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Review

Interaction of olfactory ensheathing cells with other cell types *in vitro* and after transplantation: Glial scars and inflammation

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ABSTRACT

Olfactory ensheathing cells (OECs) have been investigated extensively as a therapy to promote repair in the injured CNS, with variable efficacy in numerous studies over the previous decade. In many studies that report anatomical and functional recovery, the beneficial effects have been attributed to the ability of OECs to cross the PNS–CNS boundary, their production of growth factors, cell adhesion molecules and extracellular matrix proteins that promote and guide axon growth, and their ability to remyelinate axons. In this brief review, we focus on the interaction between OECs and astrocytes *in vivo* and *in vitro*, in the context of how OECs may be overcoming the deleterious effects of the glial scar. Drawing from a selection of different experimental models of spinal injury, we discuss the morphological alterations of the glial scar associated with OEC transplants, and the *in vitro* research that has begun to elucidate the interaction between OECs and the cell types that compose the glial scar. We also discuss recent research showing that OECs bear properties of immune cells and the consequent implication that they may modulate neuroinflammation when transplanted into CNS injury sites. Future studies in unraveling the molecular interaction between OECs and other glial cells may help explain some of the variability in outcomes when OECs are used as transplants in CNS injury and more importantly, contribute to the optimization of OECs as a cell-based therapy for CNS injury. This article is part of a Special Issue entitled: Understanding olfactory ensheathing glia and their prospect for nervous system repair.

Contents

Introduction

The primary olfactory pathway constitutes a remarkable structure in terms of its anatomy and physiology. Dendrites of individual olfactory neurons are exposed partly on the surface of the olfactory epithelium while their axons course through the cribriform plate and synapse with second order neurons in the olfactory bulbs located in the central nervous system (CNS). Olfactory neurons are regularly replaced by new neurons derived from stem cells in the olfactory epithelium, implying constant axonal regrowth from the peripheral epithelium into the CNS (Chuah et al., 2003; Mackay-Sim and Kittel, 1991). Olfactory neurons have a close relationship with an equally remarkable and unique glial cell: the olfactory ensheathing cell (OEC). Olfactory ensheathing cells bear characteristics of astrocytes and Schwann cells, and ensheath olfactory axons for their full length from the epithelium to the olfactory bulb (Chuah and West, 2002; Doucette,

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1990). Unlike Schwann cells which originate from neural crest cells, precursors of OECs reside in the olfactory epithelium and migrate with, and ahead of olfactory axons, as they grow towards the bulb during development (Chuah and Au, 1991; Tennent and Chuah, 1996). Due to the substantial evidence that OECs provide trophic support for olfactory neuron regeneration (Kafitz and Greer, 1999; Lipson et al., 2003; Woodhall et al., 2001), their possible path-finding role and their relative accessibility in humans, OECs have been proposed as candidate cells for autologous implantation into spinal cord lesions and other CNS injuries (Barnett and Chang, 2004; Mackay-Sim et al., 2008).

Many studies that utilize OECs as a therapeutic agent for repair in the CNS have focussed on their capacity to induce regeneration of the injured axons (Bartolomei and Greer, 2000; Deumens et al., 2006; Guntinas-Lichius et al., 2001). This brief review gives an appraisal of OECs as a cell-based transplantation therapy by presenting data in the context of interactions between OECs, other glial cells and fibroblasts. The possible influence of OECs on inflammation is also discussed in light of recent findings that OECs have the potential to produce cytokines and display innate immune properties and thus modify the characteristics of the constituent cells of the glial scar.

Olfactory ensheathing cells and glial scar components in experimentally induced models of spinal injury

The primary objective of studies utilizing OEC transplants was to establish whether OECs are able to induce regrowth of injured axons. Therefore, considerably less attention has been paid to the interaction between OECs and cells involved in glial scarring. Olfactory ensheathing cells have been transplanted into several spinal cord injury models with variable degrees of success (Li et al., 1998; other references in Table 1). These discrepancies could be due to a number of reasons, e.g. the source and method of purification of OECs, different injury models leading to different temporal sequences of glial scarring and inflammation and the time of transplantation (acute vs. chronic). The studies listed in Table 1 are by no means comprehensive but they demonstrate various spinal cord related injury models and the variability in functional and anatomical outcomes consequent to the use of OEC transplants in different laboratories. A more comprehensive survey of different lesion models and their outcomes is reviewed elsewhere (Richter and Roskams, 2008).

Electrolytic lesion

Electrolytic lesions induced by passing direct current for several minutes into tissues, are small, circumscribed lesions that can be precisely performed on specific tracts, such as the corticospinal tract in the spinal cord (Li and Raisman, 1995). Severance of the tract results in massive cell death of oligodendrocytes in the first few days (Li et al., 1999), followed by progressive hypertrophy of longitudinal astrocytic processes from 1 to 13 weeks after the lesion. By the end of this period, axonal sprouts from the severed tract have ramified within a macrophage-filled central area of lesion, surrounded by a dense astrocytic scar. In addition, Schwann cells were reported to migrate into the lesion and myelinate some of the severed axons (Li and Raisman, 1995). Using this injury paradigm, Li and co-workers transplanted semi-purified OECs isolated from adult rat olfactory bulbs into the lesion site (Li et al., 1997, 1998). Based on positive

Table 1
Examples of glial tissue response in spinal cord and rhizotomy injury models transplanted with olfactory ensheathing cells.

Injury model	Reported outcome	References
Electrolytic lesion of CST; immediate transplantation of OB-derived OECs + fibroblasts	3 weeks post-lesion, no astrocytic hypertrophy; no astrocytic scars. Presence of axonal regrowth.	Li and Raisman, 1995; Li et al., 1997, 1998
Unilateral electrolytic lesion of CST at cervical level; 8 weeks post-lesion transplantation of OB-derived OECs + fibroblasts	3 weeks post-transplantation, lesion site closed off by glial scar. Functional recovery and axonal regrowth if transplant is introduced precisely into lesion site.	Keyvan-Fouladi et al. 2003
Photochemical lesion of at T12/L1 level; immediate transplantation of OB-derived OECs	15 days post-lesion, reduced astrogliosis with most astrocytes showing short, thin processes. Reduction in size of cystic cavities. Axonal regrowth not assessed.	Watson et al., 1986; Bunge et al., 1994; Verdú et al., 2001, 2003
Dorsal column transection at thoracic level; immediate transplantation of encapsulated and non-encapsulated OB- and LP-derived OECs	5 weeks post-lesion, extensive necrosis, macrophage infiltration and tissue scarring. Some axonal regrowth and collateral sprouting from intact ventral CST.	Chuah et al. 2004
Dorsal column transection at cervical level; immediate transplantation of LP-derived OECs	28 days post-lesion, glial scar transformed by reorganization of astrocytic alignment and CSPG deposition. Minimal cavity formation. Shows axonal regrowth.	Ramer et al. 2004a
Complete thoracic spinal transection; immediate transplantation of OB-derived OECs	7 months post-lesion, presence of glial scar. Intermingling of OECs and astrocytes in scar. Gliotic tissue did not inhibit OEC migration. Shows functional recovery and axonal regrowth.	Ramon-Cueto et al. 2000
Complete thoracic spinal transection; immediate and 1-week delayed transplantation of OB-derived OECs	9 months post-lesion, GFAP and NG2 downregulated in perilesional cord segments. Acute transplantation of OECs produced better functional and histological outcome than delayed transplantation.	López-Vales et al. 2006
Complete thoracic spinal transection; delayed (45 days) transplantation of OB-derived OECs	5 months post-transplantation, astrogliosis is not reduced. Shows functional recovery and axonal regrowth.	López-Vales et al. 2007
Moderate thoracic spinal contusion; 1-week delayed transplantation of OB-derived OECs	12 weeks post-lesion, GFAP, CSPG and cavitation reduced, axons present in graft. In the same study, Schwann cells promoted better sparing/regeneration.	Takami et al. 2002
Moderate thoracic spinal contusion; OB-derived OECs transplanted 30 min or 7 days post-lesion	8 weeks post-lesion, OEC promoted more sparing/regeneration of supraspinal axons than non-transplanted spinal cord. OECs reduced cavitation; better integration with host tissue in delayed transplantation.	Plant et al. 2003
Mild cervical spinal contusion; OB-derived OECs transplanted at time of lesion	3 months post-lesion, OECs aggregated or formed cellular trabeculae in cysts; transplant contains mixture of astrocyte processes, neurites and OECs.	Collazos-Castro et al. 2005
Cervical rhizotomy at multiple levels; immediate transplantation of OB-derived OECs at DREZ	2 months post-transplantation, OEC injection site enclosed by reactive astrocytes. No significant axonal regeneration.	Gomez et al. 2003
Lumbar rhizotomy at DREZ; immediate transplantation of OB-derived OECs + fibroblasts	2 weeks post-lesion, astrocytic processes had been reorganized into ladder-like structure which intermingled with processes of peripheral nerve. Axonal regrowth past OEC transplant.	Li et al. 2004
Lumbar rhizotomy; immediate transplantation of OB-derived OECs	6 months post-lesion, extensive astrogliosis. No significant functional and axonal recovery	Riddell et al. 2004
Cervical or lumbar rhizotomy; immediate transplantation of LP-derived OECs	4 weeks post-lesion, presence of laminin-rich injection track. OECs transformed scar into discontinuous, GFAP-positive perimeter. No axonal regrowth.	Ramer et al. 2004b

immunostaining for $p75^{NTR}$, glial fibrillary acidic protein (GFAP), fibronectin and L1, it was reported that the OECs aggregated in the central area of the lesion surrounded by astrocytic processes which only slightly upregulated their GFAP expression. Furthermore, sprouts from the severed tracts traversed the transplant and continued in the spinal cord caudal to the lesion. Using the same injury model, a similar histological finding, accompanied by recovery of directed forepaw retrieval, was obtained when OEC transplants were placed precisely into the central lesion cavity 8 weeks after electrolytic damage of the corticospinal tract (Keyvan-Fouladi et al., 2003). Functional recovery was absent if the OEC transplant was misplaced a few hundred microns dorsal to the lesion cavity, suggesting that interaction between OECs and hypertrophic astrocyte processes was a prerequisite for functional recovery.

Photochemical lesion

A photochemical lesion of the spinal cord is induced by injecting a photosensitizing organic dye, e.g. Rose Bengal, and then irradiating with light at a specific wavelength. Excitation of the dye induces free radical production that leads to endothelial dysfunction, platelet aggregation and ultimately vascular occlusion (Bunge et al., 1994; Watson et al., 1986). The resultant oedema and tissue necrosis creates a large lesion cavity, which by 5 days contains debris-laden macrophages (Bunge et al., 1994). The lesion cavity is markedly more extensive than that induced by an electrolytic lesion and spans the white and gray matter of the dorsal half of the spinal cord. The border of the lesion cavity is marked by several layers of flattened astrocytes coated with basal lamina and surrounded by collagen fibrils (Bunge et al., 1994). By 2 weeks, meningeal cells and Schwann cells have migrated into the cavity and some of them myelinate axons at the injury site. Olfactory ensheathing cells transplanted into a photochemical spinal cord lesion surrounded the lesion cavity, which was significantly smaller with a larger area of preserved spinal cord than in non-transplanted animals (Verdú et al., 2001, 2003). Immunostaining showed that most of the astrocytes in the OEC-transplanted rats did not hypertrophy, had short, thin processes and expressed lower levels of proteoglycans. Although no neuroanatomical techniques were used to demonstrate regeneration of axonal fibers in these studies, OEC-transplanted rats showed better locomotor ability and had larger amplitudes of motor- and somatosensory-evoked potentials (Verdú et al., 2003).

Dorsal column and complete spinal transection

A common experimental model is physical sectioning of the spinal cord, either to the dorsal column or a complete transection which severs all ascending and descending tracts. Such a lesion induces massive infiltration of fibroblasts and macrophages, reorganization and hypertrophy of astrocytes into dense walls of cells and the formation of cystic cavities (Lagord et al., 2002; Ramer et al., 2004a; Silver and Miller, 2004). Table 1 provides examples of dorsal columnar spinal transection models using acute or delayed transplantation of OECs derived from the lamina propria or the olfactory bulb. Ramer et al. (2004a) reported a reduction in cavity formation and transformation of the glial scar when OECs were transplanted following dorsal column transection. The astrocytes realigned their processes to form a diffuse glial fibrillary acidic protein-positive boundary and CSPG was deposited diffusely along laminin-rich channels. This novel environment may be crucial for promoting extensive sprouting of sensory and motor axons into the lesion site (Ramer et al., 2004a). In comparison, when OECs were transplanted into the injured dorsal column in polyvinylidene fluoride capsules, cystic cavities persisted in many animals although collateral growth from the intact ventral corticospinal tract was observed (Chuah et al., 2004). The persistence of cystic cavities was likely due to compression pressure resulting from the capsule or the lack of direct cellular contact between OECs and spinal cord tissue.

Although the study by Ramer et al. (2004a) suggested that glial scar alteration could be crucial to axonal regrowth, studies by others (López-Vales et al., 2007, 2006; Ramon-Cueto et al., 2000) cast doubt on this view. In these studies, the rat spinal cord was completely transected at the thoracic level and OECs were transplanted at the time of lesion or post-lesionally by one week or 45 days. Following recovery in both OEC-transplanted and non-transplanted spinal cords, the spinal transection site was marked by a wide zone of tissue lacking GFAP staining (Ramon-Cueto et al., 2000). This zone was of meningeal origin while the cut ends of the two spinal cord stumps showed intense immunoreactivity for GFAP, both in the presence and absence of OECs. When OECs were transplanted one week post-lesion, GFAP and NG2 were downregulated in the perilesional cord segments, while transplanting OECs 45 days after the injury did not reduce post-traumatic astrogliosis as assessed by quantitative analysis of GFAP immunoreactivity (López-Vales et al., 2007, 2006). Despite these differing effects on astrogliosis, regrowth of corticospinal and raphespinal axons was demonstrated for both transplant conditions. Hence there are likely to be additional molecular signals regulated by OECs that may directly or indirectly influence neural repair.

Contusion lesion

Contusion lesions are often induced experimentally by dropping a blunt weight on to the spinal cord from different heights using devices such as the MASCIS impactor (Gruner, 1992). The resulting compression results in the gradual destruction of tissues, ultimately leading to the formation of a central fluid-filled cavity. The dura remains intact and consequently few fibroblasts are found in the scar tissue that forms around the injury (Oudega, 2007). When OECs were transplanted into moderately contused thoracic spinal cord, they appeared to aggregate or form trabeculae in the cysts, thus reducing the cystic volume (Collazos-Castro et al., 2005). Immunoreactivity for GFAP and CSPG was also reduced around the lesion site (Plant et al., 2003; Takami et al., 2002). Although the OEC transplants contained astrocytic processes and neurites, improvement in locomotor function was not a consistent outcome (Collazos-Castro et al., 2005; Plant et al., 2003; Takami et al., 2002).

Olfactory ensheathing cell transplants in the rhizotomy injury model

Following rhizotomy, the central processes of the dorsal root ganglion cells retain their regenerative capacity and regrow their axons up to the dorsal root entry zone (DREZ). However, these centrally directed axons are unable to grow through the transitional region between the PNS and CNS at the DREZ to enter the spinal cord (Carlstedt, 1997; Chong et al., 1999). At the DREZ, reactive astrocytes normally reform a continuous glial-pial membrane with an outward facing basal lamina that excludes the peripheral Schwann cells and is impenetrable to regenerating axons (Liuzzi and Lasek, 1987). The first study on OEC promotion of regeneration after rhizotomy reported that following injection of OECs into the DREZ, calcitonin gene-related peptide- and GAP-43-positive axons crossed the DREZ to enter the dorsal horn of the spinal cord (Ramon-Cueto and Nieto-Sampedro, 1994).

Li and colleagues have formulated the 'pathway hypothesis' which proposes that interactions between astrocytes and transplanted OECs in the CNS establish a permissive pathway for regenerating axons (Li et al., 2004). Experiments from Li's group showed that if OECs were transplanted into the entry zone following rhizotomy there was extensive outgrowth of astrocyte processes, which interwove with OEC and Schwann cell processes, forming bridges that allowed dorsal root axons to regenerate into the spinal cord (Li et al., 2004). Subsequently, they propose that OECs interact with astrocytes to

induce a rearrangement of tissue that creates a physical route along which new axons can grow. However, other groups utilizing the rhizotomy model have reported vastly different results. For example, Ramer and co-workers reported transformation of the glial scar following OEC transplantation after rhizotomy at the cervical and lumbar levels, but found no evidence for axon regrowth (Ramer et al., 2004b). The failure of OECs to promote regeneration of sensory afferents into the spinal cord has also been reported by other laboratories (Table 1). The reason for this discrepancy is not known but it has been proposed that OECs are able to promote regeneration only when the preferred migration of these cells coincide with the direction of new nerve sprouts (Nieto-Sampedro, 2003; Ramon-Cueto and Nieto-Sampedro, 1994). Thus a deeper understanding of the molecular signals that govern OEC orientation and behavior in the lesion site may be required to optimize their therapeutic potential. *In vitro* studies, where more precise molecular techniques can be applied on purified populations of specific types of cells could provide valuable relevant evidence.

Do OECs require assistance from Schwann cells or fibroblasts to elicit repair?

Despite positive outcomes being reported in several studies following OEC transplantation, the differences in histological and functional outcomes raises questions about their reproducibility. One complicating issue is the infiltration of Schwann cells into the injury site. Schwann cells have long been used experimentally to induce repair of the injured CNS (Martin et al., 1996; Richardson et al., 1980; Xu et al., 1997). Thus it is possible that the beneficial effects attributed to OECs may be partly due to interaction with invading Schwann cells. It has even been proposed that OECs only play an indirect role in promoting repair, by facilitating invading Schwann cells to form a matrix that is conducive to nerve growth and myelination (Boyd et al., 2005). The latter proposal is based largely on a review of ultrastructural observations drawn from several studies. Clearly, future studies involving molecular signaling between OECs and Schwann cells would help to elucidate the role of OEC and Schwann cell interaction in promoting CNS repair.

It is worth noting that lesion studies conducted by Li and co-workers have used transplants that are composed of olfactory bulb-derived, $p75^{NTR}$-expressing OECs and fibronectin-expressing fibroblasts (Li et al., 1998, 2003). They propose that the fibroblasts are crucial to the regenerative properties of OECs (Raisman, 2001). Similarly, others report that remyelination by OECs in an X-irradiation/ethidium bromide model is enhanced by the presence of meningeal cells in the transplant (Lakatos et al., 2003). Olfactory ensheathing cells appeared to form myelin sheaths around the large diameter axons in the demyelinated spinal cords and these were separated into fascicles by surrounding fibronectin-expressing fibroblasts. The presence of fibroblasts increased both the remyelination of axons and the number of myelinating cells in the lesions (Lakatos et al., 2003). It was proposed that the increase in extracellular matrix secreted by meningeal cells contributed to enhanced remyelination by OECs. Other beneficial effects may be attributed to growth factors such as fibroblast growth factor 2 secreted by meningeal cells which promote OEC proliferation, survival and migration over a larger area in the lesion site. Although how meningeal cells or fibroblasts interact with OECs in the environment of the lesion site remains a complex issue, *in vitro* studies that restrict OEC interaction to one or two specific cell populations have begun to yield some interesting findings (refer to following section).

In vitro interaction between OECs and components of the glial scar

In vitro studies that examine OEC interaction with astrocytes often include Schwann cells for comparison because these two cell types have been used as a cell-based therapy to stimulate repair (Fairless et al., 2005; Lakatos et al., 2000; Santos-Silva et al., 2007). However, the discussion here will focus primarily on the reciprocal influence between OECs and astrocytes. In co-cultures with astrocytes, OECs intermingle and migrate amongst astrocytes without forming boundaries (Fairless et al., 2005; Lakatos et al., 2000; Santos-Silva et al., 2007). Although the molecules mediating the spatial integration between OECs and astrocytes are yet to be defined, the repertoire of cadherins (cadherin-3, cadherin-5 and MT-protocadherin) and integrins (alpha 1, alpha 6, alpha 7 and beta 4) expressed by OECs are candidates for future investigations (Franssen et al., 2008). The net effect of differential expression of these proteins could determine whether specific groups of OECs remain associated with particular clusters of astrocytes or demonstrate a more motile behavior amongst the astrocytes.

A related issue is the question of directional cues regulating OEC migration. Whilst several studies have reported that OECs are capable of migrating from the injection site, over white matter tracts and gray matter (Ramon-Cueto et al., 2000, 1998; Resnick et al., 2003), problems associated with certain dyes have complicated interpretation of these claims, for example, the possibility of dye leakage or the engulfment of dead pre-labeled OECs by macrophages. In contrast, labeling of OECs by viral vector-mediated expression of GFP has revealed a more conservative migratory capability, while others have suggested that the eventual localization of OECs was more likely due to passive spreading rather than active migration (Lu et al., 2006; Ruitenberg et al., 2002). Recent *in vitro* experiments have indicated possible mechanisms determining the path of OEC migration. A Boyden migration assay revealed that OECs exhibit chemotaxis in response to tumor necrosis factor-α (TNF-α) secreted by activated astrocytes (Su et al., 2009). The same study also showed that following spinal hemisection, a linear expression gradient of TNF-α was formed in the glial scar, and this peaked at 7 days after injury. Therefore, in studies that failed to show OEC migration, the concentration of TNF-α may have been too low. In addition, OECs express other molecules that promote (collagen triple helix repeat containing 1 and tenascin-C) and inhibit migration (CD9 and receptor activity-modifying protein 3) that should be considered in future studies (Franssen et al., 2008).

The presence of OECs does not induce the stress responses of hypertrophy or increased GFAP in astrocytes (Lakatos et al., 2000). However, hypertrophic reaction in astrocytes can be induced in co-cultures with OECs if the cells are exposed to medium conditioned by Schwann cells and supplemented with heparin and fibroblast growth factor 2, suggesting that the heparin sulfate proteoglycan profile of OECs may be involved in modulating the reactive phenotype in astrocytes (Santos-Silva et al., 2007). The use of an *in vitro* model of astrogliosis induced by needle scratch injury, has also confirmed the modulating effects of OECs. The resulting reactive astrocytes expressed significantly less GFAP when cultured both in direct contact with OECs and when the two cell types were separated by a porous membrane (O'Toole et al., 2007). Immunofluorescence staining also suggested that reactive astrocytes showed decreased CSPG in the presence of OECs, although the reduction was not statistically significant.

When spinal cord injury involves the rupture of meninges, meningeal cells invade the lesion site and become a component of the glial scar (Silver and Miller, 2004). Thus, their interaction with transplanted OECs is another contributing factor to the physiological milieu. *In vitro* experiments showed that unlike Schwann cells which formed clusters when co-cultured with meningeal cells, OECs intermingled with meningeal cells and formed very small or no clusters (Franssen et al., 2009).

Thus far, many of the studies on OEC interaction with cells of the glial scar have employed morphological techniques such as immunostaining intensities to assess how the physical aspect of the scar may be altered by OECs. The elucidation of molecular mechanisms involved in OEC–astrocyte interaction in future studies, using more precise molecular biology techniques will be the new tools to biochemically modify the lesion environment to improve permissivity

Table 2
Cytokines and cytokine receptors expressed in olfactory ensheathing cells.

Cytokines and receptors	Method of detection	References
CXCL1/Gro 1	Microarray analysis, immunostaining	Vincent et al., 2005; Franssen et al., 2008
CXCL12	Microarray analysis	Franssen et al. 2008
CX3CL1	Microarray analysis	Franssen et al. 2008
CXCR4	Microarray analysis	Franssen et al. 2008
Interleukin-6/Interferon β2	Immunostaining	Nan et al. 2001
Interleukin-6 receptor	Immunostaining	Nan et al. 2001
Leukemia inhibitory factor receptor	Immunostaining	Nan et al. 2001
Interleukin-1 receptor 1	Microarray analysis	Pastrana et al. 2006
Monocyte chemotactic protein-1	Microarray analysis, immunostaining	Vincent et al. 2005
TROY	In situ hybridization, immunostaining	Hisaoka et al. 2004
Ciliary neurotrophic factor (CNTF)	Immunostaining, RT-PCR	Asan et al., 2003; Wewetzer et al., 2001
CNTF receptor α	RT-PCR	Wewetzer et al. 2001

for axon regeneration. An example of this is the recent discoveries that OECs respond to a range of scenarios by producing cytokines, chemokines and other molecules which have previously been associated with cells of the innate immune system. This response is amenable to molecular characterization and given the degree of commonality associated with stress, infection and injury models, and the likely effect of these molecules on other cells in a lesion environment, is worthy of further investigation.

OECs and neuroinflammation

The expression of cytokines and their receptors in OECs has been reported in a number of studies (Table 2), notably those employing microarray analyses (Franssen et al., 2008; Pastrana et al., 2006; Vincent et al., 2005), which have the potential to uncover even more cytokines in the future. Microarray analyses have shown that OECs express CXCL1 (Gro1), CCL2 (MCP-1), CX3CL1, CXCL12 (stromal cell-derived factor-1) and its receptor CXCR4. CXCL1, CCL2 and CXCL12 are signaling factors for recruiting neutrophils and various leukocytes (Babcock et al., 2003; Bleul et al., 1996; McKimmie and Graham, 2010). This suggests that OECs are likely to interact with immune cells in regulating inflammation, thus playing a role in the repair process. Moreover their expression of CXCR4 indicates that CXCL12 could have an autocrine effect on OEC cytokine activity.

In studies involving olfactory bulbectomy (Nan et al., 2001), OECs upregulated their expression of interleukin 6 (IL-6), IL-6 receptor and leukemia inhibitory factor receptor (LIFR) at 3 days post-surgery. This coincided with the infiltration of macrophages into the lamina propria. Thus the data suggest that OECs could interact with macrophages secreting IL-6 and LIF (Gauldie et al., 1987; Getchell et al., 2002). Consistent with this possible interaction is the finding that OECs express CX3CL1 which binds to CX3CR1 expressed by macrophages and dendritic cells (Chinnery et al., 2007).

TROY is a member of the tumor necrosis receptor superfamily, which induces activation of nuclear factor kappaB and has been localized to OECs (Hisaoka et al., 2004). Although the precise role of TROY in OECs is uncertain, it potentially has the ability to regulate cytokine production by OECs. Although the presence of the ciliary neurotrophic factor (CNTF) and

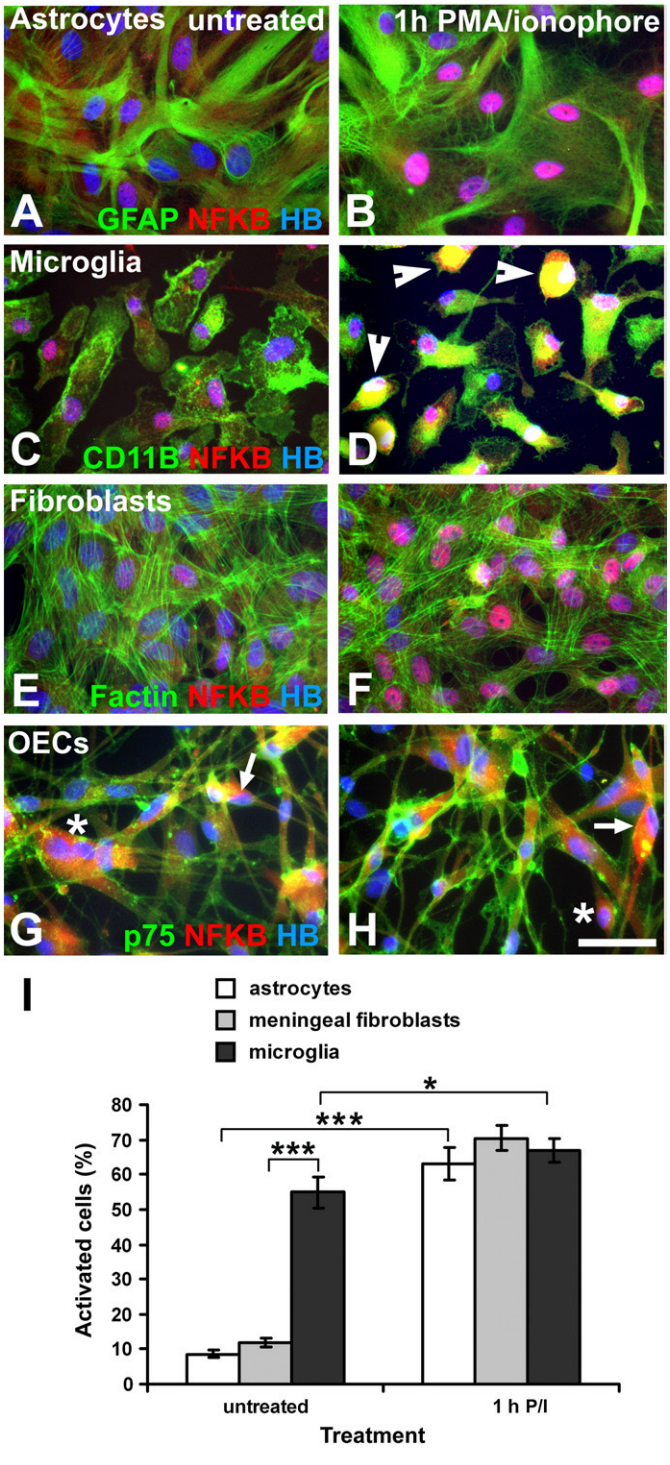

Fig. 1. PMA/calcium ionophore activate astrocytes, microglia, meningeal fibroblasts but not OECs. Cultures were immunostained for NFκB (red), GFAP for astrocytes (A, B), CD11B for microglia (C, D) and $p75^{NTR}$ for OECs (G, H). Meningeal fibroblasts (E, F) were delineated with phalloidin staining of cytoskeletal actin filaments (green). Nuclei were stained with Hoechst blue (HB). The more intense nuclear staining for NFκB following activation by PMA/calcium ionophore was observed for astrocytes (B), microglia (D) and meningeal fibroblasts (F) but not for OECs (H). After PMA/calcium ionophore stimulation, some microglia appeared to be highly activated, displaying rounded morphologies with intense NFκB and CD11B immunostaining (arrowheads in D). OECs displayed variable cytoplasmic NFκB staining in cell bodies and processes with no difference in the NFκB staining between untreated (G) and PMA/calcium ionophore treated (H) cultures. Where cytoplasmic NFκB staining was intense there was faint nuclear staining (asterisks in G and H) probably due to overlying cytoplasmic staining. This was confirmed by fortuitously orientated OECs that allowed Hoechst blue-stained nuclei, devoid of NFκB, to be discerned distinct from adjacent bright cytoplasmic NFκB staining (arrows in G and H). Scale bar is 50 μm. I: Quantitative analysis of PMA/calcium ionophore (P/I) activation of astrocytes, microglia and meningeal fibroblasts. There were significantly more activated microglia than astrocytes or fibroblasts in the untreated cultures ($p<0.001$). PMA/calcium ionophore induced significant increases in activation of the three cell types ($p<0.05$ for microglia; $p<0.001$ for astrocytes and fibroblasts). Percentages are means ± SEM; $^{*}p<0.05$, $^{***}p<0.001$.

its receptor in OECs (Asan et al., 2003; Wewetzer et al., 2001) is generally thought to be associated with their axonal growth-promoting property, its precise function is yet to be elucidated. It is known that astrocytes upregulate their expression of CNTF as part of the gliotic response in the aftermath of injury (Guthrie et al., 1997), while mice lacking CNTF demonstrate motor neuron degeneration (Masu et al., 1993).

The finding that OECs produce cytokines and can function as innate immune cells has implications for their use in CNS regeneration studies. Olfactory ensheathing cells produce cytokines that can act on other immune cells such as neutrophils, macrophages and lymphocytes and can phagocytose cellular debris and pathogens (Leung et al., 2008). These mechanisms would allow OECs to modulate inflammation at an injury site. The possibility that OECs promote neural repair by modulating inflammation has not been subject to extensive investigation. Lopez-Vales et al. (2004) showed that photochemically-injured spinal cords with transplanted OECs demonstrated a significantly higher reactivity for iNOS and IL-1β at 7 days post-injury than control injuries without transplanted OECs, while at a later time point no difference was observed between the two groups. Rats transplanted with OECs also showed a higher locomotor score and improved electrophysiological recovery (Lopez-Vales et al., 2004). However, because the OECs were not pre-labeled prior to transplantation, it was uncertain whether all the iNOS- and IL-1β-positive cells were OECs.

Cytokine production by transplanted OECs could possibly have pro- or anti-inflammatory effects, being most likely subject to spatiotemporal variation dependent on interaction with surrounding activated glial cells and their secretions. Although inflammation initiates tissue regeneration responses essential for wound healing (Farooqui et al., 2007; Kracht and Saklatvala, 2002), more severe and prolonged inflammation is associated with increased astrogliosis, secondary neuronal damage and worse functional deficits after CNS injury (Ridder and Schwaninger, 2009; Stichel and Muller, 1998; Swanson et al., 2004). A key facilitator of responses associated with injury and inflammation is the signaling pathway based on nuclear factor kappaB (NFκB), and indeed, moderation of inflammation by limiting NFκB activation can therefore produce beneficial effects in a variety of injury models (Fernandez et al., 2007; Khorooshi et al., 2008). Therefore, an important question is whether OECs, or molecules produced by OECs can impact on the NFκB pathway in astrocytes and other components of the glial scar following injury or stress.

Inflammatory activation of cellular components of the glial scar can be induced *in vitro* as indicated by nuclear translocation of p65 NFκB after treatment with the known stimulants, phorbol myristate acetate (PMA) and calcium ionophore (Fig. 1). PMA binds to the activation site of calcium-dependent protein kinase (PKC) (Amos et al., 2005; Tsuboi et al., 1994), an important intracellular signaling molecule in NFκB activation pathways (Baeuerle and Henkel, 1994; Silberman et al., 2005). The divalent cation transporter, calcium ionophore can induce increases in intracellular free Ca^{2+} (Kolber and Haynes, 1981) to activate PKC (Medkova and Cho, 1999) and other signaling pathways that can lead to NFκB activation (Baeuerle and Henkel, 1994; Brettingham-Moore et al., 2005). NFκB regulates many of the protein expression changes that characterize astrogliosis, including increased expression of cytokines (Meeuwsen et al., 2003), chemokines (Khorooshi et al., 2008), adhesion molecules (Ballestas and Benveniste, 1995), iNOS (Zhang et al., 2007), granulocyte macrophage-colony stimulating factor (GM-CSF) (Zaheer et al., 2007) and glial fibrillary acidic protein (GFAP) (Bae et al., 2006).

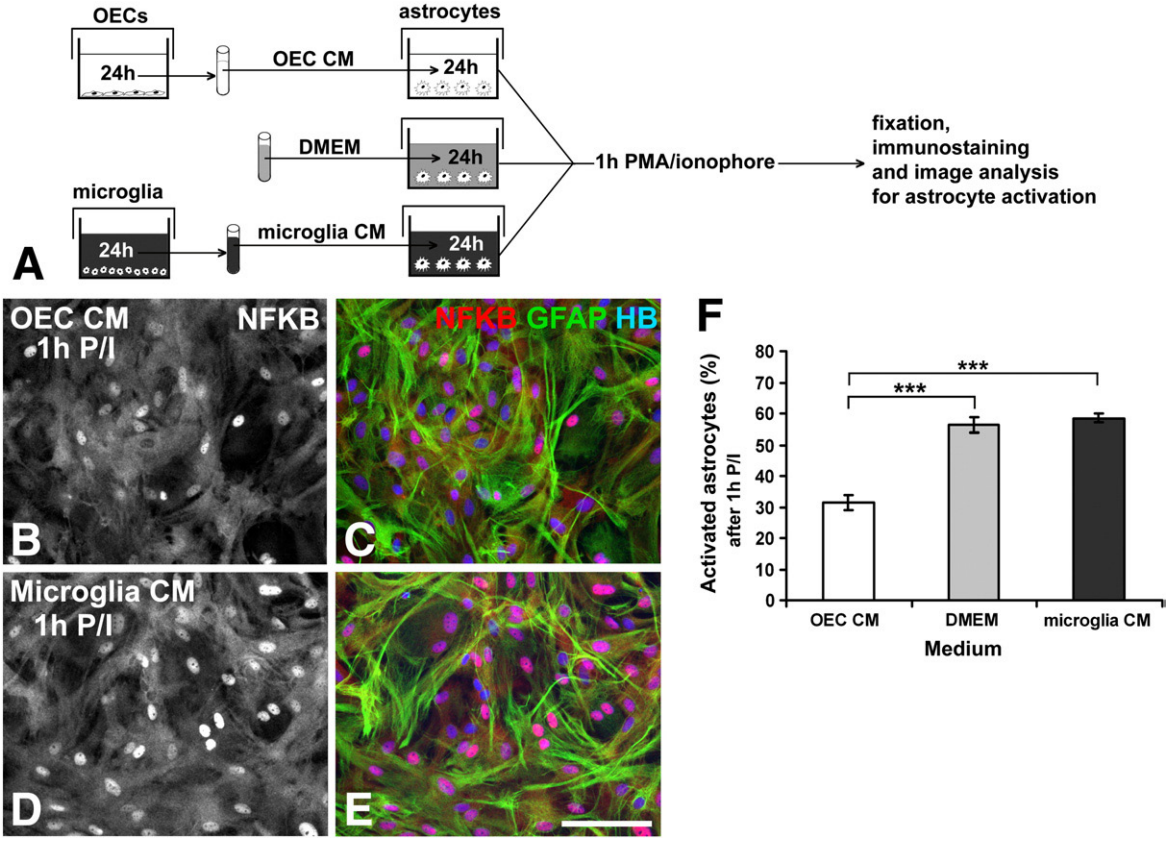

Fig. 2. OEC-conditioned medium moderates activation of astrocytes by PMA/calcium ionophore. Astrocytes in DMEM and astrocytes pre-incubated in medium conditioned by OECs or microglia, were stimulated with PMA/calcium ionophore (A). Cultures were immunostained for NFκB (B, D and red in C, E) and GFAP (green in C, E). Nuclei were counterstained with Hoechst blue (C, E). Following activation, more astrocytes in microglia-conditioned medium (D, E) had intense nuclear NFκB immunostaining than astrocytes in OEC-conditioned medium (B, C). Scale bar is 100 μm. F: Quantitative analysis of activated astrocytes after 1 h stimulation with PMA/calcium ionophore in OEC-conditioned medium, microglia-conditioned medium or DMEM. There was significantly less astrocyte activation in OEC-conditioned medium than when in microglia-conditioned medium or DMEM ($p<0.001$). Percentages are means ± SEM; ***$p<0.001$.

Because activated astrocytes are the major contributor to glial scars, it is relevant to ask whether soluble secretions from OECs could attenuate astrocyte activation. Activation of astrocytes by PMA/calcium ionophore was least if the astrocyte cultures were pre-incubated in OEC-conditioned medium compared to those exposed to microglia-conditioned medium or unconditioned medium (Fig. 2A). These findings suggest that OECs transplanted into the injury site of the CNS could have a role in downregulating inflammation. Given that different lesion models induce different degrees of tissue damage and inflammation profile, a key strategy in future studies is to determine the optimal site and time of transplantation following injury.

In summary, although OECs have clearly produced beneficial effects in promoting repair in several injury paradigms, there are also cases in which their advantages as a therapeutic agent are not apparent. These discrepancies highlight the complexity of OEC biology as well as the intricate dynamics of cell–cell interaction in the injury site. Recent studies have begun to investigate OEC interaction with astrocytes in moderating the detrimental effects of glial scar. Future studies will also need to investigate interaction between OECs and microglia, the CNS resident immune cells, particularly in relation to OEC regulation of neuroinflammation. These issues need to be addressed if OECs are to be developed as a reliable and relevant therapeutic agent for neural repair.

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