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Xenotransplantation of transgenic pig olfactory ensheathing cells promotes axonal regeneration in rat spinal cord

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Abstract

Here we describe transplantation of olfactory ensheathing cells (OECs) or Schwann cells derived from transgenic pigs expressing the human complement inhibitory protein, CD59 (hCD59), into transected dorsal column lesions of the spinal cord of the immunosuppressed rat to induce axonal regeneration. Non-transplanted lesion-controlled rats exhibited no impulse conduction across the transection site, whereas in animals receiving transgenic pig OECs or Schwann cells impulse conduction was restored across and beyond the lesion site for more than a centimeter. Cell labeling indicated that the donor cells migrated into the denervated host tract. Conduction velocity measurements showed that the regenerated axons conducted impulses faster than normal axons. By morphological analysis, the axons seemed thickly myelinated with a peripheral pattern of myelin expected from the donor cell type. These results indicate that xenotransplantation of myelin-forming cells from pigs genetically altered to reduce the hyperacute response in humans are able to induce elongative axonal regeneration and remyelination and restore impulse conduction across the transected spinal cord.

Keywords

Olfactory ensheathing cells; axonal regeneration; cell transplantation; engineered cells

There is much interest in the potential of xenotransplantation of genetically engineered pig cells as a source for human cell therapies^{1,2}. Unlike primate tissues, pigs are generally agreed to be an ethically acceptable source of cells. Moreover, relatively large quantities of pig cells can be collected for potential cell therapies. An important obstacle with xenotransplantation is the problem of natural antibody reactivity and subsequent complement activation that leads to complement-mediated cell lysis. One approach to address this problem has been to engineer pig cells to express a human complement inhibitory protein (e.g., hCD59). In the present study we demonstrate that transgenic pigs engineered to express hCD59 do indeed express this protein in olfactory ensheathing cells (OECs) and Schwann cells, and when these cells are transplanted into the transected rat spinal cord, they facilitate axonal regeneration, form new myelin and restore conduction in the injured spinal cord.

In rat models, transplants of cultured OECs into ablated corticospinal tract³, fasciculus gracilis⁴, or nerve bridges in the spinal cord⁵ enhance axonal regeneration. OECs have several

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unique properties that provide a rationale for their potential to enhance CNS axonal regeneration. OECs are specialized cells that support axons that leave the olfactory epithelium and project through the peripheral nervous system into the olfactory bulb of the central nervous system; they are pluripotential cells that can show Schwann cell or astrocyte-like cell properties⁶. The possibility that OECs or Schwann cells, which also support CNS regeneration^{4,7}, could be used as a cell therapy in humans to repair the transected^{3–5} and demyelinated^{8,9} spinal cord has been considered. Yet, attaining enough of the appropriate cells is still problematic.

Our results indicate that these genetically altered pig cells both survive and promote elongative regeneration of electrophysiologically functional axons across a site of spinal cord transection in the immunosuppressed rat. These findings are important prerequisites for consideration of these cells as candidates for xenotransplantation studies in humans.

Results

Transgenic Expression

OECs and Schwann cells were isolated from transgenic pigs expressing the human complement inhibitor, hCD59. The transgenically derived OECs and Schwann cells were cultured as described in Experimental Protocol. Cells were analyzed by flow cytometry to assess transgene expression. The antibody, PT85A, was used to detect endogenously expressed Swine Leukocyte Antigen (SLA) class I and served as a positive control (Fig. 1). It was important to demonstrate SLA class I expression because the transgenic line was produced using a murine major histocompatibility class I promoter expression construct, H2Kb-hCD59 (ref. 10). Indirect immunofluorescence analysis determined that the transgene was expressed on the cell surface of both Schwann cells and OECs isolated from the hCD59 transgenic pig (Fig. 1). Interestingly, the concentration of endogenous SLA class I expression was very high in both cell types, however, the level of transgene expression was an order of magnitude less in both the OECs and Schwann cells. This could be the result of integration site inactivation effects for these cell types, or the absence of an enhancer element, as yet unidentified in the class I gene, specific to ensheathing cells.

Conduction of regenerated axons across the transected spinal cord

Adult Wistar rats were anesthetized and laminectomies were performed to create the dorsal column lesion as described previously⁴. Lesioned animals were randomly divided into two cohorts: a sham transplant group receiving vehicle and a cell transplant group receiving either transgenic OECs or Schwann cells. The cell transplant group received injections of about 30,000 cells/ μ l as described in Experimental Protocol. Four to five weeks after transection of the dorsal columns, the spinal cords were prepared for *in vitro* electrophysiological analysis in a recording chamber (see Experimental Protocol). The completeness of the lesion was studied independently using anatomical techniques, and only those spinal cords subsequently demonstrated to have histologically complete transections of their dorsal columns were used for analysis.

The surface of the dorsal columns was stimulated with a silver wire electrode caudal to the transection site to activate ascending afferent sensory axons originating from dorsal root ganglia as they project within the fasciculus gracilis of the dorsal columns. Glass microelectrodes were used to record field potentials of the propagating action potentials near the midline surface of the dorsal columns (Fig. 2A). Virtually no electrical activity could be recorded beyond the lesion site in histologically complete non-transplanted rats indicating a lack of regeneration across the lesion site (Fig. 2B; $n = 9$). Only a stimulus artifact with no electrophysiological response can be observed in a recording obtained across a lesion site with

no cell transplantation. However, when transgenic pig OECs were transplanted near the transection site, compound action potentials could be recorded distal to the site of transection (Fig. 2C) in 7 of 10 rats indicating axonal regeneration through the lesion site. At 5 mm beyond the lesion site compound action potentials were consistently recorded in the transplant groups, although attenuated in amplitude as compared with non-transected control spinal cords.

Conduction was observed across the transection site for both transgenic pig OEC and Schwann cells transplantation. Conduction velocity was determined from the inverse slope of a plot of latency versus distance for several points beyond the lesion. Figure 3A shows conduction velocities for control, transection alone and following transplantation of either OECs or Schwann cells. Virtually no conduction was observed following transection alone. Interestingly, conduction velocity was greater for the regenerated axons following OEC (20.98 ± 5.38 m/sec; $n = 7$; $P = 0.002$) or Schwann cells (19.09 ± 6.2 m/sec; $n = 5$; $P = 0.033$) transplantation as compared with control uninjured spinal cords (12.93 ± 2.06 m/sec; $n = 11$). There was no difference in conduction velocity between OEC and Schwann cells transplanted groups ($P = 0.28$).

The rapid conduction velocities in the transplanted spinal cords indicate that the axons were myelinated as confirmed by histological analysis. Recognizable, but attenuated compound action potentials could be recorded at least 14 mm (length of recording chamber) beyond the transection site in both OEC and Schwann cells transplanted dorsal columns. Plots of compound action potential amplitude as a percent of the first wave versus distance beyond the lesion site are shown for control, OEC and Schwann cells transplantation in Figure 3B. Control and transplant groups showed attenuation of the responses with increasing conduction distance. The attenuation is probably the result of temporal and spatial dispersion of the axons. Both OEC and Schwann cells transplant groups displayed less relative attenuation with distance particularly more than the first several millimeters of conduction as compared with controls. We are not certain as to why this occurred, but given the geometric complexity of lesioned cord at and near the lesion site, the axons may have a tortuous trajectory in this region, which is reflected as greater stability of the response amplitude. These results indicate that axonal regeneration as assayed electrophysiologically occurred for a significant longitudinal distance within the spinal cord.

Axonal signaling typically occurs through the discharge of several action potentials. To determine if the regenerated axons could sustain high frequency discharge we examined their frequency-response properties. Figure 3C shows the attenuation of the compound action potential with increasing stimulus frequency for control, OEC and Schwann cells transplant groups recorded 5 mm beyond the lesion site. At frequencies up to 400 Hz there was no difference between the intact control and transplant groups indicating that the regenerated axons maintain stable high frequency discharge properties similar to normal axons. Moreover, paired pulse experiments indicated that the refractory properties of the axons were similar for all groups (Fig. 3D).

Localization of labeled donor cells and morphology of regenerated axons

A limited number of spinal cords received transplants of fluorescently labeled donor cells (see Experimental Protocol) to demonstrate their survival and presence in the host. Confocal imaging of 5-(and -6)-carboxyfluoresceine diacetate, succinimidyl ester (CFDA, SE) loaded transplanted OECs (Fig. 4) and Schwann cells (not shown) demonstrated that transplanted myelin-forming cells remained within the dorsal column region for at least 4 weeks after transplantation and migrated several millimeters along the rostral-caudal axis of the spinal cord. It is important to note that we injected the donor cells into the margins of the transected cord both rostrally and caudally, and not into the gap of the lesion. The demonstration that

viable transplanted cells were present in the spinal cord spanning the lesion gap indicates that a 'bridge' of donor cells was established.

Immediately after the *in vitro* electrophysiological studies the spinal cords were fixed and processed for histological analysis using standard plastic embedding protocols (see Experimental Protocol). Histological examination of semi-thin sections confirmed that the surgical protocol consistently produced a complete transection of the dorsal columns with minimal damage to the ventral horns. The dorsal columns in all lesioned animals used in this study were completely transected.

Histological examination of transected dorsal columns with transplanted transgenic pig OECs or Schwann cells revealed dispersed axons exhibiting Schwann cell-like patterns of myelination (large cytoplasmic and nuclear regions and the presence of a basement membrane) both within and rostral to the transection sites. The electron micrographs in Figure 5 demonstrate the presence of a peripheral pattern of myelination for both transgenic pig OEC and Schwann cell transplantation. The patterns of axonal distribution were slightly different between OEC and Schwann cell transplantation. Myelinated axons in OEC transplanted lesions were typically clustered in small bundles of 3–8 axons with a large surrounding extracellular space (Fig. 5A), whereas myelinated axonal profiles in Schwann cell transplanted lesions seemed to be more random (Fig. 5B). Electron microscopic analysis of the spinal cords indicated that both OEC and SC transplanted spinal cords established relatively thick myelin surrounded by a basement membrane (Fig. 5C–D).

Consistent with the electrophysiological evidence of myelinated axons extending across transected dorsal column lesions, semi-thin plastic-embedded sections of lesioned dorsal columns (see Experimental Protocol) revealed the presence of many axons showing the characteristic appearance of peripherally myelinated axons within and adjacent to OEC or Schwann cell transplanted lesions. Counts of myelinated axons immediately rostral to the lesion revealed an increase in the numbers of myelinated axons extending across the transection site in animals receiving transplants of transgenic pig OECs or Schwann cells. Lesions transplanted with pig OECs or Schwann cells exhibited an average of 304 ± 56 ($n = 10$) and 268 ± 61 ($n = 4$), respectively, peripheral-like myelinated axons crossing the lesion compared with only 112 ± 22 ($n = 9$) axons in control lesions. Numbers of myelinated axons extending across the lesion in OEC and Schwann cell transplanted lesions combined differed significantly from control lesions at $P < 0.003$, respectively (unpaired two tailed Student's *t* test).

Discussion

Transplantation of allogeneic OECs (refs. 9 and ¹¹) or Schwann cells (refs. 8 and ¹²) have been shown to remyelinate demyelinated axons as well as facilitate axonal regeneration of transected axons in the rat spinal cord^{3,5,13,14}. A recent study indicates that transplantation of human OECs can remyelinate the immunosuppressed rat spinal cord¹⁵ thus suggesting the feasibility of xenotransplantation to repair axons. Electrophysiological and histological examination of transected dorsal columns in this study support the conclusion that transplantation of either transgenic pig OECs or Schwann cells facilitate long distance regeneration of functionally remyelinated axons within the spinal cord.

Compound action potentials could be detected up to 14 mm (recording chamber length) rostral to histologically complete dorsal column transections that had received cell transplants. The conduction velocity and frequency-response characteristics of the regenerated axons crossing the lesion were indicative of myelinated axons, and histological sections revealed numerous Schwann cell-like (peripheral pattern) myelinated axon profiles within the dorsal column area rostral to the lesion of the rostrally projecting sensory axons. In lesioned controls (no transplant)

virtually no conduction was observed beyond the transection site. The conduction beyond the lesion site in transplanted recipients and the absence of conduction in non-transplanted animals was similar to experiments using rat donor cells⁴. This indicates that cross-species transplantation of genetically altered cells does not reduce the repair potential of the cells in immunosuppressed rats.

Although the electrophysiological data were consistent with functionally remyelinated axons, it is noteworthy that the CAPs of both OEC and Schwann cell-induced regenerated axons had conduction velocities greater than control. Similar to control axons, regenerated axons were also able to follow repetitive stimuli at frequencies up to 200 Hz. Taken together these results indicate that a rapid and secure conduction pathway is formed following transplantation of either transgenic pig OECs or Schwann cells into the transected dorsal column lesion.

Electron microscopic analysis indicated that both OECs and Schwann cells exhibited a peripheral-type of myelination pattern with large cytoplasmic and nuclear components surrounding the myelin, and the presence of a basal lamina, which are characteristic of peripheral myelin¹⁶. Moreover, extracellular collagen deposition, which is characteristic of peripheral myelin forming cells, was observed in the spinal cord. The myelinated axons following OEC transplantation tended to cluster in bundles, whereas myelinated axons were more dispersed following Schwann cell transplantation. Li et al.³ report a similar bundling of myelinated axons following transplantation of OECs in an electrolytic corticospinal lesion site. The bundling of regenerated myelinated axons that was typical in the OEC transplants suggests that there may be some differences in the pattern of axonal regeneration following transplantation of OECs versus Schwann cells. In spite of these anatomical differences, we found no difference in the electrophysiological properties following transgenic pig OEC or Schwann cell transplantation.

Histological examination of the spinal cords 4–5 weeks after injection indicated that the spinal cord was contiguous and that regenerating axons and transplanted cells were present in the transition zone and beyond. The injury site was indeed free of normal oligodendrocyte-myelinated axons and contained scattered regenerated axons that exhibited a peripheral pattern of myelination. The dorsal columns were completely transected by several cuts and sparing of residual intact axons within the lesion zone would be virtually impossible. One proposal to explain the fusion of the cut spinal cord after the severe injury is that the OECs and Schwann cells may have migrated into the lesion zone and served as guide cells for the axons to extent across the lesion by providing a hospitable regenerative environment. These cells do not express inhibitory proteins that induce growth cone collapse as do oligodendrocytes. CNS axons can regenerate through peripheral nerve grafts¹⁷ and in an environment where glial inhibitory proteins have been neutralized by antibodies¹⁸. The transplanted OECs and Schwann cells could also provide trophic support to encourage axonal regeneration; both OECs (ref. 19) and Schwann cells (ref. 20) can express NGF P75 receptors and Schwann cells are known to express NGF (ref. 21) after axotomy. It is yet to be determined whether NGF and NGF receptor interactions or other neurotrophins contribute to the enhanced regenerative capacity of CNS axons after OEC and Schwann cell transplantation.

The demonstration that OECs and SCs derived from hCD59 transgenic pigs can elicit axonal regeneration of functionally intact axons emphasizes the potential of cell therapy approaches using engineered xenogeneic cells to repair damaged CNS axonal tracts. Contusive spinal cord injury not only results in transection of axons, but can also result in demyelination of long tracts. Moreover, in addition to demyelination, axonal transection can be observed in early several sclerosis lesions²². Engineered pig OECs and Schwann cells rendered transgenic for the hCD59 gene can encourage at least some degree of axonal regeneration and form myelin.

These cells can be collected ethically in large numbers, and may be useful as a donor cell for cell therapy approaches to repair the demyelinated or injured spinal cord.

At present there are several clinical applications for the use of porcine tissues. For example, the treatment of Parkinson's disease patients with fetal porcine neural cells^{1,23} or the use of porcine hepatocytes for liver support systems in cases of acute liver failure^{1,24}. However, the success of these applications is still limited by the poor understanding of the mechanisms that lead in many cases to loss of function and rejection of the transplanted tissue. Many porcine cell types useful for transplantation, such as cardiomyocytes²⁵ or OECs (refs. 9 and 11) and Schwann cells (ref. 8) for spinal cord repair, would be expected to trigger a humoral and cellular immune response². Therefore the demonstration that genetically modified OECs and Schwann cells perform comparable to allotransplants⁴ and that these engineered cells have been modified to resist humoral immunity, enforces the use of transgenic porcine tissues for human therapy. Finally, transgenic porcine cells have several advantages over human fetal tissue, such as availability, quality control of the tissues obtained and timing of cell collection and reproducibility.

Experimental protocol

Cell preparation and culture

The anterior tips of the olfactory bulbs and the sciatic nerves of transgenic pigs were removed and cut into 1-mm pieces. Sciatic nerve pieces were then incubated for 90 min (30–40 min for OEC collections) at 37°C in DMEM with 1 mg/ml each of collagenase A and D; (Boehringer Mannheim) with continuous agitation. The resulting mixture was then centrifuged and cells were re-suspended with 2.5 mg/ml trypsin (Boehringer Mannheim, Germany) and 0.5mg/ml DNase for 15–30 min (10 min. for OEC collections). Cell suspensions were then centrifuged and resuspended in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal calf serum and triturated through a flame polished siliconized pipette. Any large undissociated pieces of tissue were then removed before washing cells twice with DMEM and resuspending at a concentration of 30,000 cells/ μ l in DMEM for transplantation. Enzymatic activity was blocked with 10% fetal calf serum containing medium and the cells were washed twice in DMEM before resuspending them at 30,000 cells/ μ l in DMEM.

Transgenic expression analysis

Schwann cell and OECs isolated from hCD59 transgenic animals were assayed for expression of the transgene by indirect immunofluorescence using the anti-hCD59 mouse monoclonal antibody (mAb), MEM-43 (Biodesign International, Kennebunkport, ME), and a polyclonal anti-hCD59 antibody, ALP-3 (ref. 26). Endogenous expression of SLA class I was assayed with the anti-SLA Class I mAb, PT85A (VARD, Inc., Pullman, WA) and served as a positive control antibody. Indirect immunofluorescence was typically performed on 1×10^4 to 1×10^5 cells with 20 μ g/ml of the polyclonal or with 10 μ g/ml of MEM 43 in $1 \times$ PBS plus 2% fetal bovine serum. Goat anti-rabbit IgG (polyclonal sera, Zymed, South San Francisco, CA) or goat anti-mouse IgG (monoclonal sera, Zymed) fluorescein isothiocyanate (FITC)-conjugated antisera were used to detect specific antibody binding to the cell surface. Cell surface expression was then measured by flow cytometry on a Becton-Dickinson FACSsort.

Surgical procedures

Adult Wistar rats were deeply anesthetized with ketamine/xylazine (75 mg/kg; 10 mg/kg, i.p.) and subjected to a standard lumbar spinal cord dorsal column transection at vertebral level T11. A sterile laminectomy was performed at T11 and dorsal column severed by making several transverse cuts with a pair of 8.5 cm Vannas-Tubbinagin spring scissors (Fine Science Tools, Forest City California) along the top of the spinal cord, but sparing the central vein. This surgery

resulted in paraparesis of the hind limbs in all animal subjected to the procedure, but did not prevent locomotion or impair eating, drinking or elimination. Immediately after spinal cord transection, 1.0 ml of freshly isolated transgenic pig OECs, Schwann cells or control DMEM (Gibco BRG) were injected into the dorsal column via a drawn glass micropipette at depths of 0.5 and 0.7 mm at two injection sites 0.5 mm rostral and caudal to the lesion. OECs and Schwann cells were injected at a concentration of 30,000 cells/ μ l for a total of 60,000 cells transplanted into each animal.

Electrophysiological analysis of axonal conduction

Four to five weeks after spinal cord transection and cell transplantation, rats were killed under sodium pentobarbital anesthesia (75 mg/kg i.p.). The vertebral column from each animal between T5 and L2 was then removed and transferred and placed in cold dissecting solution (135 mM choline chloride; 20 mM choline bicarbonate/1.0 mM KCl; 1.2 mM KH_2PO_4 ; 90 mM dextrose). The spinal cord region between T6 and L1 was removed and pinned in a recording chamber continuously perfused with zero calcium modified Krebs' solution (124 mM NaCl, 3mM KCl, 2.0 mM MgCl_2 , 26 mM NaHCO_3 , 1.3 NaHPO_4 , 10 mM dextrose) saturated with 95% O_2 /5% CO_2 at a drip rate of 4.0–5.5 ml/min at room temperature for 30 min. CaCl_2 (2.0 mM) was then added to the solution; electrophysiological recordings were obtained 2 h later at 36°C. The site of the spinal cord transection was identified by the presence of a pale yellow scar running transversely across the dorsal surface of the spinal cord. A bipolar silver stimulating electrode was placed within the dorsal column 1 mm caudal to the transection site. Electrical responses were recorded with glass micro-electrodes filled with 2M NaCl (3–10 M Ω resistance) from the surface of the dorsal column at successive points from 1 to 15 mm rostral to the lesion site. Stimulus intensity was set at 25% above the strength necessary to elicit maximal compound action potentials amplitude.

Donor cell labeling

For some experiments, cells were loaded with CFDA, SE (Molecular Probes, Eugene, OR) before transplantation to identify their location at the time of killing. Dissociated Schwann cells or OECs were incubated with 20 μ M CFDA, SE for 15 min (ref. 27) and washed 3 times with DMEM before transplantation. CFDA, SE loaded cells that were cultured for 2–4 weeks showed no evidence of dye toxicity and confirmed that the dye was retained in Schwann cells and OECs throughout the culture period. Dye also did not seem to transfer to sensory neurons when CFDA, SE loaded cells were added to cultures of rat DRG neurons.

Histology

Following electrophysiological recordings, spinal cord tissue was fixed for 24 h in 2% paraformaldehyde plus 2% glutaraldehyde (weight/volume in 0.14 M Sorensen's buffer) for routine histological analysis or, 4% paraformaldehyde for confocal fluorescence microscopy. Tissue intended for routine histology was washed 3 times, stored overnight in buffer and cut into 2-mm segments. Spinal cord segments were then post-fixed with 1% osmium (Polysciences, Warrington PA) for 4 h and embedded in Epox-812 (Ernest Fullam Inc., Latham, NY). Tissue was then serially sectioned on an ultramicrotome and 1- μ m sections were collected every 0.25 mm. Sections were then counterstained with 0.5% methylene blue, 0.5% azure II in 0.5% borax. Spinal cords examined with confocal microscopy were transferred to PBS buffer, cut into 1-cm lengths that were notched to indicate rostral/caudal orientation, and then sliced into three approximately equal longitudinal sections.

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Figure 1. FACS analysis of transgenic Schwann cells and transgenic olfactory ensheathing cells (OECs) expressing CD59. SLA class I (A and B) was detected with the mAb PT85A and the goat anti-mouse secondary control was used to determine background reactivity of a non-specific antibody. CD59 was detected with mAb MEM43 (A and B) and the polyclonal Ab ALP3 (C and D). Cells were analyzed on a Becton-Dickinson FACSsort.

Figure 2. Conduction across the transplantation zone. (A) Schematic showing transection site and positions of stimulating and recording electrodes. Stimulating electrodes were positioned 1 mm caudal to the dorsal column transection site (0 mm) and recordings were obtained at 1 mm intervals beyond the lesion site. (B) CAP recordings for control lesion taken at 2 (R1) and 3 (R2) mm rostral to the stimulating site (i.e., 1 and 2 mm beyond the transection site, respectively) in lesioned dorsal columns without cell transplantation. (C) Field potential recording from beyond dorsal column lesion of OEC transplant recipient. The peak negativities are indicated by solid squares.

Figure 3.

Conduction properties of transplant-induced regenerated spinal cord axons. (A) Comparison of conduction velocity between normal (non-lesioned), dorsal column transection alone (Cont.), and transected following transplantation of transgenic OECs or Schwann cells. (B) Although no impulse conduction was observed for transected dorsal columns without cell transplantation, both OEC- and Schwann cells-transplant groups displayed conduction across the lesion for distances comparable to controls. (C) Both OEC- and Schwann cell-transplant groups following high frequency stimulation similarly to non-lesioned controls. (D) The refractory period from paired pulse stimulation experiments was similar between control and transplant groups.

Figure 4. Presence of transgenic pig donor OECs in the recipient spinal cord. OECs labeled with the fluorescent membrane marker, CFDA, SE were present in the dorsal columns 3 weeks after cell transplantation. The calibration bar corresponds to 20 μ m.

Figure 5.

Ultrastructural characteristics of regenerated axons following transgenic pig OEC and Schwann cells transplantation. The tissue was fixed after *in vitro* electrophysiological recordings were obtained. Low power electron micrographs of regenerated myelinated axons in the fasciculus gracilis of the dorsal columns following OEC (A) and Schwann cell (B) transplantation. The micrographs were obtained rostral to the lesion site, but distal to the cell bodies (dorsal root ganglia) of the transected ascending sensory neurons. Note the presence of small clusters of regenerated axons following OEC transplantation, but individual axons are seen following Schwann cell transplantation. Higher power electron micrographs indicate formation of thick myelin for OECs (C) and Schwann cells (D), respectively, and the presence of a basement membrane and extracellular collagen deposition (D).