Transplantation of Autologous Olfactory Ensheathing Cells in Complete Human Spinal Cord Injury

Pawel Tabakow,* Wlodzimierz Jarmundowicz,* Bogdan Czapiga,* Wojciech Fortuna,†
Ryszard Miedzybrodzki,† Marcin Czyz,* Juliusz Huber,‡ Dariusz Szarek,* Stefan Okurowski,§
Pawel Szewczyk,¶ Andrzej Gorski,†# and Geoffrey Raisman**

*Department of Neurosurgery, Wroclaw Medical University, Wroclaw, Poland
†Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland
‡Department of Pathophysiology of Locomotor Organs, Karol Marcinkowski Medical University, Poznan, Poland
§Neurorehabilitation Center for Treatment of Spinal Cord Injuries AKSON, Wroclaw, Poland
¶Department of General Radiology, Interventional Radiology and Neuroradiology, Wroclaw Medical University, Wroclaw, Poland
#Transplantation Institute, Warsaw Medical University, Warsaw, Poland
**Spinal Repair Unit, UCL Institute of Neurology, Queen Square, London, UK

Numerous studies in animals have shown the unique property of olfactory ensheathing cells to stimulate regeneration of lesioned axons in the spinal cord. In a Phase I clinical trial, we assessed the safety and feasibility of transplantation of autologous mucosal olfactory ensheathing cells and olfactory nerve fibroblasts in patients with complete spinal cord injury. Six patients with chronic thoracic paraplegia (American Spinal Injury Association class A-ASIA A) were enrolled for the study. Three patients were operated, and three served as a control group. The trial protocol consisted of pre- and postoperative neurorehabilitation, olfactory mucosal biopsy, culture of olfactory ensheathing cells, and intraspinal cell grafting. Patient’s clinical state was evaluated by clinical, neurophysiological, and radiological tests. There were no adverse findings related to olfactory mucosa biopsy or transplantation of olfactory ensheathing cells at 1 year after surgery. There was no evidence of neurological deterioration, neuropathic pain, neuroinfection, or tumorigenesis. In one cell-grafted patient, an asymptomatic syringomyelia was observed. Neurological improvement was observed only in transplant recipients. The first two operated patients improved from ASIA A to ASIA C and ASIA B. Diffusion tensor imaging showed restitution of continuity of some white matter tracts throughout the focus of spinal cord injury in these patients. The third operated patient, although remaining ASIA A, showed improved motor and sensory function of the first spinal cords segments below the level of injury. Neurophysiological examinations showed improvement in spinal cord transmission and activity of lower extremity muscles in surgically treated patients but not in patients receiving only neurorehabilitation. Observations at 1 year indicate that the obtaining, culture, and intraspinal transplantation of autologous olfactory ensheathing cells were safe and feasible. The significance of the neurological improvement in the transplant recipients and the extent to which the cell transplants contributed to it will require larger numbers of patients.

Key words: Human; Olfactory ensheathing cells (OECs); Spinal cord injury (SCI); Transplantation

INTRODUCTION

Most accepted treatment protocols for spinal cord injury focus on techniques of early neuroprotection aimed at maximal prevention of secondary spinal cord injury (5–7,27,56) as well as on methods of stimulation of plasticity in the central nervous system (CNS) (39). While these methods have been shown to have functional benefit in patients with incomplete spinal cord injury, the results in cases of complete human spinal cord injuries remain very limited. Presumably the limited number of spared axons is unable to mount an adequate plastic response. Besides, injured supraspinal and spinal neuronal populations undergo progressive degeneration showing no regenerative response (50). Critical factors determining the lack of regeneration in the CNS are the growth inhibitory influence of central postrauumatic glial scar and myelin-associated proteins as Nogo, the lack of intrinsic capacity of surviving neurons for regeneration (20), and the lack of an effective glial pathway to guide regenerating axons across the lesion (31).

Results from a substantial number of animal experiments performed mainly on the model of mammalian spinal cord injury in the last three decades led to the establishment
of numerous regeneration-promoting strategies including application of neurotrophic factors, antibodies blocking the myelin-associated proteins, and transplantation of cells with neurotrophic activity (2,36,57). Among the various populations of neurotrophic cells being tested experimentally, the olfactory ensheathing cells (OECs) deserve special attention. They are a unique population of macroglia found in the lamina propria of olfactory mucosa, around the olfactory nerve fascicles, and in the two outer layers of the olfactory bulb (11,34,43,44,46). These cells have the natural ability to stimulate the regrowth of lesioned peripheral and central axons. When transplanted into complete transection lesions of the thoracic spinal cord of rats, they evoked long-distance axon regeneration and subsequent recovery of locomotion of the paralyzed limbs (45,47,49). OECs were also able to guide transected axons of the selectively lesioned corticospinal tract throughout the focus of injury which resulted in restoration of paw movements (22,29). OECs have been also shown to restore breathing and climbing after transplantation into high cervical spinal cord injuries (28).

The observed robust regeneration of various supraspinal and spinal neuronal populations in the injured spinal cord after transplantation of OECs, documented in preclinical studies, has generated a considerable interest in the application of these cells as a strategy for repair of the injured human spinal cord. Several clinical trials, focused on the effects of intraspinal grafting of olfactory tissue or OECs isolated from that tissue, have been conducted in patients with complete chronic spinal cord injury (9,15,18,32,33,38). To date, there is only one completed Phase I study evaluating the safety and feasibility of transplantation of autologous OECs in paraplegics (15,38). In a 3-year clinical trial, the authors reported that the procedure of microinjection into the spinal cord of purified OECs obtained from the olfactory mucosa was safe in the patients examined. No significant neurological recovery was detected.

In a Phase I nonrandomized controlled prospective study, we have tested the hypothesis that an approach for treatment of complete spinal cord injuries consisting of intraspinal transplantation of autologous OECs and fibroblasts isolated from the olfactory mucosa, combined with intense neurorehabilitation, is safe and feasible. In this article, we report the outcome of the trial 1 year after cell transplantation.

**MATERIALS AND METHODS**

**Patients**

For patient recruitment, personal interviews were followed by detailed review of medical records, performance of laboratory tests, and neurological examination. The inclusion criteria were a single spinal cord injury between levels C5 and L5; myelopathy not exceeding two spinal cord segments as confirmed by MRI, complete loss of sensory and motor function below the injury, confirmed in control studies (ASIA Category A); time from injury between 6 months and 5 years; age from 16 to 65 years; patient undergoing continuous rehabilitation; good patient motivation and cooperation, no mental disturbances. Exclusion criteria included a coexisting lesion of the nervous system, progressive post-traumatic syringomyelia, significant spinal stenosis or instability, muscle atrophy or joint ossifications, severe systemic disease such as neoplasm, contagious disease, diabetes, etc.

Patients who met the above-mentioned criteria underwent laryngological examination and a computerized tomography (CT) scan of the nasal cavity and paranasal sinuses. Those who were diagnosed with chronic sinusitis, tumors, or polyps of nasal cavities or presented hyposmia in repetitive smell perception tests were excluded.

Patients for this pilot trial were selected from over 50 enquiries. Recruitment took place over 2 years. Six patients were enrolled in the study. Three patients were qualified for the procedure of olfactory tissue biopsy and subsequent cell transplantation (surgery group), and three patients were qualified to the nontransplanted group (control group). All patients were males, aged 22–26 years, and were diagnosed with complete chronic thoracic spinal cord injury between T3 and T11. In five of the cases, the spinal cord injury was caused by motor vehicle accident, and in one case, a penetrating spinal cord injury was caused by knife assault (Table 1). All patients provided a written informed consent according to the Declaration of Helsinki and understood the risk of the trial and the potential for no benefit. The trial was approved by the Bioethics Committee of Wroclaw Medical University, according to the guidelines of the National Health Council of Poland.

**Rehabilitation Protocol**

All patients qualified for the trial underwent the same neurorehabilitation program at the Centre for Rehabilitation of Spine Injuries “Akson” (Wroclaw, Poland). The program was performed under the supervision of an experienced spinal injuries physiotherapist. Before being qualified for the study, the patient’s records from previous rehabilitation programs as well as the patient’s medical status were carefully examined in order to exclude those cases where

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>Years After Injury</th>
<th>Mechanism of SCI</th>
<th>Vertebrae Level of Injury</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>M</td>
<td>24</td>
<td>4</td>
<td>Transection</td>
<td>T10/T11</td>
</tr>
<tr>
<td>T2</td>
<td>M</td>
<td>24</td>
<td>5</td>
<td>Compression</td>
<td>T6/T7</td>
</tr>
<tr>
<td>T3</td>
<td>M</td>
<td>26</td>
<td>1.3</td>
<td>Compression</td>
<td>T4</td>
</tr>
<tr>
<td>C1</td>
<td>M</td>
<td>22</td>
<td>1.5</td>
<td>Compression</td>
<td>T3</td>
</tr>
<tr>
<td>C2</td>
<td>M</td>
<td>25</td>
<td>1.5</td>
<td>Compression</td>
<td>T11</td>
</tr>
<tr>
<td>C3</td>
<td>M</td>
<td>24</td>
<td>1.2</td>
<td>Compression</td>
<td>T5</td>
</tr>
</tbody>
</table>

T1–T3, transplant recipients; C1–C3, control patients; SCI, spinal cord injury; M, males.
significant muscle atrophy, joint ossifications, or significant spasticity were present. Patients from the transplant recipient group were qualified for preoperative rehabilitation that was planned to last at least 3 months. Postoperative rehabilitation was planned for 24 months. Patients from the control group were also qualified for a 24-month training.

The rehabilitation program was divided into cycles. One rehabilitation cycle lasted 3 months. The break between the cycles did not exceed 4 weeks. Patients were trained 4–5 h/day, 3–5 days a week. The training agenda consisted of 1 h range of motion and stretching exercises, 3 h locomotor training, and 1 h sensory training. The main emphasis was set on locomotor training that included overground and treadmill walking exercises as well as training for posture and balance.

Olfactory Mucosal Biopsy

After completion of the cycle of preoperative neurorehabilitation, patients from the transplant recipient group were admitted to the Department of Neurosurgery Wroclaw Medical University for the procedure of olfactory mucosa retrieval. Preoperatively, thin-slice computed tomography (CT) and magnetic resonance imaging (MRI) scans of the patient’s heads were performed and merged according to the standard protocol for image-guided navigation. The area for mucosal biopsy was chosen based on our experience with obtaining olfactory tissue from cadaver donors (41). We chose the area of the nasal septal mucosa that was located just below the cribiform plates of the ethmoid bone. This area was marked using a commercially available program for planning of cranial navigation (Stealth Station, Medtronic, Minneapolis, MN, USA). On the day before operation, standard laryngological tests for evaluation of nostril patency and for assessment of respiratory epithelium function (the saccharine test) were performed.

Then patient’s smell perception was tested. We developed a 12-point scale for evaluation of smell perception using the following fragrant oils—vanilla, coffee, and almonds. Smell perception was tested for each nostril separately.

For each tested smell, a score of 0–2 points was assigned, where 0 meant no odor perception, 1 = slight smell discrimination, and 2 = normal smell perception.

For mucosal biopsy, the patient was placed under general anesthesia in a supine position, with head slightly extended and fixed in a Mayfield clamp (Doro Cranial Stabilization, Pro Medical Instruments, Tuttingen, Germany). The nasal cavity was repeatedly rinsed with sterile isotonic sodium chloride solution (PolPharma, Gdansk, Poland) and then disininfected with 3% H2O2 solution (Hasco-Lek, Wroclaw, Poland). The transnasal transeptal submucoperiosteal microsurgical approach, which is a part of the traditional transnasal transphenoidal approach for treatment of pituitary tumors, was used for dissection of the olfactory mucosa. After partial removal of the perpendicular lamina of the ethmoid bone, the nasal mucosa was excised en bloc bilaterally to the septum under the guidance of instruments for cranial navigation (Fig. 1). Five to six mucosal fragments with a mean size of 1.75 cm² were collected from each patient. To obtain sufficient numbers of OECs Patient T1 underwent a second biopsy procedure after 7 days.

The biopsy specimens were directly placed in 3% H2O2 solution for 30 s and transported in cold mixture of complete culture medium (1:1, v/v) of Dulbecco’s modified Eagle’s medium and Ham’s F12 (DMEM/F12; Sigma, St. Louis, MO, USA) supplemented with 10% inactivated fetal bovine serum (FBS, HyClone, Logan, UT, USA), 1 mM glutamine (Sigma), 100 U/ml penicillin, and 100 µg/ml streptomycin (both from Polfa Tarchomin, Warsaw, Poland). Subsequent processing was performed under aseptic conditions. After washing the tissue in Hank’s balanced salt solution (HBSS; Institute of Immunology and Experimental Therapy, Wroclaw, Poland), the fragments of olfactory mucosa were incubated for 45 min at 37°C in a 2.4 units/ml solution of dispase II from Bacillus polymyxa (Roche, Mannheim, Germany). Digestion was stopped by adding HBSS supplemented with 10% FBS, and the tissue was washed once again in HBSS. The olfactory epithelium was carefully peeled away from the lamina propria with a microspatula under a dissection microscope. The lamina propria was finely minced with scissors and washed in HBSS, and the tissue was resuspended in 5 mg/ml collagenase H solution (Sigma) was incubated for 15 min at 37°C. Enzyme activity was stopped by adding 2 mM ethylene-diaminetetraacetic acid (EDTA; Chempur, Karlsruhe, Germany) in PBS with 10% FBS. The tissue was triturated sequentially by passing it through 19–22-gauge needles, and the cell suspension was centrifuged (SIGMA Laborzentrifugen GmbH, Osterode am Harz, Germany) for 2 min at 800 × g, resuspended in complete culture medium, triturated with 20–25-gauge needles, and placed in culture dishes. A small aliquot of cell suspension was incubated with acridine orange (15 mg/ml) and ethidium bromide (50 mg/ml) mixture (both from Sigma) (8), and cell viability was assessed under fluorescence microscopy (Zeiss, Poznan, Poland). The tissue was processed for culture within 4 h from olfactory mucosa collection.

Cell Culture

The dissociated cells were seeded on 30–70 kDa poly-L-lysine hydrobromide (0.1 mg/ml, Sigma)-coated plastic dishes (Corning, Corning, NY, USA) and cultured in complete culture medium (1:1, v/v) of DMEM/F12 supplemented with 10% FBS, 1 mM glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin. The cultures were maintained at 37°C in 95% O2/5% CO2, at 95% humidity. The cells were fed every second day by replacing half of the culture medium volume. At the time window when the cells reached confluence the cells were detached by
trypsinization (Gibco, Gaithersburg, MD, USA; at 17, 18, and 21 days for the three patients, respectively), the enzyme activity was stopped by adding HBSS with 10% FBS, and the cell suspension was transferred into 1.5-ml tubes (Sarstedt AG & Co., Nümbrecht, Germany), centrifuged for 2 min at 800 × g, and resuspended in HBSS with 10% FBS. The next steps of cell preparation took place in the operating theatre on the same day. After four rounds of washing in HBSS without serum, the supernatant was entirely discarded, and the cells were resuspended in serum-free medium and transferred to a glass syringe (World Precision Instruments, Sarasota, FL, USA) for microinjection.

Sterility Tests and Identification of Cell Phenotype

A 100-μl aliquot of supernatant from the culture medium was taken every 5 days for the assessment of development of bacterial or fungal infection. The samples were transferred to transport swabs (Hagmed, Tomaszowska, Poland) and delivered to the Microbiology Department of the Wroclaw Medical University.

The two main populations of cells present in the olfactory lamina propria, the OECs and the olfactory nerve fibroblasts (ONFs), were identified in culture by their characteristic morphology and by immunocytochemistry. OECs were characterized as bi- or multipolar cells with long processes, and the next steps of cell preparation took place in the operating theatre on the same day. After four rounds of washing in HBSS without serum, the supernatant was entirely discarded, and the cells were resuspended in serum-free medium and transferred to a glass syringe (World Precision Instruments, Sarasota, FL, USA) for microinjection.

Figure 1. Photograph taken from the cranial navigation system during the operation of olfactory mucosal biopsy. Coronal, sagittal, and axial magnetic resonance imaging (MRI) scans of the head have been merged with analogous computed tomography (CT) scans of the patient. The blue tip shows an intraoperatively navigated instrument determining the area of planned resection of nasal septal mucosa for olfactory tissue collection (outlined with color lines on sagittal scan). This area covers the region just below the cribriform plates and extends downwards to the virtual line formed by the inferior wall of the sphenoid sinus (pink line).
small nucleus, and reduced cytoplasm, expressing the cytoplasmic S100 protein and the p75 low-affinity nerve growth factor receptor (p75-LNGFR). ONFs were identified by the presence of fibronectin (FN) in their cytoplasm. The presence of specific cells populations in culture was assessed with a fluorescence microscope (magnification: 100×, analyzed field of vision of the digital camera was 0.4 mm²). Immunocytochemistry was carried out as described previously by Ramón-Cueto and Valverde (48). Briefly, the cells were fixed with 3% paraformaldehyde (Sigma) for 10 min at 37°C, then washed twice in PBS with 0.5% FBS (Hyclone). The cells from the cultures selected for S100 and FN immunostaining were permeabilized using 0.05% Triton X-100 (Serva, Heidelberg, Germany) in PBS with 10% FBS for 60 min, then incubated for 60 min at 37°C with primary polyclonal rabbit anti-FN antibody (1:100, Sigma), primary polyclonal rabbit anti-S100 antibody (1:100, DAKO, Glostrup, Denmark), or primary monoclonal mouse anti-p75 NGFR antibody (1:50, Kamiya, Seattle, WA, USA). After washing, secondary antibodies (both from Sigma) were added for 60 min at 37°C: R-phycocerythrin (R-PE) conjugated with goat anti-rabbit IgG (1:200 and 1:400) to the cells previously immunostained for S100 and FN or fluorescein isothiocyanate (FITC) conjugated with goat anti-mouse IgG (1:60) for the cells labeled with p75-LNGFR. In all assays, controls were performed by incubation of cells with secondary antibodies. DNA of cell nuclei was labeled by incubation with 5 μM of Hoechst 33342 dye (Sigma) for 5 min, washed, and visualized under fluorescence microscopy.

Cell Transplantation Procedure

Preoperative Preparation. The patients were readmitted to the Department of Neurosurgery for the operation of cell transplantation in the period between 17 and 21 days after mucosal biopsy. A detailed analysis of preoperative MRI (GE Signa HDx; GE Healthcare, Wauwatosa, WI, USA) scans of the patients enabled the determination of the approximate extent of posttraumatic myelopathy and the demarcation of the target area of the spinal cord located rostrally and caudally to the lesion epicenter, selected for cell grafting. Based on data from the axial T2-weighted MRI scans, a virtual three-dimensional (3D) model of the spinal cord lesion was built for each transplant recipient. These 3D models were used for the elaboration of a schematic grid for cell microinjection and also allowed us to establish in each case specific topographic reference points on the surface of the spinal cord for intraoperative navigation of the stereotactic injection device. In general, injections were planned to be done in a matrix pattern into the lateral columns of the normal spinal cord parenchyma, proximally and distally to the lesion epicenter. This matrix consisted of four to five rows, 2 mm apart. The posterolateral sulcus was chosen as the main entry point for cell microinjection. Other target for cell microinjection was the spinal cord localized close to the lesion epicenter (Patients T1, T2, and T3) and the dorsal columns of the spinal cord (Patient T3) (Fig. 2). The cell suspension was planned to be delivered maximally at seven depths at each injection site, 0.5 mm apart, depending on the thickness of the spinal cord at a specific level. We set the coordinates for cell injection by comparing the topography of the axial MRI scans of the spinal cord of our patients with photographs of equivalent normal spinal cord segments contained in the National Library of Medicine’s Visible Human Project (www.nlm.nih.gov/research/visible/visible_human.html).

System for Cell Microinjection. The microinjection system was composed of an automatic micropump (UltraMicro Pump II, World Precision Instruments) and a 3D micromanipulator (SM-15, Narishige, Tokyo, Japan). The injector device was fitted with a 25-μl glass syringe with 26-gauge bevelled needle (World Precision Instruments). The micropump was modified to be connected to the micromanipulator. Further modifications allowed attachment of the micromanipulator to the surgical frame during operation. Parameters for cell injection were as follows: volume of single injection 0.5 μl, velocity of injection 2 μl/min, cell concentration from 30,000–200,000 cells/μl.

The safety of the procedure of olfactory ensheathing cell and olfactory fibroblast intraspinal microinjection was tested in an experimental study in which the cells were transplanted into the intact spinal cord of adult rats (40). In this study, we showed that this method of transplantation did not have any negative effects on the neurologic state of the rats. After a minimal inflammatory reaction in the spinal cord of OEC recipients, long-term survival of injected OECs was obtained in most examined animals. No tumorigenesis was noted in the OEC-grafted rats during the entire period of 16 weeks after cell grafting.

Surgical Technique. Operations were performed under general endotracheal anesthesia. The patient was placed in a prone position. The level of operation was identified preoperatively with fluoroscopy. A midline skin incision was made at the level of the injury, followed by thoracic laminectomy (Patients T1 and T2) or removal of the spinal fixation system (Patient T3). The dura was exposed at the site of injury and for about 2 cm rostrally and caudally to the focus of injury. Using the magnification of an operating microscope (OPMI Pentero, Zeiss Company, Germany), a midline durotomy was performed, followed by sharp dissection of the posttraumatic adhesions between the spinal cord surface and the dura. The area of spinal cord lesion had different morphology depending on the mechanism of trauma. In the first operated case (Patient T1) the spinal cord had undergone a transection. It had a regular shape, becoming atrophic at the level of injury. The vasculature proximally to the lesion had normal appearance but was
Figure 2. Preoperative MRI scans of the injured spinal cords of the transplant recipients and its virtual 3D models. Black dots (1 mm apart) show the navigation plane, white dots (2 mm apart) show the entry points in the posterolateral sulcus planned for cell microinjection, and blue dots show the areas where additional microinjections were performed. (A-C) Patient T1; (D-F) Patient T2; and (G-I) Patient T3. (A) T2-weighted sagittal MRI scan of the thoracic spinal cord of Patient T1 4 years after its transection at vertebral level T10/T11. The focus of myelopathy comprised of a firm gliotic scar, 1.8 mm in diameter. (B) The glial scar (red color) was oblique and irregular and had two compartments—intraparenchymal (matt red) and dorsal exophytic (clear red). (C) Schematic grid for cell transplantation. The dorsal exophytic scar served as a landmark for intraoperative navigation of the stereotactic injection device. (D) T2-weighted sagittal MRI scan of the spinal cord of Patient T2 5 years after its compression by dislocated fractured T7 vertebra. The focus of the spinal cord injury (SCI) comprised of a thin gliotic scar, 1.8 mm in diameter (arrowhead) and a nonprogressive 22-mm-long cystic cavity proximally to the lesion epicenter. (E) 3D model of the area of the injured spinal cord. Arrowhead shows the focus of primary SCI. (F) Schematic grid for cell microinjection. The proximal cystic cavity (orange) served as a landmark for intraoperative navigation of the stereotactic injection device. Arrowhead shows the focus of primary SCI. (G) Sagittal view of the spinal cord lesion of Patient T3 on a T1-weighted MRI scan 1.5 years after its compression by fractured vertebrae T4 and T5. The area of myelopathy comprised of a 27-mm-long cystic cavity. (H) 3D model of the spinal cord. The focus of SCI was localized slightly above the level of the metal prostheses of vertebrae T4 and T5 (red color). (I) Schematic grid for cell microinjection. The lesion cavity is shown in blue. Its most superficial part (shown in green) served as a landmark for cell microinjection. In this patient after finishing of cell transplantation into the lateral columns (white dots), injections were made additionally into the dorsal columns (blue dots).
less identifiable in the distal stump. A firm 1.5-cm fibrous scar was observed at the level of spinal cord transection. This scar adhered to the dura matter, traversed through a posttraumatic tear of the dura into the epidural space, and was adherent to cicatrized fragments of the paravertebral muscles. In Patient T2, who had sustained a compressive injury, the spinal cord was much more atrophic at the level of primary injury and contained a posthemorrhagic calcification at the lesion epicenter. A cystic cavity was observed in the spinal cord stump proximal to the lesion epicenter. In Patient T3, an irregular cystic posttraumatic cavity was also observed. In neither case was the intraparenchymal scar tissue removed. After completion of the dorsal myeladhesiolysis, the system for stereotactic cell microinjection was mounted on the operating table. At this moment, the depth of anesthesia was raised to minimize the amplitude of patient’s respiratory movements. The mixture of cultured autologous OECs and ONFs suspended in serum-free culture medium was centrifuged and added to an Eppendorf vial (Eppendorf AG, Hamburg, Germany) and a 25-μl glass syringe with 26-gauge bevelled needle was filled with the cells. In all patients, the main part of the mixture of OECs and ONFs was injected through the posterolateral sulcus between the dorsal nerve rootlets into the lateral columns of the spinal cord, proximally and distally to the lesion epicenter (Fig. 2). The remaining cell suspension was injected into the dorsal columns of the spinal cord (T3) and around the lesion epicenter (T1, T2, T3).

In Patient T1, the cell suspension was delivered through 120 microinjections via 20 separate spinal cord penetrations. In Patient T2, the cell suspension was delivered through 128 microinjections via 40 injection sites. Patient T3 received 212 microinjections through 46 injection sites. The detailed parameters of cell microinjection are summarized in Table 2.

At the end of the procedure of microinjection, a small aliquot of cell suspension remaining in the microsyringe was taken for assessment of sterility and monitored in continued cell culture. The dura was closed with absorbable sutures. No duraplasty was performed. A wound drain was placed under the muscle layer, and the wound was closed in layers.

**Initial and Repeated Assessments**

Patients’ pre- and postoperative evaluation included general medical assessment; neurological, otorhinolaryngological, and physiotherapeutical examination; and radiological and neurophysiological studies. General medical assessment was conducted preoperatively in the first 2 weeks postsurgery and then every 3 months. Before surgery, tests for the presence of anti-HIV antibodies were performed, and hepatitis B and C status was assessed. Perioperatively, the transplant recipients were assessed for hematology and blood chemistry, and urine analysis. Laboratory tests were followed by an electrocardiographic study and chest X-rays. Microbiological studies of blood, urine, or cerebrospinal fluid (CSF) were planned to be conducted only in case of suspicion of sepsis or neuroinfection.

Laryngological examination concerned only transplant recipients and included functional and smell perception tests as well as evaluation of image studies of the nasal cavity and paranasal sinuses.

Neurological examination was performed monthly by the same assessor and included ASIA examination, evaluation of spasticity using the Ashworth scale, and assessment of reflex activity. The assessor was not blinded.

At 3-month intervals, a spinal injuries physiotherapist undertook a functional independence measure (FIM). Additionally, achievements in each physical exercise during the locomotor training were regularly recorded and documented.

MRI images were undertaken on a 1.5-T MR unit and included T2-weighted, T2-weighted fat saturation (FAT-SAT) images and T1-weighted images before and after administration of contrast medium (gadolinium; MultiHance, Bracco Diagnostics, BIPSO GmbH, Singen, Germany), in sagittal, coronal, and axial planes. In transplant recipients, MRI study was performed preoperatively and at 1, 3, 6–7, and 11–12 months postsurgery in the first year of observation. In control patients, MRI was conducted at the beginning and end of the procedure of microinjection, a small aliquot of cell suspension remaining in the microsyringe was taken for assessment of sterility and monitored in continued cell culture. The dura was closed with absorbable sutures. No duraplasty was performed. A wound drain was placed under the muscle layer, and the wound was closed in layers.

**Table 2. Parameters of Cell Microinjection**

<table>
<thead>
<tr>
<th>Volume of Single Injection</th>
<th>Velocity of Cell Injection</th>
<th>Total Number of Injections</th>
<th>Number of Injection Sites</th>
<th>Total Volume</th>
<th>Total Number of Grafted Cells</th>
<th>Percentage of OECs in the Cell Suspension</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 0.5 μl</td>
<td>2 μl/min</td>
<td>120</td>
<td>20</td>
<td>60 μl</td>
<td>1,800,000</td>
<td>10</td>
</tr>
<tr>
<td>T2 0.5 μl</td>
<td>2 μl/min</td>
<td>128</td>
<td>40</td>
<td>64 μl</td>
<td>1,920,000</td>
<td>25.7</td>
</tr>
<tr>
<td>T3 0.5 μl</td>
<td>2 μl/min</td>
<td>212</td>
<td>46</td>
<td>106 μl</td>
<td>21,200,000</td>
<td>12</td>
</tr>
</tbody>
</table>

T1–T3, transplant recipients.
of the observational period. At time of postoperative MRI, all three transplanted patients had diffusion tensor imaging (DTI). Patient T2 also had one preoperative DTI. In Patient T3, the posterior spinal fixation system was removed at the time of transplantation to permit the postoperative DTI. The DTI was performed as follows: 25 diffusion directions, NEX 2, axial acquisition, slab thickness 4 mm, no gap, TR 10,000 ms, TE 100 ms. DTI included an assessment of the topography of the water diffusion tracts in the spinal cord on tractography and the estimation of the values of fractional anisotropy (FA) of the spinal cord on the FA maps at 0.5 and 2 cm above and below the lesion epicenter.

Neurophysiological examinations included transcranial magnetic motor-evoked potentials (MEPs), electroneuromyography (ENG), and electromyography (EMG) using the KeyPoint Diagnostic System (Medtronic, Copenhagen, Denmark). The results of the neurophysiological investigations were additionally evaluated by a blinded assessor.

Standard single pulses of the magnetic field were used for transcranial magnetic stimulation and were induced from MagPro generator (Medtronic, Denmark) via 70-mm coil placed bilaterally on the patient’s head, over the areas of cortical motor neurons controlling the abdominal and lower extremity muscles. The optimal site for stimulation was defined with tracking stimuli delivered at 1 Hz with 40% of maximal stimulus strength (1.5 T) when the maximal amplitude MEP was recorded from the responding muscle. The highest intensity stimulus never exceeded 90% of the maximal value. The smallest MEP amplitude parameter needed for transmission within corticospinal fibers was at 50 μV. Potentials were usually recorded with time base at 5 ms/D and sensitivity of amplification at 100–1000 μV/D of the Keypoint recorder.

In all subjects, bilateral ENG M-wave-evoked potentials recordings in lower extremity nerves evaluated the transmission of peripheral motor fibers impulses. Amplitudes and conduction velocities parameters were analyzed and calculated from traces recorded with time base at 5 ms/D and sensitivity of amplification at 5,000 μV/D.

Bilateral EMG recordings with surface electrodes from the rectus abdominis, rectus femoris, gastrocnemius, anterior tibial, and extensor digiti muscles assessed the muscle motor units activity. Amplitude and frequencies of recruited motor units action potentials were analyzed in recordings with time base at 80 ms/D and sensitivity of amplification at 100–500 μV/D.

Neurophysiological testing was performed in transplant recipients before surgery and at 1, 6, and 12 months post-cell grafting. In control patients, tests were conducted at the beginning and end of the rehabilitation.

The urodynamic study was conducted according to the protocol of the International Continence Society (58) using the Duet Logic G2 system (Medtronic, Denmark). It consisted of uroflowmetry, pressure flow studies, and electromyography of anal sphincter. It was performed at the same time as the neurophysiological tests were conducted.

**RESULTS**

**Evaluation of Safety of the Procedure of Olfactory Mucosa Biopsy**

There were no intra- or postoperative complications attributed to the procedure of olfactory mucosa biopsy in any of the transplant recipients. In the first week postsurgery, a loss of smell perception was noted in the patients. Laryngological evaluation revealed edema of the nasal mucosa. During the next 7–14 days, all patients regained their normal olfactory function achieving the maximal range of 12 points in the smell perception test (Fig. 3).

**Cell Culture**

The cultures of cells isolated from the olfactory mucosa contained mainly OECs and ONFs and were not further purified. The preoperative viability of the isolated cells varied from 76.6% to 85%. In the culture dish, bi- or multipolar S100 and p75-LNGFR OECs formed networks on a monolayer of FN-positive ONFs (Fig. 4). OEC and ONF immunostaining covered more than 95% of Hoechst-stained cell populations. The percentage of S100-positive cells in culture in Patients T1, T2, and T3 was 10%, 25.7%, and 12%, respectively. After a period of 14–21 days, when OECs reached a confluent monolayer in culture, they were harvested from the culture flasks and prepared for

![Figure 3](image-url)
transplantation. The sterility of the cell culture was checked every 5 days. There was no evidence of bacterial or fungal contamination within the 3-week period of cell culture. The residual volume of cells remaining in the syringe at the end of the operation of cell grafting was seeded on to culture flasks and cultured for about 10 days for identification of cell populations and for assessment of culture sterility. In all cultures used for transplantation S100-positive cells could be identified in culture, and there was no evidence of microbial contamination (Fig. 4).

Assessment of the Safety of the Operation of Cell Transplantation

Clinical Evaluation. Some adverse events following the procedure of cell grafting were observed in all operated patients, mainly in the early postoperative period (Table 3). These adverse events were typical for patients subjected to long-lasting operations of the spine and were not aggravated in the transplanted patients. In the first postoperative week, Patients T1 and T2 were diagnosed for urinary tract infection and for mild anemia. After administration of antibiotics, the symptoms of uroinfection resolved within a period of 3–4 days. In Patient T1 decubitus ulcers on the forehead (grade I) and transient paresis of the right musculocutaneous nerve were observed as a result of prolonged maintenance of the patient in prone position during surgery. During the first 5 postoperative days, Patient T3 received catecholamines and a red cell transfusion because of symptoms of systemic hypotension and progressive anemia.

Followed for 1 year after transplantation, there were no clinical complications related to the procedures of myeloablative and microinjection of OECs and ONFs into the spinal cord; none of the patients experienced neurological deterioration, meningitis, CSF leakage, or local infection at the site of surgery. No patient complained of neuropathic pain.

Radiology. MRI studies of the spine showed no signs of postoperative spinal stenosis or instability in any of the transplanted patients. There was no evidence of pseudomeningocele or spinal cord tumors at the injection site in the first year after cell transplantation (Fig. 5). In Patient

| Table 3. Summary of the Adverse Events Observed in the Cell-Grafted Patients |
|--------------------------|-----------------|-----------------|-----------------|
| Fever (T1, T2, T3)         | Urinary tract infection (T1, T2) | Mild anemia (T1, T2) | Anemia requiring blood transfusion (T3) |
| Systemic hypotension (T3) | Decubitus ulcer (T1) | Transient apraxia of musculocutaneous nerve (T1) |

T1-T3, transplant recipients.
T1, a decrease of the area of myelomalacia was initially observed at 1 month postsurgery. At 3 months an asymptomatic enlargement of the central canal of the spinal cord proximally to the cell-grafted area and retethering of the spinal cord to the dura by fibrous adhesions was observed in this patient and remained at 1 year postsurgery. This cystic enlargement of the central canal showed a tendency towards progression in the second year of observation but still without any deleterious effect on the clinical state of the patient or on the results of his electrophysiological studies.

The results from continued long-term observation of the safety and feasibility of OEC transplantation will be presented in a separate publication.

In Patient T2, the observed posttraumatic cyst collapsed after surgery and did not reoccur in control MRI studies. Besides, a decrease of the focus of myelopathy in the distal spinal cord stump was observed in T2-weighted images (Fig. 5).

In Patient T3, the size of the spinal cord cyst did not change during the first year after transplantation.
DTI studies of the thoracic spinal cord were performed postoperatively in Patient T1 at 3, 6, and 12 months. At 3 months postsurgery, a 3-mm gap in continuity of diffusion tracts in the spinal cord was observed at the level of spinal cord injury (Fig. 6). The 6-month DTI study revealed new tracts of water diffusion about 3–4 mm wide, completely bridging the gap, as well as several thin, tapering spurs extended rostrally for about 1.8 cm from the distal stump (Fig. 6). At 12 months, the continuity was not seen and the gap had increased up to 6 mm, consistent with the cystic enlargement of the central canal seen on MRI scans. Assessment of fractional anisotropy (FA) showed a gradual decrease of the values in the proximal spinal cord stump in subsequent DTI studies (mean FA decrease was 0.24). In contrast, FA values increased in the distal stump. Mean FA increase was 0.05 (Fig. 6).

In Patient T2, preoperative DTI showed a 5-cm gap in continuity of diffusion tracts. Following myeloadhesiolysis and cell transplantation, new diffusion tracts arising from both the proximal and distal spinal cord stump demonstrated at 3 and 11 months after surgery, causing a decrease of the gap in water diffusion from 5 to 4 cm. Graphs summarizing the FA values of Patients T1 (D), T2 (H), and T3 (I). On the x-axis were shown the levels of the spinal cord above and below the lesion epicenter (measured in centimeters) that were chosen for measurement of FA values; levels 0.5 cm and 2 cm refer to the proximal spinal cord stump, whereas levels −0.5 cm and −2 cm to the distal stump.
of FA in the distal spinal cord stump remained relatively high (mean FA value was 0.60) (Fig. 6).

DTI studies performed in Patient T3 showed a tendency towards a slight increase of FA values in the proximal spinal cord stump adjacent to the cyst (mean increase was 0.04) and more evident increase of FA in the distal stump (mean increase was 0.14).

The appearance of the spine and focus of spinal cord injury did not change in time in the patients from the control group. DTI was not performed in these patients because of the presence of posterior fixation metal implants in their spines.

Neurological Assessment of the Patients at 1 Year

Neurological function improved in all three transplant recipients (T1, T2, T3) during the first year postsurgery. This included a decrease of muscle spasticity (T1, T2) as well as improvement of sensory (T1, T2, T3) and motor function (T1, T2, T3) below the level of spinal cord injury.

A marked decrease of muscle spasticity of the lower extremities was observed in Patients T1 and T2 from the first day postsurgery and remained unchanged throughout the next 12 months. In Patient T1, the mean Ashworth score decreased from 1.25 to 0, and in Patient T2, from 3.25 to 1.12. Spasticity in lower limbs did not change essentially from 2.0 to 2.5. In contrast, there was no change of the Ashworth grade in patients from the control group after 12 months of rehabilitation.

In Patient T1, the first symptoms of recovery of sensation below level of injury were noted at 6 months postsurgery. The patient reported tingling in the dermatomes S4–S5. This impaired sensation turned to a sensation of light touch or pin prick by 8 months post-cell grafting. In the same period, the patient gained voluntary adduction of lower extremities (2 points in the Medical Research Council Scale, MRC), and at 12 months, a slight voluntary flexion of the right hip (MRC 1), indicating conversion of the ASIA grade from A to C.

Patient T2 showed also symptoms of recovery of sensation in dermatomes S4–S5 at 9 months postsurgery. We also noted an increase in the strength of abdominal muscles in this patient, but as this type of motor function is not included in the ASIA score, we classified him as ASIA B.

After an initial decrease of the sensation concerning mainly the sensory level and the zone of partial preservation on the right, noted in the first 3 months after surgery, Patient T3 recovered sensation at 4 months to the state before surgery. In addition, new areas of sensation covering the dermatomes from T9 to T11 on the right side were noted 12 months after cell transplantation, and a slight increase in the strength of abdominal muscles was observed in the period from 4 to 12 months. As this type of neurological improvement is not scored in the ASIA classification, this patient was assessed as ASIA A.

No improvement of the neurological function could be observed in the control patients. There was no change in ASIA grade in these patients after a period of 1 year of

Table 4. Summary of the Results From Neurological Examination of Patient T1

<table>
<thead>
<tr>
<th>T1 (T10/T11)</th>
<th>Day 1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Preop. 06.2005</td>
</tr>
<tr>
<td>ASIA</td>
<td>A</td>
</tr>
<tr>
<td>ZPP</td>
<td>L1(R) L1(R) L1(R) L1(R) L1(R) L1(R) L1(R)</td>
</tr>
<tr>
<td>Motor function in LE</td>
<td>0 0 0 0 0 0 0</td>
</tr>
<tr>
<td>Spasticity in LE</td>
<td>R1 R1 R0 R0 R0 R0 R0</td>
</tr>
<tr>
<td>FIM</td>
<td>110 125 – – – – – – – – 125 125</td>
</tr>
</tbody>
</table>

Data shown in bold indicate the new neurological findings noted postoperatively, T10/T11, the vertebral level of injury; ZPP, zone of partial preservation; FIM, functional independence measure; LE, lower extremities; R, right side; L, left side; Add., adduction; paresth., paresthesia. Motor function was assessed in the Medical Research Council (MRC) scale. Evaluation of sensory function included the pin prick and light touch tests. As both tests gave identical results, they were not shown separately in the table. Spasticity was scored using the Ashworth scale. Note that the patient was neurologically stable for a long period of time before being qualified for OEC transplantation, and the ZPP covered a small region of one dermatome.
intense neurorehabilitation. The results from the neurological assessments performed in the operated and control patients are summarized in Tables 4–7.

**Functional Assessment**

Measurements of functional activity using the FIM scale showed no essential changes in serial observations of the patients after cell transplantation (Tables 4–6). An increase of the FIM values was seen only preoperatively in Patients T1 and T2 during the period of rehabilitation. After surgery FIM scores retained at relatively high values of 125 points (T1,T2) and 118 points (T3). FIM scores did not change in the patients from the control group (Table 7).

**Neurophysiological Evaluation.** Comparison of the results from MEP and EMG studies (Fig. 7 and Table 8) may suggest, that at the beginning of the program, no significant transmission to the lower limbs was found within the spinal cord tracts in patients from either group, apart from Patient T2, who presented weak motor unit activity in the rectus femoris muscles before transplantation. After OEC grafting, electrophysiological evidence of supraspinal activation of spinal cord motoneurons was observed mainly

### Table 5. Summary of the Results From Neurological Examination of Patient T2

<table>
<thead>
<tr>
<th>T2 (T6/T7)</th>
<th>Preop. 06.2005</th>
<th>Preop. 07.2008</th>
<th>2 Days Postop. 08.2008</th>
<th>1 Month</th>
<th>2 Months</th>
<th>3 Months</th>
<th>5 Months</th>
<th>7 Months</th>
<th>9 Months</th>
<th>13 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASIA</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>Sensory level</td>
<td>T5 (R)</td>
<td>T6 (R)</td>
<td>T6 (R)</td>
<td>T6 (R)</td>
<td>T6 (R)</td>
<td>T6 (R)</td>
<td>T6 (R)</td>
<td>T6 (R)</td>
<td>T6 (R)</td>
<td>T6 (R)</td>
</tr>
<tr>
<td>ZPP</td>
<td>T6 (L)</td>
<td>T5 (L)</td>
<td>T5 (L)</td>
<td>T5 (L)</td>
<td>T5 (L)</td>
<td>T5 (L)</td>
<td>T5 (L)</td>
<td>T5 (L)</td>
<td>T5 (L)</td>
<td>T5 (L)</td>
</tr>
<tr>
<td>Motor function in LE</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Spasticity in LE</td>
<td>R4</td>
<td>R2</td>
<td>R1</td>
<td>R1</td>
<td>R2</td>
<td>R2</td>
<td>R1</td>
<td>R0</td>
<td>R0</td>
<td>R1</td>
</tr>
<tr>
<td>FIM</td>
<td>117</td>
<td>124</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>124</td>
<td>125</td>
<td>–</td>
</tr>
</tbody>
</table>

The new neurological findings are shown in bold. T6/7, vertebral level of injury; ZPP, zone of partial preservation; FIM, functional independence measure; LE, lower extremities; R, right side; L, left side. Motor function was assessed in the MRC scale. The recovery of abdominal muscle (abd. m.) activity could not be scored according to the ASIA protocol. Evaluation of sensory function included the pin prick and light touch tests. As both tests gave identical results they were not shown separately in the table. Spasticity was scored using the Ashworth scale. Note that the patient was neurologically stable for a long period of time before being qualified for OEC transplantation and the ZPP covered a small region of one dermatome.

### Table 6. Summary of the Results From Neurological Examination of Patient T3

<table>
<thead>
<tr>
<th>T3 (T4/T5)</th>
<th>Preop. 01.2010</th>
<th>Preop. 04.2010</th>
<th>4 Days Postop. 06.2010</th>
<th>1 Month</th>
<th>3 Months</th>
<th>4 Months</th>
<th>6 Months</th>
<th>8 Months</th>
<th>10 Months</th>
<th>12 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASIA</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>Sensory level</td>
<td>T5 (R)</td>
<td>T5 (R)</td>
<td>T4 (R)</td>
<td>T4 (R)</td>
<td>T4 (R)</td>
<td>T5 (R)</td>
<td>T5 (R)</td>
<td>T5 (R)</td>
<td>T5 (R)</td>
<td>T5 (R)</td>
</tr>
<tr>
<td>ZPP</td>
<td>T8 (R)</td>
<td>T8 (R)</td>
<td>T7 (R)</td>
<td>T5 (R)</td>
<td>T5 (R)</td>
<td>T5 (R)</td>
<td>T6 (R)</td>
<td>T6 (R)</td>
<td>T6 (R)</td>
<td>T11 (R)</td>
</tr>
<tr>
<td>Motor function in LE</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Spasticity in LE</td>
<td>R1</td>
<td>R2</td>
<td>R4</td>
<td>R3</td>
<td>R3</td>
<td>R2</td>
<td>R2</td>
<td>R3</td>
<td>R3</td>
<td>R3</td>
</tr>
<tr>
<td>FIM</td>
<td>L2</td>
<td>L2</td>
<td>L3</td>
<td>L3</td>
<td>L3</td>
<td>L3</td>
<td>L2</td>
<td>L3</td>
<td>L3</td>
<td>L2</td>
</tr>
</tbody>
</table>

Data shown in bold indicate the new neurological findings. T4/5, the vertebral level of injury; ZPP, zone of partial preservation; FIM, functional independence measure; LE, lower extremities; R, right side; L, left side. Motor function was assessed in the MRC scale. The recovery of abdominal muscle (abd. m.) activity could not be scored according to the ASIA protocol. Evaluation of sensory function included the pin prick and light touch tests. As both tests gave identical results they were not shown separately in the table. Spasticity was scored using the Ashworth scale. Note that the patient was neurologically stable for a long period of time before being qualified for OEC transplantation in this patient, a larger ZPP on the right was noted covering three dermatomes.
in Patients T1 and T3. The parameters of EMG and MEPs in the abdominal muscles increased over their preoperative values in Patients T1 and T3. For the first time, a positive response mainly in the rectus femoris muscles was also noted postoperatively in both these patients, which corresponds with activation of the lumbar group of spinal cord motoneurons L2–L4. This improved spinal cord transmission was very prominent at 6 months postsurgery and also confirmed at 1 year. Parameters of MEPs did not change significantly in Patient T2.

In the three control patients, neither EMG activity nor efferent transmission in MEPs to the muscle groups of lower extremities was recorded. No significant abnormalities were found in peripheral efferent transmission of the lower extremity nerves in the control patients or in the pre- and postoperative studies of the transplanted patients. M wave amplitude parameters did not differ greatly from those found at normal conditions (with lower physiological limit at 2,500 μV) except the third trial when they suggested an axonal degeneration process in motor nerve fibers.

Table 7. Summary of the Results From Neurological Examination of the Patients From the Control Group

<table>
<thead>
<tr>
<th></th>
<th>C1 (T3)</th>
<th>C2 (T11)</th>
<th>C3 (T5/T6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2008</td>
<td>2009</td>
<td>2008</td>
</tr>
<tr>
<td>ASIA</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>Sensory level</td>
<td>T2(R)</td>
<td>T2(R)</td>
<td>T10(R)</td>
</tr>
<tr>
<td></td>
<td>T2(L)</td>
<td>T2(L)</td>
<td>T10(L)</td>
</tr>
<tr>
<td>ZPP</td>
<td>T4(R)</td>
<td>T4(R)</td>
<td>T11(R)</td>
</tr>
<tr>
<td></td>
<td>T4(L)</td>
<td>T4(L)</td>
<td>T11(L)</td>
</tr>
<tr>
<td>Motor function in LE</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Spasticity in LE</td>
<td>R 4</td>
<td>R 4</td>
<td>R 0</td>
</tr>
<tr>
<td></td>
<td>L 4</td>
<td>L 5</td>
<td>L 0</td>
</tr>
<tr>
<td>FIM</td>
<td>119</td>
<td>119</td>
<td>118</td>
</tr>
</tbody>
</table>

Data shown in bold indicate the new neurological findings. ZPP, zone of partial preservation; FIM, functional independence measure; LE, lower extremities; R, right side; L, left side.

Figure 7. Neurophysiological examples for Patient T1. Examples of motor-evoked potentials (A; upper traces) and electromyographic recordings (B; lower traces) performed in Patient T1 before OEC transplantation (a, first trial), 1 month after surgery (b, second trial), after 6 months (c, third trial), and after 1 year of rehabilitation (d, fourth trial).
### Table 8. Neurophysiological Examination Data for Control and Treated Patients

<table>
<thead>
<tr>
<th>Examined Parameter in Patients With OEC Grafts</th>
<th>Number of Trials</th>
<th>Number of Trials</th>
<th>Number of Trials</th>
<th>Number of Trials</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First Trial (Before OEC Graft)</td>
<td>Second Trial (1 Month After OEC Graft)</td>
<td>Third Trial (6 Months After OEC Graft)</td>
<td>Fourth Trial (12 Months After OEC Graft)</td>
</tr>
<tr>
<td>Patient</td>
<td>T1 T2 T3</td>
<td>T1 T2 T3</td>
<td>T1 T2 T3</td>
<td>T1 T2 T3</td>
</tr>
<tr>
<td>MEP amplitude (µV), rectus abdominis muscles recordings</td>
<td>0 50 30</td>
<td>100 60 60</td>
<td>200 50 50</td>
<td>600 50 100</td>
</tr>
<tr>
<td>MEP amplitude (µV), rectus femoris muscles recordings</td>
<td>0 20 0</td>
<td>100 20 50</td>
<td>300 20 100</td>
<td>200 0 100</td>
</tr>
<tr>
<td>EMG amplitude (µV), rectus abdominis muscles recordings</td>
<td>0 250 100</td>
<td>150 200 150</td>
<td>200 150 50</td>
<td>500 50 50</td>
</tr>
<tr>
<td>EMG amplitude (µV), rectus femoris muscles recordings</td>
<td>0 600 0</td>
<td>50 100 50</td>
<td>600 200 20</td>
<td>100 100 20</td>
</tr>
<tr>
<td>EMG amplitude (µV), gastrocnemius muscles recordings</td>
<td>0 50 0</td>
<td>20 100 0</td>
<td>10 50 50</td>
<td>10 100 50</td>
</tr>
<tr>
<td>EMG amplitude (µV), tibialis anterior muscles recordings</td>
<td>0 20 0</td>
<td>0 50 0</td>
<td>50 0 0</td>
<td>100 20 0</td>
</tr>
<tr>
<td>EMG amplitude (µV), extensor digiti muscles recordings</td>
<td>0 20 0</td>
<td>0 150 50</td>
<td>50 50 35</td>
<td>50 50 50</td>
</tr>
<tr>
<td>ENG M-wave amplitude (µV), peroneal nerve stimulation</td>
<td>2000 1500 1800</td>
<td>3000 2500 500</td>
<td>2000 3000 100</td>
<td>5500 5500 200</td>
</tr>
<tr>
<td>ENG M-wave conduction velocity (m/s), peroneal nerve stimulation</td>
<td>39.4 44.7 39.3</td>
<td>42.1 40.2 33.2</td>
<td>40.1 40.7 30.5</td>
<td>41.5 43.2 28.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Examined Parameter in Patients With no OEC Grafts (Rehabilitation Only)</th>
<th>Number of Trials</th>
<th>Number of Trials</th>
<th>Number of Trials</th>
<th>Number of Trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>First Trial (Before Rehabilitation)</td>
<td>Second Trial (During Rehabilitation)</td>
<td>Third Trial (During Rehabilitation)</td>
<td>Fourth Trial (12 Months After Rehabilitation Only)</td>
</tr>
<tr>
<td>Patient</td>
<td>C1 C2 C3</td>
<td>C1 C2 C3</td>
<td>C1 C2 C3</td>
<td>C1 C2 C3</td>
</tr>
<tr>
<td>MEP amplitude (µV), rectus abdominis muscles recordings</td>
<td>100 50 50</td>
<td>NT</td>
<td>NT</td>
<td>100 0 80</td>
</tr>
<tr>
<td>MEP amplitude (µV), rectus femoris muscles recordings</td>
<td>100 0 0</td>
<td>NT</td>
<td>NT</td>
<td>0 0 0</td>
</tr>
<tr>
<td>EMG amplitude (µV), rectus abdominis muscles recordings</td>
<td>100 50 150</td>
<td>NT</td>
<td>NT</td>
<td>200 50 50</td>
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<tr>
<td>EMG amplitude (µV), rectus femoris muscles recordings</td>
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<td>NT</td>
<td>50 0 50</td>
</tr>
<tr>
<td>EMG amplitude (µV), gastrocnemius muscles recordings</td>
<td>0 0 150</td>
<td>NT</td>
<td>NT</td>
<td>0 0 50</td>
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</table>

*(continued)*
The results from neurophysiological studies were additionally evaluated by an independent blinded assessor who confirmed the above-mentioned observations of improved spinal cord transmission observed in cell-grafted patients. Urodynamic studies performed in Patients T1, C1, C2, and C3 did not show any difference in control examinations. In these patients, a hyperreflexia of the bladder detrusor and dyssynergy between the bladder detrusor and urethral sphincter activity was noted. In contrary, a synergy between bladder detrusor and urethral sphincter activity was noted in Patient T2 at 2 months after transplantation and in Patient T3 at 12 months, which led to improved bladder emptying during urination. As this finding could not be confirmed in subsequent examinations in Patient T2, further observation will also be necessary to confirm the positive findings in Patient T3. In all patients, there was no electromyographic evidence of voluntary functional activation of the anal sphincter.

**DISCUSSION**

**Trial Design**

The main goal of this Phase I nonrandomized controlled prospective study was to assess the safety of obtaining olfactory mucosal tissue and transplantation of cultured OECs into the spinal cord of patients with a functionally complete spinal cord injury. The trial meets the requirements for the enrolment of human subjects in research as outlined by the Declaration of Helsinki as well as the regulatory requirements of the National Health Council of Poland. The design of the trial is also in accordance with most of the international guidelines for conduction of clinical studies in humans with a spinal cord injury (1,54). We chose for the cell therapy protocol patients with a functionally complete spinal cord injury (diagnosed as ASIA A) at levels below the spinal cord segment C4. In order to decrease any risk of clinically relevant neurological deterioration after transplantation, we admitted finally only patients with thoracic paraplegia. In this article, we present the first postoperative year observation of three patients receiving transplants and three unoperated control patients. The inclusion criteria required all six to be neurologically stable in at least two control examinations. The examinations were performed at intervals at least 3 months apart in order to prove that the patients continued to present a complete spinal cord injury (ASIA A). The patients for the trial were divided in two groups: transplant recipient group and nontransplanted control group. All patients had a stable psychological and social status. The surgery group was formed by patients who showed the highest degree of motivation during rehabilitation. The patients assigned to the control group, although motivated, either had unreasonable expectations and did not understand the risk of no benefit from the trial (Patients C1 and C2) or had laryngological contraindications for obtaining of olfactory mucosa (nasal polyps in Patient C3). Prior to cell transplantation, Patients T1, T2, and T3 underwent rehabilitation for at least 3 months to exclude the probability of spontaneous neurological improvement due to intense rehabilitation. Patients from both groups underwent neurorehabilitation for at least 1 year according to the same protocol. This

<table>
<thead>
<tr>
<th>Examined Parameter in Patients With no OEC Grafts (Rehabilitation Only)</th>
<th>Number of Trials</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>First Trial (Before Rehabilitation)</td>
</tr>
<tr>
<td>Patient</td>
<td>C1</td>
</tr>
<tr>
<td>EMG amplitude (μV), tibialis anterior muscles recordings</td>
<td>200</td>
</tr>
<tr>
<td>EMG amplitude (μV), extensor digiti muscles recordings</td>
<td>350</td>
</tr>
<tr>
<td>ENG M-wave amplitude (μV), peroneal nerve stimulation</td>
<td>3000</td>
</tr>
<tr>
<td>ENG M-wave conduction velocity (m/s), peroneal nerve stimulation</td>
<td>43.9</td>
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</table>

Global comparison of the data obtained from neurophysiological examinations performed in Patients T1–T3 (with OEC grafts) and Patients C1–C3 (undergoing rehabilitation only). Observations were made prior to the treatment (first trial), after 1 month of treatment (second trial), after about 6 months of treatment (third trial), and finally after 12 months of treatment (4th trial). Second and 3rd trials were abandoned in C1–C3 patients (NT: no tests were performed). 0 number marks that no recordings were obtained during the trial. Numbers refer to the mean values of parameters obtained during bilateral recordings from both sides. MEP, motor-evoked potential; ENG, electroneurography; EMG, electromyography.
allowed us to gain information about any efficacy of the procedure of exposure of the spinal cord and injection of olfactory mucosal cells combined with neurorehabilitation compared to rehabilitation alone. It was essential to have actively trained nontransplanted patients to be sure that any clinical benefits observed in the cell-grafted patients were not simply due to the intense rehabilitation program. To increase the chance for neurological improvement, we decided to admit only patients in whom the myelopathic area seen on MRI scans did not exceed two spinal cord segments (approximately 2 cm), with no progressive syringomyelia, spinal stenosis, or instability. The numerous inclusion and exclusion criteria for participation in this trial led to slow recruitment and did not enable the enrolment of a large number of participants. Within a period of 2 years of recruitment, only six patients from about 50 candidates met the criteria for participation in this study.

All patients were regularly subjected to neurological, neurophysiological, and radiological examinations. The assessment in the control group was focused on two time points—the beginning and the end of the 1-year rehabilitation program. This is because they had not received an invasive procedure. In the transplant-recipient group, we found important to have shorter intervals between the assessment periods, for evaluation of the safety of the procedure. The same assessors were used in the study to obviate any interobserver variability. All patients were regularly examined laryngologically, neurologically, urologically, neurophysiologically, and radiologically by the same persons. The operations of olfactory mucosa retrieval and olfactory mucosal cell transplantation were performed by the same neurosurgeons. We did not find necessary to blind the assessors because the primary goal of the study was to evaluate the safety of cell transplantation. An exception was the neurophysiologic investigation where the results from the tests were additionally evaluated by a blinded assessor for increasing of their reliability. For this reason, any recorded clinical benefit from cell transplantation and/or neurorehabilitation in this trial must be considered only in the category of a suggestion of efficacy and will need to be confirmed in a sufficient number of patients in phases II and III trials.

Safety Issues

Obtaining Olfactory Mucosa. Olfactory mucosa was chosen as a source of OECs because it is relatively easily accessible using an extracranial approach. We chose the transnasal submucoperiosteal approach, which is in general used for access to the sphenoid sinus and sella turcica in neurooncology (26). Choi et al. (10) showed that it can be applied for obtaining of tissue rich in OECs and ONFs. The main advantage of the microscopic endonasal submucosal approach over the alternative endoscopic transnasal approach for obtaining of olfactory mucosa (16) is that in the former approach the target tissue is the lamina propria of the olfactory mucosa, which is known to be the richest source of OECs. Our modification of the technique described by Choi et al. (10) was the introduction of the technique of intraoperative optical cranial neuronavigation for precise determination of the area for olfactory mucosa biopsy and for identification of critical anatomical landmarks of the skull base. Based on data from thin-slice computed tomography and magnetic resonance studies of the patient’s head, it was possible to obtain almost the whole area of olfactory mucosa of the septum in the vicinity of the cribriform plates of the ethmoid bone. In our previous studies, we showed that this area is rich in OECs (41). The patients showed no complications following olfactory mucosa retrieval. There was no postoperative bleeding from the nasal cavity and no local infection. Although in the first week after the operation both patients presented bilateral anosmia, they regained normal nasal patency and normal smell perception on both sides within a period of 3 weeks.

Cell Culture. The assessment of the safety of the process of cell culture included the identification of appropriate cell populations, prevention of cell overgrowth or early cell detachment from the culture flasks, and serial examinations for microbial contamination. Immunocytochemical studies confirmed that the cultures of cells isolated from the olfactory mucosa contained mainly OECs and ONFs. We did not try to characterize other cell populations in culture as this would require the usage of more culture dishes for staining and thus would entail an unacceptable decrease in the numbers of cells available for the cell treatment. In all cases the threshold of 5% of OECs in culture, we had set up as acceptable for transplantation, was reached (Table 2). The number of cells varied to a large degree between Patients T1–T2 (1,800,000 and 1,920,000, respectively) and Patient T3 (21,200,000). This was because the cell cultures were not purified, and it was very difficult to keep the same proportion of OECs and fibroblasts in each case. Specifically in Patient T3, the fibroblast overgrowth was highly noticeable. Ethically, we could not discard the larger cell population, as it could give the patient a better chance of repair.

Regular light microscopic monitoring was necessary for early detection of fibroblast overgrowth that may inhibit the growth of OECs in culture. We avoided replating and passage, because this maneuver can lead to substantial loss of OECs. The time for OEC detachment and transplantation was determined by the time they formed a confluent monolayer in culture and did not exceed 3 weeks. Serial microbiological tests confirmed that there was no bacterial or fungal contamination in the culture of the cells isolated from the nasal mucosa.

Transplantation. Initial 1-year clinical observations show that our procedure of transplantation of autologous OECs into the spinal cord is safe. Adverse events noted in Patient T1 were decubitus ulcers and transient paresis of
the right musculocutaneous nerve due to prolonged compression during the long time of operation (11 h). The main factors that substantially prolonged the operation were the time needed for cell detachment (2 h) and the time for preparation of the cell suspension and its microinjection into the spinal cord (3 h). We shortened the time of the operation in Patients T2 and T3 to 9 h mainly by synchronization of the moment of the enzymatic cell detachment with the operative procedures that were done simultaneously in the operating room, and in these patients, we did not observe the previous adverse events.

The main clinical problem in Patients T1 and T2 was the uroinfection diagnosed in the early postoperative period. It was caused by reactivation of bacteria that were chronically resident in the urinary tract of those spinal cord-injured patients and resolved within 3–4 days of administration of antibiotics. In the first days after operation, Patient T3 suffered from systemic hypotension and anemia due to increased intraoperative blood loss. After administration of vasoconstrictors and red cell transfusion, his clinical state improved.

There were no clinical complications that could be defined as a direct effect of the operation of transplantation of OECs into the spinal cord. None of the patients experienced neurological deterioration, neuropathic pain, neuroinfection, CSF leakage, or local infection at the site of surgery at 1 year posttransplantation. To assess the risk of the numerous microinjections performed into the spinal cord of the transplant recipients, we evaluated the risk of spinal cord inflammation in form of adhesive arachnoiditis using the clinical and radiological guidelines described by Morisako et al. (42). There were no complaints of postoperative neuropathic pain in our cell-grafted patients—one of the main symptoms of arachnoiditis. Also early MRI studies excluded signs of spinal cord edema or inflammation. In addition, postoperative MRI studies showed no indication of intraspinal bleeding, no spine instability or stenosis, and no evidence of tumorigenesis in the grafted area of the spinal cord. The only new radiological abnormality seen in MRI was late retethering of the spinal cord at the site of cell grafting by fibrous adhesions and enlargement of the central canal of the spinal cord proximally to the lesion epicenter observed from the 3 months postsurgery in Patient T1 (Fig. 5). The exact mechanism of this cystic enlargement is unknown. The most probable mechanism may be attributed to the inhibitory effect of the fibrous adhesions around the focus of spinal cord lesion on the circulation of the CSF. This could result in an increased diffusion of the CSF into the spinal cord parenchyma and may have led to the development of communicating syringomyelia (3,17). It may be relevant that the initial injury in Patient T1 was a transection of the spinal cord. The dural tear that persisted for 4 years after the accident served as a gate for ingrowth of scar tissue from extraspinal origin that is known to contain more fibroblasts and connective tissue and has higher proliferative potential than the host glioblastic scar that usually develops after closed compressive spinal cord injuries (33,50). However, we cannot exclude that the observed spinal cord retethering was in part caused by sustained subclinical arachnoiditis. The observed retethering and cystic enlargement of the spinal cord in Patient T1 did not exert any harmful effect on his neurological state and on the results of his neurophysiological studies at 1 year post-cell transplantation.

**Evaluation of Effectiveness of OEC Transplantation**

Clinical and neurophysiological 1-year observations suggest that all patients who underwent the operation of OEC transplantation combined with intense pre- and postoperative neurorehabilitation showed modest neurological signs of recovery. The neurological state of the three control patients remained unchanged.

We observed a substantial reduction of muscle spasticity in Patients T1 and T2 immediately after surgery. Recovery of sensation in the dermatomes S4–S5 was observed in Patient T1 at 8 months and in Patient T2 at 9 months postsurgery. An increase of sensation over three dermatomes (from Th8 to Th11) was noted in Patient T3 at 12 months. At 8 months, Patient T1 regained voluntary adduction of the lower extremities (2 points in the MRC scale), and at 12 months, a slight voluntary flexion of the right hip (MRC 1). The observed motor recovery was most probably caused by reinnervation from supraspinal neurons of the motoneuron pools of the first lumbar segments (L1–L3) that are involved in adduction and flexion of the lower limbs. Patients T2 and T3 also showed an improvement of motor function below the level of injury in form of an increase in the strength of the abdominal muscles that was not ASIA scored. This recovery also may be indicative of reinnervation of the first thoracic motoneurons below the focus of injury responsible for abdominal muscle innervation starting from the segments T7 and T8. In conclusion, Patient T1 converted from ASIA A to C, Patient T2 from ASIA A to B. Although Patient T3 remained ASIA A, the observed segmental recovery of sensory and motor function also demonstrated a clinical benefit from OEC transplantation. This fact reflects some limitations of the ASIA scoring in objective evaluation of functional recovery observed in our patients.

The observed improvement in neurological function in transplant recipients is supported by the results of neurophysiological tests. After OEC grafting, the parameters of EMG and MEPs increased their values in transplant recipients at 6 months after surgery not only at levels of T8 but also at L2–S1 innervation (Table 8). The enhancement of MEP parameters was observed mainly in the abdominal
and quadriceps muscles and was more prominent in Patient T1 and Patient T3. This improved spinal cord conductivity may indicate a functional restitution of descending motor tract fibers in the transplant recipients. The three control patients showed neither activity in recorded muscle groups in lower extremities nor afferent transmission to the lower extremity muscles. The recovery both in transmission of spinal pathways and in activity of muscle motor units below the level of injury preceded the recovery of motor function. Our findings are in accordance with the results of Knoller et al. (23) and Lima et al. (32). These authors also reported a high correlation between improvement in MEP and EMG recordings and the ASIA motor score improvement. Macdonell and Donnan (37) underline the importance of MEP findings in the prediction of motor recovery in spinal cord injured patients.

In addition to the assessment of neurological function using the standard ASIA and Ashworth scales and the neurophysiological studies, we have applied diffusion tensor magnetic resonance imaging (DTI) as a new outcome measure for structural changes of the spinal cord fiber tracts after OEC transplantation. Standard 1.5 or 3 T MRI protocols are unable to detect transplanted cells or regenerative changes in the injured spinal cord. DTI studies show the direction and extent of water diffusion along the fibers in the CNS. Thus, DTI can show indirectly the topography of white matter tracts from different origin and with different function. Parameters like fractional anisotropy (FA) can estimate quantitatively the degree of synchronization of water diffusion in the white matter. Higher FA values may indicate higher degree of integrity of axons (51). DTI was used first in brain pathology, but recently it was applied in the diagnostics of spinal cord pathologies including traumatic injuries (13,52,59). DTI was shown to be predictive in the estimation of severity of spinal cord injury (35,52). We present the first DTI data of patients with cell transplantation into chronic lesions of the thoracic spinal cord. This study revealed the formation of new tracts of water diffusion in both the proximal and distal spinal cord stumps in Patients T1 and T2 (Fig. 6). This could not be shown in Patient T3 because of the artifacts caused by the metal vertebral prosthesis. This process was detected first at 6 months after OEC transplantation and correlated with the neurological and neurophysiological improvement of the transplant recipients. FA values had an increasing tendency in the distal spinal cord stumps of all patients in subsequent studies. One year posttransplantation FA values in the distal spinal cord stump of Patients T2 and T3 were comparable to those of healthy people (12). In contrast, FA values measured in the proximal spinal cord stumps of Patients T1 and T2 gradually decreased. The observed decrease of FA values was most likely caused by the effect of local water accumulation due to cystic cavitation of the proximal spinal cord stump. This process was more prominent in Patient T1, in whom a cystic enlargement of the proximal spinal cord was observed postsurgery. The observed cyst led to an increase of the initial 3-mm gap in water diffusion observed at 1 month postsurgery to 6 mm at 1 year. Patient T2 had a preoperative spinal cord cyst proximally to the lesion epicenter that persisted postsurgery. Proximal FA values did not change significantly in time in Patient T3, which was consistent with his MRI findings, showing no evidence of cyst formation in the proximal spinal cord stump at levels where DTI measurements were undertaken.

These DTI results suggest the possibility of restitution of some white matter tracts after OEC transplantation, although the inhibiting effect of artifacts from intraspinal cysts complicated the interpretation of this phenomenon.

Possible Mechanisms of Recovery

The focus of a complete spinal cord injury contains a centrally located area of cell necrosis that usually turns into fluid-filled cyst (50). The borders of the cyst are surrounded by reactive astrocytes that change the longitudinal alignment of their processes into transverse, forming a barrier for central axon elongation (31). Even in patients scored as “complete” by neurological criteria, there is nearly always some remaining white matter tissue containing both demyelinated and myelinated axons (19,21).

The possible pattern of observed neurological recovery in our operated patients may have been a combination of remyelination of spared demyelinated axons (25), stimulation of regeneration of lesioned axons towards the target host neurons (30), and reactivation or sprouting of surviving axons.

We noted in Patients T1 and T2 an immediate decrease of muscle spasticity that persisted during the 1-year observation without the necessity for use of muscle relaxants. Possibly, this phenomenon could be the effect of decreased spinal cord traction by scar, improvement of its blood supply, and reactivation of quiescent uninjured axons after detethering (myeloadhesiolysis). Observations from studies conducted on a large number of patients confirm our observation, that late surgical adhesiolysis of the tethered posttraumatic spinal cord can cause a decrease of spasticity shortly after the operation (14).

The crucial period, when main recovery of neurological function could be observed in all three transplant recipients, was the time between the 6th and 12th months. In this period, all patients regained sensory and motor function below the level of injury. Neurophysiological tests performed 6 and 12 months postsurgery supported the findings of neurological improvement. This late recovery of function suggests enhanced plasticity, remyelination, and possibly functional reconnection of regenerating axons, mediated by transplanted OECs. At present, it is impossible to determine
what was the exact mechanism of action of the transplanted OECs and other accompanying cell populations, such as ONFs, in our patients. Our hypothesis, supported by experiments in animals (28,29), is that transplantation of OECs into both spinal cord stumps adjacent to the focus of injury enabled their interaction with host astrocytes around the lesion epicenter. Grafted OECs could change the orientation of the astrocytic processes and reestablish the longitudinal alignment of their processes, making the glial scar permissive for regrowing axons (31). Although OECs are the main cell population thought to evoke neurological improvement, their survival and function is dependent on other cells, such as ONFs, present in their natural milieu and extracellular matrix molecules (4,30). For this reason, we decided to transplant unpurified OECs. Our thesis is additionally supported by the fact that at this moment only authors who have transplanted complex olfactory tissue (32,33) but not purified human OECs (15,38) have obtained neurological improvement in their patients.

The therapeutic approach in this study included also pre- and postoperative neurorehabilitation. Neurorehabilitation itself seldom causes significant neurological improvement in patients with complete chronic spinal cord injury (56). Neither of our control patients showed any improvement in clinical and neurological tests. On other hand, rehabilitation evokes activation of neuronal circuits in the spinal cord and stimulates the endogenous production of neurotrophic factors that can positively influence the pattern regeneration of lesioned axons (53). Preclinical studies have demonstrated that task-specific training may enhance the extent of OEC-mediated axon regeneration in the completely transected spinal cord (24,55). Similar conclusions have been drawn from clinical studies conducted in completely injured patients. Lima et al. (33) did not use neurorehabilitation in their first seven patients with implantation of whole, uncultured autologous olfactory mucosa combined with surgical removal of the scar. After inclusion of neurorehabilitation in the treatment protocol, Lima et al. (32) reported in the next group of 20 patients an increase of the percentage of patients who improved neurologically from 28% to 55%.

In this study, we show that the observed neurological improvement in the transplant recipients was dependent on the surgical intervention. It consisted of myelodisectomy and cell transplantation. Although the control patients did not receive sham operations, previous experience suggests that, apart from some influence on decreasing muscle spasticity, the procedures of exposing the spinal cord and the necessary adhesiolysis in chronic spinal cord lesions alone were unlikely to lead to statistically significant improvement of sensory and/or motor function at a mean follow-up of 3.4 years (14). Thus, the observations suggest that transplantation of OECs was the most likely reason for the sensorimotor improvements in the three transplanted patients.

The cohort of transplanted and control patients will be assessed at the 2- and 3-year periods to obtain further information about the long-term safety and the possibility of later functional changes.

**CONCLUSION**

Clinical observations at 1 year confirm that the obtaining, culture and intraspinal transplantation of autologous OECs is safe. The modest improvements in neurological function occurred against a background of intense neurorehabilitation, which is probably needed for the improvements although the control patients indicate that this is not in itself sufficient.

We consider that the transplantation of OECs was the main factor contributing for the neurological improvements in the three transplanted patients. Among possible mechanisms for the functional improvement both the MEPs and DTI studies suggest that transplantation of OECs may mediate some restitution of efferent and afferent long white matter tracts in these three patients.

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