Experimental Neurology 229 (2011) 174–180

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Review

Olfactory ensheathing cells from the nose: Clinical application in human spinal cord injuries

Alan Mackay-Sim *, James A. St John

National Centre for Adult Stem Cell Research, Eskitis Institute for Cell and Molecular Therapies, Griffith Unversity, Brisbane, QLD 4111, Australia

ARTICLE INFO

Article history:
Received 28 May 2010
Accepted 22 August 2010
Available online 9 September 2010

Keywords:
Olfactory epithelium
Olfactory ensheathing glia
Spinal cord repair
Human
Cell transplantation
Cell therapy

ABSTRACT

Olfactory mucosa, the sense organ of smell, is an adult tissue that is regenerated and repaired throughout life to maintain the integrity of the sense of smell. When the sensory neurons of the olfactory epithelium die they are replaced by proliferation of stem cells and their axons grow from the nose to brain assisted by olfactory ensheathing cells located in the lamina propria beneath the sensory epithelium. When transplanted into the site of traumatic spinal cord injury in rat, olfactory lamina propria or purified olfactory ensheathing cells promote behavioural recovery and assist regrowth of some nerves in the spinal cord. A Phase I clinical trial demonstrated that autologous olfactory ensheathing cell transplantation is safe, with no adverse outcomes recorded for three years following transplantation. Autologous olfactory mucosa transplantation is also being investigated in traumatic spinal cord injury although this whole tissue contains many cells in addition to olfactory ensheathing cells, including stem cells. If olfactory ensheathing cells are proven therapeutic for human spinal cord injury there are several important practical issues that will need to be solved before they reach general clinical application. This article is part of a Special Issue entitled: Understanding olfactory ensheathing glia and their prospect for nervous system repair.

Contents

Introduction

Olfactory ensheathing cells (OECs) surround the axons of the sensory neurons whose cell bodies lie in the olfactory epithelium in the nose and which synapse in the specialised glomeruli of the olfactory bulb in the brain (Fig. 1). As the axons grow out through the basal layer of the epithelium, they penetrate the basement membrane and enter the lamina propria where they coalesce to form axon bundles that are ensheathed by OECs (Fig. 1B) (Chuah and Au, 1991). These nerve bundles run through the lamina propria where they fasciculate into multiple larger nerve bundles to penetrate the skull at the cribriform plate (Fig. 1C). The nerve bundles then penetrate the coverings of the brain to run across the surface of the olfactory bulb forming its outer layer, the nerve fibre layer (Fig. 1C) (Au et al., 2002). Within this layer the olfactory axons defasciculate, turning deeper into the bulb to enter the glomeruli where they form synapses with mitral cells and interneurons (Shipley and Ennis, 1996) (Fig. 1C). OECs are in close association with the axons from their entry into the lamina propria in the peripheral nervous system until their entry into glomeruli within the olfactory bulb of the central nervous system.

In most mammals the olfactory epithelium is not the only chemosensory organ in the nose. The vomeronasal organ is a separate chemosensory organ whose axons project in distinct nerve bundles to the accessory olfactory bulb at the caudal end of the olfactory bulb (Halpern and Martinez-Marcos, 2003). There are two additional

* Corresponding author. Fax: +61 7 3735 4255.
E-mail address: a.mackay-sim@griffith.edu.au (A. Mackay-Sim).

0014-4886/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.expneurol.2010.08.025

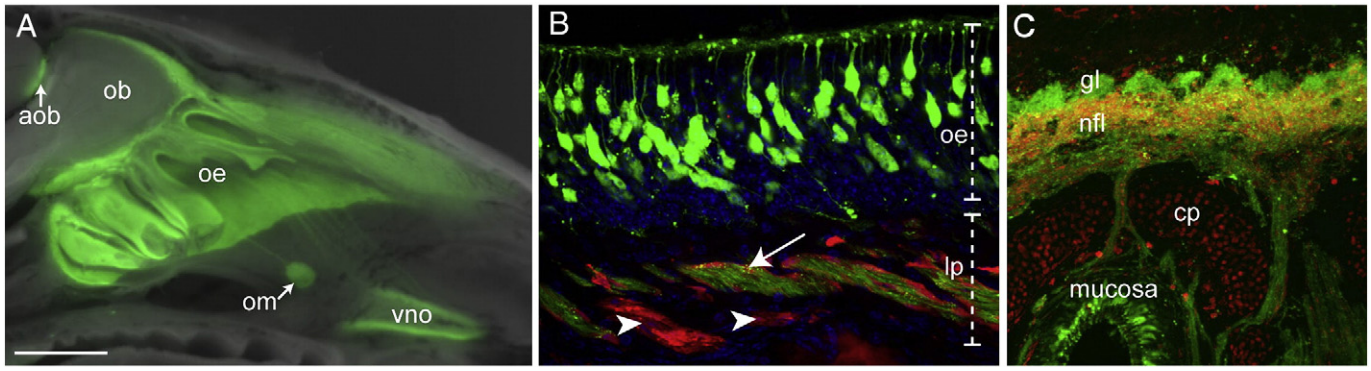

Fig. 1. Olfactory neurons and olfactory ensheathing cells in the nose and brain. A: A mouse head longitudinally dissected to expose the lateral nasal cavity and anterior forebrain. Anterior is to the right. Dorsal is up. The mouse is a transgenic strain (OMP-ZsGreen) which has a green fluorescent protein (ZsGreen) under the promoter for olfactory marker protein (OMP) that is expressed by all olfactory sensory neurons. Chemosensory epithelia are identified: olfactory epithelium (oe), vomeronasal organ (vno), and organ of Masera (om). The septal organ of Grüneberg is out of view to the right. The nerves emanating from the om and vno can be seen passing under the unlabelled respiratory mucosa and into the olfactory mucosa. The olfactory nerves form the outer layer of the olfactory bulb (ob) and the vomeronasal nerves form the outer layer of the accessory olfactory bulb (aob). B: Section through the olfactory mucosa showing the olfactory epithelium (oe) and the olfactory lamina propria beneath (lp). The mouse is a transgenic strain, a cross between the OMP-ZsGreen strain and the S100ß-DsRed strain (in which the red fluorescent protein, DsRed, is under the S100ß promoter). Olfactory sensory neurons and their axons are green while OECs and Schwann cells are red. Arrow points to a green olfactory nerve bundle surrounded by red OECs. Arrowheads point to nerve bundles, presumed trigeminal nerves with red Schwann cells. Such nerve bundles are uncommon. C: Section through the nasal cavity and skull showing the olfactory sensory neurons of the mucosa sending nerve bundles through the skull at the cribriform plate (cp) to synapse in the glomerular layer (gl) of the olfactory bulb. The olfactory nerves with associated OECs form the superficial nerve fibre layer (nfl) of the olfactory bulb. DsRed is expressed also by chondrocytes in the bone of the skull. Scale bar is 850 μm in A; 50 μm in B; and 120 μm in C.

specialised patches of olfactory sensory neurons on the nasal septum: the septal organ of Grüneberg, located close to the entrance of the nasal cavity and the septal organ of Masera, located ventrally (Fig. 1A). Axons from these sensory neurons terminate in specialised glomeruli located at the caudal end of the olfactory bulb (Pedersen and Benson, 1986; Storan and Key, 2006). It is possible that the OECs from these various chemosensory epithelia are different, providing a heterogeneity in preparations from the olfactory mucosa and bulb. The adult human nose only has an olfactory epithelium so the OEC population derived from the nasal epithelium is probably homogeneous.

Isolating OECs from the mucosa or bulb requires separation from the many potential "contaminating" cells. The olfactory epithelium contains sensory neurons, supporting cells, basal stem and progenitor cells, and Bowman's gland duct cells which are separated from the lamina propria beneath by a basement membrane (Moran et al., 1982; Morrison and Costanzo, 1990). Beneath the epithelium in the olfactory lamina propria are the olfactory nerve bundles, which are composed of axons and the surrounding OECs, but also Bowman's glands, blood vessels, trigeminal nerve fibres and the interstitial fibroblasts and tissue resident immune cells. The outer layer of the olfactory bulb comprises the olfactory nerve with attendant ensheathing cells, but cell preparations will also contain a small number of interfascicular astrocytes (Doucette, 1993), fibroblasts from the perineurium and meninges, as well as cells of the vasculature.

The olfactory system is perhaps the most plastic part of the adult nervous system. Throughout development and adult life, neurogenesis continues to occur in the olfactory epithelium in the nose where stem cells in the basal layer of the epithelium proliferate to generate new sensory neurons (Graziadei and Monti Graziadei, 1979; Leung et al., 2007; Mackay-Sim and Kittel, 1991). Within the central nervous system, proliferation of stem cells in the subventricular zone of the forebrain generates neural progenitors that migrate forward into the olfactory bulb to generate new interneurons throughout development and into adulthood (Curtis et al., 2007; Lledo et al., 2008). Severing the olfactory nerve leads to apoptosis of the sensory neurons in the epithelium which are then replaced quickly by new neurons arising from enhanced neurogenesis as demonstrated in many studies, including in primate (Graziadei and Graziadei, 1979; Graziadei et al., 1980). The remarkable ability for continual axon growth and successful targeting is attributed to the presence of the glia associated with the olfactory nerve since the OECs accompany the axons from the epithelium through the olfactory bulb in the brain (Chuah and Zheng, 1992). The combination of assisting in axon growth and associating with the central nervous system has made OECs candidates for assisting in repair of injury to the brain and spinal cord (Li et al., 1998; Ramon-Cueto et al., 2000; Smale et al., 1996).

The adult human olfactory mucosa, unlike rat and other mammals, is not contiguous but patchily distributed and interspersed with respiratory mucosa in the upper nasal cavity (Moran et al., 1982; Morrison and Costanzo, 1990; Nakashima et al., 1984; Paik et al., 1992). Olfactory mucosa can be detected more anteriorly and inferiorly in the adult human nose than in the fetus (Feron et al., 1998; Leopold et al., 2000). Consequently, "olfactory mucosa biopsies" from adult humans will virtually always contain respiratory mucosa. Biopsies will therefore contain many cell types in addition to OECs: cells of the olfactory epithelium (horizontal basal cells, globose basal cells; olfactory sensory neurons, supporting cells, and Bowman's glands duct cells), cells of the respiratory epithelium (ciliated cells, and goblet cells), and cells of the lamina propriae (Bowman's gland cells, fibroblasts, tissue macrophages, vascular cells, and Schwann cells of the trigeminal nerve). There will be stem cells of the olfactory epithelium (i.e the horizontal basal cells) (Carter et al., 2004; Leung et al., 2007; Mackay-Sim and Kittel, 1991; Roisen et al., 2001) and a multipotent stem cell in the lamina propria capable of differentiating into numerous non-olfactory cell types (Feron et al., 2009; Murrell et al., 2005; Tome et al., 2009) and with some characteristics of mesenchymal stem cells (Feron et al., 2009).

Olfactory ensheathing cells from mucosa and bulb are different

OECs from the olfactory nerve and olfactory bulb are not identical. Mucosal OECs express several antigens not expressed by bulb OECs (Au and Roskams, 2003); they differ in their ability to grow in vitro (Jani and Raisman, 2004), and they differ in their reparative effects when transplanted into the injured spinal cord (Richter et al., 2005). There is also heterogeneity among OECs of the olfactory bulb. The olfactory nerve layer in the bulb is subdivided into two layers within which the OECs express S100 but only the outer layer OECs express p75 (Au et al., 2002). We have also observed functional heterogeneity in OECs from different regions of the bulb and the olfactory nerve in the epithelium (Windus et al., 2010). Those in the mucosa mainly interact with each other by adhesion and they cause olfactory sensory

axons to fasciculate. OECs from the bulb show heterogeneous interactions with each other — either adhesion or repulsion or neither; and axons growing on the bulb-derived OECs do not form fascicles (Windus et al., 2010). The proportion of cells demonstrating these behaviours varied depending on the region of the olfactory bulb (dorsal/ventral, and rostral/caudal) from which they were derived (Windus et al., 2010). We also noted functional differences in the OECs from the olfactory bulbs of embryonic, postnatal and adult olfactory bulbs (Windus et al., 2010).

OECs also show heterogeneity when growing in vitro (Pixley, 1992). It is now established that this heterogeneity is reflective of plasticity in morphology and antigen expression that can be manipulated by growth conditions (Huang et al., 2008; Vincent et al., 2003). When monitored with time-lapse imaging, cells of each morphology are seen to change into the other during the course of an hour in a different growth medium (Huang et al., 2008). The most spindle-shaped OECs migrated three times faster than the most flattened OECs (Huang et al., 2008) so morphology may reflect a different functional state.

In summary, these studies demonstrate heterogeneity in morphology, antigen expression and function in OECs from olfactory mucosa and olfactory bulb, at developmental stages and under different culture conditions. There may be species differences as well (Smithson and Kawaja, 2009). These are all variables that will confound any comparison of outcomes of transplanting "olfactory ensheathing cells" into the injured spinal cord. It would be wise not to assume all authors are using the same cells.

The trigeminal nerve innervates the olfactory mucosa and olfactory bulb

The trigeminal nerve innervates the olfactory and respiratory mucosae, providing touch and pain sensations within the nasal cavity, as well as chemosensitivity. This nerve also innervates the olfactory bulb with some neurons branching to innervate mucosa and bulb (Finger and Bottger, 1993; Schaefer et al., 2002; Silver and Finger, 2009). Non-myelinated trigeminal motor nerves innervate blood vessels to regulate blood flow within the mucosa and myelinated sensory nerves are also present (Herrera et al., 2005; Kawaja et al., 2009). Hence it is possible that preparations of "olfactory ensheathing cells" from the olfactory mucosa and olfactory bulb could also contain Schwann cells from the trigeminal nerve (Rizek and Kawaja, 2006). Compared to the number and size of olfactory nerve bundles in the lamina propria and olfactory bulb, trigeminal innervation of the olfactory mucosa and bulb is sparse (Fig. 1B). There has not been a systematic comparison to quantify this but the proportion of OECs in any cell culture from the olfactory mucosa is expected to be far larger than the proportion of trigeminal Schwann cells. The difficulty for researchers is in distinguishing these two cell types in vitro because they both express p75, GFAP and S100. Proteomics analysis identified two potential antigens expressed by OECs and not Schwann cells, namely calponin and smooth muscle actin (Boyd et al., 2006; Jahed et al., 2007). However, those findings were dependent on the purity of the cultures and calponin has subsequently been localised to olfactory mucosa fibroblasts and olfactory bulb meningeal cells, not OECs (Ibanez et al., 2007). Fibroblasts also express smooth muscle actin (Jahed et al., 2007) so this antigen is also shared with potential contaminants of OEC cultures.

In order to assess Schwann cell contribution to OEC cultures we have previously looked for an antigen expressed by Schwann cells but not OECs. HNK-1 is an antigen expressed by myelinating Schwann cells in human and mouse sciatic nerve (Levi et al., 1994; Martini et al., 1988) and by myelinating and non-myelinating Schwann cells in canine trigeminal nerve (Bock et al., 2007). Since rat olfactory bulb OECs do not express HNK-1 in vitro (Barnett et al., 1993), we used HNK-1 immunogenicity to distinguish OECs from Schwann cells in rat and human cultures from olfactory mucosa to demonstrate that trigeminal Schwann cells but not mucosal OECs expressed this antigen in vitro (Bianco et al., 2004; Feron et al., 2005). Contrary to these studies it is argued that while HNK-1 is expressed in vivo by canine Schwann cells, it is not expressed in vitro (Bock et al., 2007) so the general utility of HNK-1 antigenicity remains unresolved because of species differences and culture methods.

It is well understood that defined media can be very selective for the cell types whose growth they support. We identified that a minimal serum-free medium containing NT3 was capable of expanding and purifying OECs from rat and human olfactory mucosa (Bianco et al., 2004; Feron et al., 2005). This medium is selective for OECs because it does not support long-term culture of Schwann cells beyond 24 h (Cheng et al., 1998; Levi et al., 1994; Lobsiger et al., 2000). In many laboratories the culture medium contains either fetal calf serum or one of the neuregulins which will support both OECs and Schwann cells (Bock et al., 2007; Rizek and Kawaja, 2006).

In summary, it is probably wise to assume that all OEC cultures may contain a small proportion of trigeminal Schwann cells unless proven otherwise. As discussed it is very difficult to distinguish the two cell types in vitro but certain culture conditions may be selective for OECs. This potential contamination with Schwann cells applies equally to OECs from mucosa and bulb but in both cases the proportion of Schwann cells is probably low. This is supported indirectly by reports of differences in gene and protein expression between OEC cultures and sciatic Schwann cell cultures (Boyd et al., 2006; Vincent et al., 2005). Differences would not be observed if there was significant Schwann cell contamination of OEC cultures.

From a pragmatic perspective for clinical application, cells will need to be grown according to Good Manufacturing Practice (GMP) protocols. They must be produced under very strict standards of quality control and comply with defined specifications, such as percentages of cells with particular immunophenotypes. Ultimately, the "purity" of the transplanted OECs is less important than a defined method of cell culture and high level of quality control to produce a predictable cell population with therapeutic efficacy. If well defined cells are generated from olfactory mucosa biopsies and they are effective clinically it may not matter from a regulatory perspective if they are "pure" OECs or are contaminated with a small percentage of trigeminal Schwann cells.

Clinical sources of olfactory ensheathing cells for human trials

There are almost as many descriptions of OEC preparations as there are laboratories exploring their biology (Kawaja et al., 2009). These differences should be considered when comparing the results of different laboratories describing OEC biology and function, including their therapeutic efficacy when transplanted into the injured spinal cord. They should also be considered when choosing sources for human therapies.

Without going into details that are covered elsewhere it can be said that the differences in OEC preparations differ along many dimensions. Sources vary — most studies use olfactory bulb preparations but an increasing number use olfactory mucosa. Of those that use olfactory bulb preparations, some use the nerve fibre layer from the entire olfactory bulb (Li et al., 1997; Lopez-Vales et al., 2006; Ramon-Cueto et al., 2000; Ramon-Cueto et al., 1998) whereas others use only the nerve fibre layer from the rostral olfactory bulb (Lankford et al., 2008), or ventral olfactory bulb (Guest et al., 2008), or others do not specify the exact region (Teng et al., 2008). Developmental stage varies — embryonic, postnatal and adult tissues have been used. Species vary — most research is on rat but mouse, canine, cat, guinea pig, and human OECs have been investigated. Selection methods vary — some use differential adhesion, some use immunopanning/FACS/MACS for p75NTR/O4; many do not select. Dissociation methods vary — different enzymes, different mechanical dissociation. Culture methods vary — presence of serum, composition of defined media, different growth

factors. Verification methods vary — 69 antigens have been used to identify OECs (Kawaja et al., 2009). The most commonly accepted markers are p75NTR, S100 and GFAP which are used to verify the relative purity of the resultant OEC cultures. A combination of these markers can eliminate all the potential contaminating cells from olfactory mucosa and bulb cell cultures except trigeminal Schwann cells, as discussed earlier.

For clinical application, OECs could be obtained from either the olfactory mucosa or the olfactory bulb. They could be obtained from the same patient, i.e. autologously, or from autopsy tissue for heterologous transplantation. Autologous transplantation is perhaps the most clinically acceptable method for transplantation because it avoids immune rejection. OECs from the peripheral olfactory nerve can be easily obtained from biopsies of human olfactory mucosa (Bianco et al., 2004; Feron et al., 1998) and enough cells for autologous transplantation can be obtained by culturing for 4–6 weeks (Feron et al., 2005). Another approach is to remove the olfactory bulb unilaterally through the nasal bone and orbit as demonstrated in primate (Rubio et al., 2008). This procedure is riskier than a nasal biopsy, with higher morbidity including the risk of CSF leak through the surgical entry into the skull, and will have a negative impact on the patient since it would eliminate olfaction unilaterally. Nasal biopsy from the medial and lateral walls of the nasal cavity does not pose a risk of CSF leaks and does not affect the sense of smell (Feron et al., 1998; Lanza et al., 1994). Despite these comparative risks, if bulbar OECs prove clinically superior to nasal cells, then the risk of bulb biopsy may be warranted.

In the acute stages of spinal cord injury, clinical use of autologous cells is not possible because of the time required to generate and culture the cells (Feron et al., 2005; Rubio et al., 2008). If acute application is required then heterologous transplantation would be necessary. One approach would be to immortalize cells from a single person that could serve as a ready source for many spinal injured patients (Lim et al., 2010), although this would require immune suppression. Another approach would be to develop a bank of OECs with appropriate HLA subtypes for cell transplantation.

Whatever the source of OECs, the regulations in most countries require that cells used for transplantation must be produced under GMP protocols. These regulations would differentially affect the relative costs of autologous or heterologous cell transplantation. In both cases the batch of cells to be transplanted requires defined properties of identification and microbiological non-contamination. The per-patient costs of GMP production and certification could be reduced for banked cells by using them for multiple patients but the costs to establish such a bank would be very high. For banked cells the treatment costs per-patient would be increased if immuno-suppressive drugs were required.

Mucosal olfactory ensheathing cells and spinal cord repair

The early evidence for the therapeutic efficacy of OEC transplantation used cells obtained from the olfactory bulb of embryonic or very young rats (Li et al., 1998; Ramon-Cueto et al., 1998). For clinical use, autologous transplantation of nasal OECs is most desirable so it was necessary to discover whether OECs from the olfactory mucosa could also promote recovery when transplanted into the injured spinal cord and also to discover whether the use of OECs from adult animals was effective. In our initial experiments in adult rats, we transplanted pieces of olfactory lamina propria into the transected spinal cord and we demonstrated that improved locomotion was achieved with evidence that descending axons from brainstem motor nuclei had crossed the injury and transplant site and entered the spinal cord cuadal to the injury site (Lu et al., 2001). Because the olfactory lamina propria contains many cells other than OECs, as a control we transplanted pieces of respiratory lamina propria and found that this failed to promote locomotor recovery and there was no evidence for axon growth in the spinal cord (Lu et al., 2001). Semi-purified OEC cultures were similarly effective as pieces of olfactory lamina propria (Lu et al., 2001). Transplantation of cells into an acute spinal cord injury places them into the early phases of the inflammed tissue and delayed transplantation may be more beneficial. As an initial test of delayed transplantation we grafted pieces of olfactory lamina propria into the injured spinal cord 3 weeks after complete transection (Lu et al., 2002). This also promoted functional recovery, with descending brainstem motor axons growing through the injury site and into the caudal spinal cord (Lu et al., 2002). Such recovery was not evident with delayed transplantation of respiratory lamina propria (Lu et al., 2002).

Transplantation of whole olfactory mucosa, which includes olfactory epithelium as well as lamina propria, also improved functional recovery after spinal cord injury (Iwatsuki et al., 2008). Transplantation of human neural progenitors from olfactory epithelium was also effective in a rat model of spinal cord injury (Xiao et al., 2005), so there may be more than one source of cells with therapeutic potential in the olfactory mucosa. For example we demonstrated that a multipotent stem cell in rodent and human olfactory mucosa was capable of differentiating into neurons and glia as well as many other non-neural cell types in vitro and after transplantation into embryos (Murrell et al., 2005).

Olfactory mucosa OECs transplanted into dorsal column lesions in rodent promoted migration of host Schwann cells into the lesion to reduce the scar and lesion cavity and promoted regeneration of sensory and motor axons into and through the lesion (Ramer et al., 2004). Interestingly, the olfactory mucosa OECs were superior in these respects compared to olfactory bulb OECs (Richter et al., 2005). When human olfactory mucosa OECs were transplanted into the contused spinal cord in rat they reduced lesion size and cavity volume and they improved locomotor function (Gorrie et al., accepted for publication). This study indicates that human olfactory mucosa OECs, like cells from other species, have the potential for spinal cord repair. They also show that olfactory mucosa OECs are effective in the contused spinal cord, a "more realistic" model for traumatic human spinal cord injury than transection injuries. Olfactory bulb OECs are also effective in the contused model (Plant et al., 2003).

After high level, complete, spinal cord injury, the patient is at risk of loss of control of blood pressure such that a strong sensory stimulus below the level of injury, including a full bowel, can cause a rapid and life threatening rise in blood pressure, known as autonomic dysreflexia. In a rat model of autonomic dysreflexia, transplantation of olfactory mucosa OECs reduced the increase in blood pressure in response to strong bowel stimulation (Kalincik et al., 2009). This was accompanied by changes in the morphology of the sympathetic motor neurons above and below the injury (Kalincik et al., 2009).

Autologous olfactory ensheathing cell transplantation in human spinal cord injury

Clinical trials are essentially well defined experiments on humans. Phase I trials are generally small studies to ensure that the new drug or procedure is safe. Phase II trials are to test efficacy in a small group of well defined, treated and control patients. Phase III trials are large multi-centre studies to test the efficacy and safety of the treatment in a large number of patients as a prelude to introducing the drug or procedure to clinical practice. Phase II and Phase III trials are always controlled and usually double-blinded to reduce biases introduced by knowledge of the treatment by patients or their clinicians. There are now published guides for clinical trials in spinal cord injury published by an international panel after an international workshop (Fawcett et al., 2007; Lammertse et al., 2007; Steeves et al., 2007; Tuszynski et al., 2007).

We undertook a Phase I/IIa clinical trial to test the safety of autologous transplantation of OECs into the injured spinal cord (Feron

et al., 2005). OECs were grown from nasal biopsies from patients with complete thoracic paraplegia according to published protocols (Bianco et al., 2004). Patients were males aged 18–55 years with injuries sustained 18–32 months prior to enrolling in the trial. Olfactory mucosa OECs derived from biopsies of the patient's nasal mucosa were grown in vitro for 4–6 weeks according to published protocols (Bianco et al., 2004) and tested to confirm there was no bacterial and yeast contamination prior to transplantation. Twelve to 20 million cells were injected into the injured spinal cord after a laminectomy to expose the extent of the injury (Feron et al., 2005). This procedure was safe with no adverse outcomes and no changes on MRI at 1 year (Feron et al., 2005). Patients were assessed for 3 years with a variety of outcome measures including MRI, AIS assessments, FIM, SCIM, neurological assessment, SSEP, MEP and psychosocial assessments (Mackay-Sim et al., 2008), with the aim of identifying any adverse outcomes.

One of the problems with longitudinal trials and clinical outcome measures is the risk of bias of the assessors. For this reason, we designed the trial using a matched group of (non-operated) controls with the same assessors throughout who were blinded to the patient/control status. The patients were examined every 3 months with treated and control groups bandaged on the back over the injury site to maintain blinded assessments. This design thus allowed some estimate of efficacy by comparison of treated and control cases, although the numbers were too few (three per group) for meaningful statistical analysis. At 3 years there were no alterations in MRI in the treated group and there were no adverse outcomes such as neuropathic pain or loss of functions above the level of injury (Mackay-Sim et al., 2008). In one of the treated patients there was increased light touch and pin prick sensation below the pre-operative level which suggests a change of sensation in the zone of partial preservation (Mackay-Sim et al., 2008).

Autologous transplantation of olfactory mucosa in human spinal cord injury

As noted earlier, the olfactory mucosa contains other cell types which have the potential to assist spinal cord injury repair, in addition to OECs. Autologous transplantation of olfactory mucosa is being undertaken in several clinics, although the procedure is not yet administered according to formal clinical trial protocols.

A pilot study described the outcomes 18 months after autologous transplantation of olfactory mucosa in 7 patients with complete, ASIA A, paraplegia from injuries at C4 to T10 spinal levels experienced 6 to 78 months prior to transplantation surgery (Lima et al., 2006). Some encouraging positive outcomes were reported with increases in ASIA scores from A to C in two patients. All patients, except one, improved in sensory and motor component scores of the ASIA score. In three patients there were “indications of voluntary control of new muscles” and two patients reported changes in bowel and bladder function or sensation. Olfaction was temporarily lost but then regained after the nasal biopsy procedure. MRI showed a reduction of the lesion cavity in all patients. There were few adverse events with one patient having a decrease in sensory component of the ASIA score. Two patients had increased trunk pain for 2–3 months (Lima et al., 2006).

A second study described the outcomes 18 months after the procedure in four patients with complete, ASIA A paraplegia, and one patient with incomplete, ASIA B paraplegia from injuries at C6 to T10 spinal levels experienced 29 to 99 months prior to transplantation surgery (Chhabra et al., 2009). There were no positive outcomes in any of the assessment criteria and the ASIA B patient had a decrease in the sensory component of the ASIA score which was not entirely regained during the study (Chhabra et al., 2009). MRI at 18 months showed increases in myelomalacia in 4 patients and formation of a syrinx in one patient. One patient experienced discharge from the surgical site that required a second operation to close the wound (Chhabra et al., 2009).

Recently a third report of autologous transplantation of olfactory mucosa described the outcomes 12–45 months after the procedure on 20 patients with ASIA A or B paraplegia or tetraplegia from injuries at C4 to T12 experienced 18–189 months previously (Lima et al., 2010). This study differed in protocol from the others with all patients undergoing rigorous pre-operative rehabilitation exercise programs for 4 months prior to surgery. In this pre-operative phase there was no improvement in outcome measures. After surgery the exercise program was maintained. In 11/20 patients there was an improvement in ASIA scores: six patients improved from ASIA A to C, two patients improved from ASIA A to B, and three patients from ASIA B to C. One patient declined from an ASIA score of B to A. Scores improved on the FIM and WISCI scales in all patients tested (13) and urodynamic responses improved in five patients. New voluntary EMG responses were recorded in 15 patients and new SSEPs were recorded in 4 patients. MRI indicated that the lesion site in all patients was filled by the transplants with no evidence of overgrowth and no syringonyelia. All patients recovered olfactory function. One patient was hospitalized with aseptic meningitis (Lima et al., 2010).

Collectively these reports represent 32 cases of autologous olfactory mucosal transplantation in C4-T12 paraplegics and tetraplegics. All were followed for 18 months. Collectively they indicate that the surgical procedure was well tolerated, although 5/32 (15%) showed evidence of syringomyelia or myelomalacia that could be attributed to the procedure. ASIA scores improved in 13 patients (40%) and declined in one patient. Two patients required a second hospitalization after the initial surgery. Taken together these reports indicate that the surgical procedure is “relatively safe” (Lima et al., 2010) with indications that it may improve outcomes, although the design of the studies is not as a Phase II clinical trial, so firm conclusions of the efficacy of the procedure are not warranted at this stage. A blinded and controlled trial would confirm the encouraging outcomes described.

There are questions raised by the reports of all three studies related to the methodology. First, the removal of the scar tissue at the injury site is contentious because of the unknown implications for any potentially surviving axon tracts. The removed tissue was examined in two patients and reported to contain a few “peripheral axons” without stating any criteria for classifying them as “peripheral” and not “central”. Second, the cellular composition of the grafts was not defined (see later). Third, the outcome measures were mostly clinical assessments and subject to assessor variability and bias and there is no description of methods to reduce this, such as using the same assessors throughout or standardizing and monitoring different assessors for consistency. Fourth, the method of olfactory function testing is not stated despite there being quantitative tests available (Hummel et al., 2007).

Olfactory ensheathing cell transplantation: what is the future for clinical trials?

Modern evidence-based medicine demands that the introduction of new procedures and drugs undergo formal clinical trials under similar principles accepted in many countries. Without this formal approach it is unlikely that OEC transplantation will receive regulatory approval in most jurisdictions and would therefore not be delivered widely to patients in need. A major barrier to this route to regulatory approval is the large cost required, especially in Phase III trials. In the development of new drugs, these costs are borne by large corporations, based on exclusive intellectual property rights and Good Manufacturing Practice batch manufacture of defined products that can be administered to many patients. These are unlikely to apply to OEC transplantation therapies because there is limited intellectual property that is patentable and attractive to commercial investors.

Furthermore, with autologous cell transplantation there is no opportunity to reduce costs through batch processing and wide distribution of the cellular product. These realities suggest that commercial backing for clinical trials is unlikely. The costs will have to be borne by governments and medical foundations. This may be appropriate since governments and the public currently bear the costs of spinal cord injury and will have the most to gain from new therapies, but can they afford it, and will they see this as a priority?

An unexpected barrier to clinical trials may be finding enough patients to participate in all phases of clinical trials. This may seem surprising given the large cumulative numbers of individuals with spinal cord injuries but Phase II trials will require matched groups of patients and controls large enough for statistical analysis (Fawcett et al., 2007) and the matching might be very selective further reducing the pool of patients from which to choose. In early trials the major focus is on safety and so conservative choices must be made in selecting participants. For this reason, for example, thoracic injuries are favoured over cervical, and complete injuries over incomplete (Fawcett et al., 2007). Complete, thoracic injuries are among the least common class of spinal cord injuries so greatly reducing the proportion of participants from which to choose. Complete, thoracic, injuries are preferred in early safety (Phase I) trials if there is a risk of damage to functional tracts and induction of syringomyelia which may affect functions above the level of lesion (and below in the case of incomplete injuries). Where cells or tissues are transplanted into the spinal cord injury one risk of the intervention is to interfere with normal recovery, so early clinical trials will be limited to chronic injuries. Our trial design used these inclusion criteria and was also highly selective for psychosocial factors which further reduced the candidate cohort (Fronek, 2004; Mackay-Sim et al., 2008). With all these considerations our selection criteria reduced the participants to six from an original pool of 600 interviewees (Mackay-Sim et al., 2008). These considerations suggest that future clinical trials of OEC transplantation will require cooperation between several centres to be able to find enough patients to fulfil the requirements for formal clinical trials, with more centres required as the trials move from Phase I to III. This requirement increases the complexity and cost of the logistics of such trials imposed by the need for training, data management and reporting, and quality control across the participating centres.

There are some technical issues that the field needs to address before the efficacy of OEC transplantation can be adequately assessed. One of these is the adequacy of the outcome measures (Steeves et al., 2007). For the clinician and patient, only clinical and everyday functional outcomes may be relevant but these are usually qualitative, not quantitative, and may not be validated for clinical trials. They may also lack sensitivity to distinguish incremental improvements that can direct the design of future trials. These issues are not confined to cell transplantation trials. The question of cell survival is crucial but tracking cells after transplantation in living beings requires new methods of cell labelling. A potential candidate is to label the cells with paramagnetic nanoparticles (Lee et al., 2004). Currently this method is not approved for labelling cells transplanted into the human spinal cord, although this would provide very useful information in determining the mechanism of any change in outcome and the survival of cells could be an outcome measure in itself. Finally, improved and validated methods of MR imaging would be beneficial for future clinical trials. In our trial we used the clinical standard of time, 1.5 Tesla MRI (Feron et al., 2005). With 3 Tesla MRI now available it should be possible to get higher resolution images, although imaging the spinal cord is problematic because of the high contrast of the bone nearby and the common use of metal stabilisation protheses. For scientific outcomes (as opposed to clinical outomes) new imaging methods to quantify aspects of the injury before and after transplantation would be a major advance. New developments in diffusion tensor MRI indicate its utility for following intraspinal tracts in living animals with spinal cord injury (Kim et al., 2010) and protocols are being developed for imaging human spinal cord (Filippi et al., 2010).

There are different ethics, regulations and allowable practices that govern medical treatment and clinical trials throughout the world. This diversity may allow practices to occur in one jurisdiction that would not be legal in another. This may help advance cell transplantation trials into clinical practice because different regulations may allow clinical trials to proceed in one country until there is enough evidence to satisfy the requirements of another. The essential point is that such clinical trials must be designed to provide reliable data and allow reliable conclusions. The main issue here is not the differences in national regulation of cell therapy, but whether the designs of the treatment and assessment regimes are capable of finding any treatment effects if they are present. This is the strength of formalised clinical trials compared to clinician-initiated individual treatments, even if those treatments are repeated in many individuals.

References

Au, E., Roskams, A.J., 2003. Olfactory ensheathing cells of the lamina propria in vivo and in vitro. Glia 41, 224–236.

Au, W.W., Treloar, H.B., Greer, C.A., 2002. Sublaminar organization of the mouse olfactory bulb nerve layer. J. Comp. Neurol. 446, 68–80.

Barnett, S.C., Hutchins, A.M., Noble, M., 1993. Purification of olfactory nerve ensheathing cells from the olfactory bulb. Dev. Biol. 155, 337–350.

Bianco, J.I., Perry, C., Harkin, D.G., Mackay-Sim, A., Feron, F., 2004. Neurotrophin 3 promotes purification and proliferation of olfactory ensheathing cells from human nose. Glia 45, 111–123.

Bock, P., Beineke, A., Techangamsuwan, S., Baumgartner, W., Wewetzer, K., 2007. Differential expression of HNK-1 and p75(NTR) in adult canine Schwann cells and olfactory ensheathing cells in situ but not in vitro. J. Comp. Neurol. 505, 572–585.

Boyd, J.G., Jahed, A., McDonald, T.G., Krol, K.M., Van Eyk, J.E., Doucette, R., et al., 2006. Proteomic evaluation reveals that olfactory ensheathing cells but not Schwann cells express calponin. Glia 53, 434–440.

Carter, L.A., MacDonald, J.L., Roskams, A.J., 2004. Olfactory horizontal basal cells demonstrate a conserved multipotent progenitor phenotype. J. Neurosci. 24, 5670–5683.

Cheng, L., Esch, F.S., Marchionni, M.A., Mudge, A.W., 1998. Control of Schwann cell survival and proliferation: autocrine factors and neuregulins. Mol. Cell. Neurosci. 12, 141–156.

Chhabra, H.S., Lima, C., Sachdeva, S., Mittal, A., Nigam, V., Chaturvedi, D., et al., 2009. Autologous olfactory [corrected] mucosal transplant in chronic spinal cord injury: an Indian Pilot Study. Spinal Cord 47, 887–895.

Chuah, M.I., Au, C., 1991. Olfactory Schwann cells are derived from precursor cells in the olfactory epithelium. J. Neurosci. Res. 29, 172–180.

Chuah, M.I., Zheng, D.R., 1992. The human primary olfactory pathway: fine structural and cytochemical aspects during development and in adults. Microsc. Res. Tech. 23, 76–85.

Curtis, M.A., Kam, M., Nannmark, U., Anderson, M.F., Axell, M.Z., Wikkelso, C., et al., 2007. Human neuroblasts migrate to the olfactory bulb via a lateral ventricular extension. Science 315, 1243–1249.

Delorme, B., Nivet, E., Gaillard, J., Haupl, T., Ringe, J., Deveze, A., et al., 2009. The human nose harbours a niche of olfactory ecto-mesenchymal stem cells displaying neurogenic and osteogenic properties. Stem Cells Dev. 19, 853–866.

Doucette, R., 1993. Glial progenitor cells of the nerve fiber layer of the olfactory bulb: effect of astrocyte growth media. J. Neurosci. Res. 35, 274–287.

Fawcett, J.W., Curt, A., Steeves, J.D., Coleman, W.P., Tuszynski, M.H., Lammertse, D., et al., 2007. Guidelines for the conduct of clinical trials for spinal cord injury as developed by the ICCP panel: spontaneous recovery after spinal cord injury and statistical power needed for therapeutic clinical trials. Spinal Cord 45, 190–205.

Feron, F., Perry, C., Cochrane, J., Licina, P., Nowitzke, A., Urquhart, S., et al., 2005. Autologous olfactory ensheathing cell transplantation in human spinal cord injury. Brain 128, 2951–2960.

Feron, F., Perry, C., McGrath, J.J., Mackay-Sim, A., 1998. New techniques for biopsy and culture of human olfactory epithelial neurons. Arch. Otolaryngol. Head Neck Surg. 124, 861–866.

Filippi, C.G., Andrews, T., Gonyea, J.V., Linnell, G., Cauley, K.A., 2010. Magnetic resonance diffusion tensor imaging and tractography of the lower spinal cord: application to diastematomyelia and tethered cord. Eur. Radiol. 20, 2194–2199.

Finger, T.E., Bottger, B., 1993. Peripheral peptidergic fibers of the trigeminal nerve in the olfactory bulb of the rat. J. Comp. Neurol. 334, 117–124.

Fronek, P., 2004. The development of a psychosocial screening model for human participants with spinal cord injury in experimental research. SCI Psychosocial Process 17, 6–11.

Gorrie C., Hayward I., Cameron N., Kailainathan G., Nandapalan N., Sutharsan R. et al., Effects of human OEC-derived cell transplants in rodent spinal contusion injury. *Brain Research* accepted for publication.

Graziadei, G.A., Graziadei, P.P., 1979. Neurogenesis and neuron regeneration in the olfactory system of mammals. II. Degeneration and reconstitution of the olfactory sensory neurons after axotomy. J. Neurocytol. 8, 197–213.

Graziadei, P.P., Karlan, M.S., Graziadei, G.A., Bernstein, J.J., 1980. Neurogenesis of sensory neurons in the primate olfactory system after section of the fila olfactoria. Brain Res. 186, 289–300.
Graziadei, P.P., Monti Graziadei, G.A., 1979. Neurogenesis and neuron regeneration in the olfactory system of mammals. I. Morphological aspects of differentiation and structural organization of the olfactory sensory neurons. J. Neurocytol. 8, 1–18.
Guest, J.D., Herrera, L., Margitich, I., Oliveria, M., Marcillo, A., Casas, C.E., 2008. Xenografts of expanded primate olfactory ensheathing glia support transient behavioral recovery that is independent of serotonergic or corticospinal axonal regeneration in nude rats following spinal cord transection. Exp. Neurol. 212, 261–274.
Halpern, M., Martinez-Marcos, A., 2003. Structure and function of the vomeronasal system: an update. Prog. Neurobiol. 70, 245–318.
Herrera, L.P., Casas, C.E., Bates, M.L., Guest, J.D., 2005. Ultrastructural study of the primary olfactory pathway in *Macaca fascicularis*. J. Comp. Neurol. 488, 427–441.
Huang, Z.H., Wang, Y., Cao, L., Su, Z.D., Zhu, Y.L., Chen, Y.Z., et al., 2008. Migratory properties of cultured olfactory ensheathing cells by single-cell migration assay. Cell Res. 18, 479–490.
Hummel, T., Kobal, G., Gudziol, H., Mackay-Sim, A., 2007. Normative data for the "Sniffin' Sticks" including tests of odor identification, odor discrimination, and olfactory thresholds: an upgrade based on a group of more than 3,000 subjects. Eur. Arch. Otorhinolaryngol. 264, 237–243.
Ibanez, C., Ito, D., Zawadzka, M., Jeffery, N.D., Franklin, R.J., 2007. Calponin is expressed by fibroblasts and meningeal cells but not olfactory ensheathing cells in the adult peripheral olfactory system. Glia 55, 144–151.
Iwatsuki, K., Yoshimine, T., Kishima, H., Aoki, M., Yoshimura, K., Ishihara, M., et al., 2008. Transplantation of olfactory mucosa following spinal cord injury promotes recovery in rats. NeuroReport 19, 1249–1252.
Jahed, A., Rowland, J.W., McDonald, T., Boyd, J.G., Doucette, R., Kawaja, M.D., 2007. Olfactory ensheathing cells express smooth muscle alpha-actin in vitro and in vivo. J. Comp. Neurol. 503, 209–223.
Jani, H.R., Raisman, G., 2004. Ensheathing cell cultures from the olfactory bulb and mucosa. Glia 47, 130–137.
Kalincik, T., Choi, E.A., Feron, F., Bianco, J., Sutharsan, R., Hayward, I., et al., 2009. Olfactory ensheathing cells reduce duration of autonomic dysreflexia in rats with high spinal cord injury. Auton. Neurosci. 154, 20–29.
Kawaja, M.D., Boyd, J.G., Smithson, L.J., Jahed, A., Doucette, R., 2009. Technical strategies to isolate olfactory ensheathing cells for intraspinal implantation. J. Neurotrauma 26, 155–177.
Kim, J.H., Loy, D.N., Wang, Q., Budde, M.D., Schmidt, R.E., Trinkaus, K., et al., 2010. Diffusion tensor imaging at 3 hours after traumatic spinal cord injury predicts long-term locomotor recovery. J. Neurotrauma 27, 587–598.
Lammertse, D., Tuszynski, M.H., Steeves, J.D., Curt, A., Fawcett, J.W., Rask, C., et al., 2007. Guidelines for the conduct of clinical trials for spinal cord injury as developed by the ICCP panel: clinical trial design. Spinal Cord 45, 232–242.
Lankford, K.L., Sasaki, M., Radtke, C., Kocsis, J.D., 2008. Olfactory ensheathing cells exhibit unique migratory, phagocytic, and myelinating properties in the X-irradiated spinal cord not shared by Schwann cells. Glia 56, 1664–1678.
Lanza, D.C., Deems, D.A., Doty, R.L., Moran, D., Crawford, D., Rowley 3rd, J.C., et al., 1994. The effect of human olfactory biopsy on olfaction: a preliminary report. Laryngoscope 104, 837–840.
Lee, I.H., Bulte, J.W., Schweinhardt, P., Douglas, T., Trifunovski, A., Hofstetter, C., et al., 2004. In vivo magnetic resonance tracking of olfactory ensheathing glia grafted into the rat spinal cord. Exp. Neurol. 187, 509–516.
Leopold, D.A., Hummel, T., Schwob, J.E., Hong, S.C., Knecht, M., Kobal, G., 2000. Anterior distribution of human olfactory epithelium. Laryngoscope 110, 417–421.
Leung, C.T., Coulombe, P.A., Reed, R.R., 2007. Contribution of olfactory neural stem cells to tissue maintenance and regeneration. Nat. Neurosci. 10, 720–726.
Levi, A.D., Guenard, V., Aebischer, P., Bunge, R.P., 1994. The functional characteristics of Schwann cells cultured from human peripheral nerve after transplantation into a gap within the rat sciatic nerve. J. Neurosci. 14, 1309–1319.
Li, Y., Field, P., Raisman, G., 1998. Regeneration of adult corticospinal axons induced by transplanted olfactory ensheathing cells. J. Neurosci. 18, 10514–10524.
Li, Y., Field, P.M., Raisman, G., 1997. Repair of adult rat corticospinal tract by transplants of olfactory ensheathing cells. Science 277, 2000–2002.
Lim, F., Martin-Bermejo, M.J., Garcia-Escudero, V., Gallego-Hernandez, M.T., Garcia-Gomez, A., Rabano, A., et al., 2010. Reversibly immortalized human olfactory ensheathing glia from an elderly donor maintain neuroregenerative capacity. Glia 58, 546–558.
Lima, C., Escada, P., Pratas-Vital, J., Branco, C., Arcangeli, C.A., Lazzeri, G., et al., 2010. Olfactory mucosal autografts and rehabilitation for chronic traumatic spinal cord injury. Neurorehabil. Neural Repair 24, 10–22.
Lima, C., Pratas-Vital, J., Escada, P., Hasse-Ferreira, A., Capucho, C., Peduzzi, J.D., 2006. Olfactory mucosa autografts in human spinal cord injury: a pilot clinical study. J. Spinal Cord Med. 29, 191–203 discussion 4–6.
Lledo, P.M., Merkle, F.T., Alvarez-Buylla, A., 2008. Origin and function of olfactory bulb interneuron diversity. Trends Neurosci. 31, 392–400.
Lobsiger, C.S., Schweitzer, B., Taylor, V., Suter, U., 2000. Platelet-derived growth factor-BB supports the survival of cultured rat Schwann cell precursors in synergy with neurotrophin-3. Glia 30, 290–300.
Lopez-Vales, R., Fores, J., Navarro, X., Verdu, E., 2006. Olfactory ensheathing glia graft in combination with FK506 administration promote repair after spinal cord injury. Neurobiol. Dis. 24, 443–454.
Lu, J., Feron, F., Ho, S.M., Mackay-Sim, A., Waite, P.M., 2001. Transplantation of nasal olfactory tissue promotes partial recovery in paraplegic adult rats. Brain Res. 889, 344–357.
Lu, J., Feron, F., Mackay-Sim, A., Waite, P.M., 2002. Olfactory ensheathing cells promote locomotor recovery after delayed transplantation into transected spinal cord. Brain 125, 14–21.
Mackay-Sim, A., Feron, F., Cochrane, J., Bassingthwaighte, L., Bayliss, C., Davies, W., et al., 2008. Autologous olfactory ensheathing cell transplantation in human paraplegia: a 3-year clinical trial. Brain 131, 2376–2386.
Mackay-Sim, A., Kittel, P., 1991. Cell dynamics in the adult mouse olfactory epithelium: a quantitative autoradiographic study. J. Neurosci. 11, 979–984.
Martini, R., Bollensen, E., Schachner, M., 1988. Immunocytological localization of the major peripheral nervous system glycoprotein P0 and the L2/HNK-1 and L3 carbohydrate structures in developing and adult mouse sciatic nerve. Dev. Biol. 129, 330–338.
Moran, D.T., Rowley 3rd, J.C., Jafek, B.W., Lovell, M.A., 1982. The fine structure of the olfactory mucosa in man. J. Neurocytol. 11, 721–746.
Morrison, E.E., Costanzo, R.M., 1990. Morphology of the human olfactory epithelium. J. Comp. Neurol. 297, 1–13.
Murrell, W., Feron, F., Wetzig, A., Cameron, N., Splatt, K., Bellette, B., et al., 2005. Multipotent stem cells from adult olfactory mucosa. Dev. Dyn. 233, 496–515.
Nakashima, T., Kimmelmann, C., Snow, J., 1984. Structure of human fetal and adult olfactory neuroepithelium. Acta Otolaryngol. 110, 641–646.
Paik, S.I., Lehman, M.N., Seiden, A.M., Duncan, H.J., Smith, D.V., 1992. Human olfactory biopsy. The influence of age and receptor distribution. Arch. Otolaryngol. Head Neck Surg. 118, 731–738.
Pedersen, P.E., Benson, T.E., 1986. Projection of septal organ receptor neurons to the main olfactory bulb in rats. J. Comp. Neurol. 252, 555–562.
Pixley, S.K., 1992. The olfactory nerve contains two populations of glia, identified both in vivo and in vitro. Glia 5, 269–284.
Plant, G.W., Christensen, C.L., Oudega, M., Bunge, M.B., 2003. Delayed transplantation of olfactory ensheathing glia promotes sparing/regeneration of supraspinal axons in the contused adult rat spinal cord. J. Neurotrauma 20, 1–16.
Ramer, L.M., Au, E., Richter, M.W., Liu, J., Tetzlaff, W., Roskams, A.J., 2004. Peripheral olfactory ensheathing cells reduce scar and cavity formation and promote regeneration after spinal cord injury. J. Comp. Neurol. 473, 1–15.
Ramon-Cueto, A., Cordero, M., Santos-Benito, F., Avila, J., 2000. Functional recovery of paraplegic rats and motor axon regeneration in their spinal cords by olfactory ensheathing glia. Neuron 25, 425–435.
Ramon-Cueto, A., Plant, G., Avila, J., Bunge, M., 1998. Long-distance axonal regeneration in the transected adult rat spinal cord is promoted by ensheathing glia transplants. J. Neurosci. 18, 3803–3815.
Richter, M.W., Fletcher, P.A., Liu, J., Tetzlaff, W., Roskams, A.J., 2005. Lamina propria and olfactory bulb ensheathing cells exhibit differential integration and migration and promote differential axon sprouting in the lesioned spinal cord. J. Neurosci. 25, 10700–10711.
Rizek, P.N., Kawaja, M.D., 2006. Cultures of rat olfactory ensheathing cells are contaminated with Schwann cells. NeuroReport 17, 459–462.
Roisen, F.J., Klueber, K.M., Lu, C.L., Hatcher, L.M., Dozier, A., Shields, C.B., et al., 2001. Adult human olfactory stem cells. Brain Res. 890, 11–22.
Rubio, M.P., Munoz-Quiles, C., Ramon-Cueto, A., 2008. Adult olfactory bulbs from primates provide reliable ensheathing glia for cell therapy. Glia 56, 539–551.
Schaefer, M.L., Bottger, B., Silver, W.L., Finger, T.E., 2002. Trigeminal collaterals in the nasal epithelium and olfactory bulb: a potential route for direct modulation of olfactory information by trigeminal stimuli. J. Comp. Neurol. 444, 221–226.
Shipley, M.T., Ennis, M., 1996. Functional organization of olfactory system. J. Neurobiol. 30, 123–176.
Silver, W.L., Finger, T.E., 2009. The anatomical and electrophysiological basis of peripheral nasal trigeminal chemoreception. Ann. NY Acad. Sci. 1170, 202–205.
Smale, K.A., Doucette, R., Kawaja, M.D., 1996. Implantation of olfactory ensheathing cells in the adult rat brain following fimbria-fornix transection. Exp. Neurol. 137, 225–233.
Smithson, L.J., Kawaja, M.D., 2009. A comparative examination of biomarkers for olfactory ensheathing cells in cats and guinea pigs. Brain Res. 1284, 41–53.
Steeves, J.D., Lammertse, D., Curt, A., Fawcett, J.W., Tuszynski, M.H., Ditunno, J.F., et al., 2007. Guidelines for the conduct of clinical trials for spinal cord injury (SCI) as developed by the ICCP panel: clinical trial outcome measures. Spinal Cord 45, 206–221.
Storan, M.J., Key, B., 2006. Septal organ of Gruneberg is part of the olfactory system. J. Comp. Neurol. 494, 834–844.
Teng, X., Nagata, I., Li, H.P., Kimura-Kuroda, J., Sango, K., Kawamura, K., et al., 2008. Regeneration of nigrostriatal dopaminergic axons after transplantation of olfactory ensheathing cells and fibroblasts prevents fibrotic scar formation at the lesion site. J. Neurosci. Res. 86, 3140–3150.
Tome, M., Lindsay, S.L., Riddell, J.S., Barnett, S.C., 2009. Identification of nonepithelial multipotent cells in the embryonic olfactory mucosa. Stem Cells 27, 2196–2208.
Tuszynski, M.H., Steeves, J.D., Fawcett, J.W., Lammertse, D., Kalichman, M., Rask, C., et al., 2007. Guidelines for the conduct of clinical trials for spinal cord injury as developed by the ICCP Panel: clinical trial inclusion/exclusion criteria and ethics. Spinal Cord 45, 222–231.
Vincent, A.J., Taylor, J.M., Choi-Lundberg, D.L., West, A.K., Chuah, M.I., 2005. Genetic expression profile of olfactory ensheathing cells is distinct from that of Schwann cells and astrocytes. Glia 51, 132–147.
Vincent, A.J., West, A.K., Chuah, M.I., 2003. Morphological plasticity of olfactory ensheathing cells is regulated by cAMP and endothelin-1. Glia 41, 393–403.
Windus, L.C., Lineburg, K.E., Scott, S.E., Claxton, C., Mackay-Sim, A., Key, B., et al., 2010. Lamellipodia mediate the heterogeneity of central olfactory ensheathing cell interactions. Cell. Mol. Life Sci. 67, 1735–1750.
Xiao, M., Klueber, K.M., Lu, C., Guo, Z., Marshall, C.T., Wang, H., et al., 2005. Human adult olfactory neural progenitors rescue axotomized rodent rubrospinal neurons and promote functional recovery. Exp. Neurol. 194, 12–30.