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#2

ORIGINAL CONTRIBUTION

Olfactory Mucosa Autografts in Human Spinal Cord Injury: A Pilot Clinical Study

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Abstract

Background/Objective: Olfactory mucosa is a readily accessible source of olfactory ensheathing and stem-like progenitor cells for neural repair. To determine the safety and feasibility of transplanting olfactory mucosa autografts into patients with traumatically injured spinal cords, a human pilot clinical study was conducted.

Methods: Seven patients ranging from 18 to 32 years of age (American Spinal Injury Association [ASIA] class A) were treated at 6 months to 6.5 years after injury. Olfactory mucosa autografts were transplanted into lesions ranging from 1 to 6 cm that were present at C4–T6 neurological levels. Operations were performed from July 2001 through March 2003. Magnetic resonance imaging (MRI), electromyography (EMG), and ASIA neurological and otolaryngological evaluations were performed before and after surgery.

Results: MRI studies revealed moderate to complete filling of the lesion sites. Two patients reported return of sensation in their bladders, and one of these patients regained voluntary contraction of anal sphincter. Two of the 7 ASIA A patients became ASIA C. Every patient had improvement in ASIA motor scores. The mean increase for the 3 subjects with tetraplegia in the upper extremities was 6.3 $\pm$ 1.2 (SEM), and the mean increase for the 4 subjects with paraplegia in the lower extremities was 3.9 $\pm$ 1.0. Among the patients who improved in their ASIA sensory neurological scores (all except one patient), the mean increase was 20.3 $\pm$ 5.0 for light touch and 19.7 $\pm$ 4.6 for pinprick. Most of the recovered sensation below the initial level of injury was impaired. Adverse events included sensory decrease in one patient that was most likely caused by difficulty in locating the lesion, and there were a few instances of transient pain that was relieved by medication. EMG revealed motor unit potential when the patient was asked to perform movement.

Conclusion: This study shows that olfactory mucosa autograft transplantation into the human injured spinal cord is feasible, relatively safe, and potentially beneficial. The procedure involves risks generally associated with any surgical procedure. Long-term patient monitoring is necessary to rule out any delayed side effects and assess any further improvements.

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Key Words: Spinal cord injuries; Human; Transplant; Olfactory mucosa; Neural regeneration; Olfactory ensheathing cells, Stem cells

INTRODUCTION

Neural transplantation has been studied over the past several decades in animal models as a repair strategy for spinal cord injury (SCI). Regenerative and reconstructive experimental cellular strategies included embryonic or adult stem cells or tissue (1–3), Schwann cells (4), genetically modified fibroblasts (5,6), bone stromal cells (7,8), and olfactory ensheathing cells (OECs) (9–11). In considering possible sources for autologous cells, the olfactory mucosa is the only part of the nervous system capable of lifelong regeneration that is readily accessible with minimally invasive techniques.

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There are several potential advantages of olfactory mucosa transplants. The olfactory mucosa is a structural unit with embryonic features that offer the possibility of promoting regeneration and reconstruction. Removing part of the olfactory mucosa does not permanently damage olfaction because it is a continuously regenerating system. By using the olfactory mucosa to fill the spinal cord cavity with solid tissue as opposed to using cell suspensions, there is decreased risk of individual cells entering the cerebrospinal fluid (CSF) circulation. In an experimental transplant study, tissue (as opposed to cell suspensions derived from that tissue) was more effective and showed greater cell viability (12). During the period of adaptation to the new environment, cells may be supported by their original surrounding cell types. Olfactory mucosa transplants avoid the artificial environment of tissue culture, which also reduces the risks of the procedure. Instead, olfactory mucosa grafts preserve the CSF environment, because CSF also bathes the olfactory mucosa through the olfactory route of CSF drainage (13). The autologous olfactory mucosa graft would not be an additional burden to the immune system, because the grafted material was previously exposed to the contemporary immunological state of the central nervous system.

Although several components of the olfactory mucosa may contribute to its effectiveness, the 2 cell types in the olfactory mucosa known to be useful in repair of the nervous system are stem-like progenitor cells and OECs. The term "stem-like progenitor cell" is used because some use the term "stem cells" only for cells with documented potential to form any cell in the body. These stem-like progenitor cells divide rapidly and can develop into supporting cells or mature neurons (14). This robust regeneration, the potential to form neurospheres that contain stem cells in culture (15), is found even in the postmortem olfactory mucosa of very elderly individuals. Differing from the brain, the stem-like cells in the olfactory mucosa are taken from a constantly regenerating system that is not exposed to the original inhibitory cues of the mature brain. Therefore, the cells from the olfactory mucosa may have greater potential to divide and differentiate, a potential that may be limited by tissue culture techniques. Olfactory mucosa cells (without prior culture) transplanted into a chick embryo give rise to typical differentiated cells of the heart, trunk muscles, liver, brain, and spinal cord, etc. (16). This means that the stem-like progenitor cells of the olfactory mucosa are capable of developing into cell types normally derived from the endoderm, mesoderm, or ectoderm, and should be considered multipotent stem cells (16). The other important cell type in the olfactory mucosa is OECs. In animal experiments, OECs, obtained from olfactory bulb (17–21) or the olfactory mucosa (22–24), have the capacity to promote axonal remyelination and/or regeneration in the damaged spinal cord. Equally favorable results were obtained using pieces of the lamina propria of the olfactory mucosa or cultured nasal OECs (23). More recent studies showed that OECs, derived from the olfactory mucosa, express a unique combination of developmentally important proteins such as CD 44, β1 integrin, P200, Notch 3, NG2, vascular endothelial growth factor (VEGF), pituitary adenylate cyclase activating peptide (PACAP), and cAMP response element binding (CREB) binding protein (CBP/p300) not reported in olfactory bulb OECs (24). Additionally, transplanted OECs from the olfactory mucosa reduce scar and cavity formation after SCI (24).

A large number of animal experiments have shown partial structural and/or functional repair of the injured spinal cord using stem cells (1,2,7,8) or OECs (9–11,19–24). Based on the encouraging results from all of these studies, the potential of the autologous olfactory mucosa as a therapy for SCI was explored in a small pilot clinical study. Although the rationale for starting this study was the many published studies of SCI repair using stem cells and OECs, a small experimental animal trial using autologous whole olfactory mucosal transplants in subacute spinal transection model revealed tissue continuity across the lesion site and no signs of overgrowth (unpublished observations). Further support for the potential of the olfactory mucosa in experimental animal studies of SCI will soon be reported.

In this study, a pilot safety and feasibility trial was performed in 7 patients with chronic SCI. The study was done in patients with stable, severe deficits to circumvent spontaneous recovery bias and to minimize chances of further neurological impairment. All of the patients were classified as American Spinal Injury Association (ASIA) A, precluding future significant recoveries (26,27). The study included surgical interventions performed in a period of 16 months, starting in July 2001. This article does not include the findings of subsequent patients because some of these patients have not reached the 18-month follow-up. The hypothesis to be tested was that olfactory mucosa grafts are safe and feasible in the treatment of severe SCI.

METHODS

Methods were developed to remove the olfactory mucosa in a safe manner using rigid endoscopes and instrumentation. Several cadaveric dissections and histopathologic studies were performed before the beginning of the study to show technical feasibility of the procedure and to ensure that olfactory mucosa was present in the chosen localization in individuals less than 35 years old.

Inclusion Criteria

Patients for this pilot trial were selected among individuals who suffered a SCI more than 6 months previously and were chronically paraplegic or tetraplegic. Sham operations were not considered in such a pilot safety and feasibility study. The inclusion criteria were grade A or B on the ASIA Impairment Scale (28); age less than 35

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Table 1. Demographic and Clinical Features of the Patients

Patient	Sex	Age	Months After SCI	P/T*	Length of the Lesion (cm)	ASIA Scale	Date of Operation
1	F	21	6	T	1.5	A	July 26, 2001
2	M	18	6	P	6	A	February 13, 2002
3	M	18	36	T	1	A	March 13, 2002
4	F	24	48	P	1.5	A	July 17, 2002
5	M	29	30	P	4	A	August 29, 2002
6	F	32	78	T	2	A	October 17, 2002
7	M	22	30	P	3	A	March 12, 2003

* P, paraplegic; T, tetraplegic.

years; presence of cervical or thoracic spinal cord lesion; absence of significant nasal and paranasal sinus pathology; and absence of an additional serious medical problem, brain disease, or psychological disturbance. Rationale for age criteria is justified by the influence of aging on olfactory mucosa area and distribution (29). The study was authorized by the Administration of the Hospital de Egas Moniz and was approved by the Ethics Committee of the Hospital. Informed consent was obtained from all of the patients. Hospital de Egas Moniz is a public hospital so patients paid no fees for this procedure.

Seven patients were enrolled in the study (4 men and 3 women). Patients were enrolled over the course of almost 2 years so that the trial could be stopped if there were serious adverse events. The mean age of the patients was 23.4 $\pm$ 5.4 years. Demographic data, clinical, imaging/radiological characteristics of the patients and dates of operations are presented (Table 1). All lesions resulted from road traffic accidents except one (patient 5), which resulted from a fall. Lesions varied between 1 and 6 cm in the maximum vertical axis as measured on both the T1- and T2-weighted magnetic resonance imaging (MRI). All patients included were ASIA scale grade A. Transplants were done from 6 months to 6 years after injury (Table 1).

Outcome Measures

Pre- and postoperative assessment protocol included ASIA neurological examination as described in *International Standards For Neurological and Functional Classification of Spinal Cord Injury Patients* (28); standard conventional electromyography (EMG); full spinal cord MRI scan; otolaryngological evaluation including a general ear, nose, and throat examination, nasal endoscopy; olfactory evaluation and computed tomography (CT) scan of the nose and paranasal sinuses; and psychological assessment. Preoperative urodynamics were performed in all patients. Postoperative urodynamic studies were performed in 2 patients who reported gain in bladder sensation. The return of anal sphincter contraction was qualitatively assessed in a rectal examination.

Neurological status of the patients was clinically monitored continuously but, for the purpose of the study, ASIA neurological examination scoring was registered every 6 months after the surgery until 18 months. Differences between preoperative and postoperative ASIA neurological examination component scores (sensory pin prick, sensory light touch, motor upper and lower limbs) were determined. The 4 components of pretreatment ASIA scores and the 6-, 12-, and 18-month posttreatment ASIA scores were examined using nonparametric (Friedman ANOVA by rank) statistics. Significance was set at 0.05. Patients were interviewed extensively every 2 to 4 weeks after the intervention while in the rehabilitation program, and questioned specifically to identify any illness or need for hospitalization and/or any subjective change in strength, sensation, mood, continence, or other acute symptoms. Interviews included specific questions about bowel and bladder function to obtain subjective data on these issues.

Transplantation Protocol and Surgical Procedure

Surgical intervention was performed under general anesthesia with endotracheal intubation. All the surgical procedures were performed by the same neurosurgical and otolaryngological team. Prophylactic antibiotics were given shortly before surgery. The patient was positioned in park bench–like position. Surgical table allowed for side positioning during the procedure. The head was stabilized using Mayfield support. A lumbar intrathecal catheter was in place during the surgery. The surgical procedure was performed in 3 steps. In the first step, the neurosurgeons exposed the damaged spinal cord by a standard midline incision, posterior laminectomy, and opening of the dura mater. The damaged spinal cord was approached by a posterior midline myelotomy. Whenever necessary and feasible, posterior and postero-lateral detethering of the spinal cord was performed. Then scar tissue of the lesion was removed (within limits as to not harm normal cord tissue) to expose the gross viable nervous tissue in both stumps. The scar tissue was later examined using Mason

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Table 2. ASIA Scale and Motor and Sensory Levels Before and 18 Months After Transplantation

Patient	Pre ASIA	Post ASIA	Preoperative Motor	Postoperative Motor (18 months	Preoperative Sensory Light Touch	Postoperative Sensory Light Touch (18 months)
1	A	C	C8(R) C7(L)	T1(R) T1(L)	C7(R) C8(L)	T4(R) T4(L) and S4-S5 (R and L)
2	A	A	T6(R) T5(L)	T6(R) T5(L)	T6(R) T5(L)	T7(R) T6(L)
3	A	A	C4(R) C4(L)	C7(R) C5(L)	C4(R) C4(L)	T2(R) T2 (L)
4	A	A	T6(R) T5(L)	T8(R) T7(L)	T6(R) T5(L)	T8(R) T7(L)
5	A	A	T5(R) T5(L)	T10(R) T10(L)	T5(R) T5 (L)	T10(R) T10 (L)
6	A	C	C6(R) C6(L)	C7(R) C7(L) and S4-S5	C6(R) C7(L)	C8(R) T2(L) and S4-S5 (R and L)
7	A	A	T6(R) T6(L)	T8(R) T8(L)	T6(R) T6 (L)	T8(R) T8 (L)

* SCI level, SCI Neurological Level for Sensory and Motor; L, left; R, right.

Trichrome stain that stains collagen green and immunohistochemical techniques for glial fibrillary acidic protein (GFAP) to reveal reactive astrocytes and their fibers (processes). The surgical wound was temporarily closed.

The second step was performed by the otolaryngologists. To harvest the olfactory mucosa graft, a transnasal endoscopic approach and instrumentation were used. After cleaning the nasal and olfactory space with povidone/iodine, vasoconstrictors were injected into the mucosa. A submucoperiosteal tunnel was created in the most posterior–superior region of the medial (septal) side of the olfactory groove, and sufficient tissue was collected to fill the spinal cord cavity and to allow for histological and microbiological examination. Reabsorbable packing was placed in the olfactory groove to avoid postoperative nasal bleeding.

The last step involved the transplantation of the olfactory mucosa into the SCI site. Before implantation, the graft was immersed in saline for the first two patients or CSF for the rest of the patients and cut into small pieces to increase the surface area of the grafted tissue. Meninges and the superficial tissue layers were sutured into place. Wound clips were used to close the skin. The patients were transferred to the surgical intensive care unit postoperatively.

RESULTS

The first surgical intervention of this study was performed on July 26, 2001, and the last on March 12, 2003. The mean time between the injury and the operation was 33.4 ± 24.9 months (minimum, 6; maximum, 78 months). The surgical intervention was well tolerated by all patients. The spinal cord level of the lesion roughly corresponded to the level of the vertebral fracture. In the present series of patients, posterior and postero-lateral detethering of the spinal cord was not needed in the cervical lesions but was in almost all of the thoracic lesions. Complete detethering could not be achieved. Difficulty in localizing the precise location of the lesion was encountered in only one patient (patient 4). In all patients, it was possible to differentiate the normal tissue from the damaged nervous tissue under microscopic surgical observation.

No significant neurological, surgical wound, nasal, or general complications were observed postoperatively. There were no indications of infection. Histological examination of part of the graft confirmed that it consisted of olfactory mucosa containing basal stem-like progenitor cells and OECs in all of the patients. There were no differences in the appearance of the olfactory mucosa that were rinsed in CSF or saline. Microbiological examination of this tissue (both consecutive direct and after culturing) yielded negative results for bacteria, parasites, and fungus.

MRI Observations

At 6 months after transplantation, MRI showed a complete or almost complete filling of the lesion site in all patients except one, patient 2, who had the largest lesion (6 cm). The MRI aspect of the grafted area has a "salt and pepper" appearance. Also, there was no MRI evidence of neoplastic tissue overgrowth in any of the patients. Figure 1a is the surgical field from patient 5 who had 2 cavities connected by fibrous scar tissue. The preoperative MRI (30 months after injury) is shown in Figure 1b, and the 6-month postoperative MRI is shown in Figure 1c. Figure 1d is the surgical field from patient 3, who had a single smaller lesion (~10 mm in length), and the preoperative and 6-month postoperative MRIs are shown in Figures 1e and f.

ASIA Scores

All 7 patients were evaluated at 6, 12, and 18 months after surgery with ASIA testing. Results from the ASIA neurological examination are presented in Figure 2 and Tables 2 and 3. Patients 1 and 6 changed from ASIA A

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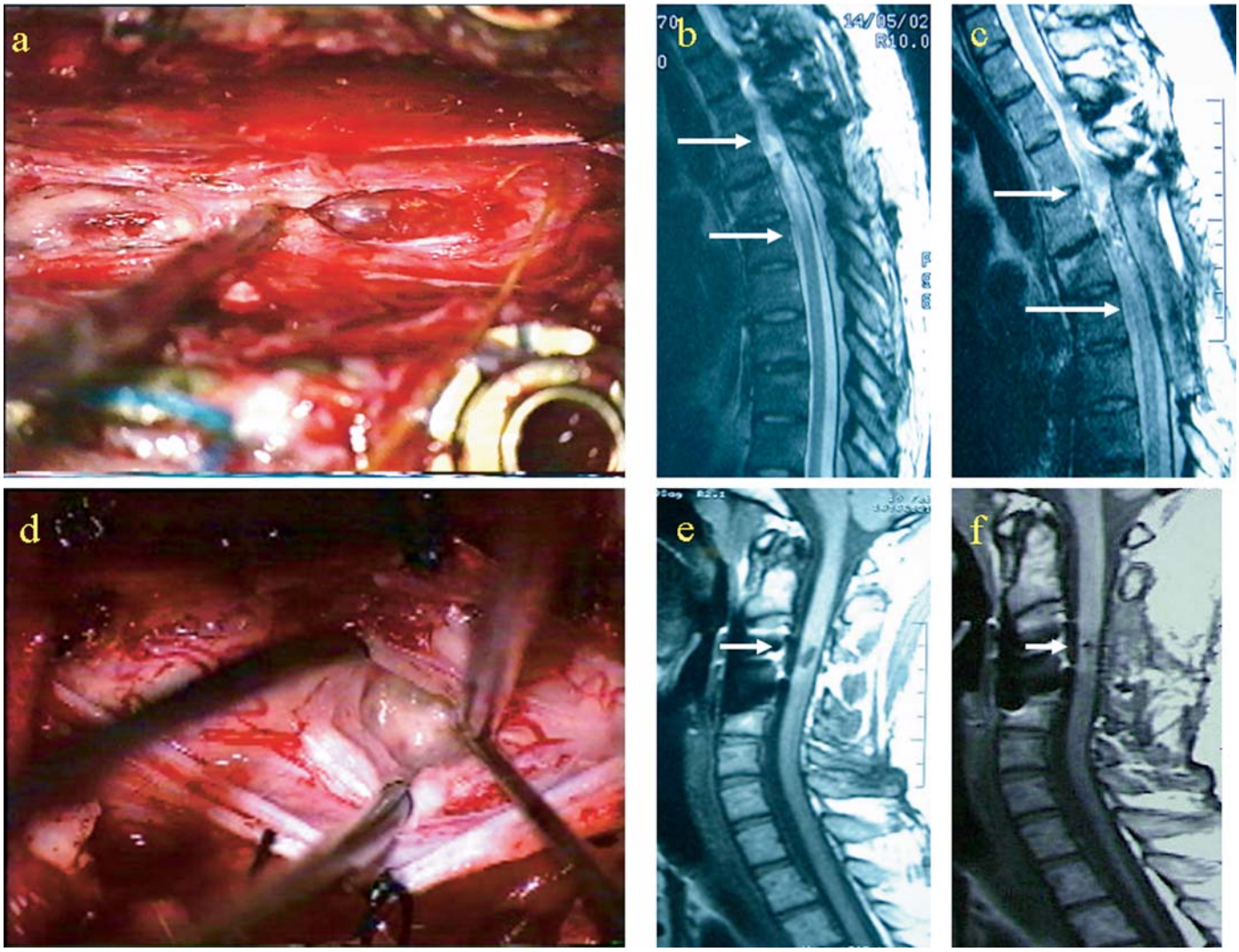

Figure 1. Surgical field, preoperative MRI, and postoperative MRI from 2 patients. (a) Surgical field from patient five revealing 2 cystic cavities that appear to be connected by scar tissue (at end of forceps). (b) Preoperative MRI at 30 months after injury shows the lesion area that extends from T4 vertebral level (upper arrow) to T6 (lower arrow) and measured about 4 cm in the T2-weighted sagittal MRIs (with artifacts from the fixing bars). (c) Six-month postoperative MRI showing presumed filling of the cavity with "salt and pepper" appearance. (d) Surgical field from patient three revealing 1 cystic cavity. (e) T1-weighted sagittal MRIs show preoperative MRI at 36 months after injury and lesion (arrow) approximately 1 cm in length at C3–C4. (f) Six-month postoperative MRI showing presumed filling of the cavity (arrow).

to ASIA C. Patient 1 had return of sensation at S4-S5 and improvement in motor function below the level of injury. Patient 6 had return of motor and sensory at S4-S5 and minor improvement in motor function below the level of injury. There was no change at the S4-S5 level in the other 5 patients. There was improvement in motor and sensory components of the ASIA scoring in all patients except for patient 4. The changes from before transplant to 18 months after transplant in ASIA scoring for sensory light-touch for patients 1 to 7 were 36, 15, 14, −13, 26, 2, and 29. These changes in ASIA scoring for pinprick for patients 1 to 7 were 34, 10, 18, −11, 25, 4, and 28. Most of the recovered sensation below the initial level of injury was impaired. Patient 4 exhibited a decrease in sensory scores but showed the greatest increase in motor scores at 6 months. For the subjects with tetraplegia, the improvement in motor index score (MIS) of the upper extremity muscle groups from before transplant to 18 months after transplant were 4, 8, and 7. The improvements from before transplant to 18 months after transplant in lower extremity muscle groups for patients 1 to 7 in MIS were 7, 2, 1, 3, 3, 1, and 7. The nonparametric tests (Friedman) for the 4 measures of ASIA testing were significant: sensory light touch ($\chi^2 = 9.857$, $P = 0.02$), sensory pinprick ($\chi^2 = 8.143$, $P = 0.043$), motor arms ($\chi^2 = 7.962$, $P = 0.047$), and motor legs ($\chi^2 = 14.288$, $P = 0.003$).

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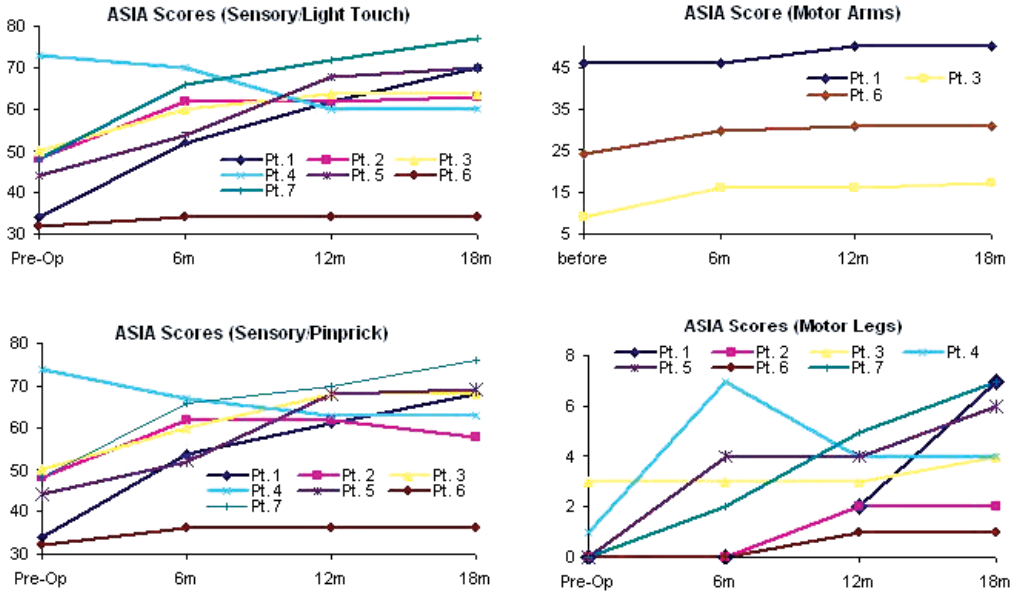

Figure 2. Graphs of the component scores of the ASIA neurological examination starting preoperatively and at 6-month intervals for each patient.

Functional Changes. For the patients with tetraplegia, there were minor increases in motor function of the hip flexors. Patient 1 gained sensory function several levels below the lesion site and also the sacral region. She recovered some sensation in her abdominal region and left leg and increased sensory function in the chest region. There were improvements in motor function of the arms and hip flexors. New movement was also noted in abdominal muscles and large adductor muscles of the thighs that is not part of the ASIA scoring. In patient 3, there was more sensation in the chest and greater ability to move his arms. Patient 6 had minor advances in sensation but now has sacral region sparing. She has greater movement of her hand and finger muscles, and contraction of a hip flexor.

The primary gains in the patients with paraplegia were also the hip flexors. Patient 2 had visible contractions of the gluteal muscles. Patient 4 was the

Table 3. ASIA Scores Given for Before Transplantation and After Transplantation at 6-Month Intervals

	Sensory Light Touch				Sensory Pinprick			
Patient	Preoperative	6 months	12 months	18 months	Preoperative	6 months	12 months	18 months
1	34[1]	52[1]	62[1]	70[1,4]	34[1]	54[1]	61[1]	68[1,4]
2	48[1]	62[1,2]	62[1,2,3]	63[1]	48[1]	62[1,2]	62[1,2,3]	58[1]
3	50[1,2]	60[1,2]	64[1,3]	64[1]	50[1,2]	60[1,2]	68[1,3]	68[1]
4	73[1,2]	70[1,2,3]	60[1]	60[1]	74[1,2]	67[1,2,3]	63[1]	63[1]
5	44[1,2]	54[1,3,4]	68[1,3,4]	70[1]	44[1,2]	52[1,3,4]	68[1,3,4]	69[1]
6	32[1,2]	34[1,2,3]	34[1]	34[1]	32[1,2]	36[1,2,3]	36[1]	36[1]
7	48[1,5]	66[1]	72[1,5]	77[1,5]	48[1,5]	66[1]	70[1,5]	76[1,5]
	Motor Arms				Motor Legs			
1	46[1]	46[1]	50[1]	50[1,4]	0[1]	0[1]	2[1]	7[1,4]
2	50[1,*]	50[1,2*]	50[1,2,3*]	50[1*]	0[1]	0[1,2]	2[1,2,3]	2[1]
3	9[1,2]	16[1,2]	16[1,3]	17[1]	3[1,2]	3[1,2]	3[1,3]	4[1]
4	50[1,2*]	50[1,2,3*]	50[1*]	50[1*]	1[1,2]	7[1,2,3]	4[1]	4[1]
5	50[1,2*]	50[1,3,4*]	50[1,3,4*]	50[1*]	0[1,2]	4[1,3,4]	4[1,3,4]	6[1]
6	24[1,2]	30[1,2,3]	31[1]	31[1]	0[1,2]	0[1,2,3]	1[1]	1[1]
7	50[1,5*]	50[1*]	50[1,5*]	50[1,5*]	0[1,5]	2[1]	5[1,5]	7[1,5]

*Normal motor score in arms for patients with paraplegia.

Evaluations done by the following: [1]Dr Carlos Lima (Hospital Egas Moniz, Lisbon, Portugal); [2]Dr Eugenia Veiga (Rehabilitation Department, Hospital Curry Cabral, Lisbon, Portugal); [3]Dr Babu Jarodiya (Int. Med., Detroit Medical Center, Wayne State University, Detroit, MI); [4]Dr Steve Hinderer (Rehab. Instit. of Michigan, Wayne State University, Detroit, MI); [5]Dr Maria João Rodrigues (Rehabilitation Department, Hospital Santo Antonio, Porto-Portugal).

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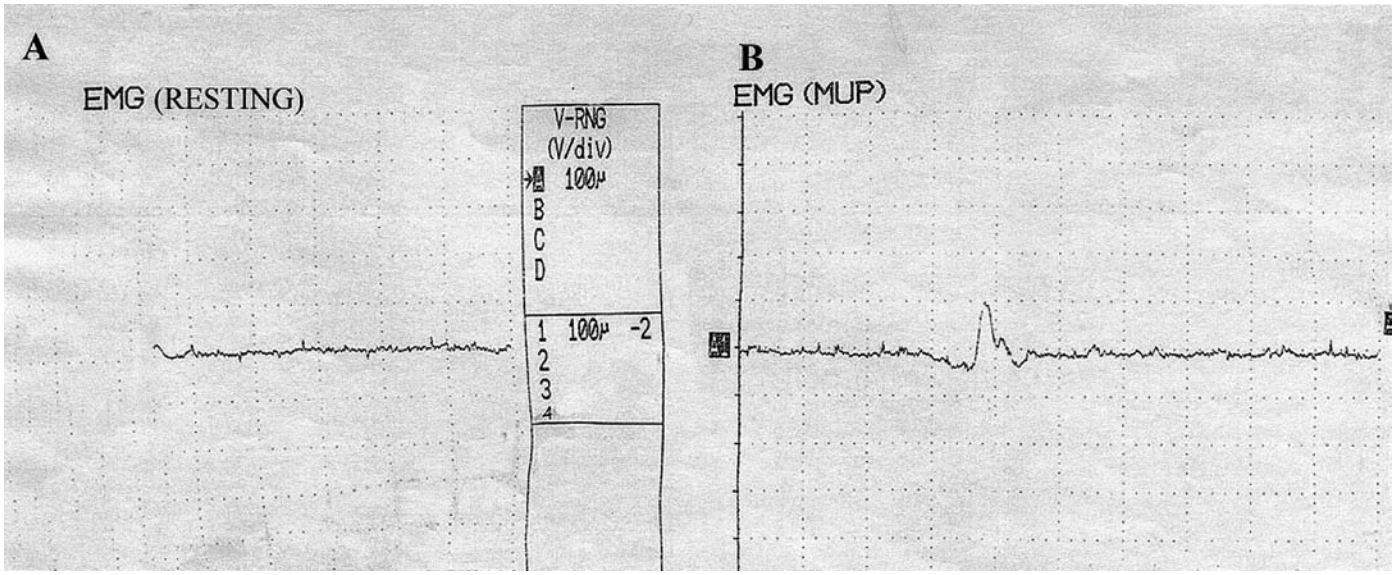

Figure 3. EMG with motor muscle potentials retrieved by voluntary control on left long adductor muscle in patient one at 10-month evaluation, preceded by no activity recording at rest.

patient in whom there was difficulty locating the lesion. She had a decrease in sensation in the lower right extremity but achieved some movement of the hip flexors. Movement of knee extensors were present at 6 months and lost when all home rehabilitative efforts were abandoned. In patient 5, there was a return of limited movement in hip flexors and increase of sensation in his legs. Patient 7 had a fair amount of improvement in sensory function primarily in the abdominal region. There was also new movement of his hip flexors.

EMG Findings

Preoperative EMG revealed signs of relatively preserved peripheral nervous system, with somewhat smaller amplitude in the muscles studied, and some borderline slow nerve conduction velocities in lower limbs were found. Patients with tetraplegia showed additional chronic neurogenic changes in the upper limbs in the myotomes consistent with SCI cervical level.

Postoperatively, in 3 patients in whom there were indications of voluntary control of a new muscles, EMG findings confirmed voluntary contraction of the muscles. This was more common in the abdominal muscles, hip flexors and large adductor muscles of the thigh, and were usually less than 3 on the motor strength score. Motor unit potential was observed in the EMG (Figure 3).

Bowel and Bladder Changes

Patient 6 reported return of bowel control, and voluntary anal sphincter contraction was confirmed in a rectal examination. Shortly after the 16th postoperative month, the patient reported recovery of sensation when the bladder was full. Urodynamic studies revealed sensation during bladder-filling cystometry and exhibited desire to void that increased during filling cystometry. Patient 6 exhibited nonspecific bladder sensation. There were signs of mild detrusor overactivity. Using Valsalva maneuver and Crédé method to void, the residual urine volume was less than 50 mL. Patient 1 also reported gaining sensation when the bladder was full, but postoperative urodynamic studies were not conclusive. Both patients decided to discontinue catheterizations and only use Valsalva maneuver and Crédé methods to void. Neither patient has had any more signs of urinary tract infections.

Subjective Impressions of Neurological Changes

From the patients' perspective, the limited changes in motor skills and any changes in bladder sensation and/or bowel function had the most impact on their lives. The recovery of limited motor function of the hip flexors sometimes allowed them to move their legs forward when braces were used or when their body weight was externally supported. For 2 of the 3 patients with tetraplegia (patients 3 and 6), the increase in ability to use their arms improved their degree of self-sufficiency, for example autonomous transfer from the wheelchair. The other patient with tetraplegia (patient 1), who already had considerable arm strength, went from total paralysis to limited movements of her hip flexors. Patient 1 showed the greatest absolute improvement in motor and sensory scores. In the paraplegics, patients 4, 5, and 7 exhibited new movement of the hip that allowed them to step with assistance while patient 2 showed lesser motor gains. In patient 4, the decrease in sensory scores seemed to have less impact than her gains in leg movements. No adverse findings were observed with regard to spasticity, dysreflexia, or temperature control.

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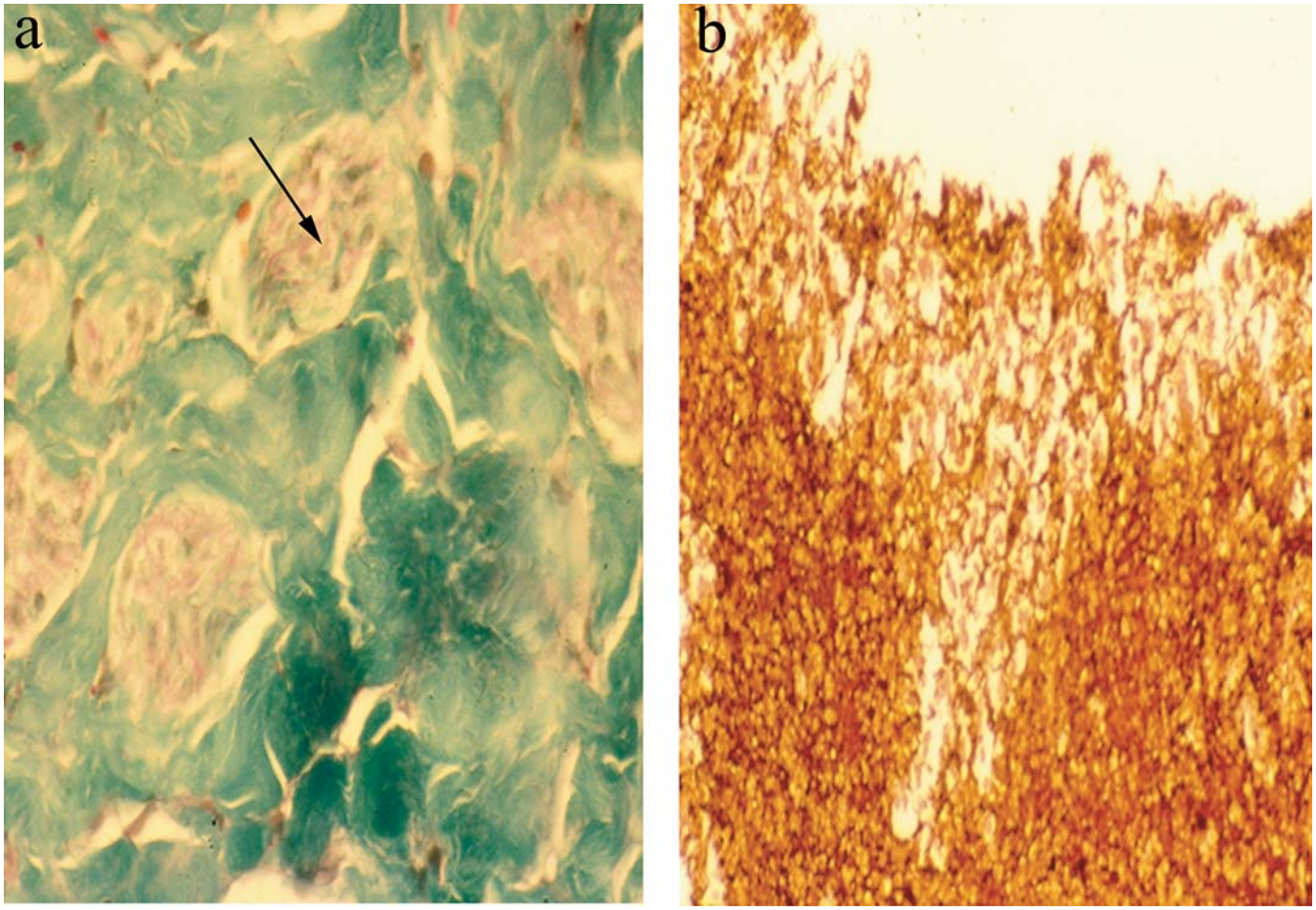

Figure 4. Sections of the scar tissue from the spinal cord removed before grafting the olfactory mucosa in patients 5 and 3. (a) Scar containing large regions of collagen with some interspersed Schwann cell ensheathed nerve fibers. Collagen bundles are stained green and an axonal bundle is marked with an arrow. Masson trichrome, ×200. (b) "Pure" glial scar that is made up of reddish-brown GFAP-positive fibers that are loosely woven close to the cavity (top) and more tightly woven at a distance (bottom). Reactive astrocytes and their fibers (processes) contain large amounts of GFAP that is stained reddish-brown. GFAP immunohistochemistry, ×200.

Olfaction

There were no definitive nasal complaints postoperatively. Nasal endoscopy documented full re-epithelization of the olfactory groove with the presence of some scar tissue and synechia. Olfaction returned to normal in all patients within 3 months.

Scar

Histopathological examination of the scar that lined the spinal cord lesion revealed variability between patients in the composition of the scar. The scar from patient 5 appeared to be primarily collagen (stained green) with some bundles of Schwann cell-ensheathed axons (Figure 4a). The scar from patient 3 was primarily glial with loosely woven astrocytic processes close to the cavity and more tightly woven farther from the cavity as revealed by GFAP immunohistochemistry that stains astrocytic processes reddish-brown (Figure 4b).

Adverse Events

There was one patient (patient 4) who had a decrease in ASIA scores (sensory component; not motor). This is also the only patient in which difficulty was encountered in finding the lesion site during surgery. In addition, a few instances of temporary pain in trunk or lower limbs occurred in the continued follow-up of patients 1 and 7, that was relieved by medication (gabapentin 300-mg tablet, 3–4 times/day as long as the complaints lasted, mean duration 2–3 months). Tingling sensations also occurred in some patients but were not characterized as painful.

DISCUSSION

The goal of this study was to determine the safety and feasibility of using a person's own olfactory mucosa in the treatment of chronic, severe SCI. This study showed that

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autologous olfactory mucosa transplantation is fairly safe, feasible, and potentially beneficial.

Advantages of Olfactory Mucosa

The primary reason for choosing olfactory mucosa as opposed to other sources of progenitor stem-like cells is that the olfactory mucosa exhibits the greatest rate of neurogenesis in adults. Neurons in the olfactory mucosa are replaced every several months (30,31). The system undergoes rapid, continuous regeneration so it is an ideal source for repairing the spinal cord and brain. Furthermore, the olfactory mucosa is a source of OECs that myelinate and promote axonal growth in the injured spinal cord and brain (9–11,17–23).

The human olfactory mucosa is located in the upper nasal cavity and consists of an epithelium and lamina propria. The epithelium contains 4 main cell types (bipolar receptor neurons, sustentacular cells, basal globose cells, and basal horizontal cells) and the underlying lamina propria (32,33). Another connective tissue layer deep to the lamina propria is the submucosa, where the largest olfactory fascicles run. The transplants actually consisted of the entire mucosa and submucosa. In the epithelium of the mucosa, the bipolar receptor neurons are terminally differentiated and axons of these neurons synapse in the brain. The basal cells represent multipotent progenitor cells because these cells can give rise both to neurons and nonneural cells (12,13,34). These basal cells with stem cell–like properties lie on a basement membrane and mature as they approach the apical surface of the epithelium. Among other cells, the sustentacular cells, which contain the intermediate filament protein nestin (used as a marker for proliferating neural progenitor cells in the nervous system), are intimately associated with the bipolar neurons and are believed to provide the scaffolding for the migration of the newly formed neurons (35). In the olfactory mucosa, cell production is under precise control so that the cells produced replace specific cell types lost from either normal attrition or certain conditions such as caustic and chemical exposure or axotomy (36,37). The mature neurons will likely exhibit apoptotic cell death shortly after transplantation because of the axotomy and loss of target that occur when the olfactory mucosa is removed (16,30,38). The absence of the inhibitory cues from mature neurons enhances the rate of neurogenesis, which is likely to occur after transplantation. Recently, the growth and differentiation factor 11 was identified in mature bipolar receptor neurons. This factor seems to contribute for this negative feedback on neurogenesis (37). Immediately beneath the olfactory epithelium are the connective tissue layers lamina propria and submucosa. The lamina propria consists of OECs that surround the olfactory nerve fibers; extracellular matrix (ECM); fibroblasts; blood and lymphatic vessels; and Bowman glands that are a characteristic feature of this area. The submucosa also contains large nerve fascicles with OECs (within a connective tissue scaffolding) that cross the openings in the cribriform plate to enter the brain. Growth factors present in these connective tissue layers support neuron production and survival (39). Progenitor cells deprived of growth factors in these layers undergo cell death by apoptosis. The ECM is generally considered to be neuroprotective and also promotes axonal outgrowth and regulates synaptic plasticity (40–42). The ECM also presents a unique combination of receptors that regulate adhesion and mitosis in nonneural stem cells and horizontal basal cells (intercellular adhesion molecule-1 [ICAM-1] and β_1, β_4, α-1, α-3, and α-6 integrins) (43). Furthermore, fibroblast proliferation might not be anticipated, because fibroblasts have been shown to express neuronal markers when exposed to neuronal precursor extracts (44).

Reason for Autologous Grafts

Reasons for using autologous transplants are avoidance of problems of rejection, overgrowth, diseases transmission, and ethical issues. In most experimental animal studies, transplants are done within the same strain of animals. In cases where very different strains (immunologically) are used, rejection of the graft is obvious when immunosuppression is not used (45). In clinical trials where embryonic/fetal tissue or cells are used, there is the possibility of uncontrolled growth of the tissue or rejection as the cells mature and are recognized as foreign by the immune system. In two different clinical trials for Parkinson disease where embryonic/fetal tissue was used, severe dyskinesia developed in several patients (46–48). Freed et al (46,47) believe that the dyskinesia in their clinical trial might be caused by overgrowth or unbalanced increases in dopaminergic levels. In the other study, Olanow et al (48) believe that the dyskinesia in their clinical trial is caused by tissue rejection, and they warn that transplants from fetuses/embryos should not be done. Very few studies have been done in animals or humans using one's own tissue or cells for neurological diseases or injuries, although there are indications that autologous transplants may be remarkably effective (49). Another advantage of using olfactory mucosa autografts as a neural stem cell source is the possibility of harvesting the donor tissue in an extracranial, easily accessible site.

Scar

The scar that forms after SCI is generally believed to be inhibitory. Support for this idea comes from experimental animal studies in which preventing scar formation or breaking down the scar is sometimes effective in encouraging axonal growth and/or recovery. The main component(s) of the scar that are responsible for this inhibition remains controversial. Among the candidate components for this inhibition are the astrocytes (glial component), type IV collagen, laminin, and chondroitin sulphate proteoglycans. Mice that are knockouts for GFAP and vimentin exhibited more sprouting and greater

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recovery than normal mice did after SCI (50). Also, methods directed at proteoglycans inhibition, such as chondroitinase ABC or decorin core protein, promoted axonal growth (51,52).

In our study, the scar was surgically removed, within limits as to not harm normal cord tissue, to provide continuity between the graft and the spinal cord above and below the injury site to encourage integration of the graft. This provided an opportunity to study the composition of chronic scar. In some cases, a dense meshwork of astrocytic fibers was observed lining the cystic cavity, thus producing an almost "pure glial scar." In other cases, type IV collagen was predominant, with little evidence of astrocytes revealed by GFAP staining. In this case, the term "glial scar" would be a misnomer. There was also variability in the amount of hemorrhagic tissue present in the scar. In most of the cases, there were peripheral type axons present in the scar that varied from a few scattered axons to several bundles. There was not an obvious difference in the composition or density of the scar from the 2 patients who received the transplant at 6 months after injury compared to the scar from more chronic injuries. The most common observation in all the cases done to date, including those in this study, was a scar of mixed composition containing both astrocytic processes, collagen, and laminin with axons interspersed. The detailed histopathological observations and results of the scar tissue removed from the patients will be the object of further studies.

The individual composition of the scar may be important in predicting how much or how quickly the scar reforms after olfactory mucosa transplantation. It is not known if the scar reforms in patients with SCI after receiving the olfactory mucosa transplants. Despite the presence of some peripheral-type axons, it is believed that the chronic scar is an important obstacle to regeneration of the spinal cord. In experimental animal studies, where autologous olfactory mucosa transplants were performed in subacute or chronic spinal cord lesions, there was little evidence of reformation of the scar (unpublished data).

MRI Findings and Filling of the Cavity

Apparently critical to the success of the treatment is the filling of the cystic cavity. MRI scans at 6 months after the transplantation reveal fairly complete filling of cavities, with a "salt and pepper" appearance of the grafted area. This aspect might be explained by the hemosiderin content of the vascular area of the graft. The obliteration of the cavities seems to indicate that the transplant survived. However, without histological examination, there is no way of knowing if this is indeed the case. If one extrapolates from the immunohistochemical results of numerous experimental animal studies where labeled cells were used (1,7,10), it is likely that this is the case. Consequently, the postoperative filling of the cavity may be predictive of improved functional outcome.

Influence of Age, Time After Injury, Length of Lesion, and Level of the Lesion

Of the patients included in this study, there was no obvious trend within the ranges used that shorter time after injury, younger age, smaller lesion, or lower spinal level resulted in greater improvement. It appeared that patients with tetraplegia might take longer before showing any improvement in their legs. However, with this small of a sample, it is difficult to draw any definitive conclusions. There may be a limit in age for this surgical treatment because, with aging, the respiratory epithelium generally replaces regions that were originally all olfactory mucosa (27). This was the rationale for including only patients less than 35 years old in the study. Also, patients were included only with lesions of 6 cm or less, as modifications of the protocol may be necessary for larger lesions and/or older patient age.

Pattern of Recovery

There was not a proximal-distal pattern of recovery that might be suggestive of a slow growth of spinal axons from the proximal to the distal part of the limbs. There was some asymmetry observed, but the reasons for this asymmetry were not clear. Both the degree and pattern of recovery differed between sensory and motor systems. Much greater sensory recovery was observed, but it is not known if this relates to the limited rehabilitation facilities or intrinsic differences caused by the olfactory mucosa transplant. The pattern of recovery between motor and sensory was quite variable. Even more variable was the amount of recovery among patients. There was some tendency for peaks of recovery in the first 3 to 6 months, and a later phase of recovery after more than a year.

There are several possible mechanisms responsible for the observed sensory and motor improvements: Our belief is that the stem-like progenitor cells are primarily responsible with added benefits from the OECs. However, there is also a possible contribution from the olfactory fibroblasts and matrix that encourage alignment and directional growth of neurites in culture (53). The stem-like progenitor cells in the human olfactory mucosa have recently been shown to be multipotent (16). The justification for using tissue containing stem cells/immature neurons in human clinical trials was recently reviewed (54). OECs may be promoting axonal growth and myelination as found in experimental animal studies (17–22). Alternatively, olfactory mucosa may simply be a substrate for growth or is modulating the immune system. The only recovery pattern that might be suggestive of a particular mechanism is the very late improvement. If reconnection to a distant target is possible by a few axons, it would be expected to be a prolonged process. On the other hand, it might be argued that this reflects a very slow maturation of some of the progenitor cells into neurons. The limited return of motor function below the level of injury might be suggestive of new connection across the lesion. However,

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myelination and/or sprouting of preexisting axons are alternative explanations.

Rehabilitation

Minimal rehabilitation was available for patients in this study, either pre- or postoperatively. We hope to be successful in our efforts to develop a rehabilitation program that includes functional electrical stimulation, weight-supported treadmill, stim bike, computer-assisted biofeedback, trunk stability exercises, and aquatics in a stimulating group therapy environment. It would be ideal if a patient could be enrolled in a rehabilitative program for at least 6 to 12 months before surgery, so that the maximal improvement afforded by rehabilitation alone could be reached. The rehabilitative program should be continued after the olfactory mucosa graft surgery. Without a program that strengthens muscles and bones, it would be difficult to get larger improvements in motor function in chronic, severe SCI. The amount of improvement observed is actually surprising given the lack of an intense rehabilitative program. If better rehabilitative facilities were available in this study, greater improvement, especially in motor leg scores, may have been seen. It is also likely that rehabilitation may need to be continued indefinitely to retain the functional gains and reach the maximal improvement possible. For example, patient four, who started with a preoperative score of 1 in motor legs on the ASIA neurological examination, reached a postoperative score of 7 at 6 months that later declined to 4 when the patient moved to a region where no rehabilitation was available.

Risks

The primary concern was whether there would be any additional decrease in neurological function as a result of the operation. We found a slight sensory decrease in one patient. No large decreases in function were detected either immediately or in the long-term follow-up (up to 42 months) in our patients, probably because surgical manipulations are focused at the damaged spinal cord tissues (cavity or scar). This decrease in sensory function in patient 4 is explained by difficulties in locating the exact site of the lesion while dissecting the spinal cord. This problem could be avoided with surgery room facilities such as surgery-assisted MRI or intraoperative sonography.

Another particular area of concern was the possibility of the introduction of pathogens in using a mucosa that is normally exposed to the air. In our study, none of the patients developed postoperative local or systemic infections with a standard antibiotic regimen and previous nasal cavities disinfection. To date, 41 patients have received transplants with no indications of infection.

An additional potential risk of the procedure was the loss or decrease of olfaction because part of the olfactory mucosa was removed. In this study, no significant olfactory loss was detected postoperatively when olfaction was evaluated 3 months after the surgery.

Positive Indicators

Bladder Sensation. The return of bladder sensation reported in 2 of the 7 patients after the 15th month may reflect some type of long-term reorganization of the spinal cord and may suggest a relatively slow spinal cord reorganization. In patient 6, return of voluntary anal sphincter contraction occurred more than 1 year after the transplantation, which was more than 7 years after her SCI. Spontaneous return of bladder sensation or bowel control is extremely unusual this late after injury; therefore, it can be presumed that the functional return is related to the transplant procedure.

ASIA Testing Results. Normally, there is little increase in function in people with complete spinal cord injuries (26,27), so changes in ASIA scores are presumed to be the result of the surgical intervention. Six of 7 patients showed some improvement in both motor and sensory function. Statistical analyses showed that these effects were significant. Although this was a small pilot trial, it seems unlikely to be caused by chance that all of these patients with a chronic, severe injury would regain movement in new muscle groups and 6 of 7 would recover a fair amount of sensation. Most of the patients exhibited improvements within the first 6 months after surgery, that, afterward, seemed to slowly increase or stabilize. Longer follow-up will reveal whether these improvements remain and whether function continues to improve or declines in the years to come. It is likely that certain pathways may have been more damaged in some patients than others even though their ASIA scores were similar. Overall, there seemed to be greater improvement in sensory ASIA scores than in motor ASIA scores that may relate to the rehabilitation.

Adverse Findings

Sensory Decrease in One Patient. There was only one patient who had a small decrease in sensory function. All other patients showed an increase in sensory and motor function as measured in ASIA neurological examination. This decrease was most likely caused by some sensory axons being damaged during the surgical procedure because of the difficulty locating the lesion site, because there was still some improvement in motor scores in this patient. Because the surgical approach was posterior, the sensory neurons that are located in the posterior part of the cord would be more likely to be injured in cases where there are difficulties finding the lesion site.

Pain and Tingling Sensations. Several patients reported a tingling sensation for some length of time before there was a return of sensation to a particular region. Some patients (1 and 7) also reported new temporary neuropathic pain, especially in the trunk and legs. This symptom was temporary and resolved with proper medication (gabapentin), and sometimes the

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transient pain seemed to precede some sort of sensory or motor recovery.

CONCLUSION

This pilot clinical study shows that autografts of olfactory mucosa are fairly safe and feasible and may possibly promote functional recovery in chronic, severe SCI in humans. When transplanted as pieces of complete mucosa, including both lamina propria and olfactory neuroepithelium, both stem-like progenitor cells and olfactory ensheathing cells are provided. All patients exhibited improvement in ASIA motor scores. In all but one patient, increases in ASIA sensory scores were observed. Two of 7 patients changed from ASIA A to ASIA C. Also, two patients reported return of sensations in the bladder; one of these patients also achieved voluntary contraction of the anal sphincter. Overall, patients exhibited a modest amount of improvement in function that is not normally observed in complete SCIs. Adverse events included a few incidents of transient pain that were relieved with medication. In addition, there was a decrease in ASIA sensory scores in one patient in whom difficulty was encountered in surgically locating the injury site. Based on the encouraging findings in this study and lack of serious adverse events, further investigational clinical trials seem to be warranted.

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REFERENCES

1. McDonald JW, Liu XZ, Qu Y, et al. Transplanted embryonic stem cells survive, differentiate and promote recovery in injured rat spinal cord. *Nat Med.* 1999;5:1410–1412.
2. Akiyama Y, Honmou O, Kato T, Uede T, Hashi K, Kocsis JD. Transplantation of clonal neural precursor cells derived from adult human brain establishes functional peripheral myelin in the rat spinal cord. *Exp Neurol.* 2001;167:27–39.
3. Reier PJ, Houle JD, Jakeman L, Winialski D, Tessler A. Transplantation of fetal spinal cord tissue into acute and chronic hemisection and contusion lesions of the adult rat spinal cord. *Prog Brain Res.* 1988;78:173–179.
4. Xu XM, Zhang SX, Li H, Aebischer P, Bunge MB. Regrowth of axons into the distal spinal cord through a Schwann-cell-seeded mini-channel implanted into hemisected adult rat spinal cord. *Eur J Neurosci.* 1999;11:1723–1740.
5. Grill RJ, Blesch A, Tuszynski MH. Robust growth of chronically injured spinal cord axons induced by grafts of genetically modified NGF-secreting cells. *Exp Neurol.* 1997; 148:444–452.
6. Liu Y, Himes BT, Murray M, Tessler A, Fischer I. Grafts of BDNF-producing fibroblasts rescue axotomized rubrospinal neurons and prevent their atrophy. *Exp Neurol.* 2002;178: 150–164.
7. Chopp M, Zhang XH, Li Y, et al. Spinal cord injury in rat: treatment with bone marrow stromal cell transplantation. *Neuroreport.* 2000;11:3001–3005.
8. Sasaki M, Honmou O, Akiyama Y, Uede T, Hashi K, Kocsis JD. Transplantation of an acutely isolated bone marrow fraction repairs demyelinated adult rat spinal cord axons. *Glia.* 2001;35:26–34.
9. Lu J, Féron F, Mackay-Sim A, Waite PM. Olfactory ensheathing cells promote locomotor recovery after delayed transplantation into transected spinal cord. *Brain.* 2002;125:14–21.
10. Ramón-Cueto A, Cordero MI, Santos-Benito FF, Avila J. Functional recovery of paraplegic rats and motor axon regeneration in their spinal cords by olfactory ensheathing glia. *Neuron.* 2000;25:425–435.
11. Ramón-Cueto A, Nieto-Sampedro M. Regeneration into the spinal cord of transected dorsal root axons is promoted by ensheathing glia transplants. *Exp Neurol.* 1994;127: 232–244.
12. Clarkson ED, Zawada WM, Adams FS, Bell KP, Freed CR. Strands of embryonic mesencephalic tissue show greater dopamine neuron survival and better behavioral improvement than cell suspensions after transplantation in parkinsonian rats. *Brain Res.* 1998;806:60–68.
13. Lowhagen P, Johansson BB, Nordborg C. The nasal route of cerebrospinal fluid drainage in man. A light-microscope study. *Neuropathol Appl Neurobiol.* 1994;20:543–550.
14. Huard JM, Youngentob SL, Goldstein BJ, Luskin MB, Schwob JE. Adult olfactory epithelium contains multipotent progenitors that give rise to neurons and non-neural cells. *J Comp Neurol.* 1998;400:469–486.
15. Roisen FJ, Klueber KM, Lu CL, et al. Adult human olfactory stem cells. *Brain Res.* 2001;890:11–22.
16. Murrell W, Féron F, Wetzig A, et al. Multipotent stem cells from adult olfactory mucosa. *Dev Dyn.* 2005;233(2):496–515.
17. Barnett SC, Alexander CL, Iwashita Y, et al. Identification of a human olfactory ensheathing cell that can effect transplant-mediated remyelination of demyelinated CNS axons. *Brain.* 2000;123:1581–1588.
18. Franklin RJ, Gilson JM, Franceschini IA, Barnett SC. Schwann cell-like myelination following transplantation of an olfactory bulb-ensheathing cell line into areas of demyelination in the adult CNS. *Glia.* 1996;17:217–224.
19. Imaizumi T, Lankford KL, Waxman SG, Greer CA, Kocsis JD. Transplanted olfactory ensheathing cells remyelinate and enhance axonal conduction in the demyelinated dorsal columns of the rat spinal cord. *J Neurol.* 1998;18:6176–6185.
20. Li Y, Field PM, Raisman G. Repair of adult rat corticospinal tract by transplants of olfactory ensheathing cells. *Science.* 1997;277:2000–2002.
21. Ramón-Cueto A, Plant GW, Avila J, Bunge MB. Long-distance axonal regeneration in the transected adult rat spinal cord is promoted by olfactory ensheathing glia transplants. *J Neurosci.* 1998;18:3803–3815.
22. Kato T, Honmou O, Uede T, Hashi K, Kocsis JD. Transplantation of human olfactory ensheathing cells elicits remyelination of demyelinated rat spinal cord. *Glia.* 2000; 30:209–218.
23. Lu J, Feron F, Ho SM, Mackay-Sim A, Waite PM. Transplantation of nasal olfactory tissue promotes partial re-

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covery in paraplegic adult rats. *Brain Res.* 2001;889:344–357.
24. Ramer LM, Au E, Richter MW, Liu J, Tetzlaff W, Roskams AJ. Peripheral olfactory ensheathing cells reduce scar and cavity formation and promote regeneration after spinal cord injury. *J Comp Neurol.* 2004;473:1–15.
25. Au E, Roskams AJ. Olfactory ensheathing cells of the lamina propria in vivo and in vitro. *Glia.* 2003;41:224–236.
26. Burns AS, Lee BS, Ditunno JF Jr, Tessler A. Patient selection for clinical trials: the reliability of the early spinal cord injury examination. *J Neurotrauma.* 2003;20:477–482.
27. Kirshblum S, Millis S, McKinley W, Tulsky D. Late neurologic recovery after traumatic spinal cord injury. *Arch Phys Med Rehabil.* 2004;85:1811–1817.
28. American Spinal Injury Association/International Medical Society of Paraplegia. *International Standards for Neurologic and Functional Classification of Spinal Cord Injury*. Chicago, IL: ASIA; 1996.
29. Paik SI, Lehman MN, Seiden AM, Duncan HJ, Smith DV. Human olfactory biopsy. The influence of age and receptor distribution. *Arch Otolaryngol Head Neck Surg.* 1992;118: 731–738.
30. Paternostro MA, Meisami E. Quantitative [3H]thymidine autoradiography of neurogenesis in the olfactory epithelium of developing normal, hypothyroid and hypothyroid-rehabilitated rats. *Brain Res Dev Brain Res.* 1994;83:151–162.
31. Graziadei PP, Karlan MS, Graziadei GA, Bernstein JJ. Neurogenesis of sensory neurons in the primate olfactory system after section of the fila olfactoria. *Brain Res.* 1980; 186:289–300.
32. Morrison EE, Costanzo RM. Morphology of the human olfactory epithelium. *J Comp Neurol.* 1990;297:1–13.
33. Morrison EE, Costanzo RM. Morphology of olfactory epithelium in humans and other vertebrates. *Microsc Res Tech.* 1992;23:49–61.
34. Chen X, Fang H, Schwob JE. Multipotency of purified, transplanted globose basal cells in olfactory epithelium. *J Comp Neurol.* 2004;469:457–464.
35. Doyle KL, Khan M, Cunningham AM. Expression of the intermediate filament protein nestin by sustentacular cells in mature olfactory neuroepithelium. *J Comp Neurol.* 2001; 437:186–195.
36. Calof AL, Hagiwara N, Holcomb JD, Mumm JS, Shou J. Neurogenesis and cell death in olfactory epithelium. *J Neurobiol.* 1996;30:67–81.
37. Wu HH, Ivkovic S, Murray RC, et al. Autoregulation of neurogenesis by GDF11. *Neuron.* 2003;37:197–207.
38. Graziadei GA, Graziadei PP. Neurogenesis and neuron regeneration in the olfactory system of mammals. II. Degeneration and reconstitution of the olfactory sensory neurons after axotomy. *J Neurocytol.* 1979;8:197–213.
39. Mumm JS, Shou J, Calof AL. Colony-forming progenitors from mouse olfactory epithelium: evidence for feedback regulation of neuron production. *Proc Natl Acad Sci U S A.* 1996;93:11167–11172.
40. Carbonetto S, Gruver MM, Turner DC. Nerve fiber growth in culture on fibronectin, collagen, and glycosaminoglycan substrates. *J Neurosci.* 1983;3:2324–2335.
41. Wallquist W, Patarroyo M, Thams S, et al. Laminin chains in rat and human peripheral nerve: distribution and regulation during development and after axonal injury. *J Comp Neurol.* 2002;454:284–293.
42. Dityatev A, Schachner M. Extracellular matrix molecules and synaptic plasticity. *Nat Rev Neurosci.* 2003;4:456–468.
43. Carter LA, MacDonald JL, Roskams AJ. Olfactory horizontal basal cells demonstrate a conserved multipotent progenitor phenotype. *J Neurosci.* 2004;24:5670.
44. Hakelien AM, Landsverk HB, Robl JM, Skalhegg BS, Collas P. Reprogramming fibroblasts to express T-cell functions using cell extracts. *Nat Biotechnol.* 2002;20:460–466.
45. Theele DP, Schrimsher GW, Reier PJ. Comparison of the growth and fate of fetal spinal iso- and allografts in the adult rat injured spinal cord. *Exp Neurol.* 1996;142:128–143.
46. Freed CR, Greene PE, Breeze RE, et al. Transplantation of embryonic dopamine neurons for severe Parkinson's disease. *N Engl J Med.* 2001;344:710–719.
47. Ma Y, Feigin A, Dhawan V, et al. Dyskinesia after fetal cell transplantation for parkinsonism: a PET study. *Ann Neurol.* 2002;52:628–634.
48. Olanow CW, Goetz CG, Kordower JH, et al. A double-blind controlled trial of bilateral fetal nigral transplantation in Parkinson's disease. *Ann Neurol.* 2003;54:403–414.
49. Lévesque MF, Neuman T. Autologous transplantation of adult human neural stem cells and differentiated dopaminergic neurons for Parkinson's disease: one year post-operative clinical and functional metabolic results. *AANS.* April 18, 2002. http://www.celmedbio.com/abstracts/Abstract_AANS_2002.pdf. Accessed May 30, 2006.
50. Menet V, Prieto M, Privat A, Gimenez y Ribotta M. Axonal plasticity and functional recovery after spinal cord injury in mice deficient in both glial fibrillary acidic protein and vimentin genes. *Proc Natl Acad Sci U S A.* 2003;100:8999–9004.
51. Bradbury EJ, Moon LD, Popat RJ, et al. Chondroitinase ABC promotes functional recovery after spinal cord injury. *Nature.* 2002;416:636–640.
52. Davies JE, Tang X, Denning JW, Archibald SJ, Davies SJ. Decorin suppresses neurocan, brevican, phosphacan and NG2 expression and promotes axon growth across adult rat spinal cord injuries. *Eur J Neurosci.* 2004;19:1226–1242.
53. Deumens R, Koopmans GC, Den Bakker CG, et al. Alignment of glial cells stimulates directional neurite growth of CNS neurons in vitro. *Neuroscience.* 2004;125: 591–604.
54. Reier PJ. Cellular transplantation strategies for spinal cord injury and translational neurobiology. *Neuro Rx.* 2004;1: 424–451.

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#3

COMMENTARY
"A Start"
Olfactory Mucosa Autografts in Human Spinal Cord Injury

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Clinical trials to improve neurological function after spinal cord injury (SCI) have been increasing throughout the world. A great deal of enthusiasm from some corners of medical researchers, injured individuals, the media, and the lay public for new techniques aimed at "cure" have led some to the expectation that a cure is imminent (1–3). To date, however, none of the recent trials has shown dramatic changes in neurological or functional recovery.

The pilot study by Lima et al, (4) published in this issue, describes a surgical intervention that has received some media attention over the last few years. In this report, 7 subjects, all initially classified as ASIA A, aged 18 to 32 years and from 6 months to 6.5 years post-injury, underwent olfactory mucosa autograft transplantation into their spinal cord with the scar tissue reportedly removed. Followup was performed at 6, 12, and 18 months after surgery, with some improvements noted in upper and lower extremity motor scores and overall sensory scores, with two subjects converting from ASIA Impairment Scale (AIS) A to C status. Improvements were also reported for some subjects in bowel and bladder function and "subjective impression of neurological changes." Adverse events were reportedly minimal. The authors conclude that "olfactory mucosa autograft transplantation into the human injured spinal cord is feasible, relatively safe and potentially beneficial."

There are a number of limitations of this preliminary study that are important to keep in mind. The first issue relates to the performance of a large-scale human study (although only 7 patients' results are detailed in this study, 41 subjects have reportedly undergone this procedure) without a more definitive safety analysis being reported. Recommended guidelines for studies on human subjects with SCI have been published that outline certain criteria for the planning, initiation, and conduct of translational research in the nervous system (5). These include:

1) Preclinical studies in which animal data should be acquired prior to human studies that would be considered predictive of lasting clinical benefit. The greater the invasiveness of the procedure, the higher the standard of preclinical safety and efficacy should be.

2) Safety assessments should be documented and should optimally include outcome measures for pain, as well as assessing maintenance of function above the lesion.

3) There should be evidence of functional benefit demonstrated on accepted objective measures of functional outcome.

4) Functional improvement in the animal model should be of sufficient magnitude and duration to justify the potential risk of a clinical trial.

5) Whenever possible, experimental trials should use placebo control groups.

The introduction of the paper describes "a small animal trial using olfactory mucosal transplants in subacute spinal transection model revealed tissue continuity across the lesion site and no signs of overgrowth." However, this is "unpublished data." The authors continue that "further support for the potential of the olfactory mucosa in experimental animal studies of SCI will soon be reported." It is surprising that human studies would be undertaken without reported animal model findings to define the functional or neurological benefits of the intervention.

The time period of inclusion into the study was from 6 months post-injury and included subjects with a neurological injury classified as AIS A and B. For clinical trials performed on individuals with chronic SCI, especially when including AIS B subjects, it is more common to wait until individuals are at least 1 year post-injury. Without intervention, patients, most especially individuals with AIS B, can show recovery in bowel and bladder function, as well as improvement to ASIA C status from 6 months to the 1-year time period postinjury. All of the subjects reported were classified as AIS A. Two subjects (Patients 1 and 2), however, who underwent the procedure at 6 months post-injury, showed significantly more recovery; and gained 35 points (the most of any subject) and 15 points (third greatest improvement), respectively, on follow-up sensory examination. For motor testing, Patient 1 improved 7 points on follow-up lower extremity motor (LEM) testing, which was equal to the greatest degree of recovery of the subjects.

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Greater detail regarding the surgical procedures would allow greater understanding by other researchers regarding the tissue implanted, for example, quantifying the amount of olfactory mucosa removed for implantation, the technique for removal of the tissue and description of the histology. Unfortunately, the examiners were not blinded and there was no mention of inter-rater reliability of the assessors. All patients reportedly had lower extremity (LE) motor recovery, including subjects who remained with complete tetraplegia (AIS A). Such large zones of partial preservation are uncommon, although possible. Quality of life (QOL) changes were described as another benefit to the subjects; however, "no measures were used to quantify this change."

Postoperative EMG findings were reported for 3 subjects who had indication of LE voluntary contraction; however, no identification of these individuals was made or whether these subjects were tested preoperatively in these same muscles to show evidence of electrodiagnostic change. Bowel and bladder changes were described for only a few patients. No comparisons were made between pre- and postoperative urodynamics, and the finding of a change from intermittent catheterization to "Valsalva voiding" is not necessarily an indication of neurologic recovery. To fully study the effect on the bladder, urodynamic testing performed in all subjects would have offered more objective data.

We are experiencing an exciting time in the field of SCI research, with so many studies currently underway and many anticipated trials in the near future [Cethrin (BA-210), HP-184, anti-nogo antibodies, minocycline, among others]. Research to discover therapies for SCI has made steady progress over the last several years (5), and continued progress is anticipated through novel approaches by individual centers, eventually leading to multi-center trials. While the limitations of this study have been elucidated, its publication has important implications. It is hoped that much can be learned from this trial and a randomized controlled trial can be discussed after analysis of the patients who have already undergone the treatment.

REFERENCES

1. Ramer LM, Ramer MS, Steeves JD. Setting the stage for functional repair of spinal cord injuries: a cast of thousands. *Spinal Cord.* 2005;1–28.
2. Steeves J, Fawcett J, Tuszynski M. Report of international trials workshop on spinal cord injury. February 20–21, 2004. Vancouver, Canada. *Spinal Cord.* 2004;42:591–597.
3. Dobkin BH, Curt A, Guest J. Cellular transplants in China: Observational study from the largest human experiment in chronic spinal cord injury. *Neurorehabil and Neural Repair.* 2006;20:5–13.
4. Lima C, Pratas-Vital J, Escada P, Hasse-Ferriera A, Capucho C, Peduzzi JD. Olfactory mucosa autografts in human spinal cord injury; A pilot clinical study. *J Spinal Cord Med.* 2006;29: 191–203.
5. Anderson DK, Beattie M, Blesch A, et al. Recommended guidelines for studies of human subjects with spinal cord injury. *Spinal Cord.* 2005;43:453–458.

AUTHORS' RESPONSE:

The advice, comments and careful evaluation of our article by Dr. Kirshblum are greatly appreciated. We strived to perform this clinical study with care and accuracy. When sufficient data are collected and analyzed on additional patients, these findings will be reported. We greatly applaud the efforts of the International Collaboration on Repair Discoveries that has recently prepared guidelines for SCI clinical trials. Last year, a large group of clinicians and scientists from across the world met in Tocha, Portugal to exchange ideas regarding the olfactory mucosa autografts (OMA) procedure.

The basis for proceeding to a small clinical study was not our study in guinea pigs, although this study did provide safety data regarding the lack of overgrowth. Rather, the justification for this clinical study was the large number of experimental animal studies that document significant functional benefit and indications of anatomical repair using stem/progenitor cells (1–3) or OECs (4–6) in SCI. Studies also indicate that tissue containing stem/progenitor cells (7) or OECs (8) acts in a similar manner to purified populations of these cell types. Evidence of the usefulness of these cell types in CNS repair continue to be reported. A recent study found that progenitor cells from adult human olfactory mucosa rescue axotomized rubrospinal neurons and promote functional recovery in SCI rats (9). Although there were a large number of studies supporting the use of stem/progenitor cells and olfactory ensheathing cells (OECs) in SCI, patients were slowly enrolled (over a 2-year period), so that the study could be stopped if significant safety concerns arose.

After it was decided to begin a small clinical study based on the experimental animal studies done by other researchers, two years were spent in the design of the OMA procedure. First, the olfactory mucosae from a large number of cadavers were examined histologically to determine the optimal region for graft removal and the age range in which this area was completely devoid of respiratory mucosa. Second, novel techniques were developed to remove pieces of olfactory mucosa using instrumentation so the graft containing progenitor cells and OECs could be obtained in a minimally invasive manner. Innovative neurosurgical procedures were also required. Third, the actual surgical procedure was practiced on cadavers to increase proficiency and work out any technical problems before beginning the clinical study.

We agree that it might have been better not to enroll any patients less than 1 year post-injury due to the occasional functional improvement that might occur from 6 to 12 months post-injury. Although it is possible that intervention at shorter times after injury may result in

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greater recovery, our limited results so far do not support this idea. Besides patient 1 (OMA at 6 months post-injury), the only other patient that changed from ASIA A to ASIA C was more than 6 years post-injury, and was actually the only patient with documented improvement in bowel and bladder function. Likewise, the other patient to improve by 7 points on follow-up lower extremity motor testing was more than 1 year post-injury at the time of the OMA procedure. In terms of sensory light-touch (although less definitive than sensory pin-prick), the increase in patient 2 (treated at 6 months) was less than other patients (patients 5 and 7) treated at 30 months post-injury. If earlier measures of outcome eventually become more predictive, it may be possible to revisit this question.

We neglected to include several points in our article that were cited by Dr. Kirshblum. Preoperative urodynamics revealed the lack of the voluntary control of bladder function in all patients. Postoperative urodynamic studies were only done in patients reporting improvements in bladder function due to logistical difficulties. Preoperative rectal examinations revealed a lack of voluntary anal sphincter contraction in every patient. Electrodiagnostic results suggesting voluntary muscle contraction were observed in patients 1, 3, and 5 postoperatively. Greater details of the OMA procedure will be presented in both otolaryngological and neurosurgical journals.

Our reason for continuing to enroll patients (beyond the first 7) was the lack of serious side effects in the long-term follow-up of these first 7 patients who were enrolled over a 2-year period. We believe that this evidence is stronger than any further experimental animal studies. However, animal studies in chronic, severe SCI-injured rodents may provide ways of improving outcome by modifying the OMA procedure or through a combination approach. Although effectiveness cannot be proven in such a small clinical study, there were indications of possible benefit in all patients that are unlikely to be due to late spontaneous recovery or placebo effects. To prove possible effectiveness, a clinical trial using blinded evaluators with more outcome measures would be required with a control group of patients possibly undergoing late decompressive surgery. Furthermore, all patients enrolled in the trial would need to be in an intense rehabilitation program for several months before and after the intervention period to maximize possible improvement and separate the effects of OMA procedure and rehabilitation. Although using one's own cells may be safer, easier, and more effective, support for such a clinical trial is difficult due to the lack of commercial product or patented procedures that would engender biotech company support.

REFERENCES:

1. Oka S, Honmou O, Akiyama Y, et al. Autologous transplantation of expanded neural precursor cells into the demyelinated monkey spinal cord. *Brain Res.* 2004;1030: 94–102.
2. Pfeifer K, Vroemen M, Blesch A, Weidner N. Adult neural progenitor cells provide a permissive guiding substrate for corticospinal axon growth following spinal cord injury. *Eur J Neurosci.* 2004;20:1695–1704.
3. Karimi-Abdolrezaee S, Eftekharpour E, Wang J, Morshead CM, Fehlings MG. Delayed transplantation of adult neural precursor cells promotes remyelination and functional neurological recovery after spinal cord injury. *J Neurosci.* 2006;26:3377–3389.
4. Radtke C, Akiyama Y, Brokaw J, et al. Remyelination of the nonhuman primate spinal cord by transplantation of H-transferase transgenic adult pig olfactory ensheathing cells. *FASEB J.* 2004;18:335–337.
5. Li Y, Decherchi P, Raisman G. Transplantation of olfactory ensheathing cells into spinal cord lesions restores breathing and climbing. *J Neurosci.* 2003;23:727–731.
6. Pascual JI, Gudino-Cabrera G, Insausti R, Nieto-Sampedro M. Spinal implants of olfactory ensheathing cells promote axon regeneration and bladder activity after bilateral lumbosacral dorsal rhizotomy in the adult rat. *J Urol.* 2002;167:1522–1526.
7. Murrell W, Feron F, Wetzig A, et al. Multipotent stem cells from adult olfactory mucosa. *Dev Dyn.* 2005;233:496–515.
8. Lu J, Feron F, Ho SM, Mackay-Sim A, Waite PM. Transplantation of nasal olfactory tissue promotes partial recovery in paraplegic adult rats. *Brain Res.* 2001;889:344–357.
9. Xiao M, Klueber KM, Lu C, Guo Z, Wang H, Roisen FJ. Human adult olfactory neural progenitors rescue axotomized rodent rubrospinal neurons and promote functional recovery. *Exp Neurol.* 2005;194:12–30.

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