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I dedicate this thesis to my mum – for everything

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List of Abbreviations

-/-	knockout
3D	three dimensional
Aβ	Amyloid beta
ACM	astrocyte conditioned medium
AQ4	aqauporin-4
ATP	adenosine triphospate
BBB	blood brain barrier
BDNF	brain derived neurotrophic factor
BMP	bone morphogenetic protein
BrdU	5-bromo-2'-deoxyuridine
Caspr	contactin associated protein
cDNA	cellular deoxyribonucleic acid
CNP	2',3'-cyclic nucleotide 3'-phosphohydrolase
CNS	central nervous system
CNTF	ciliary neurotrophic factor
CNTFRα	CNTF R alpha
CO2	carbon dioxide
CSF	cerebrospinal fluid
CSPGs	chondroitin sulphate proteoglycans
CTGF	connective tissue growth factor
Cx-	Connexin
CXCL10	C-X-C motif chemokine 10
DAPI	4'-6-diamidino-2-phenylindole
DIV	days *in vitro*
DMEM	Dulbecco's Modified Eagle Medium
dNTP	Deoxyribonucleotide triphosphate
DRG	dorsal root ganglion
E	embryonic day
EAE	experimental autoimmune encephalitis
ECM	extracellular matrix
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
E-NCAM	embryonic form of polysialic acid containing N-CAM
FBS	foetal bovine serum
FCS	foetal calf serum
FDR	false discovery rate
FGF	fibroblast growth factor
FGFR	fibroblast growth factor receptor
GalC	galactocerebroside
GDF15	growth differentiation factor 15
GFAP	glial fibrillary acidic protein
GPI	glycosylphosphatidylinositol
IGF	insulin-like growth factors
IL-	Interleukin-
IP3	Inositol trisphosphate
Kv	voltage-gated potassium channels
KO	knockout
L-15	Leibovitz medium
LIF	leukaemia inhibitory factor
LIFRß	leukaemia inhibitory factor receptor beta
LPS	Lipopolysaccharides

μM	micromoles
MAG	myelin associated glycoprotein
MBP	myelin basic protein
min	minute(s)
MOG	Myelin oligodendrocyte glycoprotein
mRNA	mitochondrial ribonucleic acid
MS	multiple sclerosis
Nav	voltage-gated sodium channels
N-CAM	neural cell adhesion molecule
Nf155	155kDa isoform of neurofascin
Nf186	186kDa isoform of neurofascin
Nrg	neuregulin
NSC	neural stem cell
OEC	olfactory ensheathing cells
OPCs	oligodendrocyte progenitor cells
OSM	oncostatin M
p75NTR	low affinity neurotrophin receptor p75
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PDGF	platelet-derived growth factor
PLL	poly-L-lysine
PLP	proteolipid protein
PNS	peripheral nervous system
PTN	pleitrophin
qRT-PCR	quantitive real time polymerase chain reaction
rpm	revolutions per minute
RT	room temperature
T12.5	12.5 cm3 tissue culture flask
T25	25 cm3 tissue culture flask
TGF-β1	Transforming growth factor beta
THBS4	thrombospondin-4
TnC	Tenascin-C
TNFα	Tumour necrosis factor-alpha
TNR	Tenascin-R
WMD	white matter disease

Abstract

Astrocytes are the most abundant cell within the central nervous system (CNS) and yet despite this, the true extent of their role in health and disease has not been fully elucidated. In the undamaged CNS, they are termed quiescent, where they maintain homeostasis. However, after injury or disease astrocytes become reactive where they are described as a physical and molecular barrier to regeneration. Emerging literature has suggested the existence of an additional phenotype of astrocyte, termed the activated astrocyte. These astrocytes are thought to enhance regeneration by creating a more growth-permissive environment for repair. In addition, it has also been reported that astrocytes may play a role in regulating myelination; however, it is unclear how the phenotype of the astrocytes may affect this process. Therefore, this thesis will focus on the variable phenotypic state of astrocytes and subsequently how this relates to their ability to support myelination.

Using an *in vitro* myelinating culture, where dissociated spinal cord cells were plated on a monolayer of astrocytes, myelination can be followed over time. Since it is hypothesised that astrocytes can affect myelination we used two protocols known to affect the reactive status of the astrocyte, i) activate the astrocytes by treating with ciliary neurotrophic factor (CNTF) or ii) induce a quiescent astrocyte state by plating them on Tenascin C (TnC).

It is hypothesised that CNTF changes the activation state of the astrocyte therefore making it more supportive to myelination. The addition of the astrocyte derived factor ciliary neurotrophic factor (CNTF) was shown to enhance myelination. My results demonstrate that CNTF addition does not lead to an increase in oligodendrocyte or microglia cell numbers or an increase in the diameter of the neurites, thus suggesting that this CNTF-induced increase in myelination is mediated via the astrocyte.

Conversely, culturing astrocytes on the extracellular matrix molecule Tenascin-C (TnC), a method to make the astrocytes quiescent (Holley et al., 2005), resulted in a reduction in myelination. Astrocytes cultured on TnC were shown have decreased expression of nestin, which is typically a marker for reactivity. A microarray gene study comparing gene expression of the various astrocyte phenotypes identified CXCL10 to be upregulated in astrocytes on TnC. Furthermore, the addition of CXCL10 into the myelination cultures resulted in a decrease in myelination. Conversely, the addition of anti-CXCL10 to myelinating cultures on quiescent astrocytes increased myelination.

Taken together, these data indicate that the astrocyte phenotype has considerable influence on myelination; where activated astrocytes support myelination whilst quiescent astrocytes do not. The identification of factors which may modify astrocyte phenotypes could lead to potential therapeutic strategies for CNS pathologies.

Author's Declaration

I declare that, except where explicit reference is made to the contribution of others, that this dissertation is the result of my own work and has not been submitted for any other degree at the University of Glasgow or any other institution.

Signature _____

Printed name _____