Molecular and Cellular Neuroscience 65 (2015) 92–101

Contents lists available at ScienceDirect

Molecular and Cellular Neuroscience

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

Differing phagocytic capacities of accessory and main olfactory ensheathing cells and the implication for olfactory glia transplantation therapies

Lynnmaria Nazareth [a,b], Johana Tello Velasquez [b], Katie E. Lineburg [c], Fatemeh Chehrehasa [a,b], James A. St John [b,*], Jenny A.K. Ekberg [a,b,**]

[a] School of Biomedical Sciences, Queensland University of Technology, Brisbane, 4000 Queensland, Australia
[b] Eskitis Institute for Drug Discovery, Griffith University, Nathan, 4111 Queensland, Australia
[c] QIMR-Berghofer Medical Research Institute, Herston, 4006 Queensland, Australia

ARTICLE INFO

Article history:
Received 2 December 2014
Revised 6 February 2015
Accepted 4 March 2015
Available online 6 March 2015

Keywords:
Olfactory nerve
Neuron
Apoptosis
Bulbectomy
Axon
Phagocytosis

ABSTRACT

The rodent olfactory systems comprise the main olfactory system for the detection of odours and the accessory olfactory system which detects pheromones. In both systems, olfactory axon fascicles are ensheathed by olfactory glia, termed olfactory ensheathing cells (OECs), which are crucial for the growth and maintenance of the olfactory nerve. The growth-promoting and phagocytic characteristics of OECs make them potential candidates for neural repair therapies such as transplantation to repair the injured spinal cord. However, transplanting mixed populations of glia with unknown properties may lead to variations in outcomes for neural repair. As the phagocytic capacity of the accessory OECs has not yet been determined, we compared the phagocytic capacity of accessory and main OECs in vivo and in vitro. In normal healthy animals, the accessory OECs accumulated considerably less axon debris than main OECs in vivo. Analysis of freshly dissected OECs showed that accessory OECs contained 20% less fluorescent axon debris than main OECs. However, when assayed in vitro with exogenous axon debris added to the culture, the accessory OECs phagocytosed almost 20% more debris than main OECs. After surgical removal of one olfactory bulb which induced the degradation of main and accessory olfactory sensory axons, the accessory OECs responded by phagocytosing the axon debris. We conclude that while accessory OECs have the capacity to phagocytose axon debris, there are distinct differences in their phagocytic capacity compared to main OECs. These distinct differences may be of importance when preparing OECs for neural transplant repair therapies.

1. Introduction

The olfactory system is characterised by its ability for the sensory neurons to regenerate continuously throughout life in the normal healthy animal as well as after injury and disease. Olfactory ensheathing cells (OECs) are the glia of the olfactory nerves and are intimately associated with the axons of the sensory neurons that line the nasal cavity. The OECs do not myelinate individual axons, but instead ensheathe large bundles of numerous axons. OECs are considered crucial for the regenerative ability of the olfactory system because they support the growth and guidance of axons by their expression of numerous molecules (Boruch et al., 2001; Chuah and Zheng, 1992; Doucette, 1990; Kafitz and Greer, 1999). Due to the continual turnover of olfactory sensory neurons, there is a constant need for removal of the debris arising from degraded axons. Studies have shown that OECs, rather than macrophages, are the main phagocytic cells responsible for the removal of cellular debris throughout development (Nazareth et al., 2015), in the adult (Su et al., 2013) and after injury (Nazareth et al., 2015; Wewetzer et al., 2005).

The numerous molecular and behavioural characteristics of OECs are seen as potentially useful for neural regeneration therapies. The transplant of OECs into the spinal cord has shown promising therapeutic outcomes in humans (Tabakow et al., 2014) and in animal models (Granger et al., 2012; Li et al., 1998, 2003; Ramon-Cueto, 2000), however, other studies have reported no functional or anatomical improvements (Chhabra et al., 2009; Collazos-Castro et al., 2005; Lu et al., 2006). One contributing reason for the variation in outcomes of OEC transplant therapies may be that the preparation of pure cultures of OECs is difficult. OECs are often purified from the olfactory epithelium lining the nasal cavity (Feron et al., 2005; Granger et al., 2012; Stamegna et al., 2011; Tharion et al., 2011) or from the nerve fibre layer of the olfactory bulb (Huang et al., 2012; Tabakow et al., 2014; Toft et al., 2007). In

* Corresponding author.
** Correspondence to: J.A.K. Ekberg, School of Biomedical Sciences, Queensland University of Technology, Brisbane, 4000 Queensland, Australia.
E-mail addresses: j.stjohn@griffith.edu.au (J.A. St John), jenny.ekberg@qut.edu.au (J.A.K. Ekberg).

http://dx.doi.org/10.1016/j.mcn.2015.03.005
1044-7431/

particular in animal models, variations in cell preparations can arise because in both the nasal and olfactory bulb regions the nerve fascicles of the accessory olfactory system and the main olfactory system project through the same anatomical regions (Fig. 1). Similar to the main olfactory system, OECs also ensheathe the accessory olfactory nerve. However, while these accessory cells express many molecules similar to OECs of the main olfactory system they also have distinct characteristics and anatomically they remain physically separate from the fascicles of the main olfactory nerve (Chehrehasa et al., 2006, 2008; Cloutier et al., 2004; Knoll et al., 2001; St John et al., 2006). The purification methods typically used to obtain OECs for neural transplant therapies do not separate out the contaminating accessory OECs and therefore cultures of OECs for neural transplant are likely to contain OECs from both the main and accessory olfactory systems. The potential mix of these different OECs may contribute to variation in outcomes of neural transplant therapies considering these cell preparations will likely contain differing proportions of accessory OECs.

The phagocytic capacity of OECs is one characteristic which may be particularly useful for neural repair therapies as the OECs can potentially increase the removal of cell debris from the injury site and thereby improve conditions for axon regrowth (Lankford et al., 2008). However, the phagocytic ability of accessory OECs has not been determined. We have previously generated a transgenic reporter line of mice, OMP-ZsGreen, in which olfactory sensory neurons of the main and accessory olfactory systems express ZsGreen fluorescent protein (Ekberg et al., 2011). In these mice, cell debris arising from olfactory axons retains expression of ZsGreen which is then able to be detected in radial glia that phagocytose the debris during development (Amaya et al., 2015) and in main olfactory OECs in the embryo (Nazareth et al., 2015). In order to determine the phagocytic capabilities of OECs of the accessory olfactory system, we have now examined the accessory olfactory nerve of OMP-ZsGreen mice and compared them to OECs of the main olfactory system.

2. Materials and methods

2.1. Animal strains

Transgenic reporter mice of two separate lines were used: S100ß-DsRed mice, expressing DsRed in cells that express S100ß including OECs (Windus et al., 2007) and OMP-ZsGreen mice, expressing ZsGreen in primary olfactory sensory neurons (Ekberg et al., 2011; Windus et al., 2011). The progeny of S100ß-DsRed mice crossed with OMP-ZsGreen mice were used for most experiments. All procedures were carried out with the approval of the Griffith University Animal Ethics Committee under the guidelines of the National Health and Medical Research Council of Australia and the Australian Commonwealth Office of the Gene Technology Regulator.

2.2. Tissue preparation

Postnatal pups were decapitated and the tissues fixed in 4% paraformaldehyde (PFA)/phosphate-buffered saline (PBS) for 1 h at room temp or overnight at 4 °C and then cryoprotected in 30% sucrose in PBS with 0.1% azide at 4 °C. For wholemount preparations, heads were bisected in the parasagittal plane to expose the septum and olfactory bulb. For sectioning, heads were embedded in mounting matrix (O.C.T., Tissue-Tek) and snap frozen by immersion into 2-methyl butane which had being cooled with liquid nitrogen. Serial sagittal or coronal sections (30 μm) were cut, mounted on slides and stored at −20 °C before processing for immunohistochemistry.

2.3. In vitro fresh dissections of OECs from OMP-Green × S100ß-DsRed pups

Accessory OECs were dissected from the accessory olfactory bulb of postnatal day 4 (P4) OMP-ZsGreen × S100ß-DsRed pups using the ZsGreen expression as a guide to accurately localise the accessory olfactory bulb. OECs from the main olfactory bulb were dissected from the lateral region of the nerve fibre layer to ensure that there was no contamination by OECs from the accessory olfactory nerve which runs along the nasal septum. Cells were dissociated using Tryple Express (Life Technologies) into single cell suspension, plated onto glass cover-slipped bottom culture dishes (Sarstedt) in medium containing DMEM, 10% foetal bovine serum, G5 supplement (Life Technologies), gentamicin, L-glutamine and incubated in 5% CO_2 at 37 °C for 1–2 h to allow attachment of cells to the plate surface and then fixed with 4% PFA. For quantification of the proportion of OECs that contained cell debris, tissue was dissected from each of 3 animals and plated separately into 3 different cultures. For quantification of the intensity of fluorescence within OECs, five separate cultures were prepared with the cells from two animals mixed together for each of the five cultures (5 × 2 animals).

2.4. In vitro phagocytosis of axon debris by OECs

For each dissociated culture of accessory and main OECs were prepared from the accessory and main olfactory bulbs of three S100ß-

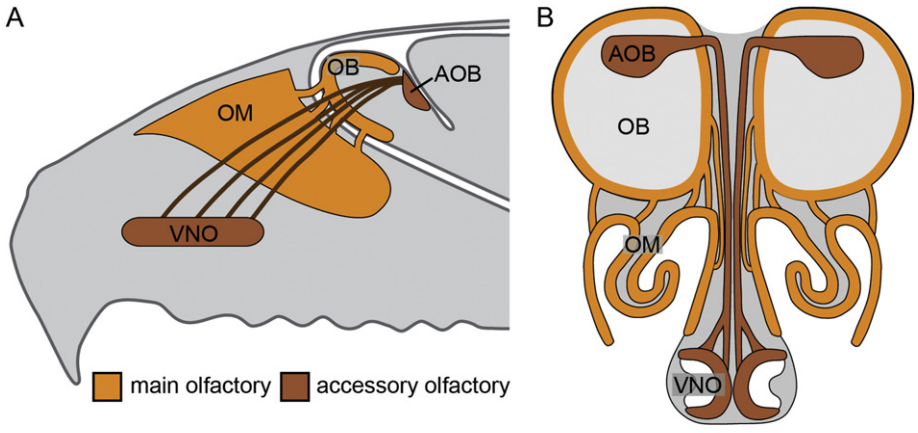

Fig. 1. The accessory and main olfactory systems in mice. (A) Schematic sagittal view of the mouse olfactory systems. In the accessory olfactory system (brown), neurons within the vomeronasal organ (VNO) project axon fascicles to the accessory olfactory bulb (AOB). The accessory olfactory axon fascicles pass through the region of the main olfactory system (orange). The main olfactory neurons populate the olfactory mucosa (OM) and their axons project to the external layer of the olfactory bulb (OB). (B) In a schematic coronal view of the olfactory systems, the close relationships between the fascicles of accessory (brown) and main (orange) olfactory axons are shown.