood 2006, 107(1):367-372.
512. Schena F, Gambini C, Gregorio A, Mosconi M, Reverberi D, Gattorno M, Casazza S, Uccelli A, Moretta L, Martini A et al: Interferon-γ -dependent inhibition of B cell activation by bone marrow-derived mesenchymal stem cells in a murine model of systemic lupus erythematosus. Arthritis Rheum 2010, 62(9):2776-2786.
513. Asari S, Itakura S, Ferreri K, Liu CP, Kuroda Y, Kandeel F, Mullen Y: Mesenchymal stem cells suppress B-cell terminal differentiation. Exp Hematol 2009, 37(5):604-615.
514. Rasmusson I, Le Blanc K, Sundberg B, Ringdén O: Mesenchymal stem cells stimulate antibody secretion in human B cells. Scand J Immunol 2007, 65(4):336-343.
515. Traggiai E, Volpi S, Schena F, Gattorno M, Ferlito F, Moretta L, Martini A: Bone marrow-derived mesenchymal stem cells induce both polyclonal expansion and differentiation of B cells isolated from healthy donors and systemic lupus erythematosus patients. Stem Cells 2008, 26(2):562-569.
516. Kim J, Hematti P: Mesenchymal stem cell-educated macrophages: a novel type of alternatively activated macrophages. Exp Hematol 2009, 37(12):1445-1453.
517. Abumaree MH, Al Jumah MA, Kalionis B, Jawdat D, Al Khaldi A, Abomaray FM, Fatani AS, Chamley LW, Knawy BA: Human placental mesenchymal stem cells (pMSCs) play a role as immune suppressive cells by shifting macrophage differentiation from inflammatory M1 to anti-inflammatory M2 macrophages. Stem Cell Rev 2013, 9(5):620-641.
518. Aggarwal S, Pittenger MF: Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood 2005, 105(4):1815-1822.
519. Arikawa T, Omura K, Morita I: Regulation of bone morphogenetic protein-2 expression by

endogenous prostaglandin E2 in human mesenchymal stem cells. J Cell Physiol 2004, 200(3):400-406.
520. Romieu-Mourez R, François M, Boivin MN, Bouchentouf M, Spaner DE, Galipeau J: Cytokine modulation of TLR expression and activation in mesenchymal stromal cells leads to a proinflammatory phenotype. J Immunol 2009, 182(12):7963-7973.
521. Meirelles LaS, Fontes AM, Covas DT, Caplan AI: Mechanisms involved in the therapeutic properties of mesenchymal stem cells. Cytokine Growth Factor Rev 2009, 20(5-6):419-427.
522. Kögler G, Radke TF, Lefort A, Sensken S, Fischer J, Sorg RV, Wernet P: Cytokine production and hematopoiesis supporting activity of cord blood-derived unrestricted somatic stem cells. Exp Hematol 2005, 33(5):573-583.
523. Croitoru-Lamoury J, Lamoury FM, Caristo M, Suzuki K, Walker D, Takikawa O, Taylor R, Brew BJ: Interferon-γ regulates the proliferation and differentiation of mesenchymal stem cells via activation of indoleamine 2,3 dioxygenase (IDO). PLoS One 2011, 6(2):e14698.
524. Nakano N, Nakai Y, Seo TB, Yamada Y, Ohno T, Yamanaka A, Nagai Y, Fukushima M, Suzuki Y, Nakatani T et al: Characterization of conditioned medium of cultured bone marrow stromal cells. Neurosci Lett 2010, 483(1):57-61.
525. Jung DI, Ha J, Kang BT, Kim JW, Quan FS, Lee JH, Woo EJ, Park HM: A comparison of autologous and allogenic bone marrow-derived mesenchymal stem cell transplantation in canine spinal cord injury. J Neurol Sci 2009, 285(1-2):67-77.
526. Pal R, Gopinath C, Rao NM, Banerjee P, Krishnamoorthy V, Venkataramana NK, Totey S: Functional recovery after transplantation of bone marrow-derived human mesenchymal stromal cells in a rat model of spinal cord injury. Cytotherapy 2010, 12(6):792-806.
527. Zhou Z, Chen Y, Zhang H, Min S, Yu B, He B, Jin A: Comparison of mesenchymal stromal cells from human bone marrow and adipose tissue for the treatment of spinal cord injury. Cytotherapy 2013, 15(4):434-448.
528. Gu W, Zhang F, Xue Q, Ma Z, Lu P, Yu B: Transplantation of bone marrow mesenchymal stem cells reduces lesion volume and induces axonal regrowth of injured spinal cord. Neuropathology 2010, 30(3):205-217.
529. Cízková D, Rosocha J, Vanický I, Jergová S, Cízek M: Transplants of human mesenchymal stem cells improve functional recovery after spinal cord injury in the rat. Cell Mol Neurobiol 2006, 26(7-8):1167-1180.
530. Lee KH, Suh-Kim H, Choi JS, Jeun SS, Kim EJ, Kim SS, Yoon DH, Lee BH: Human mesenchymal stem cell transplantation promotes functional recovery following acute spinal cord injury in rats. Acta Neurobiol Exp (Wars) 2007, 67(1):13-22.
531. Karaoz E, Kabatas S, Duruksu G, Okcu A, Subasi C, Ay B, Musluman M, Civelek E: Reduction of lesion in injured rat spinal cord and partial functional recovery of motility after bone marrow derived mesenchymal stem cell transplantation. Turk Neurosurg 2012, 22(2):207-217.
532. Himes BT, Neuhuber B, Coleman C, Kushner R, Swanger SA, Kopen GC, Wagner J, Shumsky JS, Fischer I: Recovery of function following grafting of human bone marrow-derived stromal cells into the injured spinal cord. Neurorehabil Neural Repair 2006, 20(2):278-296.
533. Menezes K, Nascimento MA, Gonçalves JP, Cruz AS, Lopes DV, Curzio B, Bonamino M, de Menezes JR, Borojevic R, Rossi MI et al: Human mesenchymal cells from adipose tissue deposit laminin and promote regeneration of injured spinal cord in rats. PLoS One 2014, 9(5):e96020.
534. Aras Y, Sabanci PA, Kabatas S, Duruksu G, Subasi C, Erguven M, Karaoz E: The Effects of Adipose Tissue-Derived Mesenchymal Stem Cell Transplantation During the Acute and Subacute Phases Following Spinal Cord Injury. Turk Neurosurg 2016, 26(1):127-139.
535. Cui B, Li E, Yang B, Wang B: Human umbilical cord blood-derived mesenchymal stem cell transplantation for the treatment of spinal cord injury. Exp Ther Med 2014, 7(5):1233-1236.
536. Ritfeld GJ, Nandoe Tewarie RD, Vajn K, Rahiem ST, Hurtado A, Wendell DF, Roos RA, Oudega M: Bone marrow stromal cell-mediated tissue sparing enhances functional repair after spinal cord contusion in adult rats. Cell Transplant 2012, 21(7):1561-1575.
537. Quertainmont R, Cantinieaux D, Botman O, Sid S, Schoenen J, Franzen R: Mesenchymal stem cell graft improves recovery after spinal cord injury in adult rats through neurotrophic and pro-angiogenic actions. PLoS One 2012, 7(6):e39500.
538. Torres-Espín A, Redondo-Castro E, Hernandez J, Navarro X: Immunosuppression of allogenic mesenchymal stem cells transplantation after spinal cord injury improves graft survival and beneficial outcomes. J Neurotrauma 2015, 32(6):367-380.
539. Kim JW, Ha KY, Molon JN, Kim YH: Bone marrow-derived mesenchymal stem cell transplantation for chronic spinal cord injury in rats: comparative study between intralesional and intravenous transplantation. Spine (Phila Pa 1976) 2013, 38(17):E1065-1074.
540. Satake K, Lou J, Lenke LG: Migration of mesenchymal stem cells through cerebrospinal fluid into injured spinal cord tissue. Spine (Phila Pa 1976) 2004, 29(18):1971-1979.
541. Chen YB, Jia QZ, Li DJ, Sun JH, Xi S, Liu LP, Gao DX, Jiang DW: Spinal cord injury in rats treated using bone marrow mesenchymal stem-cell transplantation. Int J Clin Exp Med 2015, 8(6):9348-9354.
542. Kang ES, Ha KY, Kim YH: Fate of transplanted bone marrow derived mesenchymal stem cells following spinal cord injury in rats by transplantation routes. J Korean Med Sci 2012, 27(6):586-593.
543. Dasari VR, Spomar DG, Cady C, Gujrati M, Rao JS, Dinh DH: Mesenchymal stem cells from rat bone marrow downregulate caspase-3-mediated apoptotic pathway after spinal cord injury in rats. Neurochem Res 2007, 32(12):2080-2093.

544. Novikova LN, Brohlin M, Kingham PJ, Novikov LN, Wiberg M: Neuroprotective and growth-promoting effects of bone marrow stromal cells after cervical spinal cord injury in adult rats. Cytotherapy 2011, 13(7):873-887.
545. de Almeida FM, Marques SA, Ramalho BoS, Massoto TB, Martinez AM: Chronic spinal cord lesions respond positively to tranplants of mesenchymal stem cells. Restor Neurol Neurosci 2015, 33(1):43-55.
546. Oda Y, Tani K, Asari Y, Quintanilha LF, Haraguchi T, Momota Y, Katayama M, Itamoto K, Nakazawa H, Taura Y: Canine bone marrow stromal cells promote functional recovery in mice with spinal cord injury. J Vet Med Sci 2014, 76(6):905-908.
547. Ohta M, Suzuki Y, Noda T, Ejiri Y, Dezawa M, Kataoka K, Chou H, Ishikawa N, Matsumoto N, Iwashita Y et al: Bone marrow stromal cells infused into the cerebrospinal fluid promote functional recovery of the injured rat spinal cord with reduced cavity formation. Exp Neurol 2004, 187(2):266-278.
548. Song Q, Xu R, Zhang Q, Ma M, Zhao X: Therapeutic effect of transplanting bone mesenchymal stem cells on the hind limbs' motor function of rats with acute spinal cord injury. Int J Clin Exp Med 2014, 7(1):262-267.
549. Chung HJ, Chung WH, Lee JH, Chung DJ, Yang WJ, Lee AJ, Choi CB, Chang HS, Kim DH, Suh HJ et al: Expression of neurotrophic factors in injured spinal cord after transplantation of human-umbilical cord blood stem cells in rats. J Vet Sci 2016, 17(1):97-102.
550. Cantinieaux D, Quertainmont R, Blacher S, Rossi L, Wanet T, Noël A, Brook G, Schoenen J, Franzen R: Conditioned medium from bone marrow-derived mesenchymal stem cells improves recovery after spinal cord injury in rats: an original strategy to avoid cell transplantation. PLoS One 2013, 8(8):e69515.
551. Shin DA, Kim JM, Kim HI, Yi S, Ha Y, Yoon DH, Kim KN: Comparison of functional and histological outcomes after intralesional, intracisternal, and intravenous transplantation of human bone marrow-derived mesenchymal stromal cells in a rat model of spinal cord injury. Acta Neurochir (Wien) 2013, 155(10):1943-1950.
552. Park SI, Lim JY, Jeong CH, Kim SM, Jun JA, Jeun SS, Oh WI: Human umbilical cord blood-derived mesenchymal stem cell therapy promotes functional recovery of contused rat spinal cord through enhancement of endogenous cell proliferation and oligogenesis. J Biomed Biotechnol 2012, 2012:362473.
553. Nakajima H, Uchida K, Guerrero AR, Watanabe S, Sugita D, Takeura N, Yoshida A, Long G, Wright KT, Johnson WE et al: Transplantation of mesenchymal stem cells promotes an alternative pathway of macrophage activation and functional recovery after spinal cord injury. J Neurotrauma 2012, 29(8):1614-1625.
554. Okuda A, Horii-Hayashi N, Sasagawa T, Shimizu T, Shigematsu H, Iwata E, Morimoto Y, Masuda K, Koizumi M, Akahane M et al: Bone marrow stromal cell sheets may promote axonal regeneration and functional recovery with suppression of glial scar formation after spinal cord transection injury in rats. J Neurosurg Spine 2017, 26(3):388-395.
555. Melo FR, Bressan RB, Forner S, Martini AC, Rode M, Delben PB, Rae GA, Figueiredo CP, Trentin AG: Transplantation of Human Skin-Derived Mesenchymal Stromal Cells Improves Locomotor Recovery After Spinal Cord Injury in Rats. Cell Mol Neurobiol 2016.
556. Zurita M, Vaquero J, Bonilla C, Santos M, De Haro J, Oya S, Aguayo C: Functional recovery of chronic paraplegic pigs after autologous transplantation of bone marrow stromal cells. Transplantation 2008, 86(6):845-853.
557. Morita T, Sasaki M, Kataoka-Sasaki Y, Nakazaki M, Nagahama H, Oka S, Oshigiri T, Takebayashi T, Yamashita T, Kocsis JD et al: Intravenous infusion of mesenchymal stem cells promotes functional recovery in a model of chronic spinal cord injury. Neuroscience 2016, 335:221-231.
558. Osaka M, Honmou O, Murakami T, Nonaka T, Houkin K, Hamada H, Kocsis JD: Intravenous administration of mesenchymal stem cells derived from bone marrow after contusive spinal cord injury improves functional outcome. Brain Res 2010, 1343:226-235.
559. Kim Y, Jo SH, Kim WH, Kweon OK: Antioxidant and anti-inflammatory effects of intravenously injected adipose derived mesenchymal stem cells in dogs with acute spinal cord injury. Stem Cell Res Ther 2015, 6:229.
560. Han D, Wu C, Xiong Q, Zhou L, Tian Y: Anti-inflammatory Mechanism of Bone Marrow Mesenchymal Stem Cell Transplantation in Rat Model of Spinal Cord Injury. Cell Biochem Biophys 2015, 71(3):1341-1347.
561. Matsushita T, Lankford KL, Arroyo EJ, Sasaki M, Neyazi M, Radtke C, Kocsis JD: Diffuse and persistent blood-spinal cord barrier disruption after contusive spinal cord injury rapidly recovers following intravenous infusion of bone marrow mesenchymal stem cells. Exp Neurol 2015, 267:152-164.
562. Urdzíková LM, Růžička J, LaBagnara M, Kárová K, Kubinová Š, Jiráková K, Murali R, Syková E, Jhanwar-Uniyal M, Jendelová P: Human mesenchymal stem cells modulate inflammatory cytokines after spinal cord injury in rat. Int J Mol Sci 2014, 15(7):11275-11293.
563. Cizkova D, Novotna I, Slovinska L, Vanicky I, Jergova S, Rosocha J, Radonak J: Repetitive intrathecal catheter delivery of bone marrow mesenchymal stromal cells improves functional recovery in a rat model of contusive spinal cord injury. J Neurotrauma 2011, 28(9):1951-1961.
564. Sasaki M, Radtke C, Tan AM, Zhao P, Hamada H, Houkin K, Honmou O, Kocsis JD: BDNF-hypersecreting human mesenchymal stem cells promote functional recovery, axonal sprouting, and protection of corticospinal neurons after spinal cord injury. J Neurosci 2009, 29(47):14932-14941.
565. Uchida S, Hayakawa K, Ogata T, Tanaka S, Kataoka K, Itaka K: Treatment of spinal cord injury by an advanced cell transplantation technology using brain-derived neurotrophic factor-transfected

mesenchymal stem cell spheroids. Biomaterials 2016, 109:1-11.
566. Xiong LL, Li Y, Shang FF, Chen SW, Chen H, Ju SM, Zou Y, Tian HL, Wang TH, Luo CZ et al: Chondroitinase administration and pcDNA3.1-BDNF-BMSC transplantation promote motor functional recovery associated with NGF expression in spinal cord-transected rat. Spinal Cord 2016, 54(12):1088-1095.
567. Zhao H, Cheng L, Du X, Hou Y, Liu Y, Cui Z, Nie L: Transplantation of Cerebral Dopamine Neurotrophic Factor Transducted BMSCs in Contusion Spinal Cord Injury of Rats: Promotion of Nerve Regeneration by Alleviating Neuroinflammation. Mol Neurobiol 2016, 53(1):187-199.
568. Rooney GE, McMahon SS, Ritter T, Garcia Y, Moran C, Madigan NN, Flügel A, Dockery P, O'Brien T, Howard L et al: Neurotrophic factor-expressing mesenchymal stem cells survive transplantation into the contused spinal cord without differentiating into neural cells. Tissue Eng Part A 2009, 15(10):3049-3059.
569. Wei GJ, An G, Shi ZW, Wang KF, Guan Y, Wang YS, Han B, Yu EM, Li PF, Dong DM et al: Suppression of MicroRNA-383 Enhances Therapeutic Potential of Human Bone-Marrow-Derived Mesenchymal Stem Cells in Treating Spinal Cord Injury via GDNF. Cell Physiol Biochem 2017, 41(4):1435-1444.
570. Li Z, Zhang Z, Zhao L, Li H, Wang S, Shen Y: Bone marrow mesenchymal stem cells with Nogo-66 receptor gene silencing for repair of spinal cord injury. Neural Regen Res 2014, 9(8):806-814.
571. Dong Y, Yang L, Zhao H, Zhang C, Wu D: Transplantation of neurotrophin-3-transfected bone marrow mesenchymal stem cells for the repair of spinal cord injury. Neural Regen Res 2014, 9(16):1520-1524.
572. Wang LJ, Zhang RP, Li JD: Transplantation of neurotrophin-3-expressing bone mesenchymal stem cells improves recovery in a rat model of spinal cord injury. Acta Neurochir (Wien) 2014, 156(7):1409-1418.
573. Shang AJ, Hong SQ, Xu Q, Wang HY, Yang Y, Wang ZF, Xu BN, Jiang XD, Xu RX: NT-3-secreting human umbilical cord mesenchymal stromal cell transplantation for the treatment of acute spinal cord injury in rats. Brain Res 2011, 1391:102-113.
574. Liu WG, Wang ZY, Huang ZS: Bone marrow-derived mesenchymal stem cells expressing the bFGF transgene promote axon regeneration and functional recovery after spinal cord injury in rats. Neurol Res 2011, 33(7):686-693.
575. Dooley D, Lemmens E, Vangansewinkel T, Le Blon D, Hoornaert C, Ponsaerts P, Hendrix S: Cell-Based Delivery of Interleukin-13 Directs Alternative Activation of Macrophages Resulting in Improved Functional Outcome after Spinal Cord Injury. Stem Cell Reports 2016, 7(6):1099-1115.
576. Lee SH, Kim Y, Rhew D, Kim A, Jo KR, Yoon Y, Choi KU, Jung T, Kim WH, Kweon OK: Effect of canine mesenchymal stromal cells overexpressing heme oxygenase-1 in spinal cord injury. J Vet Sci 2016.
577. Seo DK, Kim JH, Min J, Yoon HH, Shin ES, Kim SW, Jeon SR: Enhanced axonal regeneration by transplanted Wnt3a-secreting human mesenchymal stem cells in a rat model of spinal cord injury. Acta Neurochir (Wien) 2017, 159(5):947-957.
578. Tang L, Lu X, Zhu R, Qian T, Tao Y, Li K, Zheng J, Zhao P, Li S, Wang X et al: Adipose-Derived Stem Cells Expressing the Neurogenin-2 Promote Functional Recovery After Spinal Cord Injury in Rat. Cell Mol Neurobiol 2016, 36(5):657-667.
579. Chen J, Zhang Z, Liu J, Zhou R, Zheng X, Chen T, Wang L, Huang M, Yang C, Li Z et al: Acellular spinal cord scaffold seeded with bone marrow stromal cells protects tissue and promotes functional recovery in spinal cord-injured rats. J Neurosci Res 2014, 92(3):307-317.
580. Liu J, Chen J, Liu B, Yang C, Xie D, Zheng X, Xu S, Chen T, Wang L, Zhang Z et al: Acellular spinal cord scaffold seeded with mesenchymal stem cells promotes long-distance axon regeneration and functional recovery in spinal cord injured rats. J Neurol Sci 2013, 325(1-2):127-136.
581. Choi JS, Leem JW, Lee KH, Kim SS, Suh-Kim H, Jung SJ, Kim UJ, Lee BH: Effects of human mesenchymal stem cell transplantation combined with polymer on functional recovery following spinal cord hemisection in rats. Korean J Physiol Pharmacol 2012, 16(6):405-411.
582. Kang KN, Lee JY, Kim DY, Lee BN, Ahn HH, Lee B, Khang G, Park SR, Min BH, Kim JH et al: Regeneration of completely transected spinal cord using scaffold of poly(D,L-lactide-co-glycolide)/small intestinal submucosa seeded with rat bone marrow stem cells. Tissue Eng Part A 2011, 17(17-18):2143-2152.
583. Peng Z, Gao W, Yue B, Jiang J, Gu Y, Dai J, Chen L, Shi Q: Promotion of neurological recovery in rat spinal cord injury by mesenchymal stem cells loaded on nerve-guided collagen scaffold through increasing alternatively activated macrophage polarization. J Tissue Eng Regen Med 2016.
584. Han S, Wang B, Li X, Xiao Z, Han J, Zhao Y, Fang Y, Yin Y, Chen B, Dai J: Bone marrow-derived mesenchymal stem cells in three-dimensional culture promote neuronal regeneration by neurotrophic protection and immunomodulation. J Biomed Mater Res A 2016, 104(7):1759-1769.
585. Xue F, Wu EJ, Zhang PX, Li-Ya A, Kou YH, Yin XF, Han N: Biodegradable chitin conduit tubulation combined with bone marrow mesenchymal stem cell transplantation for treatment of spinal cord injury by reducing glial scar and cavity formation. Neural Regen Res 2015, 10(1):104-111.
586. Zeng X, Zeng YS, Ma YH, Lu LY, Du BL, Zhang W, Li Y, Chan WY: Bone marrow mesenchymal stem cells in a three-dimensional gelatin sponge scaffold attenuate inflammation, promote angiogenesis, and reduce cavity formation in experimental spinal cord injury. Cell Transplant 2011, 20(11-12):1881-1899.
587. Jiao G, Lou G, Mo Y, Pan Y, Zhang Z, Guo R, Li Z: A combination of GDNF and hUCMSC transplantation loaded on SF/AGs composite scaffolds for spinal cord injury repair. Mater Sci Eng C Mater Biol Appl 2017, 74:230-237.

588. Günther MI, Weidner N, Müller R, Blesch A: Cell-seeded alginate hydrogel scaffolds promote directed linear axonal regeneration in the injured rat spinal cord. Acta Biomater 2015, 27:140-150.
589. Yang EZ, Zhang GW, Xu JG, Chen S, Wang H, Cao LL, Liang B, Lian XF: Multichannel polymer scaffold seeded with activated Schwann cells and bone mesenchymal stem cells improves axonal regeneration and functional recovery after rat spinal cord injury. Acta Pharmacol Sin 2017, 38(5):623-637.
590. Wu S, Cui G, Shao H, Du Z, Ng JC, Peng C: The Cotransplantation of Olfactory Ensheathing Cells with Bone Marrow Mesenchymal Stem Cells Exerts Antiapoptotic Effects in Adult Rats after Spinal Cord Injury. Stem Cells Int 2015, 2015:516215.
591. Amemori T, Jendelová P, Růzicková K, Arboleda D, Syková E: Co-transplantation of olfactory ensheathing glia and mesenchymal stromal cells does not have synergistic effects after spinal cord injury in the rat. Cytotherapy 2010, 12(2):212-225.
592. Zhang C, He X, Li H, Wang G: Chondroitinase ABC plus bone marrow mesenchymal stem cells for repair of spinal cord injury. Neural Regen Res 2013, 8(11):965-974.
593. Lee SH, Kim Y, Rhew D, Kim A, Jo KR, Yoon Y, Choi KU, Jung T, Kim WH, Kweon OK: Impact of local injection of brain-derived neurotrophic factor-expressing mesenchymal stromal cells (MSCs) combined with intravenous MSC delivery in a canine model of chronic spinal cord injury. Cytotherapy 2016.
594. Lee SH, Kim Y, Rhew D, Kuk M, Kim M, Kim WH, Kweon OK: Effect of the combination of mesenchymal stromal cells and chondroitinase ABC on chronic spinal cord injury. Cytotherapy 2015, 17(10):1374-1383.
595. Chen L, Cui X, Wu Z, Jia L, Yu Y, Zhou Q, Hu X, Xu W, Luo D, Liu J et al: Transplantation of bone marrow mesenchymal stem cells pretreated with valproic acid in rats with an acute spinal cord injury. Biosci Trends 2014, 8(2):111-119.
596. Zhang W, Yan Q, Zeng YS, Zhang XB, Xiong Y, Wang JM, Chen SJ, Li Y, Bruce IC, Wu W: Implantation of adult bone marrow-derived mesenchymal stem cells transfected with the neurotrophin-3 gene and pretreated with retinoic acid in completely transected spinal cord. Brain Res 2010, 1359:256-271.
597. Kim KN, Oh SH, Lee KH, Yoon DH: Effect of human mesenchymal stem cell transplantation combined with growth factor infusion in the repair of injured spinal cord. Acta Neurochir Suppl 2006, 99:133-136.
598. Huang F, Wang J, Chen A: Effects of co-grafts mesenchymal stem cells and nerve growth factor suspension in the repair of spinal cord injury. J Huazhong Univ Sci Technolog Med Sci 2006, 26(2):206-210.
599. Chen D, Zeng W, Fu Y, Gao M, Lv G: Bone marrow mesenchymal stem cells combined with minocycline improve spinal cord injury in a rat model. Int J Clin Exp Pathol 2015, 8(10):11957-11969.
600. Zhou YJ, Liu JM, Wei SM, Zhang YH, Qu ZH, Chen SB: Propofol promotes spinal cord injury repair by bone marrow mesenchymal stem cell transplantation. Neural Regen Res 2015, 10(8):1305-1311.
601. Geng CK, Cao HH, Ying X, Yu HL: Effect of mesenchymal stem cells transplantation combining with hyperbaric oxygen therapy on rehabilitation of rat spinal cord injury. Asian Pac J Trop Med 2015, 8(6):468-473.
602. Ra JC, Shin IS, Kim SH, Kang SK, Kang BC, Lee HY, Kim YJ, Jo JY, Yoon EJ, Choi HJ et al: Safety of intravenous infusion of human adipose tissue-derived mesenchymal stem cells in animals and humans. Stem Cells Dev 2011, 20(8):1297-1308.
603. Park JH, Kim DY, Sung IY, Choi GH, Jeon MH, Kim KK, Jeon SR: Long-term results of spinal cord injury therapy using mesenchymal stem cells derived from bone marrow in humans. Neurosurgery 2012, 70(5):1238-1247; discussion 1247.
604. Pal R, Venkataramana NK, Bansal A, Balaraju S, Jan M, Chandra R, Dixit A, Rauthan A, Murgod U, Totey S: Ex vivo-expanded autologous bone marrow-derived mesenchymal stromal cells in human spinal cord injury/paraplegia: a pilot clinical study. Cytotherapy 2009, 11(7):897-911.
605. Satti HS, Waheed A, Ahmed P, Ahmed K, Akram Z, Aziz T, Satti TM, Shahbaz N, Khan MA, Malik SA: Autologous mesenchymal stromal cell transplantation for spinal cord injury: A Phase I pilot study. Cytotherapy 2016, 18(4):518-522.
606. Oh SK, Choi KH, Yoo JY, Kim DY, Kim SJ, Jeon SR: A Phase III Clinical Trial Showing Limited Efficacy of Autologous Mesenchymal Stem Cell Therapy for Spinal Cord Injury. Neurosurgery 2016, 78(3):436-447; discussion 447.
607. Mendonça MV, Larocca TF, de Freitas Souza BS, Villarreal CF, Silva LF, Matos AC, Novaes MA, Bahia CM, de Oliveira Melo Martinez AC, Kaneto CM et al: Safety and neurological assessments after autologous transplantation of bone marrow mesenchymal stem cells in subjects with chronic spinal cord injury. Stem Cell Res Ther 2014, 5(6):126.
608. Dai G, Liu X, Zhang Z, Yang Z, Dai Y, Xu R: Transplantation of autologous bone marrow mesenchymal stem cells in the treatment of complete and chronic cervical spinal cord injury. Brain Res 2013, 1533:73-79.
609. Jiang PC, Xiong WP, Wang G, Ma C, Yao WQ, Kendell SF, Mehling BM, Yuan XH, Wu DC: A clinical trial report of autologous bone marrow-derived mesenchymal stem cell transplantation in patients with spinal cord injury. Exp Ther Med 2013, 6(1):140-146.
610. Karamouzian S, Nematollahi-Mahani SN, Nakhaee N, Eskandary H: Clinical safety and primary efficacy of bone marrow mesenchymal cell transplantation in subacute spinal cord injured patients. Clin Neurol Neurosurg 2012, 114(7):935-939.
611. Geffner LF, Santacruz P, Izurieta M, Flor L, Maldonado B, Auad AH, Montenegro X, Gonzalez R, Silva F: Administration of autologous bone marrow stem cells into spinal cord injury patients via multiple routes is safe and improves their quality of life: comprehensive case studies. Cell Transplant 2008, 17(12):1277-1293.

612. Bhanot Y, Rao S, Ghosh D, Balaraju S, Radhika CR, Satish Kumar KV: Autologous mesenchymal stem cells in chronic spinal cord injury. Br J Neurosurg 2011, 25(4):516-522.
613. El-Kheir WA, Gabr H, Awad MR, Ghannam O, Barakat Y, Farghali HA, El Maadawi ZM, Ewes I, Sabaawy HE: Autologous bone marrow-derived cell therapy combined with physical therapy induces functional improvement in chronic spinal cord injury patients. Cell Transplant 2014, 23(6):729-745.
614. Jarocha D, Milczarek O, Wedrychowicz A, Kwiatkowski S, Majka M: Continuous improvement after multiple mesenchymal stem cell transplantations in a patient with complete spinal cord injury. Cell Transplant 2015, 24(4):661-672.
615. Kakabadze Z, Kipshidze N, Mardaleishvili K, Chutkerashvili G, Chelishvili I, Harders A, Loladze G, Shatirishvili G, Chakhunashvili D, Chutkerashvili K: Phase 1 Trial of Autologous Bone Marrow Stem Cell Transplantation in Patients with Spinal Cord Injury. Stem Cells Int 2016, 2016:6768274.
616. Lalu MM, McIntyre L, Pugliese C, Fergusson D, Winston BW, Marshall JC, Granton J, Stewart DJ, Group CCCT: Safety of cell therapy with mesenchymal stromal cells (SafeCell): a systematic review and meta-analysis of clinical trials. PLoS One 2012, 7(10):e47559.
617. Liu J, Han D, Wang Z, Xue M, Zhu L, Yan H, Zheng X, Guo Z, Wang H: Clinical analysis of the treatment of spinal cord injury with umbilical cord mesenchymal stem cells. Cytotherapy 2013, 15(2):185-191.
618. Kishk NA, Gabr H, Hamdy S, Afifi L, Abokresha N, Mahmoud H, Wafaie A, Bilal D: Case control series of intrathecal autologous bone marrow mesenchymal stem cell therapy for chronic spinal cord injury. Neurorehabil Neural Repair 2010, 24(8):702-708.
619. Vegh P, Haniffa M: The impact of single-cell RNA sequencing on understanding the functional organization of the immune system. Brief Funct Genomics 2018, 17(4):265-272.
620. Papalexi E, Satija R: Single-cell RNA sequencing to explore immune cell heterogeneity. Nat Rev Immunol 2018, 18(1):35-45.
621. La Manno G, Gyllborg D, Codeluppi S, Nishimura K, Salto C, Zeisel A, Borm LE, Stott SRW, Toledo EM, Villaescusa JC et al: Molecular Diversity of Midbrain Development in Mouse, Human, and Stem Cells. Cell 2016, 167(2):566-580.e519.
622. Matcovitch-Natan O, Winter DR, Giladi A, Vargas Aguilar S, Spinrad A, Sarrazin S, Ben-Yehuda H, David E, Zelada González F, Perrin P et al: Microglia development follows a stepwise program to regulate brain homeostasis. Science 2016, 353(6301):aad8670.
623. Paul F, Arkin Y, Giladi A, Jaitin DA, Kenigsberg E, Keren-Shaul H, Winter D, Lara-Astiaso D, Gury M, Weiner A et al: Transcriptional Heterogeneity and Lineage Commitment in Myeloid Progenitors. Cell 2016, 164(1-2):325.
624. O'Koren EG, Yu C, Klingeborn M, Wong AYW, Prigge CL, Mathew R, Kalnitsky J, Msallam RA, Silvin A, Kay JN et al: Microglial Function Is Distinct in Different Anatomical Locations during Retinal Homeostasis and Degeneration. Immunity 2019, 50(3):723-737.e727.
625. Mathys H, Adaikkan C, Gao F, Young JZ, Manet E, Hemberg M, De Jager PL, Ransohoff RM, Regev A, Tsai LH: Temporal Tracking of Microglia Activation in Neurodegeneration at Single-Cell Resolution. Cell Rep 2017, 21(2):366-380.
626. Keren-Shaul H, Spinrad A, Weiner A, Matcovitch-Natan O, Dvir-Szternfeld R, Ulland TK, David E, Baruch K, Lara-Astaiso D, Toth B et al: A Unique Microglia Type Associated with Restricting Development of Alzheimer's Disease. Cell 2017, 169(7):1276-1290.e1217.
627. Masuda T, Sankowski R, Staszewski O, Böttcher C, Amann L, Sagar, Scheiwe C, Nessler S, Kunz P, van Loo G et al: Spatial and temporal heterogeneity of mouse and human microglia at single-cell resolution. Nature 2019, 566(7744):388-392.
628. Jordão MJC, Sankowski R, Brendecke SM, Sagar, Locatelli G, Tai YH, Tay TL, Schramm E, Armbruster S, Hagemeyer N et al: Single-cell profiling identifies myeloid cell subsets with distinct fates during neuroinflammation. Science 2019, 363(6425).
629. Krasemann S, Madore C, Cialic R, Baufeld C, Calcagno N, El Fatimy R, Beckers L, O'Loughlin E, Xu Y, Fanek Z et al: The TREM2-APOE Pathway Drives the Transcriptional Phenotype of Dysfunctional Microglia in Neurodegenerative Diseases. Immunity 2017, 47(3):566-581.e569.
630. Tay TL, Sagar, Dautzenberg J, Grün D, Prinz M: Unique microglia recovery population revealed by single-cell RNAseq following neurodegeneration. Acta Neuropathol Commun 2018, 6(1):87.
631. Sousa C, Golebiewska A, Poovathingal SK, Kaoma T, Pires-Afonso Y, Martina S, Coowar D, Azuaje F, Skupin A, Balling R et al: Single-cell transcriptomics reveals distinct inflammation-induced microglia signatures. EMBO Rep 2018, 19(11).
632. Farhadian SF, Mehta SS, Zografou C, Robertson K, Price RW, Pappalardo J, Chiarella J, Hafler DA, Spudich SS: Single-cell RNA sequencing reveals microglia-like cells in cerebrospinal fluid during virologically suppressed HIV. JCI Insight 2018, 3(18).
633. Li Q, Cheng Z, Zhou L, Darmanis S, Neff NF, Okamoto J, Gulati G, Bennett ML, Sun LO, Clarke LE et al: Developmental Heterogeneity of Microglia and Brain Myeloid Cells Revealed by Deep Single-Cell RNA Sequencing. Neuron 2019, 101(2):207-223.e210.
634. Friedman BA, Srinivasan K, Ayalon G, Meilandt WJ, Lin H, Huntley MA, Cao Y, Lee SH, Haddick PCG, Ngu H et al: Diverse Brain Myeloid Expression Profiles Reveal Distinct Microglial Activation States and Aspects of Alzheimer's Disease Not Evident in Mouse Models. Cell Rep 2018, 22(3):832-847.
635. Deczkowska A, Keren-Shaul H, Weiner A, Colonna M, Schwartz M, Amit I: Disease-Associated Microglia: A Universal Immune Sensor of Neurodegeneration. Cell 2018, 173(5):1073-1081.
636. Wang Y, Cella M, Mallinson K, Ulrich JD, Young KL, Robinette ML, Gilfillan S, Krishnan GM,

Sudhakar S, Zinselmeyer BH et al: TREM2 lipid sensing sustains the microglial response in an Alzheimer's disease model. Cell 2015, 160(6):1061-1071.

637. Ahyayauch H, Raab M, Busto JV, Andraka N, Arrondo JL, Masserini M, Tvaroska I, Goñi FM: Binding of β-amyloid (1-42) peptide to negatively charged phospholipid membranes in the liquid-ordered state: modeling and experimental studies. Biophys J 2012, 103(3):453-463.

638. Zhong L, Wang Z, Wang D, Martens YA, Wu L, Xu Y, Wang K, Li J, Huang R, Can D et al: Amyloid-beta modulates microglial responses by binding to the triggering receptor expressed on myeloid cells 2 (TREM2). Mol Neurodegener 2018, 13(1):15.

639. Terwel D, Steffensen KR, Verghese PB, Kummer MP, Gustafsson J, Holtzman DM, Heneka MT: Critical role of astroglial apolipoprotein E and liver X receptor-α expression for microglial Aβ phagocytosis. J Neurosci 2011, 31(19):7049-7059.

640. Niraula A, Sheridan JF, Godbout JP: Microglia Priming with Aging and Stress. Neuropsychopharmacology 2017, 42(1):318-333.

641. Deczkowska A, Matcovitch-Natan O, Tsitsou-Kampeli A, Ben-Hamo S, Dvir-Szternfeld R, Spinrad A, Singer O, David E, Winter DR, Smith LK et al: Mef2C restrains microglial inflammatory response and is lost in brain ageing in an IFN-I-dependent manner. Nat Commun 2017, 8(1):717.

642. Butovsky O, Jedrychowski MP, Moore CS, Cialic R, Lanser AJ, Gabriely G, Koeglsperger T, Dake B, Wu PM, Doykan CE et al: Identification of a unique TGF-β-dependent molecular and functional signature in microglia. Nat Neurosci 2014, 17(1):131-143.

643. Lambert JC, Ibrahim-Verbaas CA, Harold D, Naj AC, Sims R, Bellenguez C, DeStafano AL, Bis JC, Beecham GW, Grenier-Boley B et al: Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. Nat Genet 2013, 45(12):1452-1458.

644. Boregowda SV, Krishnappa V, Phinney DG: Isolation of Mouse Bone Marrow Mesenchymal Stem Cells. Methods Mol Biol 2016, 1416:205-223.

645. Johnstone B, Hering TM, Caplan AI, Goldberg VM, Yoo JU: In vitro chondrogenesis of bone marrow-derived mesenchymal progenitor cells. Exp Cell Res 1998, 238(1):265-272.

646. Mackay AM, Beck SC, Murphy JM, Barry FP, Chichester CO, Pittenger MF: Chondrogenic differentiation of cultured human mesenchymal stem cells from marrow. Tissue Eng 1998, 4(4):415-428.

647. Phinney DG, Kopen G, Isaacson RL, Prockop DJ: Plastic adherent stromal cells from the bone marrow of commonly used strains of inbred mice: variations in yield, growth, and differentiation. J Cell Biochem 1999, 72(4):570-585.

648. Xu S, De Becker A, Van Camp B, Vanderkerken K, Van Riet I: An improved harvest and in vitro expansion protocol for murine bone marrow-derived mesenchymal stem cells. J Biomed Biotechnol 2010, 2010:105940.

649. Peister A, Mellad JA, Larson BL, Hall BM, Gibson LF, Prockop DJ: Adult stem cells from bone marrow (MSCs) isolated from different strains of inbred mice vary in surface epitopes, rates of proliferation, and differentiation potential. Blood 2004, 103(5):1662-1668.

650. Castro-Malaspina H, Gay RE, Resnick G, Kapoor N, Meyers P, Chiarieri D, McKenzie S, Broxmeyer HE, Moore MA: Characterization of human bone marrow fibroblast colony-forming cells (CFU-F) and their progeny. Blood 1980, 56(2):289-301.

651. Naglich JG, Metherall JE, Russell DW, Eidels L: Expression cloning of a diphtheria toxin receptor: identity with a heparin-binding EGF-like growth factor precursor. Cell 1992, 69(6):1051-1061.

652. Iwamoto R, Higashiyama S, Mitamura T, Taniguchi N, Klagsbrun M, Mekada E: Heparin-binding EGF-like growth factor, which acts as the diphtheria toxin receptor, forms a complex with membrane protein DRAP27/CD9, which up-regulates functional receptors and diphtheria toxin sensitivity. EMBO J 1994, 13(10):2322-2330.

653. Araña M, Mazo M, Aranda P, Pelacho B, Prosper F: Adipose tissue-derived mesenchymal stem cells: isolation, expansion, and characterization. Methods Mol Biol 2013, 1036:47-61.

654. Johansson CB, Momma S, Clarke DL, Risling M, Lendahl U, Frisén J: Identification of a neural stem cell in the adult mammalian central nervous system. Cell 1999, 96(1):25-34.

655. Basso DM, Fisher LC, Anderson AJ, Jakeman LB, McTigue DM, Popovich PG: Basso Mouse Scale for locomotion detects differences in recovery after spinal cord injury in five common mouse strains. J Neurotrauma 2006, 23(5):635-659.

656. Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, Batut P, Chaisson M, Gingeras TR: STAR: ultrafast universal RNA-seq aligner. Bioinformatics 2013, 29(1):15-21.

657. Yang Liao GKSaWS: featureCounts: an efficient general-purpose program for assigning sequence reads to genomic features. In., vol. 30: Bioinformatics; 2014: 923-930.

658. Team} RC: R: A Language and Environment for Statistical Computing. In.: R Foundation for Statistical Computing. Vienna, Austria.; 2016.

659. Smyth MERaBPaDWaYHaCWLaWSaGK: {limma} powers differential expression analyses for {RNA}-sequencing and microarray studies. In., vol. 43: Nucleic Acids Research; 2015: e47.

660. Robinson MD MDaSG: edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. In., vol. 26: Bioinformatics; 2010: 139-140.

661. Liao Y, Wang J, Jaehnig EJ, Shi Z, Zhang B: WebGestalt 2019: gene set analysis toolkit with revamped UIs and APIs. Nucleic Acids Res 2019, 47(W1):W199-W205.

662. Picelli S, Faridani OR, Björklund AK, Winberg G, Sagasser S, Sandberg R: Full-length RNA-seq from single cells using Smart-seq2. Nat Protoc 2014, 9(1):171-181.

663. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, Garimella K, Altshuler D, Gabriel S, Daly M et al: The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res 2010, 20(9):1297-1303.
664. Anders S, Pyl PT, Huber W: HTSeq--a Python framework to work with high-throughput sequencing data. Bioinformatics 2015, 31(2):166-169.
665. Stuart T, Butler A, Hoffman P, Hafemeister C, Papalexi E, Mauck WM, Hao Y, Stoeckius M, Smibert P, Satija R: Comprehensive Integration of Single-Cell Data. Cell 2019, 177(7):1888-1902.e1821.
666. Susaki EA, Tainaka K, Perrin D, Kishino F, Tawara T, Watanabe TM, Yokoyama C, Onoe H, Eguchi M, Yamaguchi S et al: Whole-brain imaging with single-cell resolution using chemical cocktails and computational analysis. Cell 2014, 157(3):726-739.
667. Fontaine MJ, Shih H, Schäfer R, Pittenger MF: Unraveling the Mesenchymal Stromal Cells' Paracrine Immunomodulatory Effects. Transfus Med Rev 2016, 30(1):37-43.
668. Mrdjen D, Pavlovic A, Hartmann FJ, Schreiner B, Utz SG, Leung BP, Lelios I, Heppner FL, Kipnis J, Merkler D et al: High-Dimensional Single-Cell Mapping of Central Nervous System Immune Cells Reveals Distinct Myeloid Subsets in Health, Aging, and Disease. Immunity 2018, 48(2):380-395.e386.
669. Hammond TR, Dufort C, Dissing-Olesen L, Giera S, Young A, Wysoker A, Walker AJ, Gergits F, Segel M, Nemesh J et al: Single-Cell RNA Sequencing of Microglia throughout the Mouse Lifespan and in the Injured Brain Reveals Complex Cell-State Changes. Immunity 2019, 50(1):253-271.e256.