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HUMAN NEURAL PRECURSOR CELLS IN SPINAL CORD REPAIR

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Cover: Neurospheres (green) and their derived neurons (red), astrocytes (blue) and oligodendrocytes ("KI-plum red"). Illustration by Jinghua Piao, Elisabet Åkesson and Marianne Grip.

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To my family with love

Obstacles are those frightful things you see
when you take your eyes off your goals.
-- Henry Ford

ABSTRACT

Traumatic spinal cord injury (SCI) often results in substantial neural cell death, axonal degeneration and demyelination, accompanied by loss of sensory and motor functions. Cell therapy may have neuroprotective effects, substitute for lost neural cells, promote regeneration and enhance remyelination in the lesioned spinal cord. The availability of neural precursor cells (NPCs) suitable for both experimental and clinical purposes may be significantly improved by expansion of these cells *in vitro*. The primary objectives of the present thesis have been to evaluate conditions for culturing and *in vitro* expansion of human NPCs and to characterize the suitability of these cells for therapeutic treatment of SCI.

NPCs derived from the human first trimester forebrain and spinal cord were cultured as neurospheres in the presence of epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF), with or without ciliary neurotrophic factor (CNTF). First trimester forebrain up to 12 weeks of gestation can serve as source for neurospheres, while spinal cord NPCs only expand successfully when the originating tissue is ≤ 9.5 weeks of gestation. Within these developmental time frames, gestational age of the donor tissue did not affect the proportion of cells with different phenotypes in culture. *In vitro* propagation enhanced the size of the cell population immunoreactive (IR) for CD133 without altering the numbers of cells expressing Tra-1-60-, Tra-1-81-, stage-specific embryonic antigen-4 (SSEA-4)-, nestin- and proliferating cell nuclear antigen (PCNA). The fraction of glial fibrillary acidic protein (GFAP)-IR cells increased, while the proportion of β-tubulin III-IR cells was reduced following five or more passages in the case of cultures originating from the spinal cord, but not from forebrain. Thus, even under the same culture conditions, the cellular composition of human neurospheres was dependent on the length of propagation *in vitro* and the regional heterogeneity of the tissue from which they were derived.

CNTF enhanced the rate of cell proliferation and expansion of human NPCs derived from the embryonic spinal cord without significantly altering the proportions of cells that expressed nestin, GFAP, β-tubulin III or O4. In contrast, this factor exerted no such influence on human NPCs derived from age-matched forebrain. This difference might reflect, at least in part, differences in the expression of the CNTF receptor-α protein in these two regions of the central nervous system during development.

In vitro cultures of human NPCs expressed human leukocyte antigen (HLA) class I and II, but almost no co-stimulatory proteins (CD40, CD80 and CD86). The level of HLA increased during expansion of both spinal cord and forebrain NPCs. This phenomenon could, at least in theory, enhance the immunogenicity of NPCs and the risk for rejection after transplantation, but human peripheral lymphocytes exhibited no response when co-cultured with NPCs expanded *in vitro*. Together, these observations indicated that expanded NPCs demonstrate a low degree of immunogenicity, despite their pronounced expression of HLA, and the incompatibility of this antigen with that of a potential recipient.

In order to study the patterns of migration of human NPCs implanted into the injured spinal cord, these cells were transplanted close to traumatic spinal cord lesions performed in rats by clip compression or partial transection. A small fraction of the grafted NPCs had migrated, always confined to the white matter, several millimeters away from the site of implantation. There was no evidence of directed migration of grafted NPCs towards any of the two spinal cord lesions at six weeks after transplantation. The proportion of cells expressing nestin, GFAP or β-tubulin III, was similar at the graft center and among the cells that had migrated furthest, suggesting that their motility was not influenced by the degree of differentiation. In agreement, pre-differentiation of NPCs *in vitro*, achieved by inhibiting γ-secretase, did not affect migration of the grafted cells. Reduction of the extracellular chondroitin sulfate proteoglycans by chondroitinase ABC promoted migration of NPCs, demonstrating that although no evidence for homing of grafted NPCs was detected, extrinsic factors can affect their migration. In conclusion, implanted human NPCs mainly remained at the site of transplantation, a feature that may be beneficial for purposes of local neuroprotection and cell replacement following focal SCI.

LIST OF PUBLICATIONS

The present thesis is based on the following articles, which will be referred to in the text by their Roman numerals:

I. **Jing-Hua Piao**, Jenny Odeberg, Eva-Britt Samuelsson, Anders Kjældgaard, Scott Falci, Åke Seiger, Erik Sundström, and Elisabet Åkesson. Cellular composition of long-term human spinal cord- and forebrain-derived neurosphere cultures. J Neurosci Res., 2006, 84: 471-82

II. **Jing-Hua Piao**, Eva-Britt Samuelsson, Anders Kjældgaard, Scott Falci, Åke Seiger, Erik Sundström, and Elisabet Åkesson. Regional-dependent proliferative response to ciliary neurotrophic factor (CNTF) by human neural stem and progenitor cells (Submitted for publication).

III. Jenny Odeberg, **Jing-Hua Piao**, Eva-Britt Samuelsson, Scott Falci, and Elisabet Åkesson. Low immunogenicity of *in vitro*-expanded human neural cells despite high MHC expression. J Neuroimmunol. 2005, 161:1-11.

IV. **Jing-Hua Piao**, Lena Holmberg, Kioumars Delfani, Åke Seiger, Anders Kjældgaard, Scott Falci, Elisabet Åkesson and Erik Sundström. Migration of human neural precursor cells grafted to the injured spinal cord (Submitted for publication).

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
APC	Antigen presenting cells
bFGF	Basic fibroblast growth factor
BrdU	5-Bromo-2-deoxyuridine
CD	Clusters of differentiation
CNS	Central nervous system
CNTF	Ciliary neurotrophic factor
CNTFRα	Ciliary neurotrophic factor receptor α
CSPG	Chondroitin sulphate proteoglycan
CXCR4	CXC chemokine receptor 4
DIV	Days *in vitro*
EGF	Epidermal growth factor
GFAP	Glial fibrillary acidic protein
GRP	Glial-restricted precursor
HLA	Human leukocyte antigen
IFN-γ	Interferon-γ
IR	Immunoreactive
L	Lumbar
MHC	Major histocompatibility complex
NF	Neurofilament
NPC	Neural precursor cell
NRP	Neuronal-restricted precursor
PBMC	Peripheral blood mononuclear cells
PCNA	Proliferating cell nuclear antigen
SCI	Spinal cord injury
SD	Standard deviation
SDF-1α	Stromal cell-derived factor-1α
SSEA-4	Stage specific embryonic antigen-4
Tra-1-60	Tumor rejection antigen 1-60
Tra-1-81	Tumor rejection antigen 1-81

INTRODUCTION

TRAUMATIC SPINAL CORD INJURY (SCI)

The worldwide annual incidence of traumatic SCI has been reported to be 15 to 40 cases per million individuals (Sosa et al., 2005) and, today, approximately 90 million people suffer from SCIs of varying severity (Jackson et al., 2004; National Spinal Cord Injury Statistical Center, NSCISC, USA, 2006). The most frequent neurological category among SCI persons is incomplete tetraplegia (30%), followed by complete paraplegia, incomplete paraplegia and complete tetraplegia (NSCISC, 2006). The most common causes are traffic accidents (which account for 47% of the cases in the United States), falls, violence and occupational and sports-related injuries. In Sweden, the incidence is about 120 SCI individuals per year. The mean age at injury is approximately 30 years and 80–85% of the SCI patients are male (Holtz and Levi, 2006).

The destruction of neural tissue caused by traumatic SCI results in loss of sensory and motor functions, loss of reflex and autonomic activity, often loss of bowel and bladder control, pain sensation, abnormal sensitivity to thermal and tactile stimuli, muscle spasms and/or sexual dysfunction. Today, there are no clinical treatments available that can restore a normal sensorimotor function after SCI. In addition, most SCI patients develop secondary complications, such as decubitus ulcers, urinary tract infections, chronic pain and depression (Levi et al., 1995; Christensen and Hulsebosch, 1997; Cairns et al., 1996). At the same time, some patients undergo spontaneous functional improvement. Thus, one year after injury, the functions of 5–20% of the SCI patients initially diagnosed as clinically complete are improved to some degree (Tuszynski et al., 2007). The number of complete SCI individuals has also decreased from 70% in 1969 to 45% in 1993. This is most probably due to the development of more safe motor vehicles and improved medical care (Holtz and Levi, 2006).

THE PATHOPHYSIOLOGY OF TRAUMATIC SCI

Both the initial mechanical damage and secondary pathophysiological changes resulting from traumatic SCI lead to lesions in descending and ascending pathways as well as the intrinsic circuitry of the spinal cord, causing loss of sensory and motor functions (Tator et al., 1991; Tator, 1995; Quencer, 2002; Schwab, 2002).

The initial mechanical insult leads to hemorrhage at the site of injury, followed by intravascular thrombosis, vasospasm and loss of microcirculation and edema that result in pronounced local hypoxia over the course of several hours (Kobrine, 1975; Sandler and Tator, 1976; Ducker et al., 1978; Stokes and Garwood, 1982; Chu et al., 2002). This local hypoxia gives rise to an inflammatory response (see below), metabolic disturbances and energy depletion, primarily in the gray matter, while the white matter is relative spared, especially in the periphery of the spinal cord lesion (Wolman et al., 1965; Kwon et al., 2004). This phenomenon is a result of the fact that neurons in gray matter are critically dependent on oxygen and glucose.

Moreover, the primary injury also initiates a cascade of vascular, biochemical and cellular processes that contributes to both necrotic and apoptotic cell death of neurons, astrocytes and oligodendrocytes within the spinal cord, axonal degeneration and demyelination (Crowe et al., 1997; Beattie et al., 2000; Quencer, 2002; Warden et al., 2001). The secondary injury phase is initiated within a few minutes after the trauma and does not cease until after a few days.

After the initial ischemia, the spinal cord may undergo reperfusion associated with a significant increase in the level of free radicals derived from oxygen that can contribute to the secondary damage (Basu et al., 2001; Lewen et al, 2000). These free radicals can inactivate key enzymes in the mitochondrial respiratory chain and alter DNA and associated proteins, a combination of effects that induces metabolic collapse and subsequent necrosis and/or apoptosis (Cuzzocrea et al., 2001; Lewen et al., 2000). Furthermore, following SCI, excitatory neurotransmitters such as glutamate are released rapidly in excessive amounts. Activation of glutamate receptors (i.e., the N-methyl-D-aspartate (NMDA), alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) and kainate receptor) induces intracellular accumulation of sodium and calcium ions (Mody and McDonald, 1995). Such accumulation of calcium can initiate a cascade of events that end in cell death, including activation of lytic enzymes, lipid peroxidation, up-regulation of cyclooxygenase-2 and inhibition of the mitochondrial respiratory chain enzymes (Choi, 1987, 1988; Xiong et al., 2007). Oligodendrocytes are particularly vulnerable to excitotoxic cell death (Matute et al. 2001; Tekkok and Goldberg, 2001), probably due to the expression of Ca^{2+}-permeable AMPA receptors (Li and Stys, 2000; Park et al., 2003).

After the acute phase, the edema regresses and necrotic tissue elements are phagocytized, leaving a cavity in the spinal cord tissue (Beattie et al., 2002). Surviving astrocytes situated in the vicinity of the injury develop a reactive phenotype, producing

many fine processes. Astrocytic processes often interact closely with one another to produce gap and tight junctions. Together with invading meningeal fibroblasts, vascular endothelial cells, microglia and, sometimes, myelin debris, these reactive astrocytes and oligodendrocyte precursors form a glial scar (Perry and Gordon, 1991; Fawcett and Asher, 1999) (Fig. 1). The cells in glial scars express and secrete molecules that inhibit axonal growth (Fawcett and Asher, 1999; Silver and Miller, 2004).

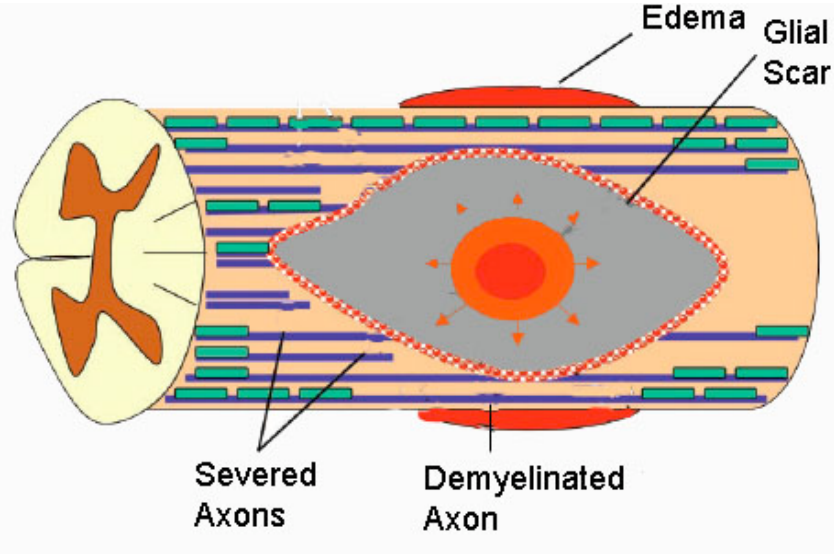


Fig. 1. Schematic illustration of pathological changes associated with traumatic SCI. The primary injury may result in tissue destruction (red circular region) and edema. The secondary injury spreads progressively and radially outward (orange circular region) until it reaches the final lesion size (gray area). This process involves neural cell death, axonal degeneration and demyelination. Finally, a cavity surrounded by a glial scar is formed. (Adapted from Hulsebosch, 2002).

INFLAMMATORY RESPONSES AFTER TRAUMATIC SCI

The inflammatory responses to a traumatic SCI involve both cellular components (e.g., neutrophils, macrophages and T cells recruited from the periphery as well as activated astrocytes and microglia), as well as non-cellular secreted components (e.g., cytokines, prostaglandins and complement proteins) (Popovich et al., 1997; Bethea et al., 2000; Kwon et al., 2004). These cellular components enter the injured cord at specific time intervals following injury. In the rodent, neurotrophils enter the injured spinal cord within 6 hours, while the recruitment of T-cells and blood-born monocytes/ macrophages (which phagocytize dead tissue) continues during the following days (Dusart and Schwab, 1994; Popovich et al., 1997; Yang et al., 2004). In both rodent and human situation, expression of the cytokines, interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF) by neurons and microglial cells is up-regulated within 30 min to

5 hours following SCI and has resided again two days later (Nakamura et al., 2003; Yang et al., 2004). At 14 days after SCI, contrasting results have been published. Low cytokine levels were reported, by some investigators (Lee et al., 2000; Nakamura et al., 2003), while another wave of up-regulation of IL-1β and TNF was reported by others (Pineau and Lacroix, 2007; Rice et al., 2007). In combination with adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and P-selectin, these cytokines mediate recruitment of immune cells to the site of injury (Klusman and Schwab, 1997; Farooque et al., 1999; Leskovar et al., 2000).

The inflammation that follows SCI may be both detrimental and beneficial. Macrophages and microglia, as well as cytokines, may promote tissue destruction and secondary necrosis by inducing the expression of additional cytokines and chemokines and elevating levels of nitric oxide and reactive oxygen (Zhang et al., 1997; Xu et al., 2001; Popovich et al., 2002). In contrast, during the later phase of the inflammatory processes, the activated macrophages and/or microglial cells exert a beneficial influence on neuronal regeneration, possibly by releasing appropriate cytokines and/or through interactions with other non-neuronal cells in the vicinity (Prewitt et al., 1997). Injection of autologous macrophages into the injured spinal cord of rats was reported to result in functional improvement (Schwartz et al., 1999). When introducing transplantation of autologous macrophages in the clinic, some SCI patients presented improvement (Knoller et al., 2005). However, Phase II trials are now halted, one reason being the difficulty to recruit patients within the set time window (Schwartz et al., Soc. Neurosci., 2007).

EXPERIMENTAL MODELS OF TRAUMATIC SCI

Experimental models are widely used to elucidate the pathophysiology of SCI, as well as the efficacy of various approaches to treatment. Obviously, to be of value, an *in vivo* model should accurately mimic at least some of the anatomical, biochemical and/or functional changes associated with traumatic SCI in humans. A feature shared by all SCI animal models is the loss of motor and sensory functions below the injury, to an extent that depends on the degeneration of descending and ascending tracts. A few of the numerous models developed in this context over the years are described below.

Contusion (impact) SCI: Traumatic SCI caused by the impact of a dropped weight was originally introduced as a model by Allen (Allen, 1911), and later modified by

Freeman and Weight (Freeman and Weight, 1953). Subsequently, other experimental techniques and devices, such as the NYU device and the PSI Infinite Horizon impactor, have been developed (Gruner et al., 1992; Rabchevsky et al., 2003). With this approach, a laminectomy is performed over the region of the spinal cord that is of interest, and then a defined weight is dropped from a specified height onto the exposed spinal cord or a rod controlled by a linear motor hits the spinal cord with a feed-back controlled force.

This results in tissue destruction with an evolving hemorrhage, ischemia and neurodegeneration with morphology and functional consequences similar to those observed in humans, including gliosis, demyelination and cyst formation. A moderate impact gives rise to relatively spared ventral funiculi that taper down laterally to a very thin rim in the dorsal funiculus (Basso et al., 1996). The size of the lesion (as determined by MRI) is correlated to the functional outcome in both human and rat (Metz et al., 2000). However, similar to other experimental SCI models, contusion impact on the spinal cord parenchyma is relatively localized, in contrast to the situation in humans, where several spinal segments are usually damaged. Furthermore, experimental contusion impact lasts for only a few milliseconds, whereas in human victims, the short impact is often followed by a prolonged compression of the cord, by displaced vertebrae.

Compression injury: Compression injury is achieved through the application of an aneurysm clip (with a jaw approximately 1 mm in width) or a wider pressure element (2-5mm) or by employing balloon inflation (Eidelberg et al., 1976; Rivlin and Tator, 1978; Khan and Griebel, 1983; Nyström et al., 1988; Martin et al., 1992; von Euler et al., 1997a). Such mechanical compression of the spinal cord leads to tissue destruction in addition to ischemia and subsequent neural degeneration. The compression injury results in a central cavity with loss of axons and demyelination in the spinal cord white matter (Fehlings and Tator, 1995; Nashmi and Fehlings, 2001). As assessed by the inclined plane and Tarlov methods, the changes in neurological function caused by compression injury are correlated to the severity of the injury (Fehlings and Tator, 1992; Nashmi et al., 1997). Compression with an aneurysm clip, which is widely used as an experimental model, always involves bilateral pressure on the spinal cord of the experimental animals, whereas dorsal pressure is most common in association with traumatic SCI in humans.

Transection injury: As an experimental model, the transection model is important for testing new treatment strategies designed to, in particular, evaluate axonal regeneration. It is not commonly used for studies on pathophysiological mechanisms. Experimental surgical transection of the spinal cord may be complete or partial, with disruption of descending and/or ascending pathways. Among other consequences, such transection results in denervation of the skeletal muscles involved (Carter et al., 1981) with progressive decreases in the somal size and in the numbers and lengths of dendrites of motor neurons affected (Kitzman, 2005). A total transection leads to loss of supraspinal and voluntary control over locomotor function with only an automatic and reflex driven ability to step or support weight with the hind limbs (Basso et al., 1996; Cheng et al., 1997). More long term, experimental animals develop knee flexion contractures (Moriyama et al., 2004). Complete transection of the spinal cord is presently uncommon in patients suffering from traumatic SCI, but the model simulates a complete lesion induced for example by knife violence.

Ischemia models: Models designed to mimic the ischemic phase of traumatic SCI have been developed for screening the possible neuroprotective effects of candidate drugs. Ischemia can be induced experimentally by occlusion of the posterior spinal vein or the distal aorta (Faden et al., 1984; Kaplan et al., 1987) as well as photochemically (Watson et al., 1986; von Euler et al., 1997b; Gaviria et al., 2002). In the latter case, an intravascular photochemical reaction is employed to produce vascular thrombosis that gives rise to ischemia and subsequent necrosis in the spinal cord tissue (Bunge et al., 1994), with the degree of functional deficit being correlated to degree of photo induction time (Prado et al., 1987). One advantage of this approach is that it does not require laminectomy, which means that this model can be used to study events, such as edema, influenced by the closed spinal compartment. Rat models of ischemic SCI demonstrate certain characteristics that resemble those associated with analogous human injuries, including edema formation, axonal degeneration and glial responses (von Euler et al., 1997b). The size and electrophysiological activity of such experimental lesions are closely related to loss of locomotor function and pain sensitivity (García-Alías et al., 2003). However, the model is not relevant for studies of pathophysiological mechanisms related to the biophysical consequences of the primary mechanical injury, which is most common among human patients.

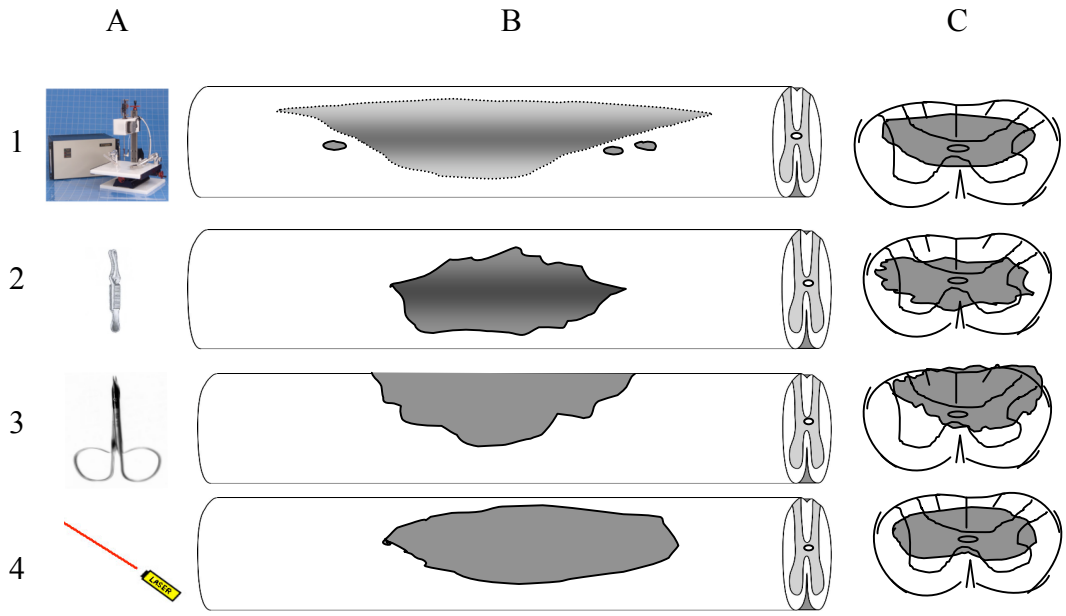


Fig. 2. A schematic illustration of four different types of SCI models. In column A) instruments employed to perform the respective lesions are shown. B) and C) indicate the affected spinal cord area (in gray). 1) A contusion injury can be performed using a weight drop device or a feed-back controlled device such as the PSI Infinite Horizon Impactor. The injury achieved mainly affects the dorsal white matter and the central gray matter. Lesion size and functional deficit depend upon the impact applied. 2) A compression lesion is achieved by employing a bilateral compression of the cord, using an aneurysm clip, bulldog clamp or similar, with a set closing pressure. A central lesion with an irregular shape is usually the result when using this lesion model. 3) A pair of eye scissors is often used to perform a transection lesion. An open, trough-shaped cavity is observed after a partial cut. 4) Irradiation by a laser in combination with an intravenous injection of a photochemically reactive dye may be employed to perform an ischemic injury in the spinal cord. This lesion model results in a smooth-shaped cyst affecting both white and gray matter. The output power of the laser and the exposure time are factors that determine the lesion size.

NEURAL PRECURSOR CELL (NPC) CULTURE AND EXPANSION

In order to replace lost cells and enhance regeneration, remyelination and functional recovery following SCI, various experimental strategies involving intraspinal cell transplantation have been employed (McDonald et al., 1999: Kato et al., 2000; Liu et al., 2000; Kohama et al., 2001; Akiyama et al., 2002; Murray et al., 2002; Windrem et al., 2004). For clinical application the neural cells must exhibit a stable karyotype and be readily available in adequate numbers. In addition, these potential donor cells should interact in an appropriate manner with the host, effectively mediate functional repair without triggering graft rejection by the immune system.

Neural stem and progenitor cells (in this thesis collectively referred to as neural precursor cells, NPCs) are potential donor cells for treatment of SCIs. These cells can

be isolated from the developing or adult central nervous system (CNS) and expanded in the form of free-floating neurospheres or as adherent cultures (sometimes with genetic modifications designed to achieve immortalization) in medium supplemented with growth factors (Reynolds and Weiss, 1992; Buc-Caron, 1995; Flax et al., 1998; Carpenter et al., 1999; Quinn et al., 1999; Bai et al., 2004; Villa et al., 2004) (Fig. 3).

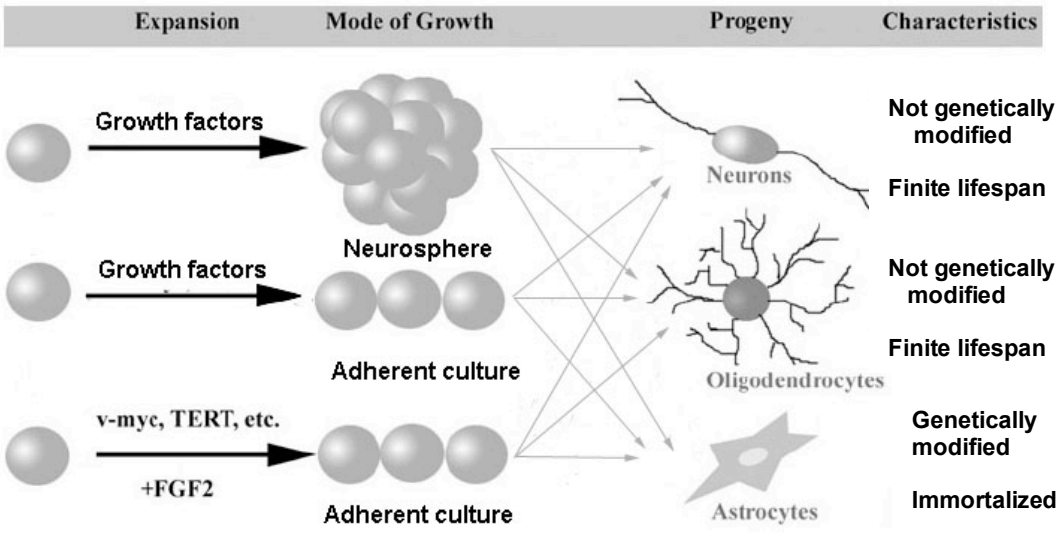


Fig. 3. Different strategies for culturing NPCs either as free-floating neurospheres or adherent cultures, with or without genetic modifications designed to achieve immortalization, in medium supplemented with growth factors. (Modified from Bithell and Williams, 2005).

Even though a finite lifespan is involved, culturing NPCs as neurospheres has proven to be an efficient means of expansion. Neurosphere cultures derived from human forebrain tissue remain multipotent and increase as much as 10^7-fold in number during a one-year period (Carpenter et al., 1999). Therefore, this approach is currently widely employed to obtain human NPCs for experimental transplantation (Akiyama et al., 2001; Imitola et al., 2004; Iwanami et al., 2005; Tarasenko et al., 2007).

In connection with attempts to repair the injured spinal cord, homotopic grafts of cells derived from the spinal cord may have advantages over implantation of cells derived from the brain. However, unlike the corresponding cultures derived from the forebrain, neurospheres originating from the spinal cord and grown in the presence of epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) have been reported to proliferate slowly and only for a short period of time *in vitro,* as well as to contain only cells of astrocytic lineage (Quinn et al., 1999; Jain et al., 2003). We previously found that additional supplementation of the medium with ciliary neurotrophic factor (CNTF) allows neurospheres derived from the human spinal cord to be expanded for at least 18 passages (223 days *in vitro*) with unaltered maintenance of

their ability to generate neurons, astrocytes and oligodendrocytes (Åkesson et al., 2007a). This discovery makes it possible to characterize such neurosphere cultures as a potential alternative source of donor cells for treatment of SCI.

THE INFLUENCE OF GROWTH FACTORS ON NPC CULTURE

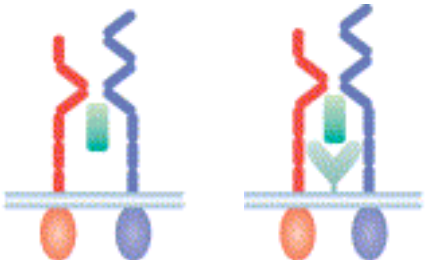
A variety of procedures for expansive culturing of NPCs from different species and developmental stages have been described. The growth factors employed most frequently in this context have been EGF in combination with bFGF (Reynolds and Weiss, 1992; Svendsen et al., 1998; Quinn et al., 1999; Akiyama et al., 2001) or together with bFGF and leukemia inhibitory factor (LIF) or CNTF (Carpenter et al., 1999; Tamaki et al., 2002; Åkesson et al., 2007a).

Growth factors can elicit significant epigenetic responses in NPCs. For instance, bFGF, which is often used together with EGF to expand both attached monolayers and free floating cultures of NPCs, not only acts as a neutral mitogen *in vitro*, but may also alter the developmental competence of the cells by influencing their dorso-ventral identity. Thus NPCs that would develop into neurons and either astrocytes or oligodendrocytes (but not both) *in vivo* may be influenced by bFGF to produce all three of these cell types *in vitro* (Gabay et al., 2003).

The cytokines CNTF and LIF are members of the interleukin-6 family (Sendtner et al., 1994; Ip, 1998). The signaling initiated by LIF involves heterodimerization of the LIF receptor-β (LIFRβ) with glycoprotein130 (gp130), while the CNTF receptor (CNTFR) complex contains these same two proteins together with CNTFR α (Fig. 4). The sequential asembly of the CNTF receptor complex involves the binding of CNTF to CNTFRα, followed by the recruitment of gp130 and LIFRβ (Ip and Yancopoulos, 1996).

LIF participates in the regulation of approximately 200 genes in human neural stem cells (Wright et al., 2003) and, like CNTF, is capable of exerting multiple effects. These factors promote neuronal survival and differentiation both *in vitro* and *in vivo* (Hagg and Varon, 1993; Sendtner et al., 1994; Ip, 1998) and enhance the proliferation and maturation of astrocytes (Bonni et al., 1997), and oligodendrocytes (Lachyankar et al., 1997). In addition, LIF and CNTF promote the maintenance of the pluripotentiality, and support the self-renewal of embryonic and adult mouse and human neural stem cells *in vitro* (Conover et al., 1993; Wolf et al., 1994; Shimazaki et al., 2001; Wright et al., 2003). Moreover, CNTF enhances the expansion of EGF-responsive neural stem

cells by suppressing their development into glial cells without directly affecting their survival (Shimazaki et al., 2001).



LIF receptor CNTF receptor

Fig. 4. Schematic illustration of the LIF and CNTF receptor complexes. Gp130 (shown in red) and LIFRβ (shown in blue) are components of both these receptors. The CNTF receptor also contains an additional, specific small subunit known as CNTF receptor-α (light green). (From Rose-John, 2002)

THE IMMUNOGENICITY OF HUMAN NPCs

Following incompatible transplantation to an injured spinal cord, the grafted cells may trigger host immune responses that result in graft rejection. Such induction of an immune response, involving proliferation and differentiation of naïve host T-cells, occurs when the human major histocompatibility complex (MHC) on antigen presenting cells (APCs) interacts with the T-cell receptor (TcR) complex as well as with a number of co-stimulatory proteins (including CD40, CD80 and CD86). In contrast, triggering of T-cells in the absence of accessory molecules may give rise to immunological unresponsiveness (Schwartz, 1990). Despite the presence of the blood–brain barrier and the low number of APCs and low level of MHC expression in the CNS, neural transplantation may elicit an immune response. Thus, following experimental implantation of neural allografts, both up-regulation of the expression of MHC and infiltration of effector cells into the graft area have been observed (Duan et al., 1995a, b, 1997; Cassiani-Ingoni et al., 2007), representing a potential risk for graft rejection.

The level of expression of MHC by neural cells derived from rodent striatum, and expanded as neurospheres for as many as three passages, remains low. However, when differentiation is evoked by exposure to cytokines (e.g., interferons), this level of expression is elevated in a GFAP-positive subpopulation of cells (McLaren et al., 2001). In addition, the expression of HLA class I molecules by human embryonic or neural stem cell cultures, which is normally low, is modestly up-regulated after differentiation. Even greater increase in HLA class I is seen upon exposure to interferon-γ (IFN-γ), TNF-α and/or IL-1β (Drukker et al., 2002; Kim et al., 2005; Lee et al., 2005). In contrast, it has also been suggested that NPCs down-regulate their expression of MHC upon differentiation *in vitro* (White et al., 1994; Modo et al., 2003).

When human neural stem/progenitor cells were xenotransplanted into traumatic brain lesions of rodents, the grafts were rapidly rejected, unless immunosuppression was employed, in which case the grafts survived for as long as 6 months (Wennersten et al., 2006). However, no significant difference in graft survival was observed with immunosuppressive treatment during 3 or 6 weeks (Al Nimer et al., 2004). Other investigations also reveal that transplantation of human neural xenografts, to rodents that are either immunosuppressed or immunoincompetent, allows long-term survival (Brundin et al., 1988; Åkesson et al., 1998; Tarasenko et al., 2007). With respect to xeno- or allografting of human NPCs into the injured spinal cords, the optimal duration or type of immunosuppression has not yet been determined.

EXPERIMENTAL THERAPY WITH NPCs FOLLOWING TRAUMATIC SCI

In order to ascertain the potential efficacy of treating SCIs with NPCs, numerous investigations involving experimental models have been designed. These studies have revealed that grafts of human NPCs derived from either fetal or adult CNS tissues and expanded *in vitro* can survive, differentiate and migrate in the injured spinal cords of rodents, reduce the size of the lesion, promote functional remyelination and improve recovery of functions (Akiyama et al., 2001; Iwanami et al., 2005; Cummings et al., 2006).

The survival of NPC grafts is, of course, essential to therapeutic efficacy. In addition to the immunological factors discussed above, this survival is influenced by procedures carried out prior to implantation, including donor cell preparation. Thus, neurospheres survive transplantation to a greater extent than implants consisting of single-cell suspensions (Johann et al., 2007), possibly due to that cells inside the spheres benefit from having neighboring cells releasing soluble factors, or the cell-cell contact itself. The microenvironment of the injured spinal cord has been suggested to be more supportive of grafted NPCs nine days after injury than in the initial acute phase after SCI. At 9 days following a SCI, cytokines were observed to be down-regulated (Nakamura et al., 2003). Furthermore, the survival of NPCs was significantly better following transplantation into sub-acute lesions than into more chronic lesions (8 weeks after the initial injury) (Karimi-Abdolrezaee et al., 2006).

A subpopulation of human NPCs grafted into the injured spinal cords of primates or mice remained undifferentiated, while some of these cells differentiated into neurons, astrocytes or oligodendrocytes (Iwanami et al., 2005; Cummings et al., 2005,

2006). In order to enhance differentiation of transplanted NPCs into neurons and/or glial cells, NPCs have been subjected to various treatments to modify their differentiation. In one such approach, culturing ("priming") of human neural stem cells in flasks coated with poly-D-lysine and medium supplemented with bFGF, heparin and laminin for 7 days prior to implantation enhanced both their survival and rates of differentiation into neurons and oligodendrocytes (Tarasenko et al., 2007). Human fetal NPCs primed *in vitro,* as above, and transplanted into rats deficient in motor neurons can differentiate into cholinergic motor neurons that integrate into spinal circuits and even send out axons to form neuromuscular junctions with peripheral muscles (Wu et al., 2002; Gao et al., 2005, 2007). As an alternative to treating NPCs, defined subsets of cells may be isolated and used for transplantation. Implantation of mixed populations of rodent neuronal-restricted precursor cells and glial-restricted precursor cells (NRPs/GRPs) has been reported to result in improved bladder and motor functions (Mitsui et al., 2005).

In connection with cell therapy of this nature, it is of considerable interest to characterize the donor cells with respect to phenotype, migratory capacity and migration pattern. Following both allo- and xeno-engraftment into the spinal cord of adult rats, both uninjured and those subjected to aneurysm clip compressions, NPCs migrate away from the site of implantation in both rostral and caudal directions (Karimi-Abdolrezaee et al, 2006; Lepore et al., 2006). However, following injection of murine NPCs into the dorsal or lateral column of the rat spinal cord, these cells were not detected in the ventral column, suggesting that they prefer to migrate along the rostro-caudal axis (Karimi-Abdolrezaee et al, 2006).

After transplantation to the injured rat CNS, both human and rat NPCs respond to chemotactic substances synthesized and released at the site of injury. The chemokine stem cell factor (SCF), stromal cell-derived factor-1α (SDF-1α) and sphingosine-1-phosphate (Sph-1-P) act via c-kit, the chemokine receptor-4 (CXCR4) and Sph-1-P receptors respectively, to regulate the migration of NPCs in both the embryonic and injured CNS (Erlandsson et al., 2004; Imitola et al., 2004; Sun et al., 2004; Kimura et al., 2007). Alternatively, the localization of cells could theoretically be determined by permanent gradients of chemotactic substances, an attractive, but perhaps unlikely mechanism by which the location of a lesion might be sensed by grafted cells. In addition to chemoattraction, transplanted cells were observed to be forced during injection along the rostro-caudal arrangement of glia and supporting tissue in the white matter of the spinal cord (Lu et al., 2006).

Additional extrinsic factors may influence the migration of NPCs in the injured spinal cord. A number of molecules in the extracellular matrix have been shown to inhibit axonal regeneration after SCI. One group of such molecules is the chondroitin sulphate proteoglycans (CSPGs). They constitute an important part of the glial scar (Höke, 2005), and pharmacological degradation of these substances by chondroitinase ABC has been reported to enhance regeneration and to potentiate transplant-mediated axonal remodeling (Kim et al., 2006). In addition to the effects on neurite growth, chondroitinase ABC has also been shown to enhance migration of Schwann cells and NPCs *in vitro* (Ikegami et al., 2005; Liu et al., 2006). Other neurite growth-inhibitory molecules such as Nogo may also affect NPC migration.

The migratory pattern of donor neural cells may also be affected by their cell-intrinsic properties. Alteration of the final phenotype attained by progenitor cells residing in the neuroepithelium of the developing CNS changes their migratory patterns (Lee and Pfaff, 2001). Clearly, further examination of the influence of both extrinsic and intrinsic factors on the fates of donor neural cells *in vivo* could provide valuable insights.

Demyelination contributes to the dysfunction associated with SCI (Bunge et al., 1993; Blight, 2002) and NPCs can promote remyelination of injured axons, as well as promote axonal regeneration and prevent further host cell death. Human NPCs can differentiate into cells of the oligodendrocyte lineage and contribute to remyelination (Cummings et al., 2005). Following injection of adult human NPCs into a demyelinated lesion in the dorsal column of the rat spinal cord (produced by X-ray irradiation and injection of ethidium bromide), remyelination and restoration of the conduction velocity of axons were observed (Akiyama et al., 2001). Remyelination induced by NPC engraftment may contribute to functional improvement in mice subjected to contusion SCI (Cummings et al., 2005).

Grafts of human NPCs have been reported to secrete neurotrophic factors, both *in vitro* and *in vivo*, including brain- and glial cell line-derived neurotrophic factors (Xiao et al., 2005; Gao et al., 2006). These growth factors are thought to prevent further secondary cell loss by counteracting perikaryal atrophy of axotomized neurons (Xiao et al., 2005) and, in addition, they support axonal growth. Following transplantation of NPCs, regenerating host axons cross the host-graft interface and more growth-associated protein-43-positive axons are detected in the lesion cavity than in injured animals not receiving grafts (Xiao et al., 2005). In addition to secreting neurotrophic factors, rodent GRP may also promote axon outgrowth by reducing extracellular levels

of proteoglycans and astrocytic scarring (Hill et al., 2004). However, to my knowledge no report of similar effects with human NPCs has been published.

CLINICAL CELL THERAPY FOR TRAUMATIC SCI

Clinical attempts to treat SCI by transplantation of human neural cells or tissues originating from the first trimester of gestation have already been performed. Grafts of fetal spinal cord tissue can survive long-term after implantation, even after immunosuppressive treatment is terminated (Falci et al., 1997; Wirth et al., 2001). Embryonic human spinal cord tissue can eliminate part of a post-traumatic cyst that had been progressing (Falci et al., 1997). Furthermore, transplantation of fetal neural or hematopoetic cells from 1 month to 6 years following SCI was reported to result in partial restoration of motor and sensory function in 11 of 15 patients (Rabinovich et al., 2003). The utilization of neural precursor cells derived from fresh fetal tissue as clinical allografts raises ethical concerns and imposes limitations on the number of cells that can be obtained. Prior to collection of appropriate human tissue, both written and verbal informed consent, based on clear and objective information concerning the material to be collected and its use, must obviously be obtained in addition to human ethical committee approval. In addition, such tissue may not be available at the time-point that is optimal for SCI treatment. Another disadvantage associated with the use of primary, non-cultured cells is the limited time available for the performance of safety tests and characterization of the donor cells prior to implantation. Such safety tests should include screening for the presence of infectious agents and quality control of the culture procedure (Sagen et al., 2003).

Although a few clinical trials involving the use of human NPCs expanded *in vitro* to treat SCI are presently being performed, few results have been published to date. Moviglia et al., (2006) described two patients with chronic SCI that received autologous grafts of transdifferentiated NPCs. The voluntary movements and potentials evoked by somatosensory stimuli improved in both of these patients. Safety evaluations were performed and no adverse effects observed.

The question, when it is time to move from experimental studies to the clinic is a challenge to answer. Whenever the step is taken, it is of utmost importance to have a valid clinical trial design with clear patient inclusion criteria, well-established assessment protocols and methods for evaluation of appropriate outcome measures (Lammertse et al., 2007; Steeves et al., 2007; Tuszynski et al., 2007).

AIMS OF THE PRESENT THESIS

The ultimate aim of the work described here was to obtain adequate numbers of human NPCs with characteristics suitable for therapeutic treatment of spinal cord injuries.

The specific aims were as follows:

- to characterize the influence of various factors – including the tissue of origin, gestational age and culture conditions – on the propagation of human NPCs *in vitro;*
- to examine specifically the effects of CNTF on cultures of human NPCs;
- to investigate the immunogenicity of human NPCs expanded *in vitro;*
- to study the pattern of migration of cultured human NPCs following their implantation into the injured spinal cord of adult rat.

MATERIALS AND METHODS

CELL AND TISSUE COLLECTION (PAPERS I-IV)

Human first trimester forebrain and spinal cord tissues (4.5–12 weeks of gestation) were collected in connection with routine elective abortions, with the informed consent of the pregnant women and the pre-approval of the Regional Human Ethics Committee in Stockholm, Sweden. The gestational age of the tissue samples was determined on the basis of anatomical landmarks, according to England (England, 1990). The forebrain, excluding the cortical anlage, and the spinal cord was dissected into DMEM: F12 medium (1:1, Life Technologies) and freed from meninges. The CNS tissue was disrupted mechanically into single-cell suspensions employing a glass-Teflon homogenizer.

Peripheral blood mononuclear cells (PBMCs) from healthy volunteer blood donors were used for immunological assays of mixed cultures (Paper III) and TERA-2 cells (human embryonic carcinoma cells; a kind gift from Prof. Lars Ährlund-Richter) were included as a positive control (Paper I) in the evaluation of Tra-1-60, Tra-1-81 and SSEA-4 immunocytochemistry.

EXPERIMENTAL ANIMALS (PAPER IV)

A total of 63 adult female athymic rats (HsdHan: RNU-rnu, Harlan, 170–200 g) were included in the thesis, after approval from the Regional Experimental Animal Ethics Committee, Huddinge Tingsrätt, Sweden. This immunoincompetent strain was used not to induce a host rejection response towards the xenografted human NPCs. The animals were housed three per cage, in ventilated cabinets in the isolated FAC unit, at Karolinska University Hospital, Huddinge. Controlled humidity (40%), temperature (22° C) and a 12-hour light/dark cycle were used in the room and the rats were provided access to autoclaved standard pellets and tap water *ad libitum* as well as wooden houses and paper tissue to enrich their environment in the cages.

NEUROSPHERE CULTURES (PAPERS I-IV)

For the establishment of neurosphere cultures, human forebrain and spinal cord tissue obtained at 4.5–12 and 4.5–9.5 weeks of gestation, respectively, were employed. Cultures derived from spinal cord tissue obtained at a later developmental stage do not expand well (Åkesson et al., 2007a). Cells were cultured at a density of 100,000–

150,000 cells/ml medium (DMEM: F12, 1:1; Life Technologies) supplemented with 0.6% glucose, 5 mM Hepes, heparin (2 μg/ml, Sigma), 1% N2-supplement (Life Technologies), EGF (20 ng/ml; R&D Systems), bFGF (20 ng/ml; R&D Systems) and CNTF (10 ng/ml; R&D Systems) to form free-floating neurospheres. Thereafter, the cells were incubated at 37° C with 5% CO_2. The neurospheres were passaged once every 7–25 days (depending on the rate of growth of each individual culture) by trituration, and subsequently re-cultured in fresh medium. In this thesis, long-term neurosphere culture refers to a culture that has been subjected to at least 5 passages.

DIFFERENTIATION OF HUMAN NEUROSPHERE CULTURES *IN VITRO* (PAPERS I AND IV)

Following mechanical dissociation of the neurospheres by trituration, the cells obtained were plated at a density of 26,000 cells/ml in DMEM: F12 (1:1) supplemented with 0.6% glucose, 5 mM Hepes, 1% N2-supplement and 10% fetal calf serum on circular glass cover slips (13 mm in diameter) coated with poly-D- lysine (0.1 mg/ml, Sigma) and fibronectin (0.1 mg/ml, Sigma) on 24-well plates (Nunc) for seven days.

In order to determine their potential for differentiation into oligodendrocytes, the dissociated neurospheres were stimulated to differentiate employing a slight modification of the protocol originally developed by Zhang and collaborators (Zhang et al., 2000). In brief, the cell suspensions were seeded as described above, except that the medium contained a lower concentration of fetal calf serum (0.5%) and was supplemented with recombinant human platelet-derived growth factor-AA (PDGF, 1 ng/ml; PeproTech Inc.). Control cells were cultured in the absence of PDGF and fresh medium was added every third day to all cultures. The cells were cultured in this manner for four weeks prior to fixation and immunocytochemical examination.

In paper IV, in order to initiate the differentiation of intact neurospheres *in vitro* prior to transplantation, the γ-secretase activity in the NPCs was inhibited by exposure to 1 μM *N*-[*N*-(3,5-difluorophenacetyl)-L-alanyl]-*S*-phenylglycine t-butyl ester (DAPT, EMD Biosciences, Inc.) for 24 hours.

IMMUNOCYTOCHEMISTRY (PAPERS I-IV)

Four individual cell and tissue preparations (a-d) were subjected to immunocytochemical evaluation employing the antibodies described in Table 1.

(a) Cytospin smears (Paper I): Cytospin smears of cell suspensions obtained by dissociation of fresh CNS tissue samples and neurospheres were prepared by centrifugation onto glass slides for 3 minutes at 800 rpm (Cytospin3, Shandon). In addition TERA-2 cells were included as a positive control. These cells were subsequently fixed with buffered 4% para-formaldehyde (pH 7.4) and then incubated with antibodies directed against Tra-1-60, Tra-1-81 or SSEA-4 (see Table 1). The nuclei were counterstained with DRAQ-5 (Alexis Biochemicals). The cells in five random fields/sample were counted using a 20 × objective lense.

(b) Sections of intact neurospheres (Papers I, II and III): Following fixation by immersion in 4% buffered para-formaldehyde (pH 7.4), the neurospheres were cryostat-sectioned at a thickness of 5 μm. These sections were incubated with primary antibodies against nestin, the proliferating cell nuclear antigen (PCNA), β-tubulin III, neurofilament (NF), glial fibrillary acidic protein (GFAP) or O4 (see Table 1). Some of the neurospheres were incubated with 10 μM bromodeoxyuridine (BrdU, Sigma) for 24 hours prior to fixation and in these cases, the sections were incubated with 1 M HCl for 20 minutes, followed by incubation with anti-BrdU in order to detect incorporation of this artificial nucleotide. The cell nuclei were counterstained with DRAQ5 and counting performed as described in (a).

(c) *In vitro* differentiated human NPCs (Paper I): After fixation with phosphate-buffered 4% para-formaldehyde (pH 7.4), differentiated cells were incubated with primary antibodies against nestin, β-tubulin III, GFAP or O4 and then counted as described in (a).

(d) Cross-sections of rat spinal cords with human NPC grafts (Paper IV): In this case, a 20-mm segment of the spinal cord was cryo-sectioned into a series of 10-μm sections for immunohistochemical processing. The human cells were identified by employing antisera against human nuclear protein (hNP). The sections were also stained immunohistochemically for nestin, GFAP, β-tubulin III, CXCR4 and SDF-1 (see Table 1) and the ratios of the number of cells expressing these proteins to those expressing hNP calculated after counting.

In all cases, incubation with primary antibodies was carried out overnight at +4° C, following which the sections or cells were rinsed with 0.1 M PBS. Detection of the primary antibodies was achieved by incubation with appropriate secondary antibodies conjugated with Alexa Fluor® 488 (1:400, Molecular Probe) or Cy3 (1:800, Jackson Immunoresearch Laboratories, Inc.). Negative control slides were processed in the

same manner, except that the primary antibodies were omitted. Immunofluorescence was assessed employing a fluorescence microscope (Zeiss, Axiophot) equipped with a CCD camera (Hamamatsu, ORCA-ER) and the Openlab software (Improvision), a Leica TCSSP2 confocal microscope and a Zeiss LSM510 Meta confocal microscope.

FLOW CYTOMETRY (PAPERS I AND III)

Aliquots (1×10^5 cells) of single-cell suspensions (1×10^6 cells/ml) derived from forebrain or spinal cord tissue, or from neurospheres were fixed with cytofix/cytoperm^{TM} Solution (Becton Dickinson Biosciences) for the subsequent detection of intracellular antigens. Following incubation with primary antibodies against nestin, PCNA, β-tubulin III or GFAP (see Table 1), the cells were washed in PBS and incubated with the appropriate conjugated secondary antibodies (described above). Separate samples incubated with control antibodies of the corresponding isotype were processed as negative controls. For the detection of membrane-bound antigens, unfixed cells were incubated with anti-CD133, - HLA class I, - HLA class II, -CD40, -CD80 and -CD86 antisera conjugated with phycoerythrin or fluorescein isothiocyanate (FITC) or the corresponding isotypic control (see Table 1). All of these samples were analyzed on a fluorescence-activated cell sorter (FACSort, Becton Dickinson) employing the Cell Quest software (Becton Dickinson Immunocytometry Systems).

IMMUNOBLOTTING (PAPER II)

Samples of spinal cord and forebrain tissue obtained at 4.5–12 weeks of gestation were homogenized in a lysis buffer containing 2 mM EDTA, 0.1% sodium dodecyl sulphate, 1% Triton X–100, complete proteinase inhibitor (Roche) and 50 mM Tris-HCl (pH 7.6). The proteins in duplicate or triplicate samples of these homogenates were separated by electrophoresis on a 10% Tris-glycine sodium dodecyl sulphate-polyacrylamide gel, transferred to a nitrocellulose filter and incubated thereafter with antibodies towards CNTFRα (see Table 1). A reference sample containing known amount of total protein was added to each gel for use as an internal standard. After detection of the primary antisera with anti-mouse immunoglobulin conjugated with horseradish peroxidase, the immunoblots were developed using the enhanced chemiluminescence (ECL^{+}) detection system (GE Health Care) and densitometric analysis carried out with the Quantity One 4.5.2 software (Bio-Rad).

Table 1. Summary of primary antibodies employed in the thesis.

Target antigen	Dilution	Isotype	Source	Employed in Paper
Tra-1-60	1:20	Mouse IgM	Chemicon	I
Tra-1-81	1:20	Mouse IgM	Chemicon	I
Stage specific embryonic antigen-4 (SSEA-4)	1:20	Mouse IgG1	Chemicon	I
CD133	1:20	Mouse IgG1	Miltenyi Biotec	I
Nestin	1:250	Mouse IgG1	Chemicon	I, II and III
	1:250	Rabbit IgG	Chemicon	IV
Proliferating cell nuclear antigen (PCNA)	1:20	Rabbit IgG	Santa Cruz Biotechnology	I
Bromodeoxyuridine (BrdU)	1:40	Rat IgG	Abcam	I and II
β-Tubulin III	1:800	Mouse IgG2b	Sigma	I
	1:1200	Rabbit IgG	Berkeley Antibody Company	I – IV
Pan neurofilament (NF)	1:1	Mouse IgG	Zymed Laboratories Inc.	I
Glial fibrillary acidic protein (GFAP)	1:20 000	Mouse IgG1	Sternberger Monoclonals Inc.	I, IV
	1:500	Rabbit IgG	DAKO	I, II and III
O4	1:50	Mouse IgM	Gift from Prof. S. E. Pfeiffer	I and II
CNTFRα	1:50	Mouse IgG	Becton Dickinson	II
HLA class I, phycoerythrin (PE)-conjugated	1:20	Mouse IgG2a	Dakopatts	III
HLA-DR, fluorescein isothiocyanate- (FITC) or PE-conjugated	1:20	Mouse IgG2a	Becton Dickinson	III
CD40, FITC-conjugated	1:20	Mouse IgG1	Becton Dickinson	III
CD80, FITC-conjugated	1:20	Mouse IgG1	Becton Dickinson	III
CD86, FITC-conjugated	1:20	Mouse IgG1	Becton Dickinson	III
Human nuclear protein (hNP)	1:250	Mouse IgG	Chemicon	IV
CXCR4	1:100	Mouse IgG	Chemicon	IV
SDF-1α	1:100	Mouse IgG	Abcam	IV

IMMUNOLOGICAL ASSAY (PAPER III)

After incubation with IFN-γ (200 U/ml, R&D systems) or vehicle alone for 3 days, human neurosphere cultures (passage 8–11) were dissociated into single-cell suspensions (0.5×10^6 cells/ml) in RPM medium containing 10% FCS. Aliquots containing 5×10^4 cells were subsequently inactivated by irradiation with 20 Gy

(Gammacell 2000; Molsgard, Denmark) and then co-cultured with 7×10^4 human PBMCs. As positive control, PBMCs were stimulated by co-culturing with irradiated PBMCs from another donor (producing a mixed lymphocyte culture, MLC). Five days after such stimulation, the cells were pulsed with 1 μCi methyl-^{3}H thymidine (Amersham, Life Science), harvested onto filters using a plate harvester (Harvester 996, Tomtec, Hamden, Connecticut, USA), and subjected to automated counting of radioactivity (with a 1450 MicroBeta Trilux, WALLAC, Sweden AB). The extent of proliferation was calculated as the mean level of radioactivity (in counts per minute) of triplicate samples.

SCI MODELS (PAPER IV)

Prior to surgery, the animals were administered atropine (0.05 mg/kg, NM Pharma AB) by intraperitoneal injection and anesthetized with rodent neurolept analgesia (fentanyl citrate (0.22 mg/kg) and fluanisone (6.75 mg/kg), Hypnorm, Jansen Pharmaceuticals) in combination with midazolam (3.38 mg/kg, Dormicum, Roche). Body temperature was maintained at 38° C throughout the surgery employing a heating pad controlled by a rectal thermometer (CMA/150, CMA/Microdialysis, Sweden). All of the rats received a subcutaneous injection of 6 ml Ringer-glucose solution both immediately before and after surgery.

Following laminectomy at the level of vertebra L1, the meninges were opened and a few drops of lidocain hydrochloride (20 mg/ml, AstraZeneca) applied on the spinal cord tissue. Partial transection was achieved by cutting the dorsal 3/4 of the spinal cord transversally. Alternatively, bilateral compression was produced by applying a bulldog clamp (5.8 cm in width, De Bakey) with a closing pressure of 35 g for 30 seconds. The urinary bladders of the animals were emptied manually 2 or 3 times each day, as required. During the initial 3-4 days after surgery, the rats were administered Temgesic (0.01 mg/kg) every 8-12 hours as analgesic treatment. Furthermore, in the event of signs of urinary infection Borgal was injected sc, 15 mg/kg per day.

TRANSPLANTATION OF NPCs DERIVED FROM HUMAN SPINAL CORD (PAPER IV)

Two weeks after performing the spinal cord lesions, neurospheres were implanted approximately 1 mm ventral to the exposed dorsal surface of the spinal cord, in all cases. In the case of partial transection, the injection was made immediately outside the

rostral or caudal end of the open cavity of the lesion. With the injuries produced by clip compression, the neurospheres were placed 7 mm rostral or caudal of the center of the lesion, which previous experience has shown to be just outside the lesion cavity.

TREATMENT WITH CHONDROITINASE ABC (PAPER IV)

Female athymic rats (HsdHan: RNU-rnu, 170–200 g, Harlan) were subjected to clip compression lesions at vertebral level L1, following which the neurospheres were injected immediately rostral to the site of the lesion. Thereafter, chondroitinase ABC was administered topically by placing a cube of gel foam (approximately 2.5 mm) that had been soaked in this enzyme (0.025 U dissolved in saline) on the dorsal surface of the injured spinal cord. In the case of the control rats, the gel foam was soaked in saline alone.

STATISTICAL ANALYSES (PAPERS I-IV)

Possible differences in the sizes of cell populations were examined for statistical significance employing analysis of variance (ANOVA, Papers I-IV) and whenever this approach revealed a significant difference, Scheffé's F test was performed *post hoc*. A Wilcoxon sign rank test was utilized for comparison of the numbers of cells in neurospheres cultured in the presence and absence of CNTF. Student's t-test was applied for comparison of CNTFRα protein levels in spinal cord and forebrain samples. In Paper I correlation analysis was used to examine the relationship between the size of the cell population and gestational age of the tissue of origin. In Paper II the relationship between levels of the CNTFRα protein and the gestational age of human spinal cord and forebrain tissue was examined utilizing both correlation and regression analyses.

All statistical analyses were performed with the Statview 4.12 software (Abacus Concepts Inc.). The data are presented as means ± standard deviations (SD) or medians and interquartile ranges. P-values of ≤ 0.05 were considered to be statistically significant.

RESULTS AND DISCUSSION

Cultures of free-floating neurospheres contain a heterogeneous population of human NPCs at different stages of differentiation, including immature, non-determined and proliferating cells, as well as cells that exhibit neuronal or glial phenotypes. There are several possible explanations for this cellular heterogeneity. For instance, different types of clone-forming stem/progenitor cells, exhibiting distinct developmental potentials, may be initially present in the neurospheres (Tropepe et al., 1999; Suslov et al., 2002). Moreover, the cells present in human neurospheres may respond differentially to growth factors present in the culture medium. For example, cells with varying responses to EGF and bFGF may be present in developing human tissue (Martens et al., 2000; Shih et al., 2001), which could influence the proliferative patterns of neural stem cells and thereby the cellular composition of neurospheres derived from such tissue. Recently, it has also been reported that neurospheres are highly dynamic structures prone to form chimeric neurospheres through fusion (Singec et al., 2006), which also may contribute to the cellular heterogeneity of individual neurospheres.

Since SCI results in the loss of a number of different types of cells, heterogeneous populations of cells demonstrating both neuronal and glial potential should ideally be utilized in the replacement treatment of such injuries. Accordingly, expansion of neural cells *in vitro* in a manner designed to yield multiple types of cells would be of value. For clinical usage, large numbers of such cells are required, the production of which entails the use of prolonged periods of *in vitro* expansion. Consequently, both intrinsic properties of human NPCs, as well as extrinsic factors present in the environments provided by the culture vessel and the intraspinal location in the host following transplantation, may exert a highly significant influence on the potential usefulness of human NPCs as donor cells in treating SCI. Below, I discuss some of the factors that we believe play more or less important roles in this context.

SIGNIFICANCE OF THE SOURCE OF THE NPCs (PAPERS I, II AND III)

Neurosphere cultures derived from the human first trimester forebrain and spinal cord contain similar proportions of cells expressing Tra 1-60, Tra 1-81 and SSEA-4 (proteins expressed by embryonic stem cells (Thomson et al., 1998; Draper et al., 2002)), CD133 (enriched in neurospheres-forming cells (Uchida et al., 2000)), nestin (neural progenitor cells) and PCNA (proliferating cells), indicating the presence of

comparable numbers of neural stem and progenitor cells in such cultures. Indeed, we found a similar pattern in cell suspensions obtained by dissociation of neurospheres originating from both forebrain and spinal cord tissue, as long as the gestational age of the fetuses was less than 12 and 9.5 weeks, respectively. These observations indicate that under the cell culture conditions we employ, immature NPCs originating from the spinal cord and forebrain proliferate and expand in a similar manner. Accordingly, not only is this procedure useful for *in vitro* propagation of NPCs from both of these tissues, but it also provides an opportunity to compare the therapeutic effects of cells from these two sources in experimental models.

The immunological HLA proteins are also expressed to similar degrees in neurosphere cultures derived from the spinal cord and forebrain, subjected to the same number of passages. In most cases, both types of culture express almost no co-stimulatory molecules CD40, CD80 and CD86. Interactions between APCs and naive T-cells involve the MHC and T-cell receptors, as well as co-stimulatory molecules and their receptors. Accordingly, the theoretical risk of recognition by host T-cells following intraspinal transplantation appear to be similar for NPCs derived from the spinal cord and forebrain.

In contrast, we did observe differences between glial and neuronal subpopulations present in neurosphere cultures originating from different regions of the fetal CNS. In the first place, during culture the number of cells that express β-tubulin III decreases in the case of spinal cord neurospheres, but remains unchanged in corresponding cultures derived from forebrain. In my opinion, this difference reflects the more mature state of the spinal cord during the first trimester, which means that this tissue most probably contains a larger population of cells that express β-tubulin III prior to culturing and thereby has a larger number of such cells to lose than forebrain cultures.

A second difference observed here is that after a relatively large number of passages, spinal cord neurospheres contain more cells that immunostain positively for GFAP than do forebrain neurospheres cultured under identical conditions. In agreement with this finding is the report that NPCs derived from different regions of the brain during the first trimester of intrauterine development also exhibit varying patterns of growth and differentiation that may arise from the molecular influences present in their regions of origin (Ostenfeld et al., 2002; Parmar et al., 2002; Jain et al., 2003; Kim et al., 2006). Also consistent with these observations is the finding that following transplantation, neural stem/progenitor cells originating from the rat forebrain were more prone to differentiate into neurons, at the expense of oligodendrocytes, than were

the corresponding cells derived from the spinal cord (Watanabe et al., 2004). This is a further indication that the region of origin in the CNS may influence the fate of NPCs following transplantation into a host.

Despite these differences, when forebrain and spinal cord neurosphere cultures are dissociated and differentiated *in vitro* in our hands, they generated similar numbers of neurons, astrocytes and oligodendrocytes. Thus, the NPCs in these cultures exhibit similar degrees of multipotency and, furthermore, the factors present in the differentiation medium exert important influences on the outcome. Together, these observations indicate that as long as the appropriate extrinsic milieu is provided, the multipotent potential of spinal cord neurospheres is as large as that of forebrain neurospheres.

The ability of factors in the microenvironment to direct the differentiation of NPCs involves region-specific alterations in gene expression. Interestingly, the levels of regional transcription factors in neurospheres may be regulated when co-cultured with slices from other CNS regions (Kim et al., 2006). Such findings suggest that the host microenvironment may alter the properties of NPCs in a region-specific manner. In this connection, further characterization of the fate of human NPCs following implantation into the injured spinal cord is required.

We reported previously that cells isolated from the forebrain at gestational ages older than 9.5 weeks demonstrate a considerably greater ability to form neurospheres and undergo long-term expansion *in vitro* than cells derived from the spinal cord at the same stage of fetal development (Åkesson et al., 2007a). This difference may reflect the presence of significantly fewer proliferating cells in the more mature spinal cord at these time-points in development.

In Paper II we also observed that the level of expression of CNTFRα is higher in spinal cord tissue than in the forebrain at 4.5–8 weeks of gestation. Furthermore, CNTF enhances the proliferation and expansion of spinal cord neurospheres obtained during this same embryonic period, but not of the corresponding neurospheres formed from forebrain tissue. This difference may reflect a higher sensitivity to and dependence on CNTF in the spinal cord during embryonic stages of development and/or the involvement of other factors in the proliferation of forebrain NPCs.

SIGNIFICANCE OF THE DEVELOPMENTAL STAGE (PAPERS I, II and III)

In connection with the present investigations we posed the question as to whether the gestational age of the tissue of origin influences the cellular composition of human neurosphere cultures. It has been speculated previously that gestational age affects at least certain properties of neurospheres propagated *in vitro*. For instance, the embryonic age influences the manner in which mouse neural stem cells proliferate in response to EGF and FGF (Tropepe et al., 1999), suggesting that different classes of neurosphere-forming cells are present at different stages of intrauterine development.

As mentioned above, human spinal cord cells derived from fetal tissue at a gestational age of more than 9.5 weeks are not able to undergo long-term expansion in the form of neurospheres. One reason for this, also discussed above, is that the number of proliferating cells present in spinal cord tissue at later stages of gestation is low (Åkesson et al., 2007a). Another possibility is that the few spinal cord NPCs present at these stages require factors in the culture medium that have not yet been identified.

However, in Paper I, we document the fact that the cellular composition of neurospheres derived from the fetal forebrain at 5–12 weeks of gestational age or from spinal cord at a gestational age of less than 9.5 weeks are not affected by the gestational age (one example shown in Fig. 5). Paper II describes both regional differences in the response to CNTF and in expression of the CNTFRα protein (as determined by immunoblotting) during embryonic development. The level of CNTFRα in the spinal cord is stable during the first trimester, whereas the corresponding level in the forebrain changes during this same period, a difference that may be related to the different responses to CNTF. Thus, after 9 weeks of gestation, the level of CNTFRα in the forebrain increases with gestational age, and neurospheres derived from this region at 9–9.5 weeks of gestational age are responsive to CNTF (Carpenter et al., 1999). In contrast, gestational age had no influence on the levels of expression of class I or II HLA or co-stimulatory factors in human neurospheres (Paper III).

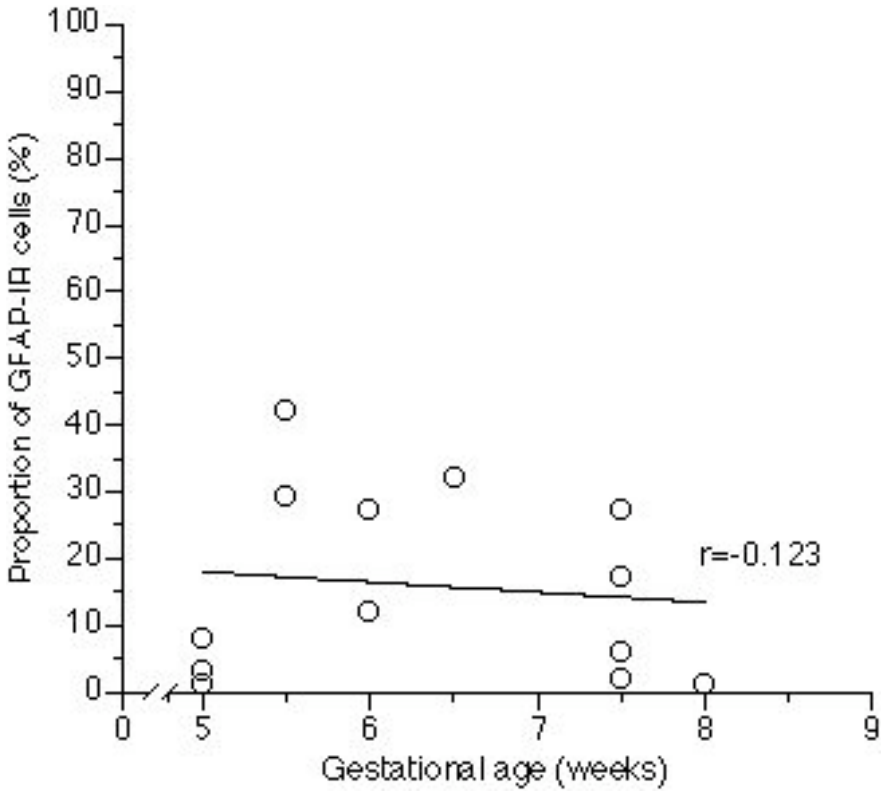


Fig. 5. Correlation analysis of the proportion of cells that express GFAP (GFAP-immunoreactive (IR) cells) in neurospheres in connection with their first passage and the gestational age of the human spinal cord tissue from which these neurospheres originate. This graph, which is representative of all of the correlation analyses performed, reveals a lack of linear correlation. r, correlation coefficient.

SIGNIFICANCE OF TIME IN CULTURE (PAPERS I AND III)

The level of cells that express Tra-1-60, Tra-1-81 and SSEA-4 in human neurospheres at passage 5 is similar to that in their corresponding donor tissue (i.e. 0.3–0.5% of the total cell number), suggesting that a small, but stable population of highly immature cells is maintained during expansion *in vitro*. This stable population of Tra-1-60-, Tra-1-81- and SSEA-4-positive cells ensures the sustained expansion and proliferation of the neurosphere cultures. Cells that express SSEA-4 and/or CD133 are known to initiate the formation of neurospheres. For example, SSEA-4^+/$CD133^+$ cells and SSEA-4^-/$CD133^+$ cells are 7- and 2-fold more effective, respectively, at initiating such formation as a general population of NPCs (Barraud et al., 2007).

The level of the CD133 protein in human neurospheres increases with the length of the culture period. For example, the proportion of CD133-positive cells increased from approximately 6% in dissociated tissue prior to culture to 31% in neurospheres at passage 10, while the proportions of cells expressing nestin and PCNA in these same

neurospheres remained stable. A lack of correlation between the number of CD133-positive cells and proliferating PCNA-positive cells has also been reported by others (Hilbe et al., 2004), suggesting that not all cells that express CD133 are undergoing proliferation and/or that some of these cells proliferate slowly. The stable populations of immature NPCs present in both human spinal cord and forebrain neurospheres following expansion *in vitro* provide a long-term source of donor NPCs that may be cryo-banked and later thawed for experimental and clinical purposes.

In the case of spinal cord neurospheres, the number of GFAP-positive cells increased, while the proportion of cells expressing β-tubulin III was decreased following five passages, but no such alterations were observed in the case of forebrain neurospheres. However, a majority of the cells that expressed GFAP also were stained positively for nestin. In response to LIF, mouse NPCs from the ganglionic eminence up-regulate their expression of GFAP and, in addition, exhibit an enhanced capacity to generate neurospheres (Pitman et al., 2004). This observation suggests that cells in which LIF has induced the expression of GFAP, or at least a subset of these cells, are immature. This is probably the case with our CNTF-treated cultures as well and could explain why spinal cord neurospheres that have been subjected to 1 or 10 passages demonstrate similar capacities to generate neurons and astrocytes upon differentiation *in vitro*, despite the elevated number of GFAP-positive cells present in the neurospheres that have undergone a large number of passages. Therefore, it appears that spinal cord neurospheres after either one or five passages and forebrain neurospheres that have been subjected to five passages can all provide donor NPCs for cell therapy.

Our examination of the expression of HLA class I and II proteins in neurospheres revealed that almost no cells in freshly dissociated spinal cord and forebrain tissue express these proteins, but that following a single passage of the corresponding neurospheres, the proportion of HLA class I -positive cells increases to 65% and 47%, respectively. Furthermore, following ten passages, most of the cells in spinal cord and forebrain neurospheres stained positively for this protein. In addition, the level of expression by each individual cells (as indicated by the fluorescence intensity detected in connection with flow cytometric analysis) is higher after this number of passages.

Class II HLA is expressed by the same neurosphere cells that express the class I protein and, thus, the level of the class II molecule is elevated after five passages. In contrast, Ubiali and co-workers (Ubiali et al., 2007) observed only low levels of class I HLA and could detect no HLA class II in human fetal forebrain neurospheres. This

apparent discrepancy may result from the presence of CNTF in our, but not their culture medium, since leukemia inhibitory factor (LIF) was reported to enhance the expression of class I MHC (Wright et al., 2003). Interestingly, this latter regulation is quite dynamic and withdrawal of LIF for only 9 days considerably attenuates the proportion of cells that express class I MHC (Wright et al., 2003). Therefore, I propose that after human spinal cord neurospheres have been expanded successfully, CNTF should be eliminated from the culture medium to see whether this leads to down-regulation of HLA class I and II and, consequently, perhaps reduced risk for graft rejection. One should remember, however, that MHC proteins are involved in maintaining synaptic plasticity, and possibly also in regenerative processes (Huh et al., 2000; Oliveira et al., 2004), so that down-regulation of their expression may interfere with these processes.

IMMUNOGENICITY OF HUMAN NPCs (PAPER III)

In order to examine the immunogenicity of human NPCs expanded *in vitro,* these cells were isolated following 8-11 passages, treated with IFN-γ and subsequently co-incubated with human peripheral lymphocytes. Treatment with IFN-γ enhances the expression of both HLA class I and II by NPCs, without affecting their levels of CD40 and CD86. However, with or without stimulation by IFN-γ, human NPCs did not elicit lymphocyte proliferation, i.e., exhibited a low degree of immunogenicity even when they expressed HLA at a high level.

This low degree of immunogenicity demonstrated by human neurosphere cultures probably reflects the presence of immature or non-functional HLA proteins on the cell surface, the small number of co-stimulatory proteins present, and/or inadequate functioning of these proteins. T-cells may become unresponsive and even prevent the activation of neighboring T-cells when MHC presentation occurs without complete and functional co-stimulatory signaling (Lombardi et al., 1994, for review see Dong and Benveniste, 2001). Another possible explanation is failure by antigen-presenting human NPCs to express other cell surface molecules required for allo-antigen-induced proliferation. As I alluded to above, expression of HLA by human NPCs may also serve purposes other than the promotion of immunological responses.

However, the absence of T-cell proliferation in our co-culture system does not definitely exclude the possibility that NPCs may elicit an immune response *in vivo* (New et al., 2004), where the microenvironment contains a variety of factors that might

activate the donor NPCs, especially during an on-going inflammatory response following SCI. Xeno-transplantation of human NPCs to immunocompetent Harlan Sprague-Dawley rats has been employed in an attempt to determine whether the degree of injury, the amount of time elapsed since the injury and the number of passages that the donor NPCs have undergone, affect the risk for rejection (Åkesson et al., 2007b). Various regimens of immunosuppressive treatment were also tested in this investigation. With no immunosuppressive treatment, numerous xenografted human NPCs were rejected but short-term treatment with cyclosporine allowed human NPCs to survive. Of course, there are significant difficulties involved in drawing conclusions regarding human allo-cell therapy from such an experimental xeno-model. There is a discrepancy between allo- and xenografts concerning both antigen recognition and effector mechanisms. Xenografts are predominantly recognized by the indirect pathway and rejected via a delayed hypersensitivity-like response, while allografts are predominantly recognized by the direct pathway and rejected by cytotoxic lymphocyte responses (Brevig et al., 2000).

In *in vitro* allograft studies, a wide variety of responses by human peripheral blood lymphocytes to human NPCs have been reported. For instance, when peripheral blood lymphocytes from eight donors were incubated with a NPC line, only four of the lymphocyte samples demonstrated a positive allo-response. In addition, no correlation between differences in the HLA loci of the donors of either the peripheral blood lymphocytes or the NPCs, and the degree of response by a mixed lymphocyte population has been observed (Ubiali et al., 2007). Therefore, in connection with future clinical practice, it would seem advisable to test the response of the host peripheral blood lymphocytes to a number of human NPC lines in order to choose a line that is as compatible as possible for cell therapy.

SIGNIFICANCE OF THE HOST ENVIRONMENT ON MIGRATION OF TRANSPLANTED NPCs (PAPER IV)

Six weeks after transplantation into lesions caused either by clip compression or partial transection, we found that only a small fraction of the grafted human NPCs had migrated a few millimeters from the site of implantation, while most of these cells remained in the center of the graft. Neither of the applied SCI models resulted in a preferential migration of the NPCs towards or away from the lesion cavity. In these experiments, we chose to inject whole neurospheres. Due to the size of the neurospheres, we could safely assume that the neurospheres were deposited at the site

of injection, and that cells found elsewhere participated in active migration. This way we avoided the risk of a pressure injection phenomena observed by Lu and co-workers (Lu et al., 2006). They found that injection of cell suspensions into the injured spinal cord forced transplanted cells along the rostro-caudal arrangement of glia and supporting tissue during the injection itself, giving a false impression of migration.

A number of studies involving transplantation of brain tissue have reported indications of selective migration of stem or progenitor cells towards different types of lesions (Imitola et al., 2004; Sun et al., 2004; Kimura et al., 2007). As a possible mechanism, chemoattractants (such as SDF-1α) secreted by surviving or invading cells in the vicinity of an ischemic injury may direct the migration of CXCR4-positive NPCs toward the ischemic core or penumbral zone (Imitola et al., 2004; Sun et al., 2004). In our experiment, the proportion of cells that expressed CXCR4 was higher among the cells that migrated than among those remaining at the graft center, suggesting that although CXCR4-expressing cells did not preferentially migrate towards the SCI, these cells are more prone to migrate.

In our system expression of SDF-1α in the injured spinal cord could not be detected immunohistochemically 6 weeks after transplantation, a time-point that corresponded to no less than 8 weeks after establishment of the experimental SCI. SDF-1α was detected in the injured mouse and rat spinal cord (Takeuchi et al., 2007 a and b). In these rodent traumatic SCI models, SDF-1α was expressed transiently 7 days after the injury, with very low levels being detected 3 and 10 days post-lesion. These observations suggest that very little or no SDF-1α may be expressed at the time-point that we chose for analysis (i.e., 8 weeks post-injury) and that the levels of this protein present at the time of transplantation (2 weeks post-injury) were too low to attract the NPCs. Therefore, directed migration (homing) of NPCs grafted into a SCI during its subacute or chronic stage may be limited. This suggests that cell therapy at these later time-points should be directed to the site of the lesion or at least close to, in order to optimize rescue, repair and replacement of the cells of the spinal cord.

We also found that the proportions of cells expressing nestin, GFAP or β-tubulin III at the center of the graft and among the cells that had migrated furthest were similar. This observation suggests that immature NPCs are not more prone to migrate than are committed glial and neuronal precursors or that the immature NPCs first migrated and then differentiated within the six-week experimental period. Pre-differentiation of NPCs by exposure to DAPT, an inhibitor of γ-secretase that promotes neuronal

differentiation *in vitro* (Kostyszyn et al., manuscript in preparation), had no effect on the migration of NPCs grafted into the lesioned spinal cord (Paper IV). These observations are in agreement with the findings by Englund and co-workers (Englund et al., 2002) that the cells that migrate demonstrate the same degree of differentiation as those remaining at the core of the graft. Together, these observations suggest that the degree of differentiation of grafted NPCs does not influence their migration.

SIGNIFICANCE OF CHONDROITINASE ABC TREATMENT ON THE MIGRATION OF HUMAN NPCs (PAPER IV)

Degradation of the CSPGs produced in response to SCI with chondroitinase ABC increased the distance of NPC migration. This illustrates how cell-extrinsic factors present in the host tissue microenvironment can influence the movement of these cells. The CSPG class of proteins, which are located in the extracellular matrix, constitutes important components of glial scars in the CNS (Höke, 2005) and are known to inhibit axonal growth (Davies et al., 2004). Our data shows that these proteins also inhibit cell migration *in vivo*. *In vitro*, the spread of NPCs from neurospheres across a plastic surface is reduced in the presence of CSPG (Ikegami et al., 2005). Another inhibitor of neurite outgrowth, Nogo was also found to inhibit migration of Nogo-66 receptor (NgR) expressing cells by preventing membrane protrusion (Su et al., 2007). However, to my knowledge, there are no reports on the occurrence of Nogo receptors in NPCs or on the effects of Nogo-blocking drugs on NPC migration.

The utilization of extrinsic factors such as chondroitinase ABC may help counteract the effects of glial scarring and enhances the migration of donor NPCs in connection with cell therapy during the subacute stages of SCI.

CONCLUSIONS

- Variations in the gestational age within the time periods of 4.5–12 weeks in the case of the forebrain and 4.5–9.5 weeks for the spinal cord do not alter the proportions of cells with different phenotypes that are present in the neurosphere cultures derived from these regions under the present conditions.

- The proportions of cells expressing Tra-1-60, Tra-1-81, SSEA-4 and nestin, as well as the fraction of proliferating cells remain stable during long term neurosphere culture when the forebrain and spinal cord is the source of origin.

- The proportion of cells expressing CD133 increases, with time *in vitro,* in human forebrain- and spinal cord-derived neurosphere cultures.

- In spinal cord cultures the proportion of cells expressing GFAP increases, while the relative number of cells that express β-tubulin III decreases with time, but no such changes are observed in forebrain cultures. Despite this difference, *in vitro* differentiation of NPCs derived from both types of cultures produces similar proportions of neurons, astrocytes, and oligodendrocytes.

- The presence of CNTF (in addition to EGF and bFGF) in the culture medium enhances the rates of proliferation and expansion of NPCs in spinal cord neurospheres, without significantly altering the proportions of cells that express nestin, GFAP, β-tubulin III or O4 in these same cultures. In contrast, CNTF exerts no such influence on NPCs derived from forebrain regions at this same gestational age. The otherwise low capacity of human spinal cord NPCs for long-term expansion *in vitro* is significantly improved in the presence of CNTF, allowing comparison of homo-and heterotopic therapy with human neural stem cells in animal models of SCI.

- The immunogenicity of human neural cells expanded for a long period *in vitro* is low, despite their relatively high expression of HLA, and there is no major difference between NPCs derived from the spinal cord and forebrain, in this respect.

- Human NPCs that are transplanted into lesions in the spinal cords of rats two weeks after SCI virtually all remain at the site of transplantation, a feature that is beneficial for purposes of local neuroprotection and/or cell replacement at a focal traumatic lesion. Among the subpopulation that migrates from the implantation site, there is no evidence for a selective migration towards or away from the spinal cord lesion. The proportions of cells expressing nestin, GFAP or β-tubulin III are similar at the center of the graft and among the cells that migrate furthest. Inhibition of γ-secretase in order to achieve pre-differentiation of NPCs *in vitro* has no effect on their migratory properties, whereas digestion of extra cellular proteoglycans containing chondroitin sulfate by chondroitinase ABC, enhances this migration.

FUTURE PERSPECTIVES

The destruction of neural tissue caused by SCI may result in severe and lifelong functional deficits. The endogenous capacity for repair of such damage is limited and, despite the advances made in modern healthcare, the relatively high prevalence of health problems related to SCI highlights both the increased vulnerability after such injuries and the lack of effective treatment. Clearly, treatment of and rehabilitation after SCI must involve a battery of potential interventions that can be tailored to meet the needs of each individual patient. Cell therapy is one such intervention strategy. Experimental models indicate that cell therapy has the potential to rescue, replace and repair injured neural tissue by supporting regeneration and remyelination (Fig. 6). However, answers to a number of questions are still of importance when making the transition from laboratory to clinical conditions.

What factors most effectively "rescue, replace and repair" in a particular type of lesion? Are allografted cells originating from embryonic stem cells with pluripotency most effective in this respect? Or will efficacy be higher using the as-yet-immature, but already committed neural precursors in the neural tube of the embryo? Perhaps regulated activation of the patient's own adult stem cells will produce the optimal outcome?

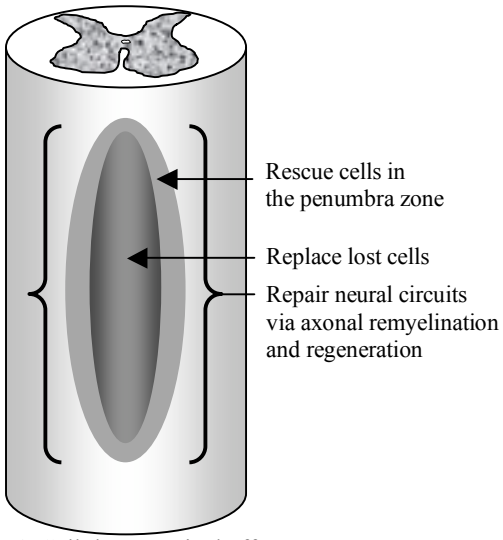


Fig. 6. Cell therapeutical effects to aim for in SCI (oval shape).

The research described here has focused on human NPCs derived from the developing neural tube of the embryo. These cells are already committed to becoming functioning neural cells and there are methods available for expanding them that do not require additional feeder cells. Our present findings help achieve long-term expansion of both forebrain- and spinal cord-derived neurospheres *in vitro,* thereby allowing us to perform studies designed to identify the cell type(s) that integrate with and function optimally in the injured spinal cord.

Such *in vitro* expansion is necessary both in order to increase the number of potential donor cells and to make these cells available at the time-point(s) when they can be used most beneficially to treat the patient. Identification of this/these time-point(s) is, of course, crucial. Within what timeframe must a SCI be treated with cell therapy in order to achieve rescue and repair? How many cells will need to be

implanted in the patient to achieve restorative effects similar to those observed in rodent models? For neuroprotection and rescue, early intervention appears to be extremely important. On the other hand, spontaneous functional improvement is often observed in the injured patient, especially soon after injury, and cell therapy must not jeopardize this process, including the regression of edema and inflammation, recovery from the so-called initial "spinal shock", and other processes that contribute to the compensatory mechanisms.

The findings in this thesis indicate that factors such as the length of the period of culture, the protocol employed for *in vitro* expansion and the tissue of origin of human fetal neural cells may influence cell characteristics. We do not yet know whether these factors are of significance for the therapeutic effects. Screening prior to both experimental and clinical usage would be of benefit in order to obtain comparable data for development of a reproducible and reliable cell therapy. This screening should include the possible presence of biological contaminants, cellular phenotypes and immunogenicity of the potential donor cells themselves.

To answer the question as to what cells actually produce the beneficial effects in rodent models of SCI, it will be necessary to test the effects of different cell populations in the same model, experiments that we are performing. Our present cultures contain only small numbers of oligodendrocyte precursor cells. If they prove to be necessary for counteracting the demyelination associated with SCIs, methods to culture these cells have to be developed.

Another issue of significance for the patient that has been touched upon here is whether immunosuppression is a necessary component of human allograft cell therapy. Following expansion *in vitro,* human NPCs express MHC proteins at high levels, but nonetheless have a low degree of immunogenicity, at least *in vitro*. However, preliminary data on xenograft models involving human NPCs derived from spinal cord tissue indicate that immunosuppression is necessary in this setting, but that short-term immunosuppression is sufficient to assure graft survival. When large numbers of NPC samples become available in biobanks, it may be possible to avoid less favorable matches between donor and host. In the future, populations of potential donor NPCs may be screened *in vitro* for immunocompatibility with the host in order to reduce the risk of rejection and immunosuppressive drugs may be administered for a short period until the blood- brain barrier is repaired.

With regard to where to implant cells for most effective treatment of SCI, we observed that in our experimental model most of the grafted NPCs remain at the site of

implantation. Therefore, for purposes of neuroprotection and replacement, we suggest transplantation directly into the site of the lesion, but a systematic comparison of different transplantation sites would be necessary for a definite answer to this question.

In summary, the work described in this thesis provides additional insight into the culturing of human NPCs, as well as into the *in vitro* and *in vivo* characteristics of these cells that are relevant to the repair of SCIs. It is my hope and belief that this information will help design more effective strategies for the reconstitution of tissue structure and function following SCIs.

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REFERENCES

Åkesson E, Kjaeldgaard A and Seiger A (1998) Human embryonic spinal cord grafts in adult rat spinal cord cavities: survival, growth, and interactions with the host. Exp Neurol. 149, 262-276.

Åkesson E, Piao JH, Samuelsson EB, Holmberg L, Kjældgaard A, Falci S, Sundström E and Seiger Å (2007a) Long-term culture and neuronal survival after intraspinal transplantation of human spinal cord-derived neurospheres. Physiol Behav. 92, 60-66.

Åkesson E, Odeberg J, Piao JH, Holmberg L, Samuelsson EB, Anderson PH, Falci S and Frykman S (2007b) Rejection response of xenografted human neural stem cells of relevance for spinal cord injury repair. Soc. Neurosci., Abstract 601.19.

Akiyama Y, Honmou O, Kato T, Uede T, Hashi K and Kocsis JD (2001) Transplantation of clonal neural precursor cells derived from adult human brain establishes functional peripheral myelin in the rat spinal cord. Exp Neurol. 167, 27-39.

Akiyama Y, Radtke C, Honmou O and Kocsis JD (2002) Remyelination of the spinal cord following intravenous delivery of bone marrow cells. Glia. 39, 229-236.

Al Nimer F, Wennersten A, Holmin S, Meijer X, Wahlberg L and Mathiesen T (2004) MHC expression after human neural stem cell transplantation to brain contused rats. Neuroreport. 15, 1871-1875.

Allen AR (1911) Surgery of experimental lesion of spinal cord equivalent to crush injury of fracture dislocation of spinal column. JAMA. 9, 878-880.

Bai Y, Hu Q, Li X, Wang Y, Lin C, Shen L and Li L (2004) Telomerase immortalization of human neural progenitor cells. Neuroreport. 15, 245-249.

Barraud P, Stott S, Møllgård K, Parmar M and Björklund A (2007) In vitro characterization of a human neural progenitor cell coexpressing SSEA4 and CD133. J Neurosci Res. 85, 250-259.

Basso DM, Beattie MS and Bresnahan JC (1996) Graded histological and locomotor outcomes after spinal cord contusion using the NYU weight-drop device versus transection. Exp Neurol. 139, 244-256.

Basu S, Hellberg A, Ulus AT, Westman J and Karacagil S (2001) Biomarkers of free radical injury during spinal cord ischemia. FEBS lett. 508, 36-38.

Beattie MS, Farooqui AA and Bresnahan JC (2000) Review of current evidence for apoptosis after spinal cord injury. J Neurotrauma. 17, 915-925.

Beattie MS, Hermann GE, Rogers RC and Bresnahan JC (2002) Cell death in models of spinal cord injury. Prog Brain Res. 137, 37-47.

Bethea JR (2000) Spinal cord injury-induced inflammation: a dual-edged sword. Prog Brain Res. 128, 33-42.

Bithell A and Williams BP (2005) Neural stem cells and cell replacement therapy: making the right cells. Clin Sci. 108, 13-22.

Blight AR (2002) Miracles and molecules–progress in spinal cord repair. Nat Neurosci. 5, Suppl., 1051-1054.

Bonni A, Sun Y, Nadal-Vicens M, Bhatt A, Frank DA, Rozovsky I, Stahl N, Yancopoulos GD and Greenberg ME (1997) Regulation of gliogenesis in the central nervous system by the JAK-STAT signaling pathway. Science. 278, 477-483.

Brevig T, Holgersson J and Widner H (2000) Xenotransplantation for CNS repair: immunological barriers and strategies to overcome them. Trends Neurosci. 23, 337-344.

Brundin P, Strecker RE, Widner H, Clarke DJ, Nilsson OG, Astedt B, Lindvall O and Björklund A (1988) Human fetal dopamine neurons grafted in a rat model of Parkinson's disease: immunological aspects, spontaneous and drug-induced behavior, and dopamine release. Exp Brain Res. 70, 192-208.

Buc-Caron MH (1995) Neuroepithelial progenitor cells explanted from human fetal brain proliferate and differentiate in vitro. Neurobiol Dis. 2, 37-47.

Bunge MB, Holets VR, Bates ML, Clarke TS and Watson BD (1994) Characterization of photochemically induced spinal cord injury in the rat by light and electron microscopy. Exp Neurol. 127, 76-93.
Bunge RP, Puckett WR, Becerra JL, Marcillo A and Quencer RM (1993) Observations on the pathology of human spinal cord injury. A review and classification of 22 new cases with details from a case of chronic cord compression with extensive focal demyelination. Adv Neurol. 59, 75-89.
Cairns DM, Adkins RH and Scott MD (1996) Pain and depression in acute traumatic spinal cord injury: origins of chronic problematic pain? Arch Phys Med Rehab. 77, 329-335.
Carpenter MK, Cui X, Hu ZY, Jackson J, Sherman S, Seiger Å and Wahlberg LU (1999) In vitro expansion of a multipotent population of human neural progenitor cells. Exp Neurol. 158, 265-278.
Carter JG, Sokoll MD and Gergis SD (1981) Effect of spinal cord transection on neuromuscular function in the rat. Anesthesiology. 55, 542-546.
Cassiani-Ingoni R, Muraro PA, Magnus T, Reichert-Scrivner S, Schmidt J, Huh J, Quandt JA, Bratincsak A, Shahar T, Eusebi F, Sherman LS, Mattson MP, Martin R and Rao MS (2007) Disease progression after bone marrow transplantation in a model of multiple sclerosis is associated with chronic microglial and glial progenitor response. J Neuropathol Exp Neurol. 66, 637-649.
Cheng H, Almström S, Giménez-Llort L, Chang R, Ove Ogren S, Hoffer B and Olson L (1997) Gait analysis of adult paraplegic rats after spinal cord repair. Exp Neurol. 148, 544-557.
Choi DW (1987) Ionic dependence of glutamate neurotoxicity. J Neurosci 7, 369-379.
Choi DW (1988) Calcium-mediated neurotoxicity: relationship to specific channel types and role in ischemic damage. Trends Neurosci. 11, 465-469.
Christensen MD and Hulsebosch CE (1997) Chronic central pain after spinal cord injury. J Neurotrauma. 14, 517-537.
Chu D, Qiu J, Grafe M, Fabian R, Kent TA, Rassin D, Nesic O, Werrbach-Perez K and Perez-Polo R (2002) Delayed cell death signaling in traumatized central nervous system: hypoxia. Neurochem Res. 27, 97-106.
Conover JC, Ip NY, Poueymirou WT, Bates B, Goldfarb MP, DeChiara TM and Yancopoulos GD (1993) Ciliary neurotrophic factor maintains the pluripotentiality of embryonic stem cells. Development. 119, 559–565.
Crowe MJ, Bresnahan J., Shuman SL, Masters JN and Beattie MS (1997) Apoptosis and delayed degeneration after spinal cord injury in rats and monkeys. Nat Med. 3, 73-76.
Cummings BJ, Uchida N, Tamaki SJ and Anderson AJ (2006) Human neural stem cell differentiation following transplantation into spinal cord injured mice: association with recovery of locomotor function. Neurol Res. 28, 474-481.
Cummings BJ, Uchida N, Tamaki SJ, Salazar DL, Hooshmand M, Summers R, Gage FH and Anderson AJ (2005) Human neural stem cells differentiate and promote locomotor recovery in spinal cord-injured mice. Proc Natl Acad Sci U S A. 102, 14069-14074.
Cuzzocrea S, Riley DP, Caputi AP and Salvemini D (2001) Antioxidant therapy: a new pharmacological approach in shock, inflammation, and ischemia/reperfusion injury. Pharmacol Rev. 53, 135-159.
Davies JE, Tang X, Denning JW, Archibald SJ and Davies SJ (2004) Decorin suppresses neurocan, brevican, phosphacan and NG2 expression and promotes axon growth across adult rat spinal cord injuries. Eur J Neurosci. 19, 1226-1242.
Dong Y and Benveniste EN (2001) Immune function of astrocytes. Glia. 36, 180-190.
Draper JS, Pigott C, Thomson JA and Andrews PW (2002) Surface antigens of human embryonic stem cells: changes upon differentiation in culture. J Anat. 200, 249-258.
Drukker M, Katz G, Urbach A, Schuldiner M, Markel G, Itskovitz-Eldor J, Reubinoff B, Mandelboim O and Benvenisty N (2002) Characterization of the expression of MHC proteins in human embryonic stem cells. Proc Natl Acad Sci U S A. 99, 9864-9869.

Duan WM, Brundin P and Widner H (1997) Addition of allogeneic spleen cells causes rejection of intrastriatal embryonic mesencephalic allografts in the rat. Neuroscience. 77, 599-609.
Duan WM, Widner H and Brundin P (1995a) Temporal pattern of host responses against intrastriatal grafts of syngeneic, allogeneic or xenogeneic embryonic neuronal tissue in rats. Exp Brain Res. 104, 227-242.
Duan WM, Widner H, Frodl EM and Brundin P (1995b) Immune reactions following systemic immunization prior or subsequent to intrastriatal transplantation of allogeneic mesencephalic tissue in adult rats. Neuroscience. 64, 629-641.
Ducker TB, Salcman M, Lucas JT, Garrison WB and Perot PL Jr (1978) Experimental spinal cord trauma, II: Blood flow, tissue oxygen, evoked potentials in both paretic and plegic monkeys. Surg Neurol. 10, 64-70.
Dusart I and Schwab ME (1994) Secondary cell death and the inflammatory reaction after dorsal hemisection of the rat spinal cord. Eur J Neurosci. 6, 712-724.
Eidelberg E, Staten E, Watkins JC, McGraw D and McFadden C (1976) A model of spinal cord injury. Surg Neurol. 6, 35-38.
Englund U, Bjorklund A and Wictorin K (2002) Migration patterns and phenotypic differentiation of long-term expanded human neural progenitor cells after transplantation into the adult rat brain. Brain Res Dev Brain Res. 134, 123-141.
Erlandsson A, Larsson J, and Forsberg-Nilsson K (2004) Stem cell factor is a chemoattractant and a survival factor for CNS stem cells. Exp Cell Res. 301, 201-210.
Faden AI, Jacobs TP, Smith MT and Zivin JA (1984) Naloxone in experimental spinal cord ischemia: dose-response studies. Eur J Pharmacol. 103, 115-120.
Falci S, Holtz A, Akesson E, Azizi M, Ertzgaard P, Hultling C, Kjaeldgaard A, Levi R, Ringden O, Westgren M, Lammertse D and Seiger A (1997) Obliteration of a posttraumatic spinal cord cyst with solid human embryonic spinal cord grafts: first clinical attempt. J Neurotrauma. 14, 875-884.
Farooque M, Isaksson J and Olsson Y (1999) Improved recovery after spinal cord trauma in ICAM-1 and P-selectin knockout mice. Neuroreport 10, 131-134.
Fawcett JW and Asher RA (1999) The glial scar and central nervous system repair. Brain Res Bull. 49, 377-391.
Fehlings MG and Tator CH (1992) The effect of direct current field polarity on recovery after acute experimental spinal cord injury. Brain Res. 579, 32-42.
Fehlings MG and Tator CH (1995) The relationships among the severity of spinal cord injury, residual neurological function, axon counts, and counts of retrogradely labeled neurons after experimental spinal cord injury. Exp Neurol. 132, 220-228.
Flax JD, Aurora S, Yang C, Simonin C, Wills AM, Billinghurst LL, Jendoubi M, Sidman RL, Wolfe JH, Kim SU and Snyder EY (1998) Engraftable human neural stem cells respond to developmental cues, replace neurons, and express foreign genes. Nat Biotechnol. 16, 1033-1039.
Freeman L and Wright T (1953) Experimental observations of concussion and contusion of the spinal cord. Ann Surg. 137, 433-443.
Gabay L, Lowell S, Rubin LL and Anderson DJ (2003) Deregulation of dorsoventral patterning by FGF confers trilineage differentiation capacity on CNS stem cells in vitro. Neuron. 40, 485-499.
Gao J, Coggeshall RE, Chung JM, Wang J and Wu P (2007) Functional motoneurons develop from human neural stem cell transplants in adult rats. Neuroreport. 18, 565-569.
Gao J, Coggeshall RE, Tarasenko YI and Wu P (2005) Human neural stem cell-derived cholinergic neurons innervate muscle in motoneuron deficient adult rats. Neuroscience. 131, 257-262.
Gao J, Prough DS, McAdoo DJ, Grady JJ, Parsley MO, Ma L, Tarensenko YI and Wu P (2006) Transplantation of primed human fetal neural stem cells improves cognitive function in rats after traumatic brain injury. Exp Neurol. 201, 281-292.
García-Alías G, Verdú E, Forés J, López-Vales R and Navarro X (2003) Functional and electrophysiological characterization of photochemical graded spinal cord injury in the rat. J Neurotrauma. 20, 501-510.

Gaviria M, Haton H, Sandillon F and Privat A (2002) A mouse model of acute ischemic spinal cord injury. J Neurotrauma. 19, 205-221.
Gruner JA (1992) A monitored contusion model of spinal cord injury in the rat. J Neurotrauma. 9, 123-128.
Hagg T and Varon S (1993) Ciliary neurotrophic factor prevents degeneration of adult rat substantia nigra dopaminergic neurons in vivo. Proc Natl Acad Sci U S A. 90, 6315-6319.
Hilbe W, Dirnhofer S, Oberwasserlechner F, Schmid T, Gunsilius E, Hilbe G, Wöll E and Kähler CM (2004) CD133 positive endothelial progenitor cells contribute to the tumor vasculature in non-small cell lung cancer. J Clin Pathol. 57, 965-969.
Hill CE, Proschel C, Noble M, Mayer-Proschel M, Gensel JC, Beattie MS and Bresnahan JC (2004) Acute transplantation of glial-restricted precursor cells into spinal cord contusion injuries: survival, differentiation, and effects on lesion environment and axonal regeneration. Exp Neurol. 190, 289-310.
Holtz A and Levi R (2006) Ryggmärgsskador- behandling och rehabilitering. Studentlitteratur.
Höke A (2005) Proteoglycans in axonal regeneration. Exp Neurol. 195, 273-277.
Hulsebosch CE (2002) Recent advances in pathophysiology and treatment of spinal cord injury. Adv Physiol Educ. 26, 238-255.
Ikegami T, Nakamura M, Yamane J, Katoh H, Okada S, Iwanami A, Watanabe K, Ishii K, Kato F, Fujita H, Takahashi T, Okano HJ, Toyama Y and Okano H (2005) Chondroitinase ABC combined with neural stem/progenitor cell transplantation enhances graft cell migration and outgrowth of growth-associated protein-43-positive fibers after rat spinal cord injury. Eur J Neurosci. 22, 3036-3046.
Imitola J, Raddassi K, Park KI, Mueller FJ, Nieto M, Teng YD, Frenkel D, Li J, Sidman RL, Walsh CA, Snyder EY and Khoury SJ (2004) Directed migration of neural stem cells to sites of CNS injury by the stromal cell-derived factor 1alpha/CXC chemokine receptor 4 pathway. Proc Natl Acad Sci U S A. 101, 18117-18122.
Ip NY (1998) The neurotrophins and neuropoietic cytokines: two families of growth factors acting on neural and hematopoietic cells. Ann NY Acad Sci. 840, 97-106.
Ip NY and Yancopoulos GD (1996) The neurotrophins and CNTF: two families of collaborative neurotrophic factors. Ann Rev Neurosci. 19, 491–515.
Iwanami A, Kaneko S, Nakamura M, Kanemura Y, Mori H, Kobayashi S, Yamasaki M, Momoshima S, Ishii H, Ando K, Tanioka Y, Tamaoki N, Nomura T, Toyama Y and Okano H (2005) Transplantation of human neural stem cells for spinal cord injury in primates. J Neurosci Res. 80, 182-190.
Jackson AB, Dijkers M, Devivo MJ and Poczatek RB (2004) A demographic profile of new traumatic spinal cord injuries: change and stability over 30 years. Arch Phys Med Rehab. 85, 1740-1748.
Jain M, Armstrong RJ, Tyers P, Barker RA and Rosser AE (2003) GABAergic immunoreactivity is predominant in neurons derived from expanded human neural precursor cells in vitro. Exp Neurol. 182, 113-123.
Johann V, Schiefer J, Sass C, Mey J, Brook G, Krüttgen A, Schlangen C, Bernreuther C, Schachner M, Dihné M and Kosinski CM (2007) Time of transplantation and cell preparation determine neural stem cell survival in a mouse model of Huntington's disease. Exp Brain Res. 177, 458-470.
Kaplan BJ, Gravenstein N, Friedman WA, Blackmore J and Curran M (1987) Thoracic aortic occlusion: somatosensory evoked potential monitoring and neurologic outcome in a canine model. Surg Neurol. 28, 351-360.
Karimi-Abdolrezaee S, Eftekharpour E, Wang J, Morshead CM and Fehlings MG (2006) Delayed transplantation of adult neural precursor cells promotes remyelination and functional neurological recovery after spinal cord injury. J Neurosci. 26, 3377-3389.
Kato T, Honmou O, Uede T, Hashi K and Kocsis JD (2000) Transplantation of human olfactory ensheathing cells elicits remyelination of demyelinated rat spinal cord. Glia. 30, 209-218.

Khan M and Griebel R (1983) Acute spinal cord injury in the rat: comparison of three experimental techniques. Can J Neurol Sci. 10, 161-165.
Kim HT, Kim IS, Lee IS, Lee JP, Snyder EY and Park KI (2006) Human neurospheres derived from the fetal central nervous system are regionally and temporally specified but are not committed. Exp Neurol. 199, 222-235.
Kim JY, Kim D, Choi I, Yang JS, Lee DS, Lee JR, Kang K, Kim S, Hwang WS, Lee JS and Ahn C (2005) MHC expression in a human adult stem cell line and its down-regulation by hCMV US gene transfection. Int J Biochem Cell Biol. 37, 69-78.
Kimura A, Ohmori T, Ohkawa R, Madoiwa S, Mimuro J, Murakami T, Kobayashi E, Hoshino Y, Yatomi Y and Sakata Y (2007) Essential roles of sphingosine 1-phosphate/S1P1 receptor axis in the migration of neural stem cells toward a site of spinal cord injury. Stem cells. 25, 115-124.
Kitzman P (2005) Alteration in axial motoneuronal morphology in the spinal cord injured spastic rat. Exp Neurol. 192, 100-108.
Klusman I and Schwab ME (1997) Effects of pro-inflammatory cytokines in experimental spinal cord injury. Brain Res. 762, 173-184.
Knoller N, Auerbach G, Fulga V, Zelig G, Attias J, Bakimer R, Marder JB, Yoles E, Belkin M, Schwartz M and Hadani M (2005) Clinical experience using incubated autologous macrophages as a treatment for complete spinal cord injury: phase I study results. J Neurosurg Spine. 3, 173-181.
Kobrine AI (1975) The neuronal theory of experimental traumatic spinal cord dysfunction. Surg Neurol. 3, 261-264.
Kohama I, Lankford KL, Preiningerova J, White FA, Vollmer TL and Kocsis JD (2001) Transplantation of cryopreserved adult human Schwann cells enhances axonal conduction in demyelinated spinal cord. J Neurosci. 21, 944-950.
Kostyszyn B, Cowburn RF, Samuelsson EB, Seiger Å, Kjældgaard A, Falci S and Sundström E (2007) The role of Notch-signaling in differentiation of human stem/progenitor cells. Manuscript in preparation.
Kwon BK, Tetzlaff W, Grauer JN, Beiner J and Vaccaro AR (2004) Pathophysiology and pharmacologic treatment of acute spinal cord injury. Spine. 4, 451-464.
Lachyankar MB, Condon PJ, Quesenberry PJ, Litofsky NS, Recht LD and Ross AH (1997) Embryonic precursor cells that express Trk receptors: induction of different cell fates by NGF, BDNF, NT-3, and CNTF. Exp Neurol. 144, 350-360.
Lammertse D, Tuszynski MH, Steeves JD, Curt A, Fawcett JW, Rask C, Ditunno JF, Fehlings MG, Guest JD, Ellaway PH, Kleitman N, Blight AR, Dobkin BH, Grossman R, Katoh H, Privat A and Kalichman M; International Campaign for Cures of Spinal Cord Injury Paralysis (2007) Guidelines for the conduct of clinical trials for spinal cord injury as developed by the ICCP panel: clinical trial design. Spinal Cord. 45, 232-242.
Lee EM, Kim JY, Cho BR, Chung WK, Yoon BW, Kim SU, Lee BC, Hwang WS, Moon SY, Lee JS and Ahn C (2005) Down-regulation of MHC class I expression in human neuronal stem cells using viral stealth mechanism. Biochem Biophys Res Commun. 326, 825-835.
Lee SK and Pfaff SL (2001) Transcriptional networks regulating neuronal identity in the developing spinal cord. Nat Neurosci. 4, 1183-1191.
Lee YL, Shih K, Bao P, Ghirnikar RS and Eng LF (2000) Cytokine chemokine expression in contused rat spinal cord. Neurochem Int. 36, 417-425.
Lepore AC, Walczak P, Rao MS, Fischer I and Bulte JW (2006) MR imaging of lineage-restricted neural precursors following transplantation into the adult spinal cord. Exp Neurol. 201, 49-59.
Leskovar A, Moriarty LJ, Turek JJ, Schoenlein IA and Borgens RB (2000) The macrophage in acute neural injury: changes in cell numbers over time and levels of cytokine production in mammalian central and peripheral nervous systems. J Exp Biol. 203, 1783-1795.
Levi R, Hultling C and Seiger Å (1995) The Stockholm Spinal Cord Injury Study: 2. Associations between clinical patient characteristics and post-acute medical problems. Paraplegia. 33, 585-594.

Lewen A, Matz P and Chan PH (2000) Free radical pathways in CNS injury. J Neurotrauma. 17, 871-890.
Li S and Stys PK (2000) Mechanisms of ionotropic glutamate receptor-mediated excitotoxicity in isolated spinal cord white matter. J Neurosci. 20, 1190-1198.
Liu J, Chau CH, Liu H, Jang BR, Li X, Chan YS and Shum DK (2006) Upregulation of chondroitin 6-sulphotransferase-1 facilitates Schwann cell migration during axonal growth. J Cell Sci. 119, 933-942.
Liu S, Qu Y, Stewart TJ, Howard MJ, Chakrabortty S, Holekamp TF and McDonald JW (2000) Embryonic stem cells differentiate into oligodendrocytes and myelinate in culture and after spinal cord transplantation. Proc Natl Acad Sci U S A. 97, 6126-6131.
Lombardi G, Sidhu S, Batchelor R and Lechler R (1994) Anergic T cells as suppressor cells in vitro. Science. 264, 1587-1589.
Lu P, Yang H, Culbertson M, Graham L, Roskams AJ and Tuszynski MH (2006) Olfactory ensheathing cells do not exhibit unique migratory or axonal growth-promoting properties after spinal cord injury. J Neurosci. 26, 11120-11130.
Martens DJ, Tropepe V and van Der Kooy D (2000) Separate proliferation kinetics of fibroblast growth factor-responsive and epidermal growth factor-responsive neural stem cells within the embryonic forebrain germinal zone. J Neurosci. 20, 1085-1095.
Martin D, Schoenen J, Delree P, Gilson V, Rogister B, Leprince P, Stevenaert A and Moonen G (1992) Experimental acute traumatic injury of the adult rat spinal cord by a subdural inflatable balloon: methodology, behavioral analysis, and histopathology. J Neurosci Res. 32, 539-550.
Matute C, Alberdi E, Domercq M, Perez-Cerda F, Perez-Samartin A and Sanchez-Gomez MV (2001) The link between excitotoxic oligodendroglial death and demyelinating diseases. Trends Neurosci. 24, 224-230.
McDonald JW, Liu XZ, Qu Y, Liu S, Mickey SK, Turetsky D, Gottlieb DI and Choi DW (1999) Transplanted embryonic stem cells survive, differentiate and promote recovery in injured rat spinal cord. Nat Med. 5, 1410-1412.
McLaren FH, Svendsen CN, Van der Meide P and Joly E (2001) Analysis of neural stem cells by flow cytometry: cellular differentiation modifies patterns of MHC expression. J Neuroimmunol. 112, 35-46.
Metz GA, Curt A, van de Meent H, Klusman I, Schwab ME and Dietz V (2000) Validation of the weight-drop contusion model in rats: a comparative study of human spinal cord injury. J Neurotrauma. 17, 1-17.
Mitsui T, Shumsky JS, Lepore AC, Murray M and Fischer I (2005) Transplantation of neuronal and glial restricted precursors into contused spinal cord improves bladder and motor functions, decreases thermal hypersensitivity, and modifies intraspinal circuitry. J Neurosci. 25, 9624-9636.
Modo M, Mellodew K and Rezaie P (2003) In vitro expression of major histocompatibility class I and class II antigens by conditionally immortalized murine neural stem cells. Neurosci Lett. 337, 85-88.
Mody I and McDonald JF (1995) NMDA receptor-dependent excitotoxicity: the role of intracellular Ca2. Trends Pharmacol Sci. 16, 356-359.
Moriyama H, Yoshimura O, Sunahori H, Nitta H, Imakita H, Saka Y, Maejima H and Tobimatsu Y (2004) Progression and direction of contractures of knee joints following spinal cord injury in the rat. Tohoku J Exp Med. 204, 37-44.
Moviglia GA, Fernandez Viña R, Brizuela JA, Saslavsky J, Vrsalovic F, Varela G, Bastos F, Farina P, Etchegaray G, Barbieri M, Martinez G, Picasso F, Schmidt Y, Brizuela P, Gaeta CA, Costanzo H, Moviglia Brandolino MT, Merino S, Pes ME, Veloso MJ, Rugilo C, Tamer I and Shuster G (2006) Combined protocol of cell therapy for chronic spinal cord injury. Report on the electrical and functional recovery of two patients. Cytotherapy. 8, 202-209.
Murray M, Kim D, Liu Y, Tobias C, Tessler A and Fischer I (2002) Transplantation of genetically modified cells contributes to repair and recovery from spinal injury. Brain Res Brain Res Rev. 40, 292-300.
Nakamura M, Houghtling RA, MacArthur L, Bayer BM and Bregman BS (2003) Differences in cytokine gene expression profile between acute and secondary injury in adult rat spinal cord. Exp Neurol. 184, 313-325.

Nashmi R, Imamura H, Tator CH and Fehlings MG (1997) Serial recording of somatosensory and myoelectric motor evoked potentials: role in assessing functional recovery after graded spinal cord injury in the rat. J Neurotrauma. 14, 151-159.
New JY, Chan SH, Yap EH and Hu H (2004) Induction of acute graft-versus-host disease by T cells that do not respond to in vitro alloantigen stimulation. Br J Haematol. 126, 828-836.
Nyström B, Berglund JE and Bergquist E (1988) Methodological analysis of experimental spinal cord compression model in the rat. Acta Neurol Scand. 78, 460-466.
Oliveira AL, Thams S, Lidman O, Piehl F, Hokfelt T, Karre K, Linda H and Cullheim S (2004) A role for MHC class I molecules in synaptic plasticity and regeneration of neurons after axotomy. Proc Natl Acad Sci U S A. 101, 17843-17848.
Ostenfeld T, Joly E, Tai YT, Peters A, Caldwell M, Jauniaux E and Svendsen CN (2002) Regional specification of rodent and human neurospheres. Brain Res Dev Brain Res. 134, 43-55.
Park E, Liu Y and Fehlings MG (2003) Changes in glial cell white matter AMPA receptor expression after spinal cord injury and relationship to apoptotic cell death. Exp Neurol. 182, 35-48.
Parmar M, Skogh C, Bjorklund A and Campbell K (2002) Regional specification of neurosphere cultures derived from subregions of the embryonic telencephalon. Mol Cell Neurosci. 21, 645-656.
Perry VH and Gordon S (1991) Macrophages and the nervous system. Int Rev Cytol. 125, 203-244.
Pineau I and Lacroix S (2007) Proinflammatory cytokine synthesis in the injured mouse spinal cord: multiphasic expression pattern and identification of the cell types involved. J Comp Neurol. 500, 267-285.
Pitman M, Emery B, Binder M, Wang S, Butzkueven H and Kilpatrick TJ (2004) LIF receptor signaling modulates neural stem cell renewal. Mol Cell Neurosci. 27, 255-266.
Popovich PG, Guan Z, Mcgaughy V, Fisher L, Hickey WF and Basso DM (2002) The neuropathological and behavioral consequences of intraspinal microglial/macrophage activation. J Neuropathol Exp Neurol. 61, 623-633.
Popovich PG, Wei P and Stokes BT (1997) Cellular inflammatory response after spinal cord injury in Sprague-Dawley and Lewis rats. J Comp Neurol. 377, 443-464.
Prado R, Dietrich WD, Watson BD, Ginsberg MD and Green BA (1987) Photochemically induced graded spinal cord infarction. Behavioral, electrophysiological, and morphological correlates. J Neurosurg. 67, 745-753.
Prewitt CM, Niesman IR, Kane CJ and Houlé JD (1997) Activated macrophage/microglial cells can promote the regeneration of sensory axons into the injured spinal cord. Exp Neurol. 148, 433-443.
Quencer RM (2002) Advances in imaging of spinal cord injury: implications for treatment and patient evaluation. Prog Brain Res. 137, 3-8.
Quinn SM, Walters WM, Vescovi AL and Whittemore SR (1999) Lineage restriction of neuroepithelial precursor cells from fetal human spinal cord. J Neurosci Res. 57, 590-602.
Rabchevsky AG, Sullivan PG, Fugaccia I and Scheff SW (2003) Creatine diet supplement for spinal cord injury: influences on functional recovery and tissue sparing in rats. J Neurotrauma. 20, 659-669.
Rabinovich SS, Seledtsov VI, Poveschenko OV, Senuykov VV, Taraban VY, Yarochno VI, Kolosov NG, Savchenko SA and Kozlov VA (2003) Transplantation treatment of spinal cord injury patients. Biomed Pharmacother. 57, 428-433.
Reynolds BA and Weiss S (1992) Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. Science. 255, 1707-1710.
Rice T, Larsen J, Rivest S and Yong VW (2007) Characterization of the early neuroinflammation after spinal cord injury in mice. J Neuropathol Exp Neurol. 66, 184-195.

Rivlin AS and Tator CH (1978) Effect of duration of acute spinal cord compression in a new acute cord injury model in the rat. Surg Neurol. 10, 38-43.
Rose-John S (2002) GP130 stimulation and the maintenance of stem cells. Trends Biotechnol. 20, 417-419.
Sagen J (2003) Cellular therapies for spinal cord injury: What will the FDA need to approve moving from the laboratory to the human? J Rehab Research Dev. 40, 71-80.
Sandler AN and Tator CH (1976) Review of the effect of spinal cord trauma on the vessels and blood flow in the spinal cord. J Neurosurg. 45, 638-646.
Schwab ME (2002) Increasing plasticity and functional recovery of the lesioned spinal cord. Prog Brain Res. 137, 351-359.
Schwartz M, Lazarov-Spiegler O, Rapalino O, Agranov I, Velan G and Hadani M (1999) Potential repair of rat spinal cord injuries using stimulated homologous macrophages. Neurosurg. 44, 1041-1045.
Schwartz RH (1990) A cell culture model for T lymphocyte clonal anergy. Science. 248, 1439-1456.
Sendtner M, Carroll P, Holtmann B, Hughes RA and Thoenen H (1994) Ciliary neurotrophic factor. J Neurobiol. 25, 1436-1453.
Shih CC, Weng Y, Mamelak A, LeBon T, Hu MC and Forman SJ (2001) Identification of a candidate human neurohematopoietic stem-cell population. Blood. 98, 2412-2422.
Shimazaki T, Shingo T and Weiss S (2001) The ciliary neurotrophic factcor/leukemia inhibitory factor/gp130 receptor comlex operates in the maintenance of mammalian forebrain neural stem cells. J Neurosci. 21, 7642–7653.
Silver J and Miller JH (2004) Regeneration beyond the glial scar. Nat Rev Neurosci. 5, 146-156.
Singec I, Knoth R, Meyer RP, Maciaczyk J, Volk B, Nikkhah G, Frotscher M and Snyder EY (2006) Defining the actual sensitivity and specificity of the neurosphere assay in stem cell biology. Nat Methods. 3, 801-806.
Sosa I, Reyes O and Kuffler DP (2005) Advances in spinal cord repair techniques. P R Health Sci J. 24, 313-322.
Steeves JD, Lammertse D, Curt A, Fawcett JW, Tuszynski MH, Ditunno JF, Ellaway PH, Fehlings MG, Guest JD, Kleitman N, Bartlett PF, Blight AR, Dietz V, Dobkin BH, Grossman R, Short D, Nakamura M, Coleman WP, Gaviria M and Privat A; International Campaign for Cures of Spinal Cord Injury Paralysis. (2007) Guidelines for the conduct of clinical trials for spinal cord injury (SCI) as developed by the ICCP panel: clinical trial outcome measures. Spinal cord. 45, 206-221.
Stokes BT and Garwood M (1982) Traumatically induced alterations in the oxygen fields in the canine spinal cord. Exp Neurol. 75, 665-677.
Su Z, Cao L, Zhu Y, Liu X, Huang Z, Huang A and He C (2007) Nogo enhances the adhesion of olfactory ensheathing cells and inhibits their migration. J Cell Sci. 120, 1877-1887.
Sun L, Lee J and Fine HA (2004) Neuronally expressed stem cell factor induces neural stem cell migration to areas of brain injury. J Clin Invest. 113, 1364-1374.
Suslov ON, Kukekov VG, Ignatova TN and Steindler DA (2002) Neural stem cell heterogeneity demonstrated by molecular phenotyping of clonal neurospheres. Proc Natl Acad Sci U S A. 99, 14506-14511.
Svendsen CN, ter Borg MG, Armstrong RJ, Rosser AE, Chandran S, Ostenfeld T and Caldwell MA (1998) A new method for the rapid and long term growth of human neural precursor cells. J Neurosci Methods. 85, 141-152.
Takeuchi H, Natsume A, Shimato S, Ito M, Mizuno M, Kim SU and Yoshida J (2007a) Soc. Neurosci. Abstract 345.14
Takeuchi H, Natsume A, Wakabayashi T, Aoshima C, Shimato S, Ito M, Ishii J, Maeda Y, Hara M, Kim SU and Yoshida J (2007b) Intravenously transplanted human neural stem cells migrate to the injured spinal cord in adult mice in an SDF-1- and HGF-dependent manner. Neurosci Lett. 426, 69-74.
Tamaki S, Eckert K, He D, Sutton R, Doshe M, Jain G, Tushinski R, Reitsma M, Harris B, Tsukamoto A, Gage F, Weissman I and Uchida N (2002) Engraftment of

sorted/expanded human central nervous system stem cells from fetal brain. J Neurosci Res. 69, 976-986.
Tarasenko YI, Gao J, Nie L, Johnson KM, Grady JJ, Hulsebosch CE, McAdoo DJ and Wu P (2007) Human fetal neural stem cells grafted into contusion-injured rat spinal cords improve behavior. J Neurosci Res. 85, 47-57.
Tator CH (1995) Update on the pathophysiology and pathology of acute spinal cord injury. Brain Pathol. 5, 407-413.
Tator CH and Fehlings MG (1991) Review of the secondary injury theory of acute spinal cord trauma with emphasis on vascular mechanisms. J Neurosurg. 75, 15-26.
Tekkok SB and Goldberg MP (2001) Ampa/kainate receptor activation mediates hypoxic oligodendrocyte death and axonal injury in cerebral white matter. J Neurosci. 21, 4237-4248.
Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS and Jones JM (1998) Embryonic stem cell lines derived from human blastocysts. Science. 282, 1145-1147.
Tropepe V, Sibilia M, Ciruna BG, Rossant J, Wagner EF and van der Kooy D (1999) Distinct neural stem cells proliferate in response to EGF and FGF in the developing mouse telencephalon. Dev Biol. 208, 166-188.
Tuszynski MH, Steeves JD, Fawcett JW, Lammertse D, Kalichman M, Rask C, Curt A, Ditunno JF, Fehlings MG, Guest JD, Ellaway PH, Kleitman N, Bartlett PF, Blight AR, Dietz V, Dobkin BH, Grossman R and Privat A; International Campaign for Cures of Spinal Cord Injury Paralysis (2007) Guidelines for the conduct of clinical trials for spinal cord injury as developed by the ICCP Panel: clinical trial inclusion/exclusion criteria and ethics. Spinal Cord. 45, 222-231.
Ubiali F, Nava S, Nessi V, Frigerio S, Parati E, Bernasconi P, Mantegazza R and Baggi F (2007) Allorecognition of human neural stem cells by peripheral blood lymphocytes despite low expression of MHC molecules: role of TGF-β in modulating proliferation. Int Immunol. 19, 1063-1074.
Uchida N, Buck DW, He D, Reitsma MJ, Masek M, Phan TV, Tsukamoto AS, Gage FH and Weissman IL (2000) Direct isolation of human central nervous system stem cells. Proc Natl Acad Sci U S A. 97, 14720-14725.
Villa A, Navarro-Galve B, Bueno C, Franco S, Blasco MA and Martinez-Serrano A (2004) Long-term molecular and cellular stability of human neural stem cell lines. Exp Cell Res. 294, 559-570.
von Euler M, Seiger Å and Sundström E (1997a) Clip compression injury in the spinal cord. A correlative study of neurological and morphological alterations. Exp Neurol. 145, 1-9.
von Euler M, Sundström E and Seiger Å (1997b) Morphological characterization of the evolving rat spinal cord injury after photochemically induced ischemia. Acta Neuropathol. 94, 232-239.
Warden P, Bamber NI, Li H, Esposito A, Ahmad KA, Hsu CY and Xu XM (2001) Delayed glial cell death following wallerian degeneration in white matter tracts after spinal cord dorsal column cordotomy in adult rats. Exp Neurol. 168, 213-224.
Watanabe K, Nakamura M, Iwanami A, Fujita Y, Kanemura Y, Toyama Y and Okano H (2004) Comparison between fetal spinal-cord- and forebrain-derived neural stem/progenitor cells as a source of transplantation for spinal cord injury. Dev Neurosci. 26, 275-287.
Watson BD, Prado R, Dietrich WD, Ginsberg MD and Green BA (1986) Photochemically induced spinal cord injury in the rat. Brain Res. 367, 296-300.
Wennersten A, Holmin S, Al Nimer F, Meijer X, Wahlberg LU and Mathiesen T (2006) Sustained survival of xenografted human neural stem/progenitor cells in experimental brain trauma despite discontinuation of immunosuppression. Exp Neurol. 199, 339-347.
White LA, Keane RW and Whittemore SR (1994) Differentiation of an immortalized CNS neuronal cell line decreases their susceptibility to cytotoxic T cell lysis in vitro. J Neuroimmunol. 49, 135-143.
Windrem MS, Nunes MC, Rashbaum WK, Schwartz TH, Goodman RA, McKhann G 2nd, Roy NS and Goldman SA (2004) Fetal and adult human oligodendrocyte

progenitor cell isolates myelinate the congenitally dysmyelinated brain. Nat Med. 10, 93-97.
Wirth ED 3rd, Reier PJ, Fessler RG, Thompson FJ, Uthman B, Behrman A, Beard J, Vierck CJ and Anderson DK (2001) Feasibility and safety of neural tissue transplantation in patients with syringomyelia. J Neurotrauma. 18, 911-929.
Wolf E, Kramer R, Polejaeva I, Thoenen H and Brem G (1994) Efficient generation of chimaeric mice using embryonic stem cells after long term culture in the presence of ciliary neurotrophic factor. Transgenic Res. 3, 152–158.
Wolman L (1965) The disturbances of circulation in traumatic paraplegia in acute and late stages: a pathological study. Paraplegia. 2, 213-226.
Wright LS, Li J, Caldwell MA, Wallace K, Johnson JA and Svendsen CN (2003) Gene expression in human neural stem cells: effects of leukemia inhibitory factor. J Neurochem. 86, 179-195.
Wu P, Tarasenko YI, Gu Y, Huang LY, Coggeshall RE and Yu Y (2002) Region-specific generation of cholinergic neurons from fetal human neural stem cells grafted in adult rat. Nat Neurosci. 5, 1271-1278.
Xiao M, Klueber KM, Lu C, Guo Z, Marshall CT, Wang H and Roisen FJ (2005) Human adult olfactory neural progenitors rescue axotomized rodent rubrospinal neurons and promote functional recovery. Exp Neurol. 194, 12-30.
Xiong Y, Rabchevsky AG and Hall ED (2007) Role of peroxynitrite in secondary oxidative damage after spinal cord injury. J Neurochem. 100, 639-649.
Xu J, Kim GM and Chen S (2001) iNOS and nitrotyrosine expression after spinal cord injury. J Neurotrauma. 18, 523-532.
Yang L, Blumbergs PC, Jones NR, Manavis J, Sarvestani GT and Ghabriel MN (2004) Early expression and cellular localization of proinflammatory cytokines interleukin-1beta, interleukin-6, and tumor necrosis factor-alpha in human traumatic spinal cord injury. Spine. 29, 966-971.
Zhang SC, Ge B and Duncan ID (2000) Tracing human oligodendroglial development in vitro. J Neurosci Res. 59, 421-429.
Zhang Z, Krebs CJ and Guth L (1997) Experimental analysis of progressive necrosis after spinal cord trauma in the rat: etiological role of the inflammatory response. Exp Neurol. 143, 141-152.