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PERSPECTIVE

Olfactory ensheathing cells for spinal cord repair: crucial differences between subpopulations of the glia

OECs for spinal cord repair: Is repairing the injured spinal cord by olfactory ensheathing cell (OEC) transplantation possible? A recent human trial in which a paralysed man regained some function after transplantation of partially purified OECs suggests that this therapy may be a successful approach (Tabakow et al., 2014). In another human trial in which olfactory mucosa lamina propria was transplanted, patients recovered some motor and sensory function (Wang et al., 2015). While these results show promise, it is clear that improvements are needed to provide patients with increased functional output. Strategies to improve the therapeutic use of OECs may include improving the purification of the OECs used for transplantation, using them in combination with growth factors to combat the inhibitory environment and improve axon growth, the use of nerve bridges, advanced physiotherapy and the use of exoskeleton robotics to reinforce functional connections. Of all these approaches, it is probably crucial that the purity of OECs is primarily addressed to ensure consistency in outcomes.

What makes OECs useful for spinal cord repair? OECs are the glia of the peripheral olfactory nerve and provide support to the olfactory sensory neurons. The OECs do not myelinate individual olfactory sensory axons, but instead they wrap up numerous bundles of axons to form the olfactory nerve fascicles. OECs ensheathe the olfactory sensory axons from the base of the olfactory epithelium that lines the olfactory nasal cavity, to the outer layer of the olfactory bulb within the cranial cavity. Perhaps of therapeutic importance, within the outer layer of the olfactory bulb, OECs interact with astrocytes from the central nervous system. Also of potential significance is that during development when the olfactory axons are first projecting out of the olfactory placode that lines the embryonic nasal epithelium, OECs migrate ahead of axons. This has also been demonstrated using live cell *in vitro* assays in which the motility of OECs directly influenced the movement of the axons (Windus et al., 2011).

The olfactory nervous system constantly regenerates throughout life. As the primary sensory neurons are responsible for detecting odors, they are exposed to pathogens and toxic substances that are inhaled into the nasal cavity. Thus primary sensory neurons frequently die off and are replaced by stem cells that line the base of the olfactory epithelium. Due to the numerous growth factors that OECs express, the newly generated axons successfully grow up into the olfactory bulb and make connections with the second order neurons.

The ability of OECs to promote axon growth, migrate ahead of axons and interact with astrocytes has led them to become leading candidates for cell transplantation therapy to repair the injured spinal cord. After injury to the spinal cord, the inflammatory response and subsequent secondary degeneration creates a hostile environment for regenerating axons and the development of the fibrotic/astrocytic scar creates a physical barrier. By transplanting OECs into the injury site, they can interact with astrocytes to reduce the astrocytic scar and then the OECs can migrate to form a glial bridge to promote the growth of axons across the injury site. The ability of OECs, or OECs together with olfactory nerve fibroblasts, to facilitate the repair of the injured spinal cord has been demonstrated in rats (Li et al., 1997), dogs (Granger et al., 2012) and human (Tabakow et al., 2014).

OECs are not all the same – which subpopulation is best for spinal repair? There are several subpopulations of OECs based on their anatomical locations, functions and the molecules that they express. In the main olfactory system, there are three subpopulations: (1) the peripheral OECs that ensheathe the bundles of axons as they project from the epithelium, (2) the OECs of the outer layer of the nerve fibre layer of the olfactory bulb, where the olfactory sensory axons defasciculate, sort out and project towards their topographic target, and (3) the OECs of the inner layer of the nerve fibre layer where the axons refasciculate and then terminate in their target glomeruli where they form synaptic connections with the second order neurons. The OECs of the peripheral nerve and the outer nerve fibre layer express the molecules S100β and p75ntr which are often used as identifying markers for OECs; OECs of the inner nerve fibre layer do not express, or only express low levels of p75ntr and S100β. We have previously shown using live cell imaging that subpopulations of OECs have behavioural differences that are consistent with their roles *in vivo* (Windus 2010). OECs of the peripheral nerve are a uniform population that promote cell-cell interactions, while OECs of the olfactory bulb are heterogeneous and either promote or inhibit cell-cell interactions (Windus et al., 2010). While some researchers favour the use of OECs from the olfactory bulb (Tabakow et al., 2014), the ease of access to obtain OECs from the nasal cavity is favoured by others (Granger et al., 2012). While the different subpopulations of OECs each have potentially favourable characteristics, it is not yet certain which subpopulation is most effective for repairing the injured spinal cord.

In animals, there are also other subpopulations of OECs. For example, the accessory olfactory system is responsible for the detection of pheromones. In rodents, the vomeronasal organ lies rostral-ventral to the main olfactory epithelium and houses the vomeronasal neurons that detect the pheromones. The axons from these neurons project up along the septum and the medial surfaces of the main olfactory bulbs to terminate in the accessory olfactory bulb which lies on the caudal surface of the main olfactory bulb. Thus the axon fascicles of the main and accessory olfactory nerves intermingle and yet remain distinctly separate despite being ensheathed by OECs (**Figure 1**). This indicates that the main and accessory OECs have at least some differing properties.

In order to isolate OECs for transplantation therapies, the olfactory mucosa on the septum or the nerve fibre layer of the olfactory bulb are commonly dissected out. Often these biopsies include portions of the medial surface in which the accessory olfactory fascicles and their OECs are located. Thus such OEC preparations are likely to contain a mix of OECs from both the main and accessory olfactory systems. If the different OECs have differing characteristics and functions, then the unregulated inclusion of accessory OECs could be a source of unwanted variation that will lead to confounding the outcomes of spinal cord repair trials in animal models.

Phagocytosis by OECs: a crucial role for repairing the spinal cord: OECs not only promote the growth of axons, but they are also the principal phagocytic cells of the olfactory nerve. Macrophages are largely excluded from the olfactory nerve even after major injury and instead it is OECs that remove debris that arises from the degenerated axons (Nazareth et al., 2015b), and OECs also phagocytose bacteria (Panni et al., 2013). The ability of OECs to not only phagocytose cell debris but to also potentially exclude macrophages is of therapeutic use for spinal cord repair. After spinal cord injury, the inflammatory response and secondary degeneration can exacerbate the damage with the consequence that the affected area becomes much larger over time. OECs are known to modulate the inflammatory response and they express some immune markers. It is not clear how OECs exert their influence on the immune response within the spinal cord. After transplantation of OECs into the injured spinal cord,

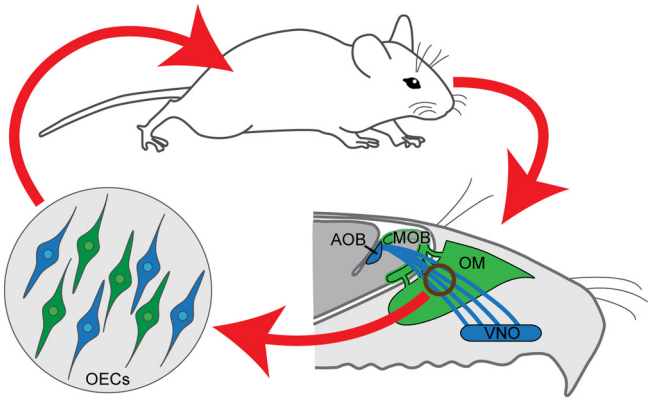

Figure 1 The mouse olfactory system consists of the main olfactory system and the accessory olfactory system.
Within the main olfactory system (green), the olfactory sensory neurons are located in the olfactory mucosa (OM) and project their axons to the main olfactory bulb (MOB). Within the accessory olfactory system (blue), the sensory neurons are located in the vomeronasal organ (VNO) and project their axons to the accessory olfactory bulb (AOB). Olfactory ensheathing cells (OECs) obtained from the septum could contain a mixed population of main and accessory OECs which have differing phagocytic activities. When transplanted into the injured spinal cord, having a mixed population of OECs may lead to variable outcomes.

the phagocytic activity of OECs could rapidly remove cell debris and thereby minimize the recruitment of immune cells and the induction of the secondary degeneration responses. Whether this important aspect of OEC function is consistent across the different subpopulations of OECs needs to be considered particularly if preparations contain mixtures of the different OECs.

To determine the relative capacity of the main and accessory OECs for phagocytosis of axon debris, we examined the phagocytic activity of OECs by tracking the fate of the reporter molecule ZsGreen that is expressed in OMP-ZsGreen transgenic mice that we previously generated (Ekberg et al., 2011). In these mice, the ZsGreen fluorescent protein is strongly expressed by main and accessory olfactory sensory axons. *In vivo* and *in vitro*, the fate of the degenerated axon debris can be easily visualized as it becomes incorporated into vesicles within the phagocytic OECs. We found that *in vivo* the accessory OECs phagocytosed around 20% less axon debris than main OECs. When the OECs were isolated and cultured *in vitro*, the phagocytic activity of the accessory OECs was found to be 20% greater than that of the main OECs (Nazareth et al., 2015a). Thus while they phagocytose less debris within their endogenous environment, when assayed *in vitro* they have a higher capacity to phagocytose cell debris than do main OECs. We further examined the ability of the accessory OECs to respond to injury *in vivo* and found that after a major surgically induced injury to the olfactory nerve, that the accessory OECs rapidly responded by phagocytosing the axon debris. Importantly, macrophages continued to be excluded from the accessory olfactory nerve (Nazareth et al., 2015a), similarly to what we observed in the main olfactory nerve (Nazareth et al., 2015b).

Uniform population of OECs for consistent results: The therapeutic potential for OECs to repair the injured spinal cord is encouraging, however to clarify the efficacy of the therapy it is important that uniform and consistent purity of OECs are obtained. Considering that OECs from the peripheral nerve and from the olfactory bulb have distinctly different behavioural characteristics (Windus et al., 2010) it is clear that the different subpopulations can exert varying effects. When the potential inclusion of accessory OECs is also considered in animal models of spinal cord repair, their differing capacity for phagocytosis of axon debris will introduce another variable that will likely produce variable outcomes and confound the analysis of the therapeutic effect of OECs. Therefore, in order to achieve a more thorough understanding of the therapeutic potential of OECs and to achieve consistent outcomes in spinal injury models, it is crucial that strategies are developed to optimize the purification of the different subpopulations of OECs. As the accessory olfactory nerve bundles project along the septum and medial surfaces of the olfactory bulb, one simple strategy to minimise the potential contamination by the accessory OECs is to avoid harvesting cells from the septum/medial nerve fibre layer and instead harvest cells from the turbinates and lateral margins of the olfactory bulb. By improving the purity of the OEC preparations, we are likely to achieve more consistent outcomes in animal spinal injury models.

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