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Review

Transplantation of Olfactory Ensheathing Cells as Adjunct Cell Therapy for Peripheral Nerve Injury

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Traumatic events, such as work place trauma or motor vehicle accident violence, result in a significant number of severe peripheral nerve lesions, including nerve crush and nerve disruption defects. Transplantation of myelin-forming cells, such as Schwann cells (SCs) or olfactory ensheathing cells (OECs), may be beneficial to the regenerative process because the applied cells could mediate neurotrophic and neuroprotective effects by secretion of chemokines. Moreover, myelin-forming cells are capable of bridging the repair site by establishing an environment permissive to axonal regeneration. The cell types that are subject to intense investigation include SCs and OECs either derived from the olfactory bulb or the olfactory mucosa, stromal cells from bone marrow (mesenchymal stem cells, MSCs), and adipose tissue-derived cells. OECs reside in the peripheral and central nervous system and have been suggested to display unique regenerative properties. However, so far OECs were mainly used in experimental studies to foster central regeneration and it was not until recently that their regeneration-promoting activity for the peripheral nervous system was recognized. In the present review, we summarize recent experimental evidence regarding the regenerative effects of OECs applied to the peripheral nervous system that may be relevant to design novel autologous cell transplantation therapies.

Key words: Wallerian degeneration; Cell transplantation; Olfactory ensheathing cells (OECs); Regeneration; Remyelination; Cell expansion

TRAUMATIC NERVE INJURY

Peripheral nerve injury associated with trauma is a common and devastating complication that can cause irreversible impairment and complete functional loss of the affected limb. Axonal regeneration and functional recovery may occur after surgical reapposition, but the clinical outcome is often not optimal. Important factors contributing to the poor functional recovery are the localization of the lesion site and the delay in nerve repair (19). It was shown that prolonged target deprivation reduces the capacity of injured motoneurons to regenerate (21). Chronic denervated Schwann cells are known to display reduced regenerative capacity (75). Thus, any therapeutical treatments that accelerate axonal regeneration may improve the functional recovery. Besides postsurgical electric stimulation (25,38), transplantation of regeneration-promoting glia is promising approach to accelerate the regenerative process (58,61).

If the proximal end of the disrupted nerve is blocked from the peripheral target associated with the distal stump, sprouting axons do not find the correct way, which results in aberrant axonal growth within the proximal stump (1,38), leading to neuroma formation associated with pain and dysesthesia (14). Achieving optimal regrowth into the distal stump is, therefore, not only essential for gaining functional recovery, but also to prevent the potentially devastating sensory hyperexcitability associated with neuroma formation. Besides the correct reinnervation of the original targets (e.g., muscle

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or sensory organs), axons also have to induce remyelination and proper formation of the nodes of Ranvier with appropriate clustering of sodium channels that allow rapid impulse conduction. Transplantation of myelin-forming cells into a nerve defect site has been shown to facilitate this complex repair process (15,56,71,72).

Reapposition of transected nerve by surgical reapposition represents the current primary clinical approach to nerve repair. An early study evaluated the efficacy of nerve coadaptation techniques and application of biological materials to nerve stumps to evaluate their role in promoting nerve regeneration (77). It is well established that minimal tissue manipulation, attenuation of inflammatory responses, and minimization of tension at suture sites are crucial for successful anastomosis of peripheral nerves (26). Extensive scar tissue formation at the lesion site that can lead to mechanical obstruction for axonal regeneration and nerve conduction block may require surgical neurolysis.

Cell transplantation approaches are being used in experimental models of spinal cord and peripheral nerve injury to enhance nerve regeneration (61,63). The cell types subject to intense investigation include Schwann cells (SCs) and olfactory ensheathing cells (OECs) either derived from the olfactory bulb or the olfactory mucosa, stromal cells from bone marrow (mesenchymal stem cells, MSCs), or adipose tissue-derived cells (60). OECs are specialized glial cells of the olfactory system that are of particular interest in this context (63). OECs do not only reside in both the peripheral nervous system (PNS) and the CNS under normal conditions, but they have also been shown to promote neural regeneration in a variety of different lesion paradigms. Although initially used for promoting central regeneration, it has become apparent in the recent years that OECs may also promote PNS regeneration (56,79). In this review, we will focus on the potential of OECs for improving regeneration when used as an adjunct to peripheral nerve suture repair.

OECs DISPLAY SPECIAL REGENERATION-PROMOTING PROPERTIES

Olfactory epithelial neurons in the nasal mucosa are continuously replaced by progenitor cells and send their axons across the PNS–CNS boundary into the olfactory bulbs even in the adult (27,52). Olfactory nerve transection leads to acceleration of olfactory epithelial neuron differentiation in the olfactory epithelium (9). OECs are glia cells located in the olfactory mucosa, olfactory nerve, and the outer nerve layer of the olfactory bulb (64). The lifelong neurogenesis of olfactory receptor neurons and the entry of their axons together with the well-established role of glial cells in axon growth were the basis to propose that OECs closely associated with olfactory neurons guide and promote their axons into and within the CNS.

Based on the observations that the olfactory system displays neurogenesis and entry of axonal processes into the central nervous system throughout life time, it was proposed that OECs display unique regenerative properties. In situ, OECs have been shown to share characteristics with both SCs and astrocytes (13). The majority of investigations so far have used OECs harvested from the olfactory bulb (63). However, OECs can also be prepared from the nasal mucosa (35,66). Harvesting OECs from biopsies of the nasal mucosa is providing an easy possibility of autologous transplantation and could reduce the risk of immunological rejection (51).

Gliotic tissue (produced by astrocytes) is an inhibitory substrate for axonal regeneration (47,67,70). However, OECs are permissive for regenerating axons and can navigate growing axons through the glial scar. Like SCs, OECs possess the ability to remove degenerating axons via phagocytosis (4,39,83) and can produce channels along which newly formed axons can regenerate (40). Thus, OECs have the ability to navigate glial scars and have been shown to enhance axonal regeneration (67), thus utilizing a mechanism employed during embryogenesis in the navigation of olfactory nerves to the olfactory bulb (65). OECs, like SCs, have been shown to synthesize a number of neurotrophic factors, such as nerve growth factor, brain-derived neurotrophic factor (BDNF), and ciliary neurotrophic factor (51,71,82), platelet-derived growth factor, and neuropeptide Y (78), suggesting that trophic factor production by OECs might enhance the survival and promote regeneration of damaged axons. While OECs normally do not form myelin in the olfactory system, they can produce myelin after transplantation into demyelinated lesions of the PNS and CNS (18,34,56).

The mature PNS generally shows a better regeneration of injured axons compared to the CNS. But complete functional recovery is rarely achieved because aberrant reinnervation frequently occurs due to extensive branching of regrowing axons. This might be even in case of morphologically good axonal regeneration (11). Facial synkinesis has been described as a pathological consequence of aberrant reinnervation (3). Reduction of hyperinnervation and precisely directed target reinnervation is an important aim of therapeutic interventions in peripheral nerve repair and might include the application of antagonizing molecular factors like neutralizing antibodies or treatment with cells secreting neurotrophic factors, although major progress has still to be achieved (2).

After nerve injury, fibroblasts are known to invade the lesion site and producing scar tissue that is a mechanical barrier to axonal growth across the lesion site. This is one factor that contributes to the reduced func-

tional outcome. Scar tissue is thought to impede the migration not only of regenerating axons but also of migrating SCs that support regeneration. Although adequate studies that compare the effects of SCs and OECs are still low in number (81,84), experimental evidence suggests that OECs but not SCs are able to cross the lesion site (18,42). OECs have a greater migratory potential than SCs (39,41,67) and they produce neurotrophic factors (82). Furthermore, OECs do not accumulate proteoglycans, which cause growth cone collapse (5,55), as do SCs. Therefore, OECs may be a suitable candidate for cell-based regenerative therapies. Clinical trials have been initiated using OECs to repair spinal cord lesions in humans (16,31,46,50) and to improve functional outcome after cerebral palsy in adolescence and children (10).

TRANSPLANTATION OF OECs IN PERIPHERAL NERVE REPAIR

The PNS is generally considered a permissive environment with respect to neurite growth (20,24). This is, at least in part, due to the low levels of myelin-associated growth inhibitory molecules that are abundant in the CNS (7). Another important consideration for successful peripheral nerve regeneration is the presence of SCs. In the PNS, SCs in the distal segment of a cut nerve dissociate from the degenerating axons, upregulate expression of nerve growth factor (NGF) and its low affinity receptor , the neurotrophin receptor p75 ($p75^{NTR}$) (37). Axons in the proximal nerve stump initiate sprouting and regeneration through SC-enriched basal lamina tubes prior to reestablishing functional connections to peripheral targets such as skin and muscle under optimal conditions with varying degrees of functional recovery. A number of issues, such as the delayed expression of NGF and $p75^{NTR}$, the dying back of the injured axons, the navigation of axons across the lesion site, and the appropriate targeting to peripheral end structures are major clinical concerns.

The prototype lesion paradigm used to determine axon growth-promoting properties of OECs is the dorsal root rhizotomy of adult rat (Table 1). However, whereas early in vivo studies reported facilitated entry of peripheral sensory dorsal root ganglionic neurons by transplantation of OECs (53,67), recent studies failed to demonstrate similar effects (23,66,69). The reason for these discrepancies still has to be clarified. Recently, Li et al. (44) reported beneficial effects of transplanted OECs to the reanastomosed ventral S1 root. Very recently, Ibrahim et al. (32) reported transplantation of OECs in a brachial plexus injury model. Here, OECs increased regeneration at both the anatomical and functional level.

OECs have been considered as an adjunct for peripheral nerve repair because they may not only provide a scaffold for regrowing axons but also because they could provide trophic factors and directional cues (12,15,56). OECs were initially transplanted to the transected peripheral nerve by Verdu et al. (79) and shown to promote axonal growth across a 15-mm gap. Engraftment of OECs into the axotomized facial nerve produced controversial results. Whereas axonal sprouting-promoting effects of OECs were initially reported (12,28), reinvestigation by Angelov et al. (2) revealed no significant alterations in axonal sprouting by transplanted OECs. This is in line with a study by Choi and Raisman (11) that reported no OEC-induced effects on the aberrant sprouting but an increase in the eye closure rate. Interestingly, transplantation of olfactory mucosa explants in the same lesion model minimized axonal branching and promoted the functional recovery of vibrissae movements (29).

Previously, we reported that both SCs (57) and OECs (15) transplanted into the transected sciatic nerve invaded the injury site and formed peripheral type myelin with correct clustering of sodium channels (Nav1.6). Moreover, engrafted OECs were found to accelerate and improve functional recovery following nerve injury (33). OECs transplanted in the adult rat sciatic nerve at the time of microsurgical intervention, improved both axonal regeneration and the functional outcome, as measured by the sciatic functional index (SFI) (56). Interestingly, we noted a significantly reduced die-back of the axons proximal to the transection site as well as an increased number of regenerated axons in the distal nerve stump. The microsutured nerves lacking OECs displayed a thinner caliber (i.e., constricted) at the repair site than nerves that received OEC transplants (56). This may suggest that the transplanted OECs were primed to produce neurotrophins, such as NGF, and, therefore, provided immediate trophic support to the injured axons. This may provide an explanation for reduced axonal die-back and the accelerated regeneration rate of the injured axons. Usually, axons die-back a couple of millimeter after nerve transection and with a delay of a several days; axonal growth rate is not higher than 1–2 mm per day. OECs, therefore, may be a tool to reduce this delay and to promote axonal growth across the injury site well before significant scar tissue is formed.

CHALLENGES IN CELL THERAPY APPROACHES FOR PERIPHERAL NERVE REPAIR

Harvesting sufficient numbers of cells is an important concern with regard to cell transplantation therapies. In the laboratory, several cell types can be expanded in culture using trophic factors and mitogens. However, in vitro cell expansion could alter the physiologic properties of the cells. Another concern with expanded cells is the potential risk of tumor formation. It is essential to determine if experimental in vivo transplantation of ex-

Table 1. Summary of In Vivo Studies Including the Transplantation of OECs or Olfactory Mucosa to Either the Dorsal Root Entry Zone (DREZ), the Dorsal Root Ganglia (DRG), or the Peripheral Nerve [Modified According to (84)]

In Vivo Lesion Model	Cellular Composition of the Ttransplant	Morphological Analysis	Functional Analysis	Reference
Dorsal horn rhizotomy implantation in DREZ	Immunopurified OECs (anti-$p75^{NTR}$ antibodies)	Increased axonal regeneration into the spinal cord	Not performed	67
Dorsal horn rhizotomy implantation in DREZ	Immunopurified OECs (anti-$p75^{NTR}$ antibodies)	Increased axonal regeneration	Improved withdrawal reflex	53
Sciatic nerve transaction implantation in sciatic nerve	Immunopurified OECs (anti-$p75^{NTR}$ antibodies)	Increased axonal regeneration	Increased compound muscle and nerve action potential	79
Dorsal horn rhizotomy	Immunopurified OECs (anti-$p75^{NTR}$ antibodies)	No increased axonal regeneration	Not performed	23
Dorsal horn rhizotomy implantation in DRG, DREZ	Purified OECs* (Thy1.1 Antibody mediated lysis of fibroblasts)	No increased axonal regeneration	Not performed	66
Dorsal horn rhizotomy implantation in DREZ	Immunopurified OECs (anti-O4 antibodies)	No increased regeneration	Not detectable cord dorsum or field potentials	69
Transection of and implantation in facial nerve	Immunopurified OECs (anti-$p75^{NTR}$ antibodies)	Increased aberrant sprouting	No improvement in vibrissae movements	28
Transection of and implantation in facial nerve	Olfactory mucosa explants	Minimized aberrant sprouting	Improvement of vibrissae movements	29
Transection of and implantation in facial nerve	Nonpurified	No alterations in aberrant sprouting	Improvement of vibrissae movements	11
Transection of and implantation in facial nerve	$p75^{NTR}$-purified OECs	No alterations in aberrant sprouting	No alterations in vibrissae movements	2
Crush of and implantation in sciatic nerve	Purified SCs	Increased myelin sheath thickness, Nav Expression at nodes of Ranvier	Not performed	57
Sciatic nerve crush implantation in sciatic nerve	Purified OECs	Increased myelin sheath thickness, NaV expression at nodes of Ranvier	Not performed	15
Ventral root injury implantation at the ventral root CNS–PNS transition zone	Nonpurified	Increased axonal regeneration	Not performed	44
Sciatic nerve transaction	Purified OECs	Thicker myelin sheaths	Improved sciatic functional index (SFI)	56
Dorsal horn rhizotomy implantation in DREZ	Nonpurified OECs	Increased axonal regeneration	Increased forepaw function	32

OECs in the studies were isolated from rats except *mice.

panded cells not only retained their ability to carry out neural repair, but also that the cells are not tumorigenic. With increased cell passages, the properties of expanding oncogenic subclones increase and rigorous testing of the expanded cells is critical. It cannot be assumed that observed safety in animal models will also apply to humans.

The neurotrophin production of transplanted OECs may alter axonal sprouting and synapse formation. One concern is that the newly organized neural structures could be maladaptive and elicit neurological problems such as pain or allodynia (30). Transplantation of NGF-producing keratinocytes into injured peripheral nerve leads to extreme hyperexcitability and pain (62). Care

must be taken to study functional properties of regenerated axons and their parent cell bodies elicited by interventional approaches such as cell transplantation does not result in maladaptive responses. Importantly, we found that nodal sodium channels are appropriate following transplantation in injured nerve (15,57) and spinal cord (71). Design of functional experiments to assess efficacy will be essential for evaluating these approaches in clinical studies. Along this line is the recent observation that SC $p75^{NTR}$ prevents spontaneous reinnervation of the adult spinal cord (74). Contrary to the widely held view, these authors postulate that $p75^{NTR}$ on glia may restrict the availability of NGF for neurons and recommend to block $p75^{NTR}$ expression on SCs and OECs prior to therapeutic application. In this context it is interesting to note that adult canine OECs in vitro display significantly lower levels of $p75^{NTR}$ than SCs (Techangamsuwan, unpublished observation).

Another challenge is the time delay that currently is required for preparation of OECs if they are prepared from biopsy of nasal mucosa. However, these autologous sources of cells would require days in culture for preparation and thus a critical therapeutic window could be missed. The development of immunologically compatible cells that could be produced in high quantity and banked for later use is a critical experimental challenge. OECs derived from porcine olfactory bulb show poor survival in long-term culture and lose their ability to myelinate axons after a few weeks in culture (59).

Although cell transplantation is a promising option for current and future clinical use, there are natural restrictions regarding cell sources and in vitro expansion that are still a major challenge to the development of autologous cell transplantation. Research focuses on two major directions to overcome this limitation: immortalization of primary cells, allowing indefinite expansion, and reprogramming of somatic cells to generate large numbers of pluripotent stem cells. Both techniques largely depend on genetic modifications often making use of viral transductions.

Immortalization of primary cells is frequently achieved by circumventing cellular senescence. This is typically done by preventing the progressive telomere shortening via telomerase reverse transcriptase (TERT) (85) alone or in combination with factors stimulating cell growth. In an approach to immortalize OECs from the human olfactory bulb, transduction with lentivectors encoding Bmi-1 and TERT transgenes flanked by loxP sites was used (45). Polycomb group transcription factor Bmi-1 can inhibit growth-inhibitory functions of both p53 and Rb pathways, via its repressive effect on the Ink4a/Arf tumor suppressor locus (36) while it is alone not able to suppress telomere shortening. Importantly, this strategy allowed excision of the floxed transgenes by lentivector-mediated Cre recombinase delivery. At every investigated state as primary, immortalized, and deimmortalized cells maintained their phenotype characterized by S100, GFAP, vimentin, ErbB2 receptor, neuroligin-3, APP, and nestin expression. Additionally, they were able to promote axonal regeneration of adult rat retinal ganglion neurons (RGN) in coculture assays (45).

García-Escudero et al. investigated different combinations of transduction with TERT and Bmi with SV40 large T antigen (TAg) and a short hairpin RNA directed against p53 (shp53). Neuroregenerative capacity was observed in all cell populations but the deimmortalzation process was best tolerated in Bmi/TERT cells (22).

In a comparative study, adult canine SCs and OECs were transfected with human TERT and analyzed with regard to antigenic expression and growth rates. No significant differences between both cell types were demonstrated underscoring the close relationship between the two cell types (76). Ectopic expression of TERT allowed escape from growth arrest without changing the cellular phenotype. Interestingly, late passage cells maintained constant proliferation rates only in the presence of fibroblast growth factor-2 (FGF-2) (76).

COMBINATORIAL THERAPIES

Treatment of peripheral nerve injury may require the development of combinatorial strategies promoting neuroprotection (pharmacological blockade of secondary damage), axonal regeneration (electrical stimulation, cell transplantation combined with genetic engineering aiming to modulate trophic factor and/or growth inhibitory molecules, minimized scar formation), and rehabilitation as proposed for the CNS (8). OECs have been reported to promote neuroprotection of spinal cord parenchyma at least in part by modulating the expression of COX-2 and iNOS (48). OECs have been shown to reduce loss of corticospinal tract neurons after spinal cord transection possibly by production of BDNF (71). Moreover, transplantation of MSCs genetically modified to produce BDNF has neuronal protective effects in this model system (73). It was recently demonstrated that genetically modified OECs were capable of surviving and producing NT-3 in vivo and to significantly improve locomotor functional recovery after spinal cord injury (49). OECs can remyelinate regenerated and demyelinated axons. Thus, OECs may exert multiple therapeutic effects including neuroprotection, neurotrophism, and remyelination.

Future research might look into effective combination strategies to improve functional outcome after injury. Combination therapies tested so far in the CNS include the application of OECs together with SCs (54), with chondroitinase ABC or trophic factors, such as BDNF, as well as the application of cyclic AMP level-

increasing reagents (6,17,54,68). Moreover, cotransplantation of neural stem cells (NSCs) and OECs resulted in greater recovery of locomotion function after spinal cord injury than a single graft either by NSCs or OECs, indicating a synergistic effect (80). Although combinatorial applications always have the potential risk of eliciting undesired effects (6), the combination of microsuture nerve repair with adjunct OEC transplantation may provide a useful combinatorial approach to enhance peripheral nerve repair.

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