BRAIN RESEARCH 1595 (2015) 29–42

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Brain Research

Research Report

Associated occurrence of p75 neurotrophin receptor expressing aldynoglia and microglia/macrophages in long term organotypic murine brain slice cultures

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ARTICLE INFO

Article history:
Accepted 7 November 2014
Available online 20 November 2014

Keywords:
Aldynoglia
Adult
Central nervous system
Low affinity nerve growth factor receptor
Mouse
Organotypic slice culture
p75 neurotrophin receptor
Schwann cell

ABSTRACT

Growth-promoting aldynoglia, characterized by the expression of the prototype immature Schwann cell marker p75 neurotrophin receptor (NTR) have been shown to occur in some demyelinating diseases. However, the mechanisms determining the emergence and fate of such cells are largely unknown. This study aimed at the identification of such cells and potential triggering factors using an *in vitro* slice culture approach. Organotypic cerebrum and brain stem slices of adult mice were cultivated for up to 18 days *in vitro*. Immunohistochemistry for the detection of $p75^{NTR}$, CD107b, periaxin, growth associated protein (GAP)-43, and glial fibrillary acidic protein (GFAP) was performed. The results for $p75^{NTR}$ were substantiated by the use of *in situ* hybridization. Cultivation was associated with a progressively increasing spontaneous occurrence of bi- to multipolar $p75^{NTR}$-positive, but periaxin-negative glia, indicative of aldynoglial Schwann cell like cells. Similar cells stained intensely positive for GAP-43, a marker for non-myelinating Schwann cells. The number of $p75^{NTR}$ positive glia did not correlate with GFAP expression, but showed a strong correlation with a remarkable spontaneous response of CD107b positive phagocytic microglia/macrophages. Moreover, aldynoglial $p75^{NTR}$ immunoreactivity negatively correlated to neuronal $p75^{NTR}$ expression, which was lost during culturing. The present results demonstrate that the cultivation of organotypic murine brain slices is accompanied by a spontaneous response of both microglia/macrophages and $p75^{NTR}$ positive cells, suggestive of Schwann cell like aldynoglia. The findings highlights the role of microglia/macrophages, which seem to be an important triggering factor, facilitating the occurrence of this unique type of macroglia.

Abbreviations: MS, multiple sclerosis; SCI, spinal cord injury; p75NTR, p75 neurotrophin receptor; GFAP, glial fibrillary acidic protein; CNS, central nervous system; OPCs, oligodendrocyte precursor cells; OECs, olfactory ensheathing cells; GAP-43, growth associated protein 43; HE, haematoxylin and eosin; PBS, phosphate buffered saline; ABC, avidin–biotin-peroxidase complex; DAB, 3,3′-diaminobenzidine-tetrahydrochloride; PCR, polymerase chain reaction; (ISH), *in situ* hybiridization

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http://dx.doi.org/10.1016/j.brainres.2014.11.027
0006-8993/

1. Introduction

Various neuropathologic conditions are characterized by a complex interplay of resident central nervous system (CNS) cells, which may either contribute to lesion progression or facilitate neuroregeneration, respectively. Besides infiltrating immune cells from the periphery, endogenous CNS cells such as microglia, astrocytes, endothelial cells, and as highlighted recently, aldynoglia, play a crucial role in neurodegenerative diseases such as trauma, stroke, and demyelinating diseases (Barreto et al., 2011; Burda and Sofroniew, 2014; Gudino-Cabrera and Nieto-Sampedro, 2000; Imbschweiler et al., 2012; Seehusen et al., 2007; Zawadzka et al., 2010).

As a receptor for neurotrophins, $p75^{NTR}$ is expressed by various cell types of the CNS in order to control proliferation, migration, differentiation, and apoptosis during development (Arevalo and Wu, 2006; Chen et al., 2009; Cragnolini and Friedman, 2008; Orlando et al., 2008). In adulthood however, $p75^{NTR}$ expression in the CNS becomes strongly down-regulated and is limited to cholinergic neurons of the basal forebrain and a specific type of glial cells, collectively referred to as aldynoglia (Friedman et al., 1991; Gudino-Cabrera and Nieto-Sampedro, 2000; Ibanez and Simi, 2012; Imbschweiler et al., 2012). Following CNS injury, there is a significant up-regulation of $p75^{NTR}$ in various cell types, and investigations have mainly focussed on its role in cell death and the promotion of neuropathology (Chen et al., 2009; Cragnolini and Friedman, 2008; Dechant and Barde, 2002). Up-regulation of $p75^{NTR}$ has for instance been observed in oligodendrocytes in multiple sclerosis (MS) lesions and in experimental autoimmune encephalomyelitis (Dowling et al., 1997, 1999; Nataf et al., 1998), cuprizone-induced demyelination in mice (Copray et al., 2005), and dying oligodendrocytes during spinal cord injury (SCI) (Beattie et al., 2002).

$P75^{NTR}$ has additionally been linked to CNS regeneration and growth-promotion; however, hints for pro-regenerative functions of $p75^{NTR}$ in the CNS are fragmentary so far. A specific population of growth-promoting macroglia, collectively referred to as aldynoglia, is characterized by sharing striking morphological and molecular properties with peripheral pre-myelinating Schwann cells, which is substantiated by the conjoint expression of $p75^{NTR}$ (Gudino-Cabrera and Nieto-Sampedro, 1999, 2000; Jessen and Mirsky, 2002). Moreover, many investigations point out that Schwann cells themselves may play a so far underestimated role in CNS regeneration. Interestingly, a comparatively high proportion of remyelination following CNS injury is done by Schwann cells as shown in many experimental animal models for demyelinating CNS diseases such as Theiler's murine encephalomyelitis as well as MS and human SCI (Dal Canto and Lipton, 1980; Guest et al., 2005; Itoyama et al., 1983). Interestingly, the absence of astrocytes seems to be a pivotal factor that favours Schwann cell remyelination as shown in spinal cord MS lesions (Itoyama et al., 1983) and in several experimental animal models of demyelination (Blakemore, 1975; Dusart et al., 1992; Felts et al., 2005; Zawadzka et al., 2010). Moreover, axonal damage has been proposed as a triggering mechanism, facilitating the occurrence of $p75^{NTR}$ expressing aldynoglia in canine distemper (Imbschweiler et al., 2012). The exact origin of these cells remains elusive so far, however, the hypothesis of a dominating peripheral origin of Schwann cells in terms of invasion from meninges, and peripheral nerves was recently challenged by the demonstration that certain CNS precursor cells are capable of giving rise to Schwann cells *in vitro* and even after transplantation into the demyelinated spinal cord (Akiyama et al., 2001; Keirstead et al., 1999; Mujtaba et al., 1998). In fact, at least in the spinal cord, the majority of Schwann cells in lysolecithin-induced demyelinated lesions in mice derives from oligodendrocyte precursor cells (OPCs), which may differentiate into astrocytes, oligodendrocytes, and lastly Schwann cells (Zawadzka et al., 2010). Usage of the term "central Schwann cells" or "Schwann cell like brain glia" has been proposed in order to classify this unique type of aldynoglial macroglia (Orlando et al., 2008).

An occurrence of bi- to multipolar $p75^{NTR}$ positive cells suggestive of such endogenous aldynoglial Schwann cell like cells was recently described in demyelinated lesions in canine distemper leukoencephalitis, which could additionally be recapitulated in organotypic slice cultures of the adult canine olfactory bulb (Imbschweiler et al., 2012). In dissociated cell cultures of canine origin, certain glial populations such as olfactory ensheathing cells (OECs) and Schwann cells have shown to up-regulate $p75^{NTR}$ expression during *in vitro* cultivation (Bock et al., 2007; Brandes et al., 2011; Orlando et al., 2008; Ziege et al., 2013).

The interdependence and complexity of resident glial cell responses following CNS injury hamper the development of *in vitro* models that adequately mimic those pathologic conditions. Here, organotypic CNS slices are characterized by a preserved organotypic architecture that allows to investigate different cell populations in their multi-cellular *in vitro* environment (Casha et al., 2005; Krassioukov et al., 2002; Pan et al., 2002; Spitzbarth et al., 2011). Organotypic slice cultures have thus been referred to represent an intermediate model between dissociated cell cultures and animal models, thus offering some advantages over dissociated cell cultures in specific questions (Bock et al., 2013; Cho et al., 2007; Spitzbarth et al., 2011). Recently, cultivated murine organotypic CNS slices have been proposed as a model of MS that mimics processes of demyelination and remyelination, respectively (Zhang et al., 2011). Most of these studies are based on the use of fetal or neonatal tissue, which is characterized by high resistance to ischemic damage and mechanical trauma, thus displaying a comparatively long lasting viability in culture (Stavridis et al., 2005). However, neonatal tissue lacks many physiological properties of adult cell-tissues and may exhibit a significantly differing response to stress and injury (Cho et al., 2007; Mewes et al., 2012). Cultivation of CNS slices of adult animals may thus be more appropriate to study pathological events in response to tissue injury and stress without the complex interference of peripheral immune cells (Spitzbarth et al., 2011). In fact, cultivation of spinal cord and olfactory bulb slices of dogs and has been used as an *in vitro* model for canine SCI and demyelinating disease, respectively (Bock et al., 2013; Imbschweiler et al., 2012; Spitzbarth et al., 2011). Recently, cultivation of canine olfactory bulb slices has shown to be associated with emerging $p75^{NTR}$ positive bi- to multipolar glial cells, suggestive of centrally derived Schwann cell like glia (Imbschweiler et al., 2012). However, if this