Significant clinical, neuropathological and behavioural recovery from acute spinal cord trauma by transplantation of a well-defined somatic stem cell from human umbilical cord blood

Jessica Schira,1 Marcia Gasis,1 Veronica Estrada,1 Marion Hendricks,1 Christine Schmitz,1 Thorsten Trapp,2 Fabian Kruse,1 Gesine Kögler,2 Peter Wernet,2 Hans-Peter Hartung1 and Hans Werner Müller1

1 Molecular Neurobiology Laboratory, Department of Neurology, Heinrich-Heine-University Medical Centre Düsseldorf, Moorenstr. 5, 40225 Düsseldorf, Germany
2 Institute for Transplantation Diagnostics and Cell Therapeutics, Heinrich-Heine-University Medical Centre Düsseldorf, Moorenstr. 5, 40225 Düsseldorf, Germany

Correspondence to: Hans Werner Müller, Molecular Neurobiology Laboratory, Department of Neurology, Heinrich-Heine-University Medical Centre Düsseldorf, Moorenstr. 5, 40223 Düsseldorf, Germany
E-mail: hanswerner.mueller@uni-duesseldorf.de

Stem cell therapy is a potential treatment for spinal cord injury and different stem cell types have been grafted into animal models and humans suffering from spinal trauma. Due to inconsistent results, it is still an important and clinically relevant question which stem cell type will prove to be therapeutically effective. Thus far, stem cells of human sources grafted into spinal cord mostly included barely defined heterogeneous mesenchymal stem cell populations derived from bone marrow or umbilical cord blood. Here, we have transplanted a well-defined unrestricted somatic stem cell isolated from human umbilical cord blood into an acute traumatic spinal cord injury of adult immune suppressed rat. Grafting of unrestricted somatic stem cells into the vicinity of a dorsal hemisection injury at thoracic level eight resulted in hepatocyte growth factor-directed migration and accumulation within the lesion area, reduction in lesion size and augmented tissue sparing, enhanced axon regrowth and significant functional locomotor improvement as revealed by three behavioural tasks (open field Basso–Beattie–Bresnahan locomotor score, horizontal ladder walking test and CatWalk gait analysis). To accomplish the beneficial effects, neither neural differentiation nor long-lasting persistence of the grafted human stem cells appears to be required. The secretion of neurite outgrowth-promoting factors in vitro further suggests a paracrine function of unrestricted somatic stem cells in spinal cord injury. Given the highly supportive functional characteristics in spinal cord injury, production in virtually unlimited quantities at GMP grade and lack of ethical concerns, unrestricted somatic stem cells appear to be a highly suitable human stem cell source for clinical application in central nervous system injuries.

Keywords: axon growth; chemotaxis; locomotor recovery; hepatocyte growth factor; tissue sparing
Abbreviations: BDA = biotinylated dextrane amine; GFAP = glial fibrillary acidic protein; HGF = hepatocyte growth factor; MSC = mesenchymal stem cell; USSC = unrestricted somatic stem cells; USSC-CM_FBS = USSCfoetal bovine serum-conditioned medium
Introduction

Traumatic spinal cord injury results in breakdown of the blood–spinal cord barrier, influx of inflammatory cells, activation of glial cells, loss of oligodendrocytes and axonal degeneration leading to permanent motor and sensory deficits. Stem cell grafting has been suggested as a therapeutic strategy for spinal cord repair. Human adult mesenchymal stem cell populations derived from adult bone marrow or peripheral blood stem cell mobilization have been transplanted in animal models and in pilot clinical studies (Cizkova et al., 2006; Himes et al., 2006; Cristante et al., 2009; Pal et al., 2009; Sasaki et al., 2009). However, reports on functional outcome were rather inconsistent, partly, because the majority of the published results reporting functional outcome were solely based on a single behavioural test. While improvement of sensory and motor activity was reported in some studies (Neuhuber et al., 2005), or unless human mesenchymal stem cells (MSC) were genetically modified (Sasaki et al., 2006; Himes et al., 2006; Cristante et al., 2009), no recovery was observed in others due to donor variations (Neuhuber et al., 2005), or unless human mesenchymal stem cells (MSC) were genetically modified (Sasaki et al., 2009). Therefore, no consensus exists at present regarding the stem cell type that will prove to be effective therapeutically (Sahni and Kessler, 2010).

Pre-clinical studies with human MSC isolated from bone marrow or human umbilical cord blood in rodent spinal cord injury suggested variable mechanisms underlying the observed effects, such as differentiation into oligodendroglia (Cizkova et al., 2006; Dasari et al., 2007), reduced scarring (Veeravalli et al., 2009) and enhanced neuroprotection (Dasari et al., 2009). Most studies used human MSC separated from the haematopoeietic cell fraction by their ability to adhere to plastic. Consequently, the transplanted cell population contained a mixture of stromal cells and human MSC leaving it unclear which cell was effective (Tetzlaff et al., 2011). In addition to human MSC, human umbilical cord blood contains unrestricted somatic stem cells (USSC; Kögler et al., 2004). Although USSC have several features in common with human MSC from bone marrow (bone marrow MSC) and human umbilical cord blood (cord blood MSC), e.g. the osteogenic and chondrogenic differentiation potential, they clearly differ in their immunological behaviour (van den Berk, 2009; Winter et al., 2009) and their transcriptome (Jansen et al., 2010). Further, we have recently identified the expression of delta-like 1/preadipocyte factor 1 (DLK-1/PREF-1) and a USSC-specific HOX gene expression profile as markers to discriminate USSC from cord blood MSC and bone marrow MSC (Kluth et al., 2010, Liedtke et al., 2010). Favourably, USSC can be purified in a good manufacturing practice (GMP)-grade status (Aktas et al., 2010) without any ethical concerns and invasive interventions, and USSC can be easily expanded on a clinical scale. Importantly, transplantation of USSC into different animal models revealed that USSC do not induce tumour formation (Aktas et al., 2010). Here, we transplanted the well-defined population of USSC from human umbilical cord blood into a rodent model of acute spinal cord injury and investigated their survival, migration and neural differentiation potential as well as their influence on axonal regeneration, lesion size and protection from spinal tissue loss. Finally, we extensively investigated the functional outcome using three different locomotor tasks (open field Basso–Beattie–Bresnahan locomotion score, horizontal ladder walking test and CatWalk gait analysis). Our investigation provides the first proof that transplantation of a well-characterized and defined human somatic stem cell (USSC) significantly improves the clinical, neuropathological and functional outcome after transplantation into an animal model of spinal cord injury.

Materials and methods

Animals

Adult female Wistar rats weighing 180–210 g were used. During the experiments, animals were housed under standard conditions with a 12 h light/12 h dark cycle. Water and food were available ad libitum. All surgical interventions and pre- and post-surgical animal care were performed in compliance with the German Animal Protection law (State Office, Environmental and Consumer Protection of North Rhine-Westphalia, LANUV NRW). The number of rats used in each experimental group are listed in Table 1.

Surgical procedures: dorsal hemisection and unrestricted somatic stem cell grafting

Adult female Wistar rats were injured as previously described (Klapka et al., 2005) with slight modifications. In brief, all surgeries were performed in compliance with the German Animal Protection law (State Office, Environmental and Consumer Protection of North Rhine-Westphalia, LANUV NRW). The number of rats used in each experimental group are listed in Table 1.

Table 1 | Experimental groups

<table>
<thead>
<tr>
<th>Survival time</th>
<th>Analysis</th>
<th>Animal groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lesion</td>
</tr>
<tr>
<td>1 day</td>
<td>In vitro</td>
<td>+ and –</td>
</tr>
<tr>
<td>2 days</td>
<td>In vivo</td>
<td>+</td>
</tr>
<tr>
<td>1 week</td>
<td>In vivo</td>
<td>+ and –</td>
</tr>
<tr>
<td>3 weeks</td>
<td>In vivo</td>
<td>+</td>
</tr>
<tr>
<td>5 weeks</td>
<td>In vivo</td>
<td>+</td>
</tr>
<tr>
<td>16 weeks</td>
<td>In vivo</td>
<td>+ and –</td>
</tr>
</tbody>
</table>

Number of rats for each group used in the presented study are given. (+) indicates lesioned spinal cord, (–) indicates non-lesioned intact spinal cord. Sham-operated rats received a laminectomy and opening of the dura but no spinal cord injury. Rostral tracing was performed in transplanted and control groups (5 weeks survival time) at 1 week prior to sacrifice.
performed under isoflurane anaesthesia. After removal of the vertebral arch at thoracic level eight (Th8), the dura was opened and the dorsal corticospinal tract, the rubrospinal tract and the dorsal columns were cut with a Scouten wire knife (Bilaney) to the depth of the central canal. That dorsal hemisection leads to a highly reproducible lesion allowing comparison between different animal groups (Hermanns et al., 2001; Klapka et al., 2005; Schiwy et al., 2009). After hemisection, the dura was sutured and the following USSC grafting was performed with a glass capillary 2 mm rostral and 2 mm caudal to the lesion site at 1.2 and 0.8 mm depth. Two microlitres, containing a suspension of 100 000 cells in Dulbecco’s modified Eagle’s medium, were injected slowly within 4 min at each injection site. The animal groups receiving either USSC or Dulbecco’s modified Eagle’s medium (control) were immune suppressed with cyclosporine A (15 mg/kg s.c.; Novartis), beginning 1 day prior to surgery and thereafter daily for 3 weeks post-operation. Sham-operated rats received a laminectomy and opening of the dura but no spinal cord injury. Postoperative care included treatment with antibiotics (Baytril, Bayer Health Care) and manual bladder emptying for 1 week. Further, rats received an analgesic (Rimadyl, Pfizer) for 2 days.

Rostral axon tracing

For labelling of regenerating fibres extending into the lesion site, anterograde axon tracing was performed rostral to the lesion at thoracic level seven (Th7) by microinjections of biotinylated dextrane amine (BDA) (10%, molecular weight 10 000; Molecular Probes) into the spinal cord as described elsewhere (Bamber et al., 2001; Hsu and Xu, 2005). Three injections (0.3 μl each) with glass capillaries at 1.5-, 1.2- and 0.8-mm depth at the midline and three injections at 1.2-, 0.8- and 0.4-mm depth bilateral to the corticospinal tract were carried out at 4 weeks after hemisection. Animals were perfused 1 week after rostral tracing (in total: 5 weeks post-hemisection).

Tissue preparation and histological procedures

Anaesthetized rats were trancardially perfused first with ice-cold phosphate-buffered saline for 2 min and then 4% paraformaldehyde (Merck) for 15 min. Spinal cord segments of ~2-cm length including the lesion site were collected and post-fixed in 4% paraformaldehyde for 24 h at 4°C. After cryopreservation in 30% sucrose at 4°C, spinal cord tissue was cut into serial 18 μm parasagittal sections. Every 10th section was used to characterize USSC with respect to localization, migration and neural differentiation as well as to quantify BDA-traced fibres in the lesion area.

For immunohistochemical stainings, sections were blocked with 10% normal goat serum (Sigma Aldrich) and permeabilized with 0.03% Triton X-100 (Merck) for 1 h. Incubation with primary antibodies directed to neurofilament protein (recognizing NEF-L, NEF-M and NEF-H) 1:1300 (rabbit, polyclonal, BioTrend, NA1297), glial fibrillary acidic protein (GFAP) 1:1000 (mouse, monoclonal, Millipore, MAB3402), GFAP 1:1500 (rabbit, polyclonal, Dako, Z0334), S100 1:300 (rabbit, polyclonal, Sigma Aldrich, S2644), oligodendrocyte-specific protein 1:750 (rabbit, polyclonal, Abcam, AB53041), doublecortin 1:2000 (rabbit, polyclonal, Abcam, AB18723), nestin 1:500 (rabbit, polyclonal, Sigma, N5413), tyrosine hydroxylase 1:750 (rabbit, polyclonal, Biozol, AB112), serotonin (5-HT) 1:50 (rabbit, polyclonal, Biozol, SE100) and human nuclei 1:500 (mouse, monoclonal, Millipore, MAB1281) was carried out overnight at 4°C. After washing with phosphate-buffered saline, a secondary antibody 1:1000 (goat anti-mouse conjugated with Alexa 488, goat anti-rabbit conjugated with Alexa 594 or goat anti-rabbit conjugated with Alexa 350, Invitrogen) was applied and incubated for 2 h at room temperature. Cell nuclei were labelled with 4,6-diamidino-2-phenylindoline (DAPI, Roche Diagnostics). Streptavidin Oregon green 488 1:500 (Invitrogen) was applied for BDA visualization after rostral tracing. Sections were mounted either with Citifluor (Citifluor) or Fluoromount G (SouthernBiotech). Images were taken either with a fluorescence microscope (Axioplan2, Zeiss) or a LSM 510 confocal microscope (LSM 510, Zeiss). Figures 2A–F, 3B, C and I–N are projections from z-stacks assembled by the software LSM image browser (Zeiss).

Axon quantification

For quantification of BDA-traced fibres, every 10th parasagittal section was stained for BDA (6–8 sections per animal). Only labelled elongated structures localized within the lesion area, which could be clearly identified as nerve fibres, were counted. The number of axon profiles was normalized to the lesion area (axons per square millimetre), which was measured using ImageJ software.

Assessment of lesion area and spared tissue

Parasagittal spinal cord sections were stained for GFAP to identify and outline the lesion area in which GFAP-positive astrocytes were markedly reduced or absent (Klapka et al., 2005; Schiwy et al., 2009). The lesion and spared tissue areas were determined as described elsewhere (Iannotti et al., 2010) with slight modifications. Briefly, from each spinal cord, the section with the largest lesion size plus two additional sections taken at a distance of 0.2 mm on each side were used for evaluation. The total area of spinal cord reaching from 1-mm rostral to 1-mm caudal of the midline of the lesion was outlined in composed images of the spinal cord sections and used as reference area. The percentile of the lesion zone within the outlined reference area was determined by Image J software. Spared tissue area (square millimetre) is defined by the reference spinal cord area minus the area covered by the lesion.

Behavioural analyses

For behavioural testing, pre-training of 35 rats started 4 weeks prior to surgery in all behavioural tasks. The animals were housed in groups of three with food ad libitum throughout the post-surgery testing period of 16 weeks. Five animals died after surgery and/or during the testing period. We evaluated both hindlimbs separately, because of a slight asymmetry in our Scouten wire knife lesion, where the right rubrospinal tract is less impaired than the left rubrospinal tract (Schiwy et al., 2009). All behavioural tests have been performed and analysed “blinded” to the treatment groups.

Open field (Basso–Beattie–Bresnahan) locomotion score

Hindlimb function was evaluated weekly in an open field test using the Basso–Beattie–Bresnahan score (Basso et al., 1995). The rats were observed for 4 min by two individuals. Forelimb–hindlimb coordination was determined separately by analysis of the walking pattern using the CatWalk device and software (Hamers et al., 2001). For this evaluation, every rat had to cross the walkway without any interruption or hitch. A minimum of three correct crossings per animal was required (Koopmans et al., 2005). The CatWalk-based coordination was
defined as follows: (i) animals with a regularity index of 100% in all three CatWalk crossings were assigned consistent coordination; (ii) animals with a regularity index of 100% in two of three crossings were scored with frequent coordination; and (iii) animals with a regularity index of 100% in only one crossing were scored with occasional coordination. Animals with a regularity index lower than 100% in all three crossings were scored as uncoordinated (Koopmans et al., 2005). The 7-point locomotor subscore, which allows evaluation of finer aspects of locomotor control, was also assessed.

**Horizontal ladder walking test**
The horizontal ladder walking test (gridwalk) was performed as described previously (Metz et al., 2000; Metz and Whishaw, 2002). Briefly, bar distances of the horizontal ladder were irregular and frequently changed to avoid animals memorizing the location of the bars. The walking of each rat was recorded with a conventional video camera and subsequently analysed in slow motion. Steps and missteps were counted for each condition, in five uninterrupted crossings per testing day for each hindlimb separately. A misstep was counted when the foot slipped or fell off the bar. The number of missteps per trial was counted, given as percentile of the total number of steps and averaged for five trials. When a lesioned rat was unable to cross the ladder, a maximum error of 100% was scored. Animals were tested every week starting at 2 weeks after surgery. Prior to that time, lesioned rats were unable to cross the ladder. Baseline data were collected 1 week prior to surgery. The horizontal ladder walking error rates were not expressed as a percentage of the baseline scores for the following surface antigens: CD31, CD34, CD45, CD56, CD106, AC133, CD184 and HLA-DR, but expressed high levels of CD13, CD29, CD44, CD71, CD73, CD105, CD146, CD166 and HLA-ABC. Briefly, the mononuclear cell fraction was obtained by a standard ficoll-gradient separation from umbilical cord blood followed by ammonium chloride lysis of red blood cells. After two washing steps, the cells were seeded in culture flasks and grown in the presence of dexamethasone. USSC growing as adherent cell colonies were selected. For expansion, USSC were incubated in Dulbecco’s modified Eagle’s medium (Lonza) supplemented with 30% heat-inactivated foetal bovine serum (Lonza), 2 mM glutamine (Gibco) and penicillin/streptomycin (100 U/ml, Gibco). USSC were incubated at 37°C in 5% CO₂ in a humidified atmosphere. USSC at passage 5–8 were used for all experiments. The data were obtained with a USSC preparation from the umbilical cord blood of a single individual. To exclude donor variations, additional USSC batches were investigated regarding the migratory behaviour and differentiation potential in vivo and the stimulation of neurite outgrowth in vitro. No differences could be observed between the batches (data not shown).

**Cell cultures**

**Unrestricted somatic stem cell isolation and expansion**
USSC were isolated as described previously (Kägler et al., 2004) and extensively characterized (Aktas et al., 2010; Jansen et al., 2010; Kluth et al., 2010; Liedtke et al., 2010). All USSC lines tested were negative for the following surface antigens: CD31, CD34, CD45, CD56, CD106, AC133, CD184 and HLA-DR, but expressed high levels of CD13, CD29, CD44, CD71, CD73, CD105, CD146, CD166 and HLA-ABC. Briefly, the mononuclear cell fraction was obtained by a standard ficoll-gradient separation from umbilical cord blood followed by ammonium chloride lysis of red blood cells. After two washing steps, the cells were seeded in culture flasks and grown in the presence of dexamethasone. USSC growing as adherent cell colonies were selected. For expansion, USSC were incubated in Dulbecco’s modified Eagle’s medium (Lonza) supplemented with 30% heat-inactivated foetal bovine serum (Lonza), 2 mM glutamine (Gibco) and penicillin/streptomycin (100 U/ml, Gibco). USSC were incubated at 37°C in 5% CO₂ in a humidified atmosphere. USSC at passage 5–8 were used for all experiments. The data were obtained with a USSC preparation from the umbilical cord blood of a single individual. To exclude donor variations, additional USSC batches were investigated regarding the migratory behaviour and differentiation potential in vivo and the stimulation of neurite outgrowth in vitro. No differences could be observed between the batches (data not shown).

**Primary astrocyte cultures**
Primary astrocytes were isolated from P0 to P1 Wistar rats (Schmalenbach and Müller, 1993). Cerebral cortical tissue was dissected and cultured in Dulbecco’s modified Eagle’s medium (Gibco) supplemented with 10% foetal bovine serum and 2 mM glutamine. After centrifugation (720 g, 30 s), cells were resuspended in 10 ml culture medium, passed through a 60 μm nylon gauze and cultured in standard cell culture flasks. Primary astrocyte cultures were used for two passages.

**Preparation of conditioned medium**
Confluent layers of USSC and primary astrocytes were incubated in Dulbecco’s modified Eagle’s medium Glutamax® supplemented with 15% foetal bovine serum or in serum-free N₂-medium for 48 h. Conditioned media of USSC and astrocytes were collected, centrifuged at 1050 g for 5 min and then applied to dorsal root ganglia or to cortical neurons in order to test neurite outgrowth.

**Neurite outgrowth assays**

**Dorsal root ganglia**
Dorsal root ganglia were prepared as described elsewhere (Murphy et al., 1996). Briefly, dorsal root ganglia were dissected from E12.5 to E14.5 rat embryos. Tissue samples were cut into 2–4 smaller explants and plated on coverslips pre-coated with poly-α-lysine (0.5 mg/ml, Sigma) and laminin (13 μg/ml, Sigma). The dorsal root ganglia explants either received the non-conditioned control medium, Dulbecco’s modified Eagle’s medium Glutamax® (Gibco) supplemented with 15% foetal...
bovine serum (PAA Laboratories), or the same culture medium after conditioning by exposure to USSC for 48 h (USSC-CM<sub>FBS</sub>). Dorsal root ganglia explants were fixed after 24 h of incubation and then immunocytochemically analysed.

**Neurite quantification of dorsal root ganglia**

For quantification of axon outgrowth, immunofluorescence microscope images were taken from neurofilament-stained dorsal root ganglia. The inner dorsal root ganglia cell cluster and the maximal centrifugal axon spread were outlined using tools of ImageJ software to measure the area of the core of the explant and the region of axonal outgrowth, respectively. For each condition, 10 explants were quantified in three independent experiments (n = 30).

**Cortical neurons**

Primary neocortical neurons were isolated from E15 Wistar rats as previously described (Kappler et al., 1997). Briefly, after dissection of cerebral cortex, the tissue was incubated in 0.05% trypsin (10 min, 37°C), rinsed with 10% foetal bovine serum (PAA Laboratories) in Dulbecco’s modified Eagle’s medium to block protease activity, washed with Dulbecco’s modified Eagle’s medium and resuspended in serum-free N₂-medium (Bottenstein and Sato, 1979). N₂-medium was a 4:1 mixture of Dulbecco’s modified Eagle’s medium with Ham’s F12 (Gibco, Invitrogen Company) supplemented with 5 μg/ml bovine insulin, 100 μg/ml human transferrin, 20 nM progesterone, 30 nM sodium selenite, 100 μM putrescine and 3.9 mM glutamine (all Sigma Aldrich). Cells were then triturated, passed through a 30 μm nylon gauze and seeded on coverslips pre-coated with 1 mg/ml poly-<sub>D</sub>-lysine and 13 μg/ml laminin in 24-well cell culture dishes, at a density of ~10,000 cells/cm<sup>2</sup> for 48 h for subsequent quantification of neurite length per neuron. Cortical neurons were incubated either in N₂-medium (non-conditioned control medium), astrocyte-conditioned medium (N₂ medium pre-conditioned by astrocytes for 48 h) or USSC<sub>N₂</sub>-conditioned medium (N₂ medium pre-conditioned by USSC for 48 h).

**Neurite quantification of cortical neurons**

For quantification of the mean neurite length per cell, immune fluorescence microscope images were taken from MAP2-stained cortical neurons. The length of each neurite was measured using tools of NeuronJ software. The mean neurite length of each neuron was defined by the total lengths of all neurites divided by the cell number. We performed two independent experiments, each with three coverslips per condition and three random microscopic images per cover slip.

**Immunocytochemical analysis of cell cultures**

For immunocytochemical analyses, dorsal root ganglia explants and cortical neurons were fixed with 4% formaldehyde (Merck), blocked with 10% normal goat serum, permeabilized with 0.03% Triton X-100 for 1 h and incubated with the primary antibody overnight at 4°C. The primary antibodies used were anti-neurofilament 1:1300 (rabbit, polyclonal, Biotrend, NA1297) and MAP2 1:250 (rabbit, polyclonal, Millipore, AB5622). After washing with phosphate-buffered saline, incubation with the secondary antibody (goat anti-rabbit conjugated with Alexa 488 or goat anti-rabbit conjugated with Alexa 594, Invitrogen) followed for 2 h at room temperature. Cell nuclei were stained with DAPI after rinsing with phosphate-buffered saline. Coverslips were then mounted in Citifluor (Citifluor).

**Under-agarose chemotaxis assay**

The under-agarose chemotaxis assay was performed as previously described (Trapp et al., 2008). Briefly, 1% agarose in Roswell Park Memorial Institute (RPMI) medium (Sigma) was poured into 35 mm plastic dishes. Three 2-mm wide parallel wells were cut 1.5 mm apart using a template. USSC were added to the central well and allowed to adhere for 2 h. The target was added to the left well and the migration buffer serving as control was added to the right well. As targets for USSC migration, extracts (prepared by homogenizing in RPMI medium on ice, 100 mg tissue/ml of medium) generated from spinal cord lesions 1 day post-hemisection and uninjured spinal cord were used. Six hours after incubation, cells were fixed for 30 min in methanol followed by 37% formaldehyde for 30 min. USSC migrating for at least 100 μm into the agarose towards a target were counted. The number of cells migrating towards control buffer (negative control) was subtracted from the number of cells migrating towards the tissue extracts. Data are expressed as fold increase in cell migration, normalized to the number of cells migrating towards the tissue extract from uninjured spinal cord. For characterization of the chemoattractant responsible for USSC migration, extracts were incubated with 5 or 10 μg/ml of anti-hepatocyte growth factor antibody (AF2207, polyclonal, R&D Systems). Specificity of the antibody was tested by pre-incubation with the immunogen (recombinant hepatocyte growth factor (HGF), R&D Systems). Additionally, recombinant HGF was added to extracts from healthy spinal cord.

**Statistical analysis**

All behavioural data were analysed using the non-parametric Mann–Whitney U-test for paired comparison. In order to verify data quality for quantitative analysis of migration, axonal growth, dorsal root ganglia neurite outgrowth and spared tissue, significance was assessed by Student’s t-test. For the quantification of mean neurite length per neuron statistical differences were established using one-way ANOVA followed by a Tukey’s post hoc test. The experimental groups were considered significantly different at P < 0.05. All data are presented as a mean ± SEM.

**Results**

**Migration of unrestricted somatic stem cells in the injured spinal cord**

Immune suppressed adult rats received a highly reproducible dorsal hemisection injury of the spinal cord at thoracic level Th8 as described previously (Hermanns et al., 2001; Klapka et al., 2005; Schiwy et al., 2009). Immediately after hemisection, 2 × 10<sup>5</sup> (2 × 2 μl) USSC from human umbilical cord blood were transplanted at a distance of 2 mm rostral and 2-mm caudal to the site of injury, respectively. The human stem cells could be clearly identified by immunostaining using a specific anti-human nuclei antibody. At 2 days after implantation, grafted USSC were located at the injection site (data not shown). This observation excludes the diffusion of implanted cells towards the lesion site due to pressure resulting from the injection. One week after transplantation, however, USSC were detectable in the lesion centre...
which shows very little immunoreactivity for the pan axonal marker neurofilament. Moreover, large numbers of USSC migrating from the injection site (red arrow) to the lesion centre (white arrow), which is virtually depleted of neurofilament (NF) immunopositive axons. In contrast, no directed migration occurred after USSC transplantation into the uninjured spinal cord (B). (C–E) Photomicrographs of migrating USSC in the under-agarose chemotaxis in vitro assay. USSC were exposed for 6 h to either control buffer (C), extract from the uninjured spinal cord (D) or extract from the spinal cord lesion site (E). The extract from uninjured spinal cord induced only a moderate migration, whereas USSC were strongly attracted by the extract from the spinal cord lesion. Control buffer did not attract USSC. (F) Quantification of migrating USSC revealed that significantly more USSC migrated to the spinal cord lesion (2) extract compared to extracts from uninjured spinal cord (1). Migration could be inhibited to levels of uninjured spinal cord tissue by incubation with an anti-hepatocyte growth factor (HGF) antibody in a dose-dependent manner (3: 5 μg/ml antibody; 4: 10 μg/ml antibody). Specificity of the anti-HGF antibody has been shown by pre-incubation of the antibody with the heat-inactivated immunogen. Incubation of the lesion extract with the antibody–immunogen complex (HGF-AB-IC) resulted in significantly enhanced USSC migration demonstrating specificity of the antibody to HGF (5). Migration of USSC towards the extract of the healthy spinal cord could be initiated by the addition of HGF to the latter tissue extract (6). Results derived from four independent experiments are shown as mean values ± SEM. ***P < 0.001 (Student’s t-test). wpt = week after transplantation.

To identify the spinal cord injury-derived chemoattractant drawing USSC towards the lesion site, an under-agarose chemotaxis in vitro assay (Nelson et al., 1975; Laevsky and Knecht, 2001) was performed. This assay, in contrast to cell motility assays, allows the formation of a stable gradient of a given chemoattractant. Tissue extracts from intact and injured spinal cord collected at 1 day post-surgery were investigated and compared with respect
to their chemoattractive activity for USSC. As shown in Fig. 1E, USSC were strongly attracted by extracts from the spinal cord lesion, whereas extracts from the uninjured spinal cord induced only a moderate USSC migration (Fig. 1D). There was nearly no attraction of the control buffer serving as a negative control (Fig. 1C). Quantification revealed that 11-fold more USSC migrated to the lesion extract compared with extracts from uninjured spinal cord after 6 h of incubation (Fig. 1F). As shown recently, the hepatocyte growth factor (HGF) is a potential chemoattractant for USSC in a rat model for focal cerebral ischaemia (Trapp et al., 2008). To verify the hypothesis that HGF is also involved in USSC migration towards a traumatic spinal cord injury, extracts from the lesion site were incubated for 2 h with a HGF-neutralizing antibody. The neutralization of the HGF bioreactivity led to a dose-dependent inhibition of USSC migration to levels of uninjured spinal cord tissue (Fig. 1F). On the other hand, pre-incubation of the HGF-neutralizing antibody with heat-inactivated immunogen resulted in a significant loss of migration-inhibitory activity of the antibody demonstrating its specificity for HGF. When, in turn, recombinant HGF was added to the extract of healthy spinal cord tissue, which shows very little chemoattraction for USSC, the cells responded by a significantly enhanced rate of migration (Fig. 1F). Taken together, these results provide evidence that HGF is the major chemoattractant for USSC migration towards a spinal cord lesion.

Unrestricted somatic stem cells lack neural differentiation after transplantation into the injured spinal cord

Histological examination revealed that USSC survived for 3 weeks after transplantation, until withdrawal of the immunosuppression (data not shown). To investigate the neural differentiation potential of USSC in the spinal cord environment, the expression of neuronal and glial marker proteins was immunohistochemically analysed. Stainings revealed that USSC neither within nor outside the lesion zone expressed the premature neuronal marker doublecortin (Fig. 2A) nor the neural progenitor protein nestin (Fig. 2B). Furthermore, USSC were negative for the neuronal marker neurofilament (Fig. 2C) and the glial proteins GFAP (Fig. 2D), oligodendrocyte-specific protein (Fig. 2E), or S100 protein (Fig. 2F) at 3 weeks after grafting. Apparently, USSC did not
differentiate into neurons, astrocytes, oligodendrocytes or Schwann cells after transplantation into the acutely injured spinal cord.

Unrestricted somatic stem cells promote axonal regrowth

Three weeks after transplantation into the injured spinal cord, USSC were mainly confined to the injury site (Fig. 3A). In close proximity to the grafted cells, numerous elongated neurofilament-positive fibres were present in the lesion centre (Fig. 3B). In control rats lacking USSC grafts, neurofilament labelling was virtually absent in the lesion area (Fig. 3C). The finding that USSC enhance the ingrowth of neurofilament-positive fibres in the lesion area led to further investigation of the axon growth-promoting activity of USSC. As axon debris could be false positively stained for neurofilament, we performed rostral tracing experiments at 4 weeks after USSC transplantation. Descending axons rostral to the lesion were traced at thoracic level Th7 by anterograde labelling with biotinylated dextrane amine (BDA), allowing the identification of regrowing axons of different fibre tracts invading the lesion zone. Immunohistochemical staining revealed that numerous BDA-labelled fibres had entered the lesion centre in USSC-grafted rats (Fig. 3D, higher magnification in Fig. 3F), whereas BDA positive fibres rarely grew into the lesion zone of control rats 5 weeks after transplantation (Fig. 3E, higher magnification in Fig. 3G). The qualitative finding that USSC promote axon regrowth was verified by quantitative image analysis determining the density (axon profiles per millimetre) of traced fibres in the GFAP-negative lesion centre. Only elongated traced structures within the lesion area, which could be clearly identified as ingrowing fibres, have been counted as indicated in Fig. 3F and G. As shown in Fig. 3H, transplantation of USSC significantly enhanced the number of regenerating axons within the lesion area by 2.3-fold as compared with control animals 5 weeks after transplantation. Among regrowing BDA-labelled fibres, subpopulations of descending serotonergic (5-HT, Fig. 3I–K) and catecholaminergic (tyrosine hydroxylase, Fig. 3L–N) fibres have been identified.

Unrestricted somatic stem cell-conditioned medium promotes neurite outgrowth in vitro

To examine whether secreted molecules could account for the neurite growth-promoting activity of USSC, two different in vitro neurite outgrowth assays were performed. Dorsal root ganglia explants prepared from embryonic rats were incubated with USSC-conditioned medium (USSC-CM_{FBS}) for 24 h. USSC-CM_{FBS} was freshly collected from confluent USSC exposed to serum-free N2-conditioned medium for 48 h (Fig. 4D). USSC-N2-conditioned medium was freshly collected from confluent USSC exposed to serum-free N2 medium for 48 h and subsequently centrifuged to remove cell debris. As positive control, astrocyte-conditioned medium (Fig. 4F) was used, which is long known to promote extensive neurite outgrowth when compared with non-conditioned control medium (Fig. 4E) (Müller et al. 1984). Neurites were visualized immunocytochemically by anti-MAP2 staining. USSC-N2-conditioned media-stimulated neurite outgrowth similar to the positive control astrocyte-conditioned medium (Fig. 4D), whereas neurons incubated with non-conditioned control medium developed shorter neurites (Fig. 4E). To confirm these findings, we quantified the mean neurite length per cell revealing that USSC-N2-conditioned medium significantly enhances neurite elongation to levels comparable with astrocyte-conditioned medium (Fig. 4G). Thus the effect of USSC-N2-conditioned medium on neurite outgrowth of cortical neurons is very similar to that of astrocytes.

Unrestricted somatic stem cell transplantation reduces tissue loss following spinal cord injury

In addition to support of neurite growth, USSC grafting into lesioned spinal cord resulted in improved tissue preservation as indicated in parasagittal sections stained with an anti-GFAP antibody to outline the lesion area (Fig. 5). Animals treated with USSC showed a smaller lesion size (white line, Fig. 5A) than controls (Fig. 5B). The lesion size (Fig. 5C) and the area of spared tissue (Fig. 5D) were examined and quantified using ImageJ software. Quantification revealed that in USSC-transplanted rats, the relative lesion size was significantly smaller (24.7 ± 5.2% versus 43.4 ± 4.2%) and the area of spared tissue was significantly larger (2.1 ± 0.33 mm² versus 1.15 ± 0.1 mm²) than in the control group, respectively. These data demonstrate that USSC treatment reduces tissue loss after transplantation in a model of acute spinal cord injury.

Unrestricted somatic stem cell transplantation leads to improved locomotor function after spinal cord injury

In order to unequivocally assess whether USSC promote functional recovery after transplantation into the injured spinal cord, three different locomotor tests were performed in a 16 week after transplantation long-term study. Rats were tested under blinded experimental conditions, starting at 1 week after transplantation with
Figure 3 Enhanced axonal growth after USSC transplantation. (A) Immunohistochemical staining of a parasagittal section 3 weeks after USSC transplantation (3 wpt). USSC (human nuclei, hNuc) were identified in the lesion centre in close proximity to numerous neurofilament (NF) positive fibres. (B) Close-up view of elongated fibres in the USSC-rich lesion core. (C) Neurofilament staining was nearly absent in the GFAP-negative lesion zone of control rats. (D and E) Immunohistochemical stainings for anterogradely biotinylated dextran amine (BDA) traced fibres of parasagittal sections 5 weeks after USSC transplantation (D and F) and control grafting (E and G). The lesion border is indicated by a white line. (F and G) Higher magnifications of (D) and (E), respectively. Arrows indicate BDA traced fibres, arrowheads indicate debris or macrophages which have been excluded from quantification. USSC transplantation increased the extent of axonal regrowth within the lesion site. (H) Quantification of the density of traced axon profiles (axons/mm²) in the lesion zone of USSC grafted and control animals at 5 weeks after transplantation. USSC grafting resulted in a significantly larger number of growing axons as compared to control (ctrl) animals. Results derived from five animals per group are shown as mean ± SEM. ***P < 0.001 (Student’s t-test). (I–K) Among BDA-traced fibres descending serotonergic (5-HT) and (L–N) catecholaminergic (tyrosine hydroxylase; TH) fibre subpopulations have been identified.
the open field Basso–Beattie–Bresnahan locomotor score to analyse the overall locomotor behaviour. The forelimb–hindlimb coordination was assessed separately by means of CatWalk gait analysis (Koopmans et al., 2005). Horizontal ladder testing to study fine locomotor performance and coordination, and CatWalk gait analysis were carried out starting at 2 weeks after transplantation. Both hindlimbs were analysed separately in all three locomotor tasks.

As shown in Supplementary Fig. 1A, the Basso–Beattie–Bresnahan score initially decreased to scores between 8 and 9 at 1 week after transplantation both in the USSC transplanted and the control group, respectively. Within 3 weeks, both animal
groups rose to Basso–Beattie–Bresnahan scores of ~11, but the score did not improve further due to the lack of coordination as revealed by CatWalk gait analysis (regularity index, Supplementary Fig. 1B). Sham-operated animals were not impaired by the surgical procedure and showed a constant Basso–Beattie–Bresnahan score of 21 (data not shown).

Fine locomotor control as assessed by the 7-point Basso–Beattie–Bresnahan subscore revealed that both hindlimbs improved in the USSC-transplanted group, reaching significance in the right hindlimb starting at 6 weeks after transplantation (Fig. 6A and B). Improved fine locomotor control in the right hindlimb of USSC-transplanted rats remained significantly enhanced until, at least, 16 weeks after transplantation. At this time point, the right hindlimb of USSC-transplanted rats reached a mean subscore that was 2.6 points higher than in control rats. At the end of the study, the score for the left hindlimb of USSC-transplanted animals was ~0.8 points higher than in control rats and just missed significance (Fig. 6A and B). Sham animals showed a constant locomotor subscore of 7 (data not shown).

We further performed the horizontal ladder walking test to assess deficits in descending fine motor control and impairment of forelimb–hindlimb coordination after spinal cord injury. Rats had to cross the horizontal ladder with irregular bar distances for at least five times per testing day. The relative error rate per hindlimb was expressed as number of missteps (slips and falls) per total number of steps given in per cent. While the uninjured sham animals performed close to zero placement errors on this task, dorsal hemisection caused major deficits as reflected in the high error rate of both right and left hindlimb (Fig. 6C and D). Interestingly, USSC-transplanted animals made significantly and consistently less missteps with both hindlimbs compared with the control group (Fig. 6C and D). At 6 weeks after transplantation, USSC-transplanted rats showed an error rate ranging from 43% ± 3% for the right hindlimb to 64% ± 4% for the left hindlimb, respectively, whereas the error rate was much higher in the right hindlimb (66% ± 4%) and left hindlimb (82% ± 3%) of the control animals. The significantly improved locomotor performance of the USSC-transplanted group remained until the end of the study at 16 weeks after transplantation, reaching reduction in placement errors compared with controls of 23% (right hindlimb) and 12% (left hindlimb), respectively.

CatWalk gait analysis was performed for quantitative assessment of interlimb coordination (regularity index), different static (relative paw placement, base of support, maximal print and paw area) and dynamic (swing speed and swing duration) gait parameters for individual paws. In Fig. 6E–H, data of the first (2 weeks after transplantation) and the last testing day (16 weeks after transplantation) after surgery are shown. All values, except relative paw placement, were normalized to the baseline, which was measured prior to surgery (relative values).

The CatWalk parameter ‘relative paw placement’ defines the distance between the placement of the ipsilateral fore- and hindpaws. While rats place their hindpaws at or close to the previous

Figure 5 USSC transplantation rescues spinal cord tissue. (A and B) Parasagittal spinal cord sections (16 weeks after transplantation) stained for GFAP to outline the lesion centre in which GFAP-positive astrocytes were markedly reduced or absent. The white line indicates the lesion area; the dashed line delineates the reference spinal cord area (for details see the ‘Materials and methods’ section). Compared to the USSC-grafted rat (A), the control animal (B) shows a larger size of the lesion and a smaller region of spared tissue. Quantification of the lesion area (C, in per cent) and the area of spared tissue (D, in square millimetre) shows that USSC transplantation significantly improves tissue sparing. Results are shown as mean ± SEM; **P < 0.01 (Student’s t-test).
position of the forepaw, this ability was largely lost after dorsal hemisection as indicated by the large distance between paw placement positions, respectively (Fig. 6E). Importantly, already at 2 weeks after transplantation USSC-grafted animals placed their right hindpaw significantly closer to the previous position of the right forepaw than control animals. At 16 weeks after transplantation, both hindlimbs were placed significantly closer to the previous forepaw positions in grafted animals than in controls. As an
additional static gait parameter, hindlimb ‘base of support’ was analysed to assess, e.g. trunk stability (Hamers et al., 2006). As shown in Fig. 6F, hindlimb base of support was increased in both animal groups at 2 weeks after dorsal hemisection. However, in the control group, hindlimb base of support further increased, whereas it remained constant in USSC-transplanted animals resulting in a significant difference between the two animal groups at 16 weeks after transplantation (Fig. 6F). Moreover, while the contact area of the hindpaws to the floor was reduced in both animal groups, as reflected by the decreased size of the ‘paw print area at maximal contact’, the maximum paw print area of the USSC-transplanted animals remained significantly higher than in controls (Fig. 6G). In addition, the total paw size as indicated by the parameter ‘print area’ was significantly larger for both hindpaws of the grafted animal group (Supplementary Fig. 1C). The dynamic gait parameters ‘swing speed’ (Fig. 6H) and ‘swing duration’ (Supplementary Fig. 1D), which normally show only little variation between individual animals (Hamers et al., 2006), were affected in both control and USSC-transplanted groups. Compared with sham animals, the relative swing speed was markedly reduced in the hindlimbs of both animal groups, whereas the relative swing speed remained significantly lower in the non-transplanted control group than in the USSC-grafted rats (Fig. 6H). On the other hand, the swing duration, which was low in sham animals, increased after spinal cord injury, but largely recovered in both grafted and control groups within 16 weeks after transplantation (Supplementary Fig. 1D). In USSC-transplanted animals, both dynamic gait parameters, ‘swing speed’ and ‘swing duration’, recovered better than in the control group.

Taken together, the behavioural data shown in Fig. 6 and Supplementary Fig. 1C and D demonstrate that USSC transplantation significantly improved the locomotor performance compared with spinal injured rats lacking the stem cell graft.

## Discussion

Here, we demonstrate that transplantation of native USSC, a well-characterized and defined somatic stem cell derived from human umbilical cord blood, into acute spinal cord injured rats is an effective strategy to promote axon growth, tissue sparing and significant functional locomotor improvement.

We have shown that USSC, when grafted into close proximity to a traumatic spinal cord injury in an immune-suppressed rat, survive for 3 weeks until withdrawal of immunosuppression and migrate into the lesion area where the stem cells accumulate. As demonstrated by specific anti-HGF antibody, USSC migration towards tissue extracts from a traumatic spinal cord lesion are predominately driven by HGF. The latter growth factor appears to be enriched in injured spinal cord but low in non-injured spinal cord tissue (Fig. 1). These data are in accordance with the recent observations that HGF is upregulated in lesioned segments of spinal cord (Shimamura et al., 2007; Takeuchi et al., 2007), which USSC express the HGF receptor c-met and that HGF promotes migration of USSC in an animal model of focal cerebral ischaemia (Trapp et al., 2008). The present data emphasize the important role of the HGF/c-met axis in regulating (stem) cell migration in CNS injuries.

In recent years, numerous studies with adult stem cells from bone marrow were published reporting contradictory observations with respect to neural differentiation potential. Differentiation into neural cells was reported by some groups (Akiyama et al., 2002; Cizkova et al. 2006; Park et al., 2010), whereas others could not detect neural lineage differentiation (Wu et al., 2003; Sheth et al., 2008). The ability of bone marrow-MSC to differentiate into neural cells still remains to be investigated (Brass 2006; English et al., 2006; Phinney and Prockop, 2007). Moreover, in vivo differentiation in the injured brain of stem cells from human umbilical cord blood is controversially discussed. Interestingly, supportive effects of transplanted human umbilical cord blood in spinal cord injury have been described both in presence (Kuh et al., 2005; Dasari et al., 2007) or absence of neural differentiation (Saporta et al., 2003). In the present investigation, we have no evidence for USSC differentiation into neuronal or glial precursors or mature cells after transplantation into the intact or injured rat spinal cord (Fig. 2). Consequently, cell replacement by USSC as the mechanism underlying the observed functional improvement is very unlikely. Previous studies with neural stem cells isolated from either foetal or adult material, or differentiated from embryonic stem cells (for review see Tetzlaff et al., 2011) described beneficial effects due to neural differentiation (Karimi-Abdolrazaee et al., 2006; Parr et al., 2007, 2008). However, the use of neural stem cells harvested from human foetal tissue, or derived from human embryonic stem cells raises severe ethical issues and technical difficulties. Our results encourage and support the idea to transplant cells that may promote regeneration via paracrine regulation rather than replacement mechanisms.

Three weeks after transplantation into the injured spinal cord, large numbers of neurofilament-positive fibres were found in close proximity of USSC, which became predominantly enriched at the injury site (Fig. 3). As shown by rostral anterograde axon tracing experiments, grafted USSC support axon growth including, e.g. serotonergic and catecholaminergic fibres, presumably through USSC-released neurite-promoting factors. We provide in vitro evidence that secreted molecules in USSC-conditioned medium very efficiently support neurite outgrowth of embryonic dorsal root ganglia explants and cortical neurons, respectively (Fig. 4).

Further, we have investigated potential influences of grafted USSC on tissue preservation in spinal cord injury. We compared the lesion size and spared tissue area in USSC-transplanted rats and control animals. Grafting of USSC into traumatic spinal cord injury significantly reduced the lesion size and enhanced the amount of spared tissue (Fig. 5) indicating a strong neuroprotective function of USSC similar to bone marrow MSC (Ankeny et al., 2004; Gu et al., 2010; Osaka et al., 2010). As shown previously (Kögler et al., 2005), native USSC release cytokines and growth factors with known neuroprotective and axon growth-promoting functions, such as leukaemia inhibitory factor, vascular endothelial growth factor, stromal cell-derived factor-1 and granulocyte macrophage colony-stimulating factor. Moreover, comparing cytokine production between USSC and bone marrow MSC revealed that certain growth factors are released at higher concentrations by USSC than by bone marrow MSC including vascular endothelial
growth factor with known support of neuronal survival (Jin et al., 2000; Sun et al., 2003; Tovar-y-Romo and Tapia, 2010), neurite outgrowth (Khaibullina et al., 2004) and tissue sparing in spinal cord injury (Kim et al., 2009). Furthermore, USSC release stromal cell-derived factor-1, which induces homing of neural stem cells in the ischaemic or injured brain (Limotila et al., 2004) and stimulates axonal sprouting in spinal cord injury (Opacz et al., 2009). Interestingly, USSC also release HGF, which is known to promote motoneuron survival and axonal regrowth, and is further considered as a guidance and survival factor during neural development (Ebens et al., 1996; Giacobini et al., 2007). The amount of endogenous USSC-derived HGF apparently is insufficient to disturb the external HGF gradient that originates from injured spinal cord tissue and strongly attracts the USSC as shown here (Fig. 1). The different growth factors or combinations thereof are likely to participate in the beneficial effects observed after USSC transplantation into the injured spinal cord.

Finally, our behavioural data reveal that, besides axon growth and tissue sparing, USSC promote functional locomotor recovery after transplantation into a model of acute spinal cord injury (Fig. 6 and Supplementary Fig. 1). In addition to the open field Basso–Beattie–Bresnahan locomotor score that has certain limitations in the determination of forelimb–hindlimb coordination (Koopmans et al., 2005), we performed the horizontal ladder walking (grid walk) test and the CatWalk gait analysis. The grid walk test was used to assess hindlimb placing and stepping, while the CatWalk gait analysis allowed us to determine interlimb coordination and individual paw parameters. At the beginning of the study (1 week after transplantation), all injured animals showed equivalent deficits as indicated by similar open field Basso–Beattie–Bresnahan locomotor scores and locomotor subscores. With regard to the open field Basso–Beattie–Bresnahan subscore, USSC-grafted animals performed better than the non-grafted control group reaching significance for the right hindlimb at 6 weeks after transplantation (Fig. 6A and B). Furthermore, at 6 weeks after transplantation and thereafter, USSC-transplanted animals made significantly fewer missteps with both hindlimbs in the horizontal ladder walking test (Fig. 6C and D). In addition, the assessment of static (relative paw placement, base of support, maximal contact area and maximal paw print area) as well as dynamic (swing speed, swing duration) gait parameters by means of CatWalk analysis revealed significant locomotor improvement for all gait parameters tested (Fig. 6E–H; Supplementary Fig 1C and D). The improved locomotor performances of USSC-transplanted rats were observed in all three behavioural tasks and remained until the end of the long-term (16 weeks after transplantation) study (Fig. 6A–H), indicating a long-lasting functional benefit resulting from transplantation of USSC into traumatic spinal cord injury.

The controversial reports in the literature about functional outcome after transplantation of somatic stem cells derived from bone marrow or umbilical cord blood (Lu et al., 2005; Neuhuber et al., 2005; Cizkova et al., 2006; Himes et al., 2006; Sheth et al., 2008; Sasaki et al., 2009) could be explained by the heterogeneity of the origin and insufficient characterization of the stem cell populations used, which mostly included the entire mononuclear cell fraction (for review see Tetzlaff et al., 2011). Furthermore, the functional outcomes reported for the latter cell preparations must be cautiously interpreted, as most of the published results were solely based on a single functional test.

Here, we have transplanted a well-defined somatic stem cell type from human umbilical cord blood into an animal model of acute traumatic spinal cord injury and demonstrated significant long-lasting functional outcome using three different behavioural tests to investigate several task-specific locomotor parameters. The observed functional improvement correlates well with reduced tissue loss or augmented tissue sparing and stimulation of regenerative axon growth. To accomplish the beneficial effects, neither neural differentiation nor long-lasting persistence of the cell graft is required. The secretion of neurite outgrowth-promoting factors by non-differentiated USSC suggests a paracrine mechanism underlying the beneficial effects of USSC in spinal cord injury. Based on the remarkable functional properties without adverse effects in spinal cord injury as well as the availability of GMP-grade cells in virtually unlimited quantities and the lack of ethical concerns, the well-defined neonatal USSC appear to be a highly suitable human cell source for clinical application in CNS injuries.

Acknowledgements

The authors thank Brígida Ziegler for excellent technical assistance and Nicole Brazda for helpful advice in the CatWalk gait analyses.

Funding

German Research Council Deutsche Forschungsgemeinschaft (DFG) (grant number MU 630/10-1); Christiane and Claudia-Hempel Foundation for Clinical Stem Cell Research.

Supplementary material

Supplementary material is available at Brain online.

References


