Uncovering the role of Neuregulin-1 in regulating microglia properties: *in vitro* studies

By

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Abstract

Microglia are resident immune cells of the central nervous system, which in concert with astrocytes and peripherally recruited immune cells initiate a pro-inflammatory response after injury and disease that results in oligodendroglial death and myelin damage. We have shown that down-regulation of a neuronally-derived growth factor, neuregulin-1, in demyelinating lesions of the spinal cord is an underlying mechanism for insufficient spontaneous oligodendrogenesis and remyelination. Recent evidence suggests that Nrg-1 treatment positively regulates the repair process and remyelination by modulating neuroinflammation. The goal of the present study was to determine the role of Nrg-1 in regulating microglia response in normal and injury state. In primary *in vitro* systems, we demonstrate a positive role for Nrg-1 in regulating microglia activity and the impact of Nrg-1 treatment on the effects of microglia on the behavior of neural precursor cells (NPCs). Using an array of cellular and molecular assays, we found that Nrg-1 attenuated the transcript expression of several pro-inflammatory markers such as tumor necrosis factor- (TNF-) α, interleukin- (IL-) 6, CD86 and the production of nitric oxide (NO). In addition, Nrg-1 restored the suppressed phagocytic ability in M1-polarized microglia cultures. Our findings showed that microglia conditioned media (MCM) from Nrg-1 treated M1-polarized microglia cultures promoted migration and proliferation of NPCs. Hence, our findings suggest that Nrg-1 therapy could be exploited to foster a pro-regenerative phenotype in microglia, which is supportive of repair and regeneration following CNS injuries and diseases.

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Dedication

This work is dedicated to my beloved husband, **MohamadReza Aghanoori**.

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Contributions

This thesis is a collation of *in vitro* experiments from 3 years of laboratory work. I performed all the experimental procedures on microglia and neural precursor cells (NPCs) cultures and data analysis under the supervision of **Dr. Soheila Karimi**. I cultured and maintained pure microglia; however, the previous research associate, **Dr. Santhosh T. Kallivalappil** and the post-doc fellow, **Dr. Hardeep Kataria**, in the laboratory cultured and maintained NPCs.

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List of abbreviations

Acetylcholine receptor	AChR
Acetylcholine-receptor-inducing activity	ARIA
Acquired immune deficiency syndrome	AIDS
Adenosine triphosphate	ATP
Alzheimer's disease	AD
α-amino-3-hydroxyl-5-methyl-4-isoxazole propionic acid	AMPA
β-amyloid peptide	Aβ
Antigen presenting cells	APC
Arginase-1	Arg-1
Artificial cerebrospinal fluid solution	aCFS
Blood brain barrier	BBB
Brain-derived neurotrophic factor	BDNF
Bromodeoxyuridine	BrdU
Central nervous system	CNS
C-C motif ligand	CCL
CD200 receptor	CD200R
Chondroitin sulfate proteoglycans	CSPGs
Colony-stimulating factor 1	CSF1
Complement component 5a	C5a
Conditioned media	CM
Connexin 43	Cx43
CX3 chemokine receptor 1	CX3CR1

C-X-C motif ligand 1	CXCL1
Cysteine-rich domain	CRD
Damage-associated molecular patterns	DAMPs
Dentate gyrus	DG
15-Deoxy-Delta-12,14-prostaglandin J2	15d-PGJ_2
Dominant-negative	DN
Endoplasmic reticulum	ER
Epidermal growth factor	EGF
Experimental autoimmune encephalomyelitis	EAE
Fibroblast growth factor 2	FGF2
Food and Drug Administration	FDA
γ-aminobutyric acid	GABA
Glial fibrillary acidic protein	GFAP
Glial growth factor	GGF
Heregulin	HRG
Human recombinant Nrg-1b1	rhNrg-1b1
Inducible nitric oxide synthase	iNOS
Insulin-like growth factor 1	IGF-1
Interferon	IFN
Interleukin	IL
Leukemia inhibitory factor	LIF
Lipopolysaccharide	LPS
Lysophosphatidyl-choline	LPC

Major histocompatibility complex class II	MHC-II
Mannose receptor	MR
Maternal immune activation	MIA
Matrix metalloproteinase	MMP
Metalloproteinase-1	TIMP-1
Microglia CM	MCM
Mitogen-activated protein kinase	MAPK
Multiple sclerosis	MS
Nerve growth factor	NGF
Neu differentiation factor	NDF
Neural stem/progenitor cells	NPCs
Neuregulins	NRGs
Neuregulin-1	Nrg-1
Nitric oxide	NO
N-methyl-D aspartate	NMDA
Norepinephrine	NE
Nuclear factor-kappaB	NF-κB
Oligodendrocyte precursor cells	OPCs
Oligodendrocyte-type-2 astrocyte	O2A
One-way repeated measures analysis of variance	ANOVA
Parkinson’s disease	PD
Paraformaldehyde	PFA
Peripheral nervous system	PNS

Perivascular extracellular matrix	ECM
Peroxisome proliferator-activated receptor gamma	PPARγ
Polyethylenimine	PEI
Post-natal days 1-10	P1-P10
Programmed cell death	PCD
Reactive oxygen species	ROS
Regulatory B cells	B_{regs}
Regulatory T cells	T_{regs}
Rostral migratory stream	RMS
Scavenger receptors	SR
Sensory and motor neuron-derived factor	SMDF
Serum free media	SFM
Signal transducer and activator of transcription 3	STAT3
β-site of amyloid precursor protein cleaving enzyme	BACE
Spinal cord injury	SCI
Sprague Dawley	SD
Standard error of the mean	SEM
Stromal cell-derived factor 1 alpha	SDF-1α
Subgranular zone	SGZ
Subventricular zone	SVZ
T helper 2	Th2
Tight junctions	TJs
TNF-α converting enzyme	TACE

TNF receptor I	TNFRI
Toll-like receptors	TLRs
Transforming growth factor	TGF
Triggering receptor expressed on myeloid cells 2	TREM-2
Tumor necrosis factor	TNF
Tween Tris buffered saline	TTBS
Vascular endothelial growth factor	VEGF
Vasoactive intestinal peptide	VIP
Ventricular zone	VZ
Yellow fluorescent protein-tagged	YFP-Tg

Chapter I: Introduction

1. Microglia in the central nervous system

Overview

Microglia are the resident immune cells of the central nervous system (CNS). They reside within the brain and spinal cord parenchyma and are closely associated with the perivascular extracellular matrix (ECM), and blood vessels (perivascular microglia) (Antony et al., 2011). Microglia were first identified in 1899 by Pio del Rio-Hortega as reactive neuroglia and named "Staebchenzellen" meaning cells with the rod-shaped nucleus (Nissl, 1899; Robertson, 1900). He described microglia as a distinct population in the CNS with the capacity to adopt both ramified and amoeboid morphologies (Del Rio-Hortega, 1920; Del Rio-Hortega and Penfield, 1927; Del Rio-Hortega, 1932). Moreover, microglia are distinguished from other CNS cell types such as neurons, astrocytes and oligodendrocytes due to their distinct origin, morphology and function (Ransohoff and Perry, 2009; Kettenmann et al., 2011). Depending on the CNS region, microglia constitute 5–20% of the total number of glial cells in rodents (Lawson et al., 1990; Perry and Gordon, 1991). Given the pivotal role of microglia in normal and pathologic CNS, efforts have been made to unravel the underlying mechanisms of microglia regulation (Saijo and Glass, 2011).

Microglia origin

Microglia are considered the CNS macrophages as they share several structural and functional characteristics (Prinz et al., 2014). Microglia serve as the front line of the host defense in the CNS due to their ability for detecting foreign antigens and communication with other immune cells (Olson et al., 2001; Gottfried-Blackmore et al., 2009). While macrophages have a myeloid origin, microglia are originated from yolk sac-derived erythromyeloid precursors that invade the CNS

parenchyma via leptomeninges and lateral ventricles, and migrate into the all CNS regions with varying proliferation rates (Kettenmann et al., 2011; Swinnen et al., 2013). At later stages of the development, microglia are preferentially found in the areas of cell death, near developing vessels and the radial glial cells (Cuadros et al., 1993; Rezaie et al., 1999; Rigato et al., 2011). Proliferating myeloid-derived cells can be identified in the mice CNS at embryonic day 8 prior to vasculature development (Alliot et al., 1999). In Zebrafish, tissue-specific macrophages derived from progenitor cells in the yolk sac populate the brain and retina and undergo further cellular changes to differentiate into immature microglia. Interestingly, differentiation of microglia is through a pathway independent of hematopoietic transcription factor PU.1 which controls myeloid cells differentiation (Lichanska et al., 1999; Herbomel et al., 2001).

Although the origin of microglia was identified in developing CNS during embryogenesis, it was not clear how these cells are maintained and proliferate in the adult CNS. In 1997, studies on bone marrow-transplanted adult mice showed that microglia can originate derive from hematopoietic cells outside the CNS (Eglitis and Mezey, 1997). A study in 2004 demonstrated that bone marrow-derived stem cells in transplanted irradiated mice have the capacity to migrate across the blood brain barrier (BBB) and reside in the tissue with morphological characteristics of ramified microglia (Simard and Rivest, 2004). Subsequent studies also supported these findings and showed that circulating monocytes can preferably enter the CNS lesions and differentiate into functional microglia in the adult brain following tissue irradiation (Mildner et al., 2007).

Since transplant models cannot accurately recapitulate physiological states, long-term studies were performed on parabiotic mice in which circulatory systems of two adult congenic mice are physically linked to investigate microglia origin under hemostasis conditions (Ajami et al., 2007; Ginhoux et al., 2010; Ajami et al., 2011; Hashimoto et al., 2013). These studies unraveled

that microglia undergo cell division throughout adulthood (homeostasis or disease) to maintain their own population. Thus, the current evidence supports the view that blood monocytes or bone marrow-derived cells do not considerably contribute to repopulation of microglia in adult CNS (Ajami et al., 2007; Ginhoux et al., 2010; Ajami et al., 2011; Hashimoto et al., 2013). However, these cells have shown the capacity to contribute to microglia repopulation under non-physiological conditions such as knock-out mice, irradiation, myeloablation or CNS pathology in which the integrity of BBB is compromised (Beers et al., 2006; Mildner et al., 2007; Ajami et al., 2011). Therefore, the ability of blood monocyte/ bone marrow-derived stem cells in microglia repopulation can provide invaluable therapeutic approaches for CNS-related diseases in which microglia are dysfunctional or depleted (Ginhoux et al., 2013).

1.2. Microglia functions in the normal CNS

In healthy CNS, microglia are described as quiescent cells of the nervous tissue with amoeboid morphology (Ashwell, 1990). The amoeboid microglia have the capacity for migration, proliferation and phagocytosis. Upon reaching their final destination in CNS parenchyma, amoeboid microglia differentiate into mature microglia and acquire a ramified morphology with a small cell body, thin cytoplasm and several long cellular processes (Davalos et al., 2005; Nimmerjahn et al., 2005; Harry and Kraft, 2012). The resting microglia with ramified morphology actively survey the CNS microenvironment through their processes and physically monitor different aspects of the CNS (Nimmerjahn et al., 2005; Kettenmann et al., 2011). Thus, microglia play a key role in physiological conditions throughout CNS development and adulthood.

1.2.1. Microglia functions in the developing CNS

Microglia populate all CNS regions before BBB is completely formed during early embryogenesis and before other neural cells are born (Ajami et al., 2007; Kriegstein and Alvarez-Buylla, 2009). This early colonization indicates the importance of microglia in building the developing CNS (Tremblay et al., 2011; Kettenmann et al., 2013; Schafer et al., 2013; Wake et al., 2013; Salter and Beggs, 2014). For example, microglia are involved in programmed cell death (PCD) in the developing CNS (Bessis et al., 2007; Logan and Freeman, 2007; Peri and Nusslein-Volhard, 2008; Kurant, 2011; Sierra et al., 2013). During CNS maturation, nearly 50% of newly born neurons undergo PCD (Schafer and Stevens, 2015). Early imaging evidence revealed that microglia engulf apoptotic neurons and remove cellular debris during development (Ferrer et al., 1990; Bessis et al., 2007). Subsequently, the active role of microglia in initiating PCD was shown *in vitro* where the absence of microglia in the optic cups of the chick retina attenuated cell death (Frade and Barde, 1998). In these retinal cultures, addition of purified microglia induced cell death in optic cups through the binding of microglial-derived nerve growth factor (NGF) to the neurotrophin receptor P75 (Frade and Barde, 1998). In similar *in vitro* studies, presence of microglia in cerebellar and spinal cord cultures initiated PCD of purkinje cells and motoneurons by releasing superoxide and tumor necrosis factor- (TNF-)α (Marin-Teva et al., 2004; Sedel et al., 2004). Moreover, *in vivo* studies suggest that microglia regulate PCD during neurogenesis (Sierra et al., 2010). Microglia progressively proliferate within the developing CNS, especially in the ventricular zone (VZ) and subventricular zone (SVZ) of the brain where neural stem/progenitor cells (NPCs) differentiate into neurons in the process of neurogenesis in rodents, primates and humans (Antony et al., 2011; Cunningham et al., 2013; Swinnen et al., 2013; Squarzoni et al., 2014). Microglia are known to shape the CNS development through phagocytosis. Phagocytosis is defined as a cellular

process of recognition, engulfment and digestion of whole cells or cellular parts (Wolf et al., 2017). Microglia perform different types of phagocytosis in the developing CNS, including phagocytosis of synapses (known as synaptic pruning or synaptophagy), axons and dendrites, and neuronal precursors (Cunningham et al., 2013; Bahrini et al., 2015; Schuldiner and Yaron, 2015). Synapse pruning or elimination contributes to refinement of neuronal networks and learning from birth until late adolescence in humans (Neniskyte and Gross, 2017). On the other hand, phagocytosis of viable axons and dendrites results in reorganization of neuronal architecture and connections during development (Riccomagno and Kolodkin, 2015; Schuldiner and Yaron, 2015). Microglia also phagocytize apoptotic and live neural precursors residing within the proliferative areas of the primate and rat cortex in both neurogenic and gliogenic stages of cortical development (Cunningham et al., 2013). Inactivation or depletion of activated microglia *in utero* using doxycycline and *in vitro* using minocycline induces the number of NPCs in the CNS (Cunningham et al., 2013). Conversely, activation of microglia using lipopolysaccharide (LPS) reduces the number of precursors in the model of maternal immune activation (MIA) (Cunningham et al., 2013). In this study, progesterone pretreatment of animals resulted in an increase in the number of neural progenitors back to the normal by reducing microglia activation (Tronnes et al., 2016). In addition to neurogenesis, microglia have important roles in shaping laminar cortex structure through regulation of new neuronal migration and positioning (Squarzoni et al., 2014).

Microglia-derived secretory factors also regulate NPC properties such as survival, proliferation and maturation in the developing CNS. For instance, microglia conditioned media induces the proliferation of NPCs and promotes the survival and maturation of neurons (Nagata et al., 1993; Chamak et al., 1994; Morgan et al., 2004). Depletion of resting microglia during embryogenesis in PU.1-deficient mice results in decreased NPC proliferation and differentiation

into astrocytes (Antony et al., 2011). Conversely, addition of resting microglia restored the reduced proliferation and astrogenesis in the transgenic mice (Antony et al., 2011). Microglia have been also suggested to modulate development of other glial cells in the CNS. *In vitro* studies have shown that IL-6 and leukemia inhibitory factor (LIF) expressed by microglia promotes NPC differentiation into astrocytes (Nakanishi et al., 2007). Furthermore, resting microglia stimulate activation of signal transducer and activator of transcription 3 (STAT3)-mediated astrogliogenesis in embryonic NPCs through paracrine effects, but not through their physical interactions (Zhu et al., 2008). In another study, pharmacological inhibition of microglia activation reduced cytokine secretion and inhibited oligodendrogenesis in the SV (Shigemoto-Mogami et al., 2014). Microglia also enhance survival of oligodendrocyte precursor cells (OPCs) and their differentiation into mature oligodendrocytes by activation of insulin-like growth factor 1 (IGF-1), IL-1β and IL-6 and nuclear factor-kappaB (NF-κB) expression (Nicholas et al., 2001; O'Kusky and Ye, 2012; Lu et al., 2013; Shigemoto-Mogami et al., 2014). In addition, microglia have been implicated in regulating myelination through iron supplementation to oligodendrocytes (Cheepsunthorn et al., 1998; Zhang et al., 2006; Clemente et al., 2013). However, the accumulation of Iron in the brain can cause neuronal degeneration through the generation of ROS (e.g. hydroxyl radicals, hydroxyl anions and peroxyl/alkoxyl radicals) and consequently damage to DNA, proteins and lipids (Mills et al., 2010).

Live-cell imaging techniques have identified a regulatory role for microglia in neural activity through expression of neurotransmitter receptors (Pocock and Kettenmann, 2007; Tremblay, 2011; Schafer and Stevens, 2015; Wu et al., 2015). For instance, mobility and growth of microglia processes are modulated by the amount of ATP released from neurons (Davalos et al., 2005; Nimmerjahn et al., 2005; Li et al., 2012; Dissing-Olesen et al., 2014). Regulation of

neural activity in the visual cortex of the brain results in dramatic changes in microglia communication with synapses (Wake et al., 2009; Tremblay et al., 2010). Furthermore, reduced microglia number in the brain of CX3 chemokine receptor 1 (CX3CR1) knock-out mice delays synapse maturation (Paolicelli et al., 2011; Hoshiko et al., 2012). Thus, microglia appear to regulate development and maturation of synapses via activity-mediated mechanisms (Frost and Schafer, 2016). Microglia also phagocytize axonal debris in the embryonic brain which supports axonal remodeling and the initial wiring of the CNS (Berbel and Innocenti, 1988). Accordingly, microglia play pivotal roles in maturation and remodeling of neural circuits throughout the developing CNS (Paolicelli et al., 2011; Schafer et al., 2012). Recent studies have also unraveled that altered microglia reactivity during development can influence memory and social behavior in adulthood. These observations emphasize the importance of microglia regulatory roles in the developing CNS that can ultimately modulate behaviors in adults (Bilbo et al., 2005; Bilbo et al., 2007; Williamson et al., 2011; Giovanoli et al., 2013).

1.2.2. Microglia functions in the adult CNS

Microglia are a self-renewing cell population with the ability to divide throughout the adulthood (Ajami et al., 2007). In the normal adult CNS, resting microglia exert regulatory effects on hippocampal neurogenesis by pruning newborn neurons and removing apoptotic cells without inducing inflammation (Figure 1A). Thus, resting microglia play critical roles in learning and memory by continuous shaping of the hippocampus through phagocytosis of apoptotic neuroblasts in the SGV (Altman and Das, 1965; Eriksson et al., 1998; Roy et al., 2000; Deng et al., 2010; Sierra et al., 2010). Resting microglia engulf apoptotic neurons in phagocytic pouches formed in

terminals or en passant branches in a cell body-independent process, different from phagocytosis during CNS diseases (Sierra et al., 2010).

In the healthy CNS, activated microglia have been found as a morphologically and immunologically distinct subpopulation of cells accommodating the SVZ and rostral migratory stream (RMS). In these CNS regions, microglia are activated by anti-inflammatory cytokines, IL-4, IL-10, and IL-13, to express STAT6-regulated genes, which supports NPC survival, proliferation, migration and differentiation into neurons and glial cells (Biswas and Mantovani, 2010; Boche et al., 2013; Ribeiro Xavier et al., 2015). *In vitro* studies have also supported the idea that microglia can induce the differentiation of adult mouse NPCs into neurons (Aarum et al., 2003). In another study, microglia conditioned media increased the formation of new neuroblasts in SVZ cultures (Walton et al., 2006). In support of the role of microglia in NPC proliferation, addition of microglia conditioned media to NPC cultures results in an increase in the number of newborn neurons as compared to non-treated cultures (Aarum et al., 2003). Furthermore, the conditioned media of non-activated microglia cultures increases the survival of cerebellar granule neurons *in vitro* (Figure 1A) (Morgan et al., 2004). Interestingly, evidence has shown that microglia can promote neurogenesis in the hippocampus of rodents exposed to environmental enrichment (Ziv et al., 2006; Choi et al., 2008).

In the adult CNS, microglia also regulate synapses (Blinzinger and Kreutzberg, 1968). Microglia activation in facial motor nerves injury results in ensheathment of injured neurons and synapse displacement, a process termed as synaptic stripping (Figure 1A) (Blinzinger and Kreutzberg, 1968). Unlike phagocytosis, Microglia do not internalize neuronal components during synaptic stripping (Chen et al., 2014). In addition, synapse stripping is reversible and synapses can be re-established when microglia are polarized to a resting state (Chen et al., 2012).

1.3. Microglia in pathologic CNS

1.3.1. Neuroinflammation

The CNS is an immune privileged site with effective strategies to prevent the invasion of immune components and activation of immune response within the nervous tissue (Klein and Hunter, 2017). This concept was first discovered by Sir Peter Medawar in the 20th century. CNS immune privilege is now considered to be a more relevant phenomenon to the CNS parenchyma and less relevant to other areas of the CNS including the choroid plexus, meninges and ventricles (Amor et al., 2010; Becher et al., 2017). Immune privilege refers to the tight regulation of immune responses in the brain and spinal cord which partially depends on the BBB as a diffusion barrier composed of endothelial cells, astrocyte end-feet, and pericytes (Janzer and Raff, 1987; Armulik et al., 2010). In the BBB, tight junctions (TJs) of endothelial cells block the penetration of blood-derived materials into the brain (Amor et al., 2010). The end-feet at the terminus of astrocyte processes cover the walls of blood vessels and secrete vasoactive agents onto the smooth muscle cells of blood vessels for regulation of vascular tone to preserve the TJs (Watkins et al., 2014). Thus, the connection between BBB components provides a proper environment for optimizing CNS function (da Fonseca et al., 2014). However, following systemic immunization, aging and CNS-related disorders, the BBB becomes compromised that results in many pathological conditions (Obermeier et al., 2013; Becher et al., 2017).

Neuroinflammation is defined as the inflammation of the brain and spinal cord in response to disturbed hemostasis (DiSabato et al., 2016). Neuroinflammation is a pathological hallmark of several leading neurological conditions of the CNS including neurotrauma, stroke and multiple sclerosis (MS) (Alexander and Popovich, 2009b; David and Kroner, 2011; Alizadeh et al., 2015).

Neuroinflammation is a common phenomenon in many different pathological disorders, ranging from changes in glial cell morphology to a large invasion and destruction of the nervous tissue by leukocyte infiltration (Streit and Graeber, 1993; Heppner et al., 2015). Since CNS is a stress-sensitive area with limited self-renewal potential and high vulnerability to injury, acute neuroinflammatory response, including microglia activation, occurs in the nervous tissue to minimize neuronal damage. However, long-term activation of microglia and sustained production of pro-inflammatory mediators cause chronic neuroinflammation which is most often detrimental to the CNS (Chen et al., 2016).

1.3.2. Microglia functions in neuroinflammation

Microglia play crucial roles in CNS pathology through their mobilization at early stages of the disease. Microglia mobilization causes major changes in their phenotype (named microglia activation) which allows them to adapt to an altered environment, react in a particular manner and exert either neuroprotective or neurotoxic effects (Streit, 2002; Mosser et al., 2017). Activation of microglia is the first indication of neuroinflammation in the brain or the spinal cord (Carson et al., 2006). Upon exposure to a stimulus, microglia undergo an activation stage which induces dramatic changes in their morphology from ramified to amoeboid (Taves et al., 2013). Following activation, microglia migrate to the site of injury and undergo microgliosis. Activation of microglia enable them to respond to the injury, conduct phagocytosis of cellular debris, and secrete inflammatory factors which commence and propagate neuroinflammation (Taves et al., 2013; Li and Zhang, 2016).

Acutely activated microglia perform beneficial functions, including scavenging neurotoxins which attenuates inflammation and releasing trophic factors which support neuronal

survival (Neher et al., 2011b). Activated microglia also serve as phagocytic cells (Stence et al., 2001). The phagocytic microglia highly proliferate and migrate towards the injury site to accommodate the areas with massive neuronal death in a process called chemotaxis (Eugenin et al., 2001). Activated microglia have the capacity to engulf and internalize apoptotic cells and debris in a wide range of neurological conditions such as Alzheimer's disease (AD) Parkinson's disease (PD), MS, stroke and CNS trauma (Gehrmann et al., 1995; Long-Smith et al., 2009; Neumann et al., 2009; Loane and Byrnes, 2010; Napoli and Neumann, 2010; Yenari et al., 2010; Prokop et al., 2013). To engulf the particles, activated microglia express different types of receptors, including toll-like receptors (TLRs), triggering receptor expressed on myeloid cells 2 (TREM-2), scavenger receptor (SR) and mannose receptor (MR) (Fu et al., 2014). Acute activation of microglia can be regulated in pathologic CNS through pro-inflammatory cytokines and mediators released by infiltrating immune cells, demyelination-derived myelin debris and various pathogens or cellular proteins which then cause the activation of TLR pathway (Sriram, 2011). The chronic activation of microglia may compromise the permeability of the BBB and augment infiltration of peripheral immune cells which lead to chronic neuroinflammation and disease progression (Schmid et al., 2009; Lyman et al., 2014). Furthermore, chronically activated microglia are implicated in demyelination by presenting antigen to infiltrating immune cells and producing inflammatory mediators (Alizadeh et al., 2015). Demyelination, which is loss of myelin sheaths around the axons, results in axonal and neuronal degeneration and functional deficits in conditions such as MS and SCI (Caprariello et al., 2012). Since chronic activation of microglia acts as a convergence point for divergent factors that cause demyelination (Lyman et al., 2014), immunomodulatory therapies with the potential to target neuroinflammation can be promising for attenuating the progression of demyelinating conditions (Frank-Cannon et al., 2009).

1.3.3. M1 versus M2 microglia

Activation of microglia can be classified into two phenotypes: classical proinflammatory (M1) phenotype and alternative anti-inflammatory (M2) phenotype (Taves et al., 2013). In response to TLR ligands such as LPS or pro-inflammatory cytokines such as IFN-γ and TNF-α, resting microglia (termed as M0) polarize to an M1 phenotype (Wang et al., 2014). Once stimulated, M1-polarized microglia secrete pro-inflammatory cytokines such as TNF-α and IL-1β, produce reactive oxygen species (ROS) and nitric oxide (NO) and express a variety of chemokines to recruit immune cells from periphery to the site of injury or insult. M1-polarized microglia also have the capacity to present antigens to other immune cells to assist in the adaptive immune response and to amplify inflammatory responses (Kettenmann et al., 2011; Carniglia et al., 2017). M1-polarized microglia upregulate respective receptors such as major histocompatibility complex class II (MHC-II) and cluster of differentiation 86 (CD86) to act as antigen presenting cells (APC) and to communicate with other immune cells (Taylor et al., 2005). To distinguish M1 microglia from M2 microglia and other immune cells, their expression level of cell surface markers and cytokines is used, for example, M1 microglia express high levels of IL-12 and low levels of IL-10 (Skeen et al., 1996; Mantovani et al., 2004). There is accumulating evidence showing that M1-polarized microglia are involved in the pathophysiology of many neurodegenerative disorders such as PD, MS and dementia, in which prolonged inflammatory response is associated with neuronal dysfunction (Figure 1B) (Zhang et al., 2017; Du et al., 2018; Luo et al., 2018; Zhang et al., 2018).

The resolution phase of inflammation is an essential step for tissue repair and regeneration after a stimulus. During this step, M1-polarized microglia adopt an alternative M2 phenotype, which was originally classified based on the expression of mannose receptor (Stein et al., 1992; Franco and Fernandez-Suarez, 2015). The M2-polarized microglia have been implicated in tissue

repair and wound healing through phagocytosis of death or dying cells and myelin debris (Miron et al., 2013). Although phagocytic ability of microglia is critical for reconstructing neuronal networks and axonal regeneration after injury, it has still remained controversial whether phagocytosis is beneficial or detrimental in different CNS disorders (Weldon et al., 1998; Ito et al., 2007; Sierra et al., 2010).

The M2-polarized microglia are also characterized by secretion of several anti-inflammatory mediators such as transforming growth factor- (TGF-) β, IL-10, and Arginase-1 (Arg-1) (Miron et al., 2013; Taylor et al., 2017). The enzyme Arg-1 competes with inducible nitric oxide synthase (iNOS) for the amino acid l-arginine and converts it to polyamines, ornithines and proline that contribute to tissue repair and matrix deposition (Rath et al., 2014). M2 microglia can also delay or mitigate glial scar formation and thereby further support regeneration or repair in CNS injury (Figure 1B) (Rohl et al., 2007).

Phenotype and function of M2 microglia can be sub-classified into M2a, M2b and M2c based on the type of cytokine(s) that is used for their polarization. The microglia exposed to IL-4 or IL-13 are characterized as M2a cells, which suppress inflammatory response through NF-κB inhibition and phagocytosis activity (Gadani et al., 2012; Sica and Mantovani, 2012). Upon exposure to IL-10, TGF-β and glucocorticoids, activated microglia are polarized to M2c phenotype, which appears to be important in wound healing and matrix remodeling after down-regulation of inflammatory responses (Cherry et al., 2014). Following activation with immune complexes, microglia/macrophages switch into M2b phenotype that express high IL-10 and low IL-12 (Filardy et al., 2010). Although M2b microglia release MHCII and CD86 molecules to stimulate T cells (Edwards et al., 2006), they appear to promote T helper 2 (Th2) differentiation, which further supports the suppression of immune responses (Figure 1B) (Filardy et al., 2010).

Collectively, M1 microglia are mainly characterized by their pro-inflammatory response, whilst M2 microglia are involved in the resolution of pro-inflammatory response and clearance of debris that is required for CNS homeostatic balance (Franco and Fernandez-Suarez, 2015). Thus, M2 microglia have neuroprotective properties and regenerative capacity, which mitigate excessive tissue degeneration in the injured and diseased CNS (Kigerl et al., 2009; Liao et al., 2012; Miron et al., 2013; Franco and Fernandez-Suarez, 2015). Given the fact that M2-polarized microglia are critical players for tissue repair and regeneration, redirecting microglia response towards a M2 phenotype can be a pivotal therapeutic approach in neuroinflammatory disorders.

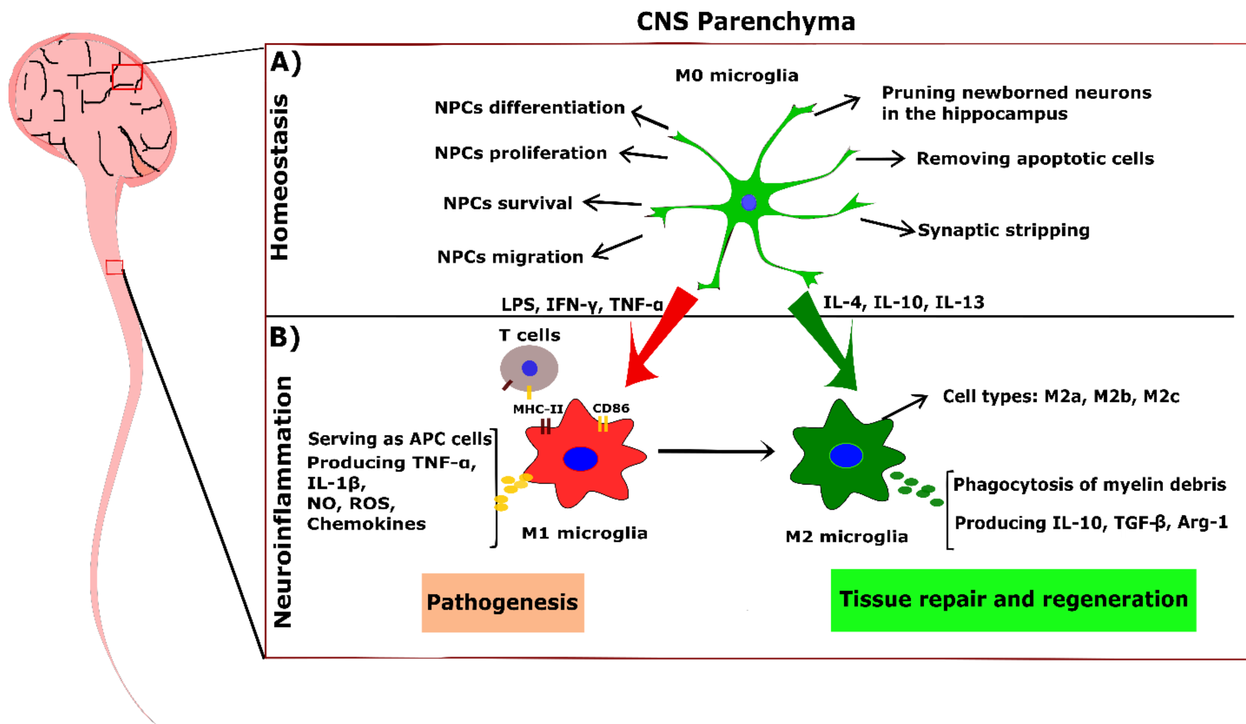


Figure 1. Role of microglia in the normal and pathologic adult CNS. (A) Schematic diagram illustrates the role of resting microglia (termed as M0) in the normal brain and spinal cord. M0 microglia prune newborn neurons, remove apoptotic cells, and regulate synaptic stripping in the adult brain (Altman and Das, 1965; Blinzinger and Kreutzberg, 1968; Eriksson et al., 1998; Roy et al., 2000; Deng et al., 2010; Sierra et al., 2010). Microglia can induce differentiation, proliferation, survival and migration of adult NPCs (Aarum et al., 2003; Morgan et al., 2004; Liu et al., 2013a). **(B)** Schematic diagram shows role and characteristics of M1/M2 microglia in neuroinflammation. LPS, IFN-γ or TNF-α induces M1 polarization resulting in production of TNF-α, IL-1β, ROS, NO and chemokines to recruit peripheral immune cells to the site of injury (Kettenmann et al., 2011; Wang et al., 2014; Carniglia et al., 2017). M1 microglia upregulate MHC-II and CD86 to act as APC cells and to communicate with T cells (Taylor et al., 2005). Thus, M1 microglia are considered to be involved in the pathogenesis of CNS-related diseases (Zhang et al., 2017; Du et al., 2018; Luo et al., 2018; Zhang et al., 2018). In contrast, IL-4, IL-10 or IL-13

diverts microglia polarization toward M2 phenotype, which is characterized by production of TGF-β, IL-10, Arg-1, and phagocytosis of cell and myelin debris (Miron et al., 2013). Thus, M2 microglia are implicated in tissue repair and regeneration (Kitayama et al., 2011). M2 microglia phenotype and function can be sub-classified into M2a, M2b and M2c (Mosser and Edwards, 2008; Gadani et al., 2012; Sica and Mantovani, 2012; Cherry et al., 2014). **Abbreviations:** CNS, central nervous system; NPCs, neural precursor cells; LPS, lipopolysaccharide; IFN-γ, interferon gamma; TNF-α, tumor necrosis factor alpha; IL-1β, interleukin beta; ROS, reactive oxygen species; NO, nitric oxide; MHC-II, major histocompatibility complex II; CD86, cluster of differentiation 86; APC, antigen presenting cells; IL-4, interleukin 4; IL-10, interleukin 10; IL-13, interleukin 13; TGF-β, transforming growth factor beta; Arg-1, arginase 1.

1.3.4. Role of microglia in cell death in CNS injuries and diseases

Following injury, microglia play an important role in wound healing by removing cell debris and myelin clearance in the injured CNS (Neumann et al., 2009; Walter and Neumann, 2009). Microglia phagocytosis is critical for maintenance of CNS homeostasis and stimulation of tissue repair (Neumann et al., 2009; Walter and Neumann, 2009). Studies have shown that impaired microglia phagocytosis in the aged CNS diminishes neural regeneration and increases the frequency of pathological conditions (von Bernhardi et al., 2010). In addition to their traditional role in phagocytosis, microglia have been implicated in cell life/death processes in the injured CNS. Depending on the type of neurotoxic or neurotrophic factors released by microglia, these cells can promote or attenuate neuronal death, respectively (Mallat and Chamak, 1994; Hanisch and Kettenmann, 2007). Complementary *in vitro* and *in vivo* studies have shown that depletion of microglia or inhibition of their inflammatory response can mitigate or completely block neuronal death in Parkinson's disease (Mount et al., 2007; Gao et al., 2011). Microglia activation is required for producing damage in pathological conditions such as retinal detachment or LPS exposure (Qin et al., 2004; Nakazawa et al., 2007; Liu and Bing, 2011; Neher et al., 2011b). Moreover, activated microglia can induce degeneration in neurons *in vitro* and following ischemia (Flavin et al., 2000; Hur et al., 2010). It has been suggested that damaged neurons initiate a degeneration cascade through activation of microglia and other glial cells, such as astrocytes (Ilieva et al., 2009; Cameron and Landreth, 2010). Indeed, an initially-damaged neuron releases damage-associated molecular patterns (DAMPs) and TNF-α through necrosis, which result in chronic activation of microglia and reactive microgliosis (Block and Hong, 2007; Lull and Block, 2010; Gao et al., 2011). Neuronal degeneration can also be attributed to direct phagocytosis of neurons by activated microglia (Figure 2) (Neher et al., 2011a; Neniskyte et al., 2011). Moreover, defects in regulatory

mechanisms of microglia activation have shown to exaggerate neurodegeneration in CNS diseases (Marin-Teva et al., 2011).

Microglia can also affect cell life/death by releasing inflammatory mediators such as ROS, NO, pro-/anti-inflammatory cytokines and chemokines after injury (Hanisch, 2002; Block et al., 2007; Brown, 2010; Brown and Neher, 2010). Microglia-derived TNF-α and IL-1β are shown to induce cell death in CNS injuries and disease (Hanisch and Kettenmann, 2007; Loane and Byrnes, 2010). It has been shown that MS disease severity is correlated with the level of TNF-α that can be detected in the serum, cerebrospinal fluid, and demyelinating lesions of affected patients (Maimone et al., 1991; Rieckmann et al., 1995). TNF-α can activate pro-apoptotic proteins to induce neuronal death, while it also stimulates NF-κB signaling to promote neuronal survival (Liu et al., 1996; Mattson, 2005; Mettang et al., 2018). It is proposed that initial expression of pro-inflammatory cytokines prevents further injury to the nervous tissue; however long-term microglia activation and pro-inflammatory cytokines expression contribute to neurotoxicity in chronic inflammatory response (Smith et al., 2012). Microglia have also been shown to diminish neurodegeneration by releasing anti-inflammatory cytokines such as IL-4 and IL10 (Figure 2) (Park et al., 2005; Henry et al., 2009; Xiong et al., 2011).

In addition to neurons, excessive activation of microglia results in oligodendroglial death, myelin defects and demyelination (Pfeiffer et al., 1993). Since oligodendrocytes are metabolically active and require high energy for their function in the CNS, they are highly susceptible to inflammatory factors produced by M1 microglia (Pang et al., 2003; Chew et al., 2013). Several pro-inflammatory cytokines such as IL-1β, IL-2, IL-3, IFN-γ, and TNF-α expressed by microglia have been found in MS lesions, showing a close correlation between microglia activity and oligodendrocyte cell death in MS pathology (Benveniste, 1997). For example, IL-1β binding to

IL-1R1 stimulates the mitogen-activated protein kinase (MAPK) signaling leading to reduced number of developing oligodendrocytes and hypomyelination in neonatal rats (Han et al., 2017). Moreover, microglia-derived IL-1β delays the remyelination processes during CNS disease (Gosselin and Rivest, 2007; Deng et al., 2008). Ectopically expression of IFN-γ in transgenic mice can provoke oligodendrocytes cell death and prevent remyelination through endoplasmic reticulum (ER)-modulated signaling pathways (Lin et al., 2006). *In vitro* studies have shown that IFN-γ-exposure of oligodendrocytes induces mRNA expression of caspases 1, 4, 7 and 8, and Fas leading to cell death and increases TNF-R1 expression in oligodendrocytes making them more responsive to TNF-α (Buntinx et al., 2004; Lin et al., 2006; Lin and Lin, 2010). The activation of iNOS gene was reported to be one of the underlying mechanisms of IFN-γ, TNF-α or IL-1β-induced oligodendrocyte damage. During inflammation, iNOS catalyzes NO production from L-arginine which results in cytolysis, cytostasis, inhibition of Krebs's cycle, and eventually oligodendrocyte death (Merrill et al., 1993; Nave, 2010). NO-induced CNS damage is also mediated by ROS production and oxidation during oxidative stress (Figure 2) (Peferoen et al., 2014).

It has been proposed that early activation of microglia mainly eliminates damaged cells and probably some healthy cells, whilst the late microglia activation predominantly contributes to tissue repair and reconstitution. This dual role of microglia has been attributed to the balance between M1 and M2 activation state of microglia. Accordingly, M1-polarized microglia would be mainly involved in early stages of inflammation, whereas M2-polarized cells would participate in later stages (Loane and Byrnes, 2010; David and Kroner, 2011). However, the mechanisms of microglia activation are complicated that can be simply explained by M1 (neurotoxic)/M2 (neuroprotective) phenotypes (Marin-Teva et al., 2011).

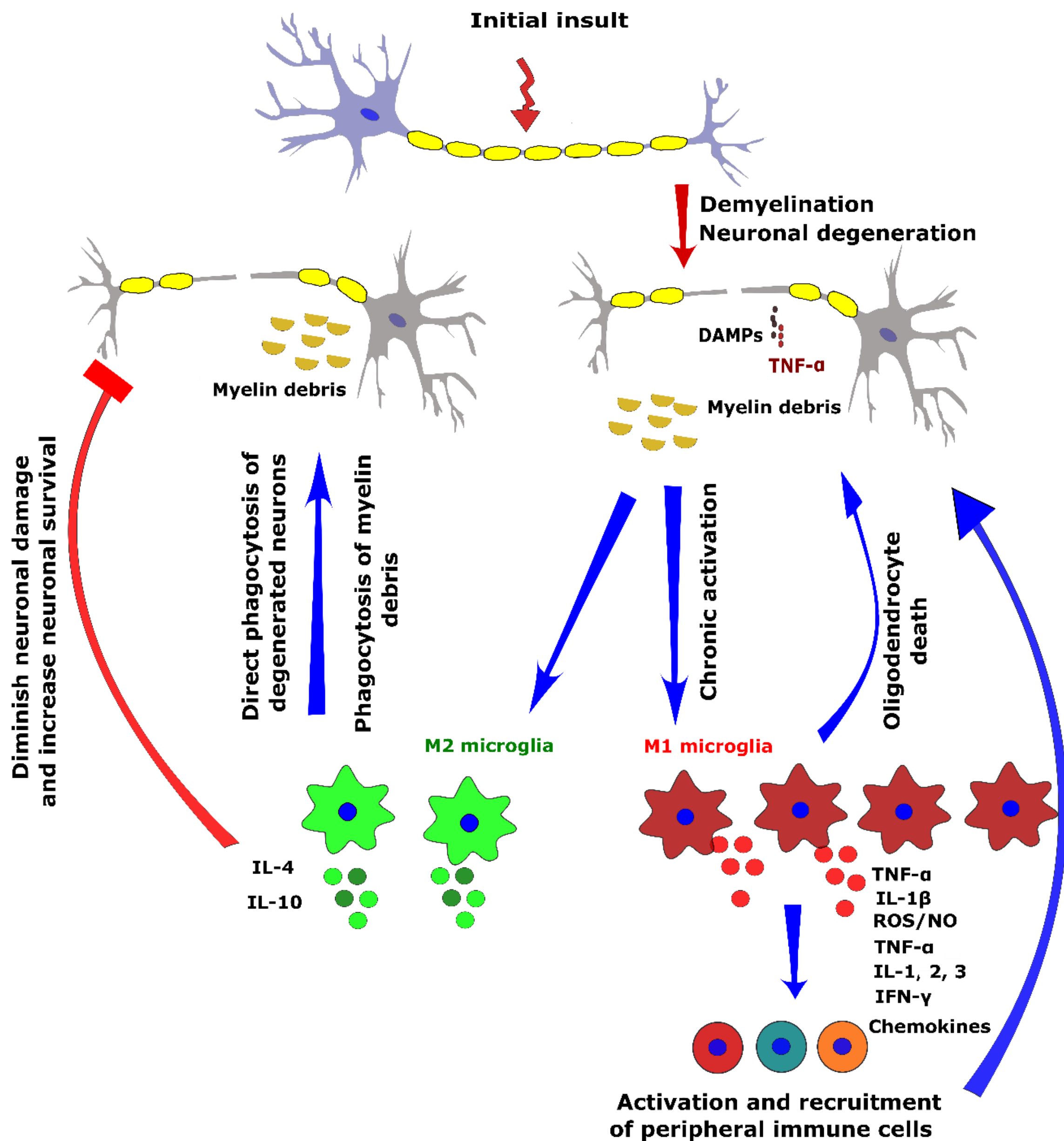


Figure 2. Role of microglia in cell death in CNS injuries and disease. Schematic diagram shows that an initial damage to a neuron initiates a degeneration cascade through activation of microglia and other glial cells leading to neuronal degeneration and demyelination (Ilieva et al., 2009; Cameron and Landreth, 2010). Indeed, the demyelinated neuron releases DAMPs and TNF-α through necrosis which results in chronic activation of microglia and reactive microgliosis (Block and Hong, 2007; Lull and Block, 2010; Gao et al., 2011). Neuronal degeneration can also be

attributed to direct phagocytosis of neurons by activated microglia (Neher et al., 2011a; Neniskyte et al., 2011). M1 microglia induce peripheral immune cells activation and recruitment leading to further neuronal damage by releasing ROS, NO, IL-1β, TNF-α, and chemokines after injury (Hanisch, 2002; Block et al., 2007; Brown, 2010; Brown and Neher, 2010). TNF-α can activate pro-apoptotic proteins to induce neuronal death, while it also stimulates NF-κB signaling to promote neuronal survival (Liu et al., 1996; Mattson, 2005; Mettang et al., 2018). M2 microglia can also diminish neurodegeneration by expressing IL-4 and IL10 and phagocytosis of myelin debris (Park et al., 2005; Henry et al., 2009; Xiong et al., 2011). In addition to neurons, excessive activation of microglia results in oligodendroglial death and demyelination through the expression of IL-1β, IL-1, IL-2, IL-3, IFN-γ, TNF-α and iNOS (Pfeiffer et al., 1993),(Merrill et al., 1993; Benveniste, 1997; Buntinx et al., 2004; Lin et al., 2006; Gosselin and Rivest, 2007; Deng et al., 2008; Lin and Lin, 2010; Nave, 2010; Kaur et al., 2013). **Abbreviations:** DAMPs, damaged-associated molecule patterns; TNF-α, tumor necrosis factor alpha; ROS, reactive oxygen species; NO, nitric oxide; IL-1β, interleukin 1 beta; NF-κB, nuclear factor-kappa beta; IL-1, 2, 3, 4, 10, IFN-γ, interleukin 1, 2, 3, 4,10; interferon gamma; iNOS, inducible nitric oxide synthase.

1.3.5. Role of microglia in endogenous cell replacement in CNS injuries and diseases

Following demyelination, spontaneous remyelination occurs to form new myelin sheaths around demyelinated axons and to protect neurons from degeneration (Franklin and Goldman, 2015) OPCs and NPCs are two endogenous cell populations with the capacity for oligodendrocyte replacement and remyelination (Beattie et al., 1997; Eftekharpour et al., 2008; Meletis et al., 2008; Barnabe-Heider et al., 2010). In adulthood, NPCs reside along the neuraxis notably in the SVZ of the lateral ventricles of the brain, the subgranular zone (SGZ) of the dentate gyrus (DG) in the hippocampus, ependymal layer of the spinal cord and white matter tissue of the brain and spinal cord (Martino and Pluchino, 2006; Jandial and Chen, 2012). Under normal conditions, NPCs are quiescent in most areas of the adult CNS indicating their slow turnover (Palmer et al., 1999). However, in the SVZ and SGZ regions, neurons can be continuously generated from NPCs that contribute to neurogenesis in these regions (Ihrie and Alvarez-Buylla, 2011; Morrens et al., 2012). A small subpopulation of NPCs produces OPC, which migrate towards the white matter and cortex (Suzuki and Goldman, 2003; Menn et al., 2006). These OPCs contributes to the turnover of oligodendrocytes, which maintain myelin sheaths around the axons to allow rapid saltatory conduction and to provide support to axons (Nave and Werner, 2014). Following injury, proliferation of NPCs is significantly induced in the SVZ and SGZ of the injured brain (Chirumamilla et al., 2002; Gao et al., 2009). In ischemic conditions, the proliferating NPCs predominantly differentiate into neurons (Sun, 2014). Moreover, the potential of the SVZ NPCs-derived OPCs for migration and oligodendrocytes formation is considerably enhanced to compensate for oligodendrocytes and myelin loss (Menn et al., 2006). In addition to NPCs-derived OPCs, the resident population of adult OPCs in the brain parenchyma contributes to oligodendrogenesis in the injured brain (Gensert and Goldman, 1997; Menn et al., 2006). In

contrast to the brain, NPCs residing within the ependymal/subependymal regions of the intact spinal cord only have self-renewal capacity to maintain their own population during adulthood (Barnabe-Heider et al., 2010). Following injury, spinal cord-derived NPCs become activated and increase their proliferation, however, they primarily differentiate into astrocytes with limited number giving rise to new oligodendrocytes (Meletis et al., 2008; Karimi-Abdolrezaee et al., 2012). Importantly, in contrast to the brain, neurogenesis is a rare event in the spinal cord and NPCs in the spinal cord they mainly have gliogenic capacity (Barnabe-Heider et al., 2010; Karimi-Abdolrezaee et al., 2012). Interestingly, when brain derived NPCs with intrinsic neurogenic potential were transplanted into the injured spinal cord, they mainly differentiated into glial cells with rare neurogenesis indicating the critical role of the stem cell niche in instructing the fate specification of NPCs (Karimi-Abdolrezaee et al., 2006).

Although neurons and oligodendrocytes can be endogenously replaced from the SVZ of the damaged brain, the regenerative potential of the brain is not enough to improve functional recovery in neurological disorders. For example, only 0.2% of the dead neurons in the striatum can be replaced by newly formed neurons after stroke (Arvidsson et al., 2002). In the injured spinal cord, regenerative response of NPCs is not balanced and constructive for repair. Majority of NPCs differentiate to astrocytes and contribute to astrogliosis and glial scar formation (Barnabe-Heider et al., 2010; Karimi-Abdolrezaee and Billakanti, 2012; Karimi-Abdolrezaee et al., 2012). Reactive astrocytes highly express chondroitin sulfate proteoglycans (CSPGs), which inhibit oligodendrogenesis and favor astrocyte differentiation leading to insufficient remyelination after SCI (Karimi-Abdolrezaee et al., 2010; Karimi-Abdolrezaee et al., 2012). In summary, adult brain and spinal cord contain precursor cells with the capacity for cell replacement and spontaneous remyelination after injuries or diseases; however, the efficacy and quality of remyelination is

compromised and limited in a hostile microenvironment surrounding the demyelinating lesions (Alizadeh et al., 2015).

Studies have shown that post-injury microenvironment influences microglia phenotype and function, which leads to altered properties of progenitor cells (Morrens et al., 2012). For example, the expression of connexin 43 (Cx43) and the presence of gap junctions in both NPCs and activated microglia in the lesion provide anatomical coupling between these two cell types that facilitates cellular communication (Talaveron et al., 2014). In addition to cell-cell interactions, microglia products can regulate fate specification of adult NPCs through paracrine mechanisms (Butovsky et al., 2006). Following injury, M2 polarization of microglia exerts positive effects on remyelination through the secretion of IGF-I and TGF-β1 which promote oligodendrocyte differentiation (Hinks and Franklin, 1999; Ousman and David, 2000). Exposure of Microglia to IL-4 or a low dose of IFN-γ can favour the fate of NPCs towards oligodendrogenesis or neurogenesis through IGF-I expression, while LPS-induced M1 microglia block NPCs differentiation via TNF-α expression (Butovsky et al., 2006). Microglia-derived IGF-1 is also a potent regulatory factor for NPC proliferation. IGF-1 knock-out mice show a significant reduction in the number of granule cells resulting in the reduced size of the brain, whereas IGF-1 over-expression induces neurogenesis and synaptogenesis in the hippocampal dentate gyrus (Beck et al., 1995; O'Kusky et al., 2000). *In vitro* studies have shown that the beneficial role of IGF-1 in the proliferation of precursor cells is mediated through the MAPK pathway (Aberg et al., 2003). In addition to IGF-I, microglia affect NPCs properties via a combination of cytokines (IL-1β, IL-6, TNF-α, and IFN-γ) in an IGF-1-independent manner (Shigemoto-Mogami et al., 2014).

There is also a supportive role of M2-polarized microglia in promoting endogenous NPC activation and migration. The expression of IL-10 by M2 microglia promotes neurogenesis and

oligodendrogenesis leading to functional recovery in experimental autoimmune encephalomyelitis (EAE) model (Pluchino and Martino, 2005; Butovsky et al., 2006; Guan et al., 2008; Yang et al., 2009). It has been shown that the supernatant of IL-4-induced M2 microglia promotes NPC differentiation into neurons and oligodendrocytes with a lower number of NPC differentiating into astrocytes (Yuan et al., 2017). Indeed, M2-polarized microglia highly express an anti-inflammatory prostaglandin, 15-Deoxy-Delta-12,14-prostaglandin J2 (15d-PGJ_2), which activates peroxisome proliferator-activated receptor gamma (PPARγ) signaling pathway in NPCs to induce their differentiation (Yuan et al., 2017). In contrast, LPS+IFN-γ activated microglia promotes astrogenesis with limited neurogenesis and oligodendrogenesis (Yuan et al., 2017). Inhibition of neurogenesis by M1 microglia leads to cognitive dysfunction in the hippocampal areas of adult rats with AD, chronic MS or LPS-induced inflammatory injuries (Ekdahl et al., 2003). *In vitro* studies have also shown that chronically activated microglia express lower level of proinflammatory cytokines such as IL-1α, IL-1β, IL-6 and TNF-α, but higher level of anti-inflammatory cytokines like IL-10 and immunomodulatory prostaglandin E2 (PGE2) compared to acutely activated microglia (Cacci et al., 2008). In the presence of chronically activated microglia or their conditioned medium, there is a strong increase in neurogenesis by embryonic and adult NPCs accompanied by a significant reduction in gliogenesis, while acutely activated microglia increase gliogenesis and reduce neurogenesis (Cacci et al., 2008). Recently, our group has shown that conditioned media of microglia exposed to IFN-γ and TNF-α (M1-polarized cells) significantly reduce the proliferation of spinal cord NPCs and significantly induce their differentiation into astrocytes (Dyck et al., 2018). In contrast, conditioned media of microglia exposed to IL-10 (M2-polarized cells) promote a significant increase in NPC proliferation and induces oligodendrogenesis (Dyck et al., 2018). Furthermore, our group has also found that the

paracrine effect of M2 microglia on NPC differentiation into oligodendrocytes is IL-10 dependent, whereas the effect on NPC proliferation seems to be IL-10 independent (Dyck et al., 2018).

Microglia also regulate OPC differentiation. Conditioned media of non-activated microglia stimulate differentiation of OPCs into oligodendrocytes (Pang et al., 2013). Non-activated microglia produce and release high levels of IGF-1, E-selectin, fractalkine (CX3CL1 chemokine, neurotactin), neuropilin-2, IL-2, IL5, and vascular endothelial growth factor (VEGF) (Pang et al., 2013). M2 microglia-derived oligodendrogenesis has been shown to be essential for effective remyelination in cuprizone-induced demyelinating model that is partially mediated through activin-A, a member of TGF-β superfamily (Miron et al., 2013). Conversely, LPS-induced microglia activation prevents OPCs differentiation by NO-mediated oxidative stress and TNF-α expression (Pang et al., 2010).

Collectively, microglia can have diverse effects on cell differentiation depending on their phenotype and the state of their activity (Morrens et al., 2012; Shigemoto-Mogami et al., 2014). Current evidence indicates that while an uncontrolled local pro-inflammatory response prevents remyelination and repair processes, a properly controlled inflammation can promote repair and recovery (Hauben and Schwartz, 2003; Butovsky et al., 2005). Therefore, it is important to understand how microglia are regulated in their environment to identify new immunomodulatory strategies that can harness the potential of microglia for CNS repair.

1.3.6. Regulation of microglia in CNS injuries and diseases

Several pathways and mediators have been identified that regulate microglia activity following CNS injury. Among the mediators controlling microglia activity in the CNS, neurons, glial cells and progenitor cells are discussed below.

1.3.6.1. The interactions between microglia and neurons

Previously, we discussed how a damaged neuron plays a critical role in microglia-mediated cell life/death processes following CNS injury. Here, we discuss the role and mechanisms of neurons in modulating microglia activity via paracrine effects or direct cellular contact (Tian et al., 2009). Paracrine effects of neurons on microglia are mediated via cytokines, chemokines, neuropeptides, neurotrophins, neurotransmitters and purines that can influence microglia activity and chemotaxis (de Haas et al., 2007; Biber et al., 2008). Neurons constitutively express TGF-β in the CNS (Lacmann et al., 2007; Wu et al., 2007). TGF-β is a cytokine important for down-regulating microglia pro-inflammatory response, which is substantially increased in TGF-β-deficient mice (Brionne et al., 2003). In addition, neurons express fractalkine, which controls microglia function by binding to microglia CX3CR1 receptor (Harrison et al., 1998). Studies on CX3CR1-deficient mice revealed that fractalkine down-regulates LPS-induced microglia response and thereby modulates inflammatory neurotoxicity (Cardona et al., 2006). Neurons also produce neurotrophins, which induce M2 phenotypes in microglia and consequently reduce damage to the CNS. As examples, brain-derived neurotrophic factor (BDNF) has been shown to reduce the expression of B7 and CD40 as co-stimulatory molecules in microglia, and NGF prevents the expression of MHC class II in microglia (Neumann et al., 1998; Wei and Jonakait, 1999).

Neuropeptides are another group of regulatory factors expressed by neurons to modulate microglia function. For example, vasoactive intestinal peptide (VIP) prevents the release of pro-inflammatory mediators such as IL-6, TNF-α, IL-12 and NO in microglia following CNS trauma and cerebral ischemia (Delgado et al., 2008). Moreover, the biophase level of norepinephrine (NE), a hormone and neurotransmitter in the catecholamine family, has been shown to be negatively correlated with LPS-induced TNF-α production by microglia (Szelenyi and Vizi, 2007). This study showed higher expression of TNF-α in NE-deficient depression model and lower levels of TNF-α when NE was over-expressed in chemically-disrupted NE reuptake model (Szelenyi and Vizi, 2007). Furthermore, the major inhibitory neurotransmitter in the CNS, γ-aminobutyric acid (GABA), attenuates IL-6 production in LPS-activated microglia (Figure 3) (Bjurstom et al., 2008).

Microglia function can also be modulated through direct interactions with neurons (Tian et al., 2012). Evidence shows that neuronal glycoproteins regulate microglia activity through microglia receptor-mediated mechanisms (Hoek et al., 2000; Meuth et al., 2008). Amongst neuronal glycoproteins, CD200 is predominately expressed in the cell bodies of neurons that interacts with CD200 receptor (CD200R) on the membrane of microglia and myeloid cells (Shrivastava et al., 2012). Microglia-neuron interactions through CD200/CD200R play an important role in maintaining microglia in their resting non-activated state (Denieffe et al., 2013). The reduced expression of CD200 has been associated with microglia activation and pro-inflammatory cytokines expression in the CNS (Denieffe et al., 2013). Studies on CD200-defiecient mice revealed induced expression of TLR4 and NFκB pathway and M1 microglia polarization following LPS challenge compared to wild-type mice (Denieffe et al., 2013). Interestingly, alternatively activated M2 microglia also express CD200 glycoprotein to maintain their M2 phenotype through an autocrine pathway (Figure 3) (Yi et al., 2012). It has been proposed that IL-4 induces M2

microglia polarization by increasing CD200 expression in microglia (Yi et al., 2012). Likewise, the up-regulation of CD200R expression in activated microglia exert neuroprotection through attenuating IL-1β and IL-6 secretion and inducing IL10 levels in the injured brain (Hernangomez et al., 2012). Studies have also shown the anti-inflammatory role of CD200/CD200R signaling pathway in modulating neuroinflammation in EAE model and the aged rat hippocampus (Chitnis et al., 2007; Cox et al., 2012).

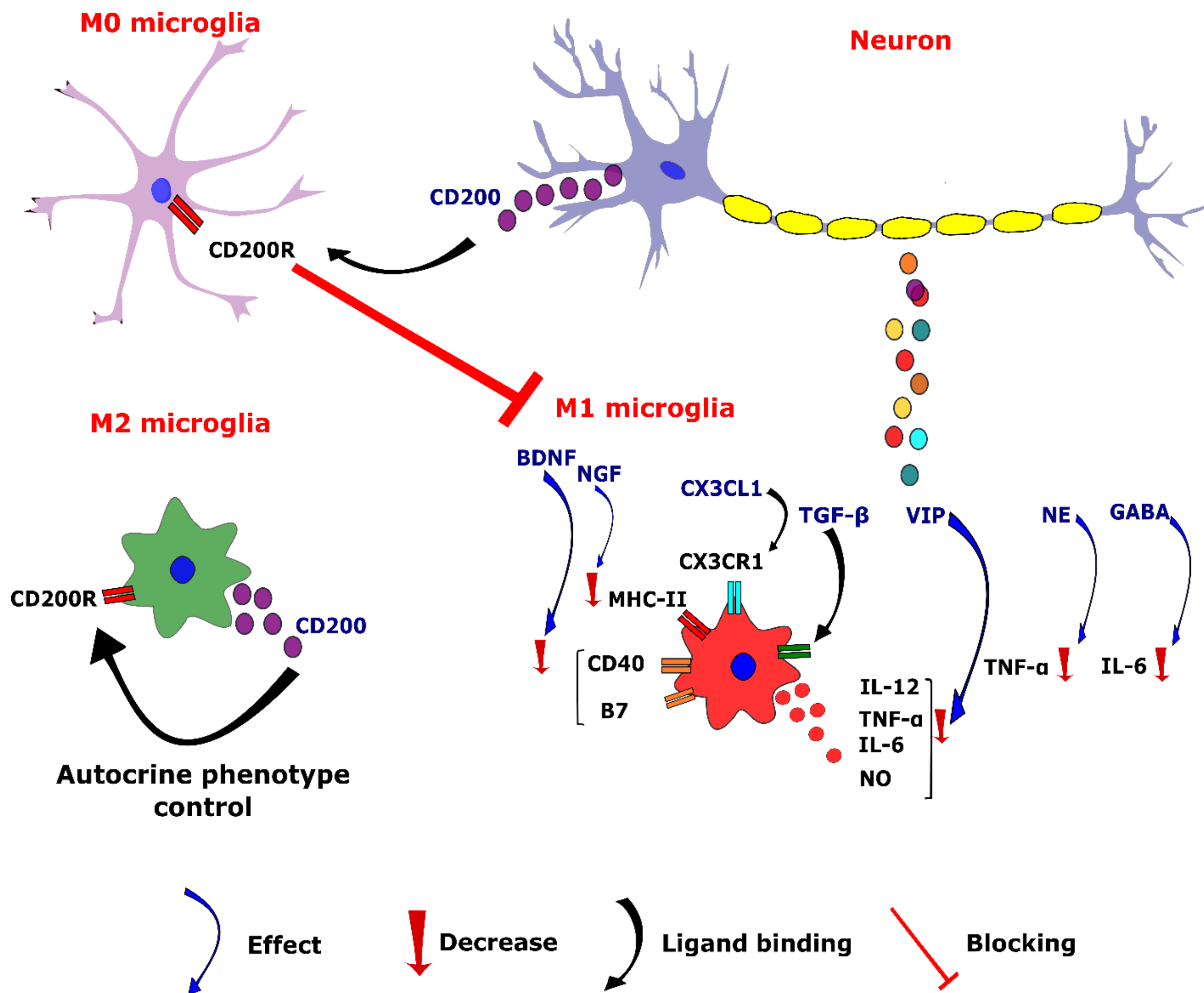


Figure 3. Neuronal mechanism of microglia regulation. Neuron-derived mediators, including anti-inflammatory cytokines, chemokines, neuropeptides, neurotransmitters and neurotrophins regulate microglia activation (Reinke and Fabry, 2006; Wahl et al., 2006; Kerschensteiner et al., 2009). Neurons express TGF-β and CXCL1 which down-regulate microglia pro-inflammatory response through binding to their respective receptors (Lacmann et al., 2007; Wu et al., 2007) (Harrison et al., 1998). In addition, neuronally-derived BDNF reduces the expression of B7 and CD40 as co-stimulatory molecules in microglia, and NGF prevents the expression of MHC class II in microglia (Neumann et al., 1998; Wei and Jonakait, 1999). Neurons also express vasoactive intestinal peptide (VIP) which prevents the release of IL-6, TNF-α, IL-12 and NO by M1 microglia

following CNS injury (Delgado et al., 2008). The neurotransmitters, NE and GABA expressed by neurons attenuate TNF-α and IL-6 production in M1 microglia, respectively (Delgado et al., 2008) (Bjurstom et al., 2008). CD200, predominately expressed by neuronal cell bodies, interacts with CD200 receptor (CD200R) on the membrane of M0 and M2 microglia (Shrivastava et al., 2012). Microglia-neuron interactions through CD200/CD200R play an important role in maintaining microglia in their resting state (Denieffe et al., 2013). Following injury, reduced expression of CD200 by neurons is associated with induced M1 microglia polarization (Denieffe et al., 2013). M2 microglia also express CD200 glycoprotein to maintain their M2 phenotype through an autocrine pathway (Yi et al., 2012). **Abbreviations:** TGF-β, transforming growth factor beta; CXCL1, chemokine (C-X-C motif) ligand 1, BDNF, Brain-derived neurotrophic factor; CD40, cluster of differentiation 40; NGF, nerve growth factor; MHC-II, major histocompatibility complex II, VIP, vasoactive intestinal peptide; IL-6, interleukin 6; TNF-α, tumor necrosis factor alpha; IL-12, interleukin 12; NO, nitric oxide; CNS, central nervous system; NE, Norepinephrine; GABA, γ-aminobutyric acid; CD200, cluster of differentiation 200; CD200R, cluster of differentiation 200 receptor.

1.3.6.2. The crosstalk between microglia and other glial cells

Astrocytes and oligodendrocytes play important roles in the normal and pathologic CNS (Petrovic-Djergovic et al., 2016). It has been shown that astrocytes and oligodendrocytes modulate neuroinflammation by direct expression of inflammatory mediators or communication with microglia and neurons (Siglienti et al., 2007). For example, astrocyte-mediated TGF-β signaling reduces expression of MHC-II and co-stimulatory molecules in activated microglia and subsequently affects their antigen presenting function (Siglienti et al., 2007). In Alzheimer's disease, β-amyloid peptide (Aβ) aggregation within neurons triggers secretion of complement C3 by astrocytes and its binding to microglia C3a receptors resulting in impaired phagocytic activity of microglia (Lian et al., 2016). In response to a stimulus, activated astrocytes produce TNF and colony-stimulating factor 1 (CSF1), which further stimulate M1 microglia polarization and proliferation (Saijo and Glass, 2011). The cross-talk between microglia and astrocytes further induce pro-inflammatory mediators contributing to the CNS pathology (Saijo et al., 2009; Saijo and Glass, 2011).

Oligodendrocytes also contribute to microglia regulation. One example of oligodendrocyte regulatory effect is through oligodendrocyte-derived CD200 glycoprotein and microglia CD200R receptor signaling (Koning et al., 2009). Spontaneous activation of microglia has been shown in CD200-deficient mice, which are more susceptible to develop EAE (Koning et al., 2009). This study provided evidence that CD200/CD200R interactions between oligodendrocytes and microglia can also regulate the activation of microglia in addition to neuron-microglia interactions (Koning et al., 2009). Furthermore, oligodendrocyte-derived cytokines such as CCL2, IL-6 and IL-8 have an important impact on microglia and other immune cells in CNS infection and diseases such as Lyme Neuroborreliosis, MS, Alzheimer's disease and schizophrenia (Ramesh et al., 2012).

For example, IL-8 and CCL2 production by oligodendrocytes influence microglia recruitment during the acute stage of neuroinflammation (Ramesh et al., 2012). A study in 2007 demonstrated that oligodendrocytes exposed to IFN-γ express CCL2, CCL3, CCL5 and CXCL10 chemoattractants (Balabanov et al., 2007). Studies on MS have shown that IL-18 expressed by oligodendrocytes binds to IL-18R on microglia in which stimulate IFN-γ production causing damage to oligodendrocytes (Cannella and Raine, 2004). Additionally, oligodendrocytes undergoing necrosis/apoptosis-mediated cell death produce extracellular nucleotides, which bind to purinergic receptors expressed on microglia resulting in microglia activation (Figure 4) (Peferoen et al., 2014). Altogether, this evidence suggests that microglia are regulated by the CNS network.

1.3.6.3. Microglia interactions with neural precursor cells

Microglia and NPCs regulate each other's activity and function through paracrine and cell-cell interactions. For example, addition of NPCs conditioned media to primary microglia cultures and BV2 microglia cell line induces microglia phagocytosis in a dose-dependent manner (Mosher et al., 2012). Complementary *in vivo* studies on C57BL/6 mice also revealed that injection of primary mouse NPCs into the striatum improved microglia proliferation and phagocytosis (Mosher et al., 2012). NPCs are shown to modulate the immune response through various mechanisms. Intrathecal injection of NPCs into the CSF of EAE mice results in a switch in microglia activation profile with significant decrease in M1 microglia and parallel increase in M2 microglia (Peruzzotti-Jametti et al., 2018). This study showed that interactions between microglia derived-succinate and succinate receptor 1 on transplanted NPC stimulate the secretion of PGE2, which induces an anti-inflammatory response (Peruzzotti-Jametti et al., 2018). VEGF expression has shown to be a

critical mechanism for NPC regulation of microglia proliferation, chemotaxis and phagocytosis (Mosher et al., 2012). Depletion of VEGF from the conditioned media of NPCs mitigates the effects of NPCs on the properties of microglia (Mosher et al., 2012). The impact of VEGF on microglia was supported by another study in which VEGF injection into the striatum was sufficient to induce microglia proliferation (Mosher et al., 2012). NPCs also mitigate LPS-induced-microglia activation by attenuating the release of TNF-α, IL-1β, NO and chemokines, and reducing phagocytosis activity (Yan et al., 2013). Similar to oligodendrocytes, NPCs also modulate microglia activity through CD200/CD200R signaling pathway. Co-culturing of NPCs with microglia results in upregulation of CD200 in NPCs accompanied by CD200R over-expression in microglia (Liu et al., 2013a). Inhibition of CD200/CD200R interactions between NPCs and microglia reduces TGF-β expression by NPCs, which subsequently decrease the survival of phagocytic microglia (Figure 4) (Clark et al., 2008; Ryu et al., 2012; Memarian et al., 2013).

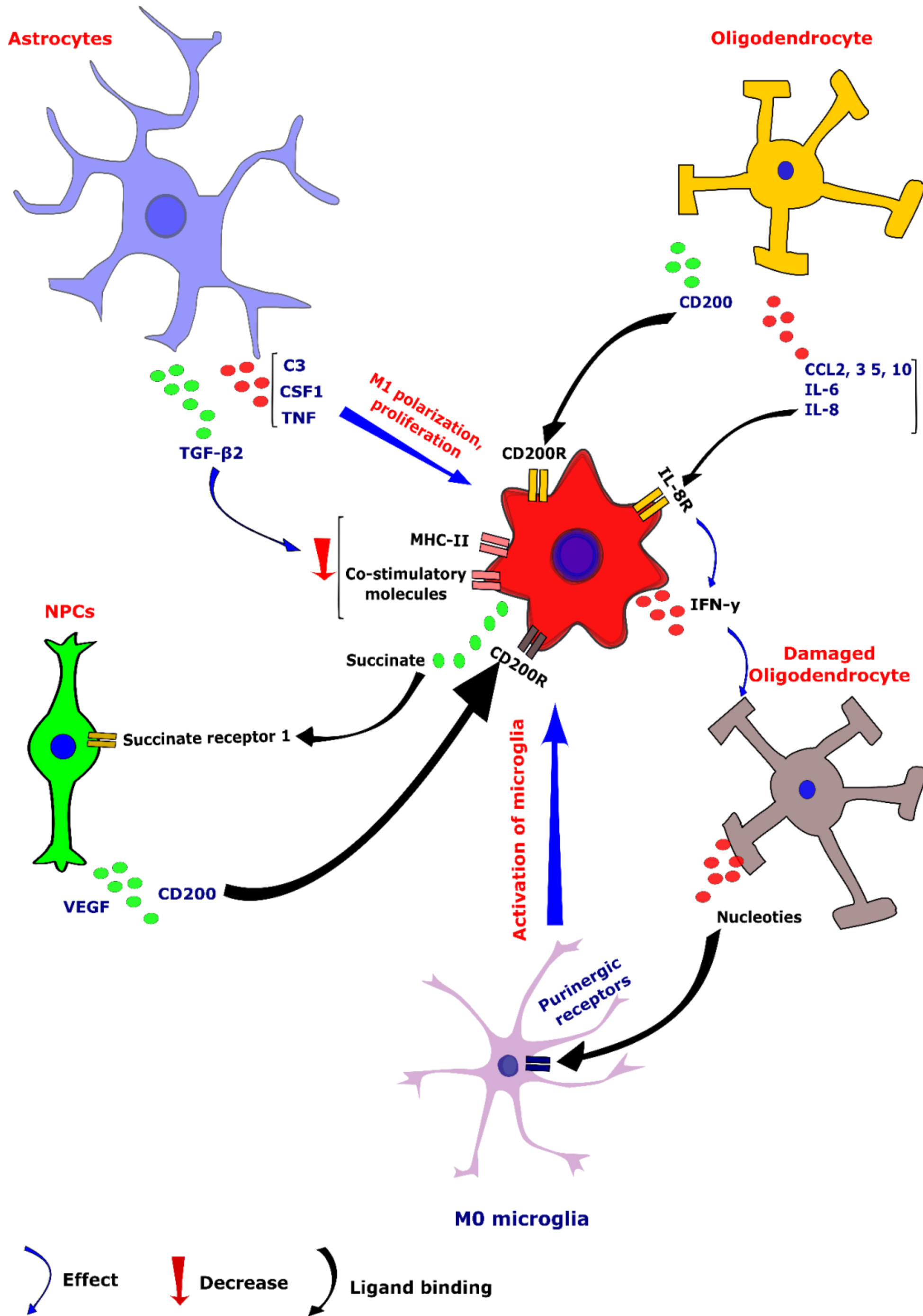


Figure 4. Astrocytes, oligodendrocytes and NPCs regulate microglia activity. Astrocyte-mediated TGF-β signaling reduces expression of MHC-II and co-stimulatory molecules in M1 microglia and subsequently affects their antigen presenting function (Siglienti et al., 2007). Activated astrocytes produce C3, CSF1, and TNF, which further stimulate M1 microglia

polarization and proliferation (Saijo et al., 2009; Saijo and Glass, 2011). Oligodendrocyte-derived CD200 glycoprotein and microglia CD200R receptor down-regulate microglia activity (Koning et al., 2009). Oligodendrocytes exposed to IFN-γ express CCL2, CCL3, CCL5 and CXCL10 chemoattractants, which influence microglia recruitment during the acute stage of neuroinflammation (Balabanov et al., 2007; Ramesh et al., 2012). IL-18 expressed by oligodendrocytes binds to IL-18R on microglia in which stimulate IFN-γ production causing damage to oligodendrocytes (Cannella and Raine, 2004). Damaged oligodendrocytes produce extracellular nucleotides, which bind to purinergic receptors expressed on M0 microglia and activate microglia (Peferoen et al., 2014). Interactions between microglia derived-succinate and transplanted NPC succinate receptor 1 stimulate the secretion of PGE2, which induces an anti-inflammatory response (Peruzzotti-Jametti et al., 2018). VEGF expression is a critical mechanism for NPC regulation of microglia proliferation, chemotaxis and phagocytosis (Mosher et al., 2012). NPCs also modulate microglia activity through CD200/CD200R signaling pathway (Liu et al., 2013a). **Abbreviations:** CCL2,3,5,10, the chemokine (C-C motif) ligand 2,3,5,10; TNF, tumor necrosis factor alpha; C3, complement component 3; CSF1, colony stimulating factor 1; IFN-γ, interferon gamma; IL-8, interleukin 8; IL-8R, interleukin 8 receptor; TGF-β2, transforming growth factor beta 2; VEGF, vascular endothelial growth factor; MHC-II, major histocompatibility complex II; CD200, cluster of differentiation 200; CD200R, cluster of differentiation 200 receptor.

1.4. Neuregulin-1 and ErbB network

Neuregulins (NRGs) are a large family of proteins consisting of four members, Nrg-1, Nrg-2, Nrg-3 and Nrg-4. NRGs are related to the epidermal growth factor (EGF) protein superfamily since they share an EGF-like domain (Lemke, 1996). Structurally-related NRG proteins are expressed in the CNS, heart, gastrointestinal tract and mammary glands (Burden and Yarden, 1997; Garratt et al., 2000; Garratt, 2006; Zhao, 2013). For cell-cell communication in the CNS, NRGs signal through the transmembrane ErbB tyrosine kinase receptors (Lemke, 1996; Burden and Yarden, 1997). NRG-ErbB receptor signaling stimulates intracellular pathways, which elicit different cellular responses including proliferation, differentiation, migration and survival (Burden and Yarden, 1997; Adlkofer and Lai, 2000).

Nrg-1, -2, -3 and -4 are encoded by individual genes which undergo alternative splicing to generate different isoforms for an isolated protein (Falls, 2003). All the NRG isoforms share an extracellular EGF-like domain for activation of ErbB receptors and the consequent cellular responses. However, the affinity of the EGF-like domain differs among different types of proteins due to an alternative splicing of a single gene which gives rise to EGF-like domain variants, including α and β (Burden and Yarden, 1997). The extracellular motifs in NRG proteins consist of a single peptide, N-terminal domain variants including cysteine-rich domain (CRD) or kringle motif-related domain, transmembrane and glycosylation domains and an Ig-like loop (Figure 5). NRG proteins can be transmembrane or secretory based on the presence or absence of the transmembrane domain (Burden and Yarden, 1997).

Among the members of NRG family, Nrg-1 is the best characterized protein. In the CNS, and peripheral nervous system (PNS), Nrg-1 is a neuronally-derived growth factor (Mei and

Xiong, 2008). Nrg-1 consists of six proteins (I-VI) and 31 isoforms including Neu differentiation factor (NDF), heregulin (HRG) and acetylcholine-receptor-inducing activity (ARIA) as type I Nrg-1, glial growth factor (GGF) as type II, and sensory and motoneuron-derived factor (SMDF) and CRD Nrg-1 as type III (Bao et al., 2003; Mei and Xiong, 2008).

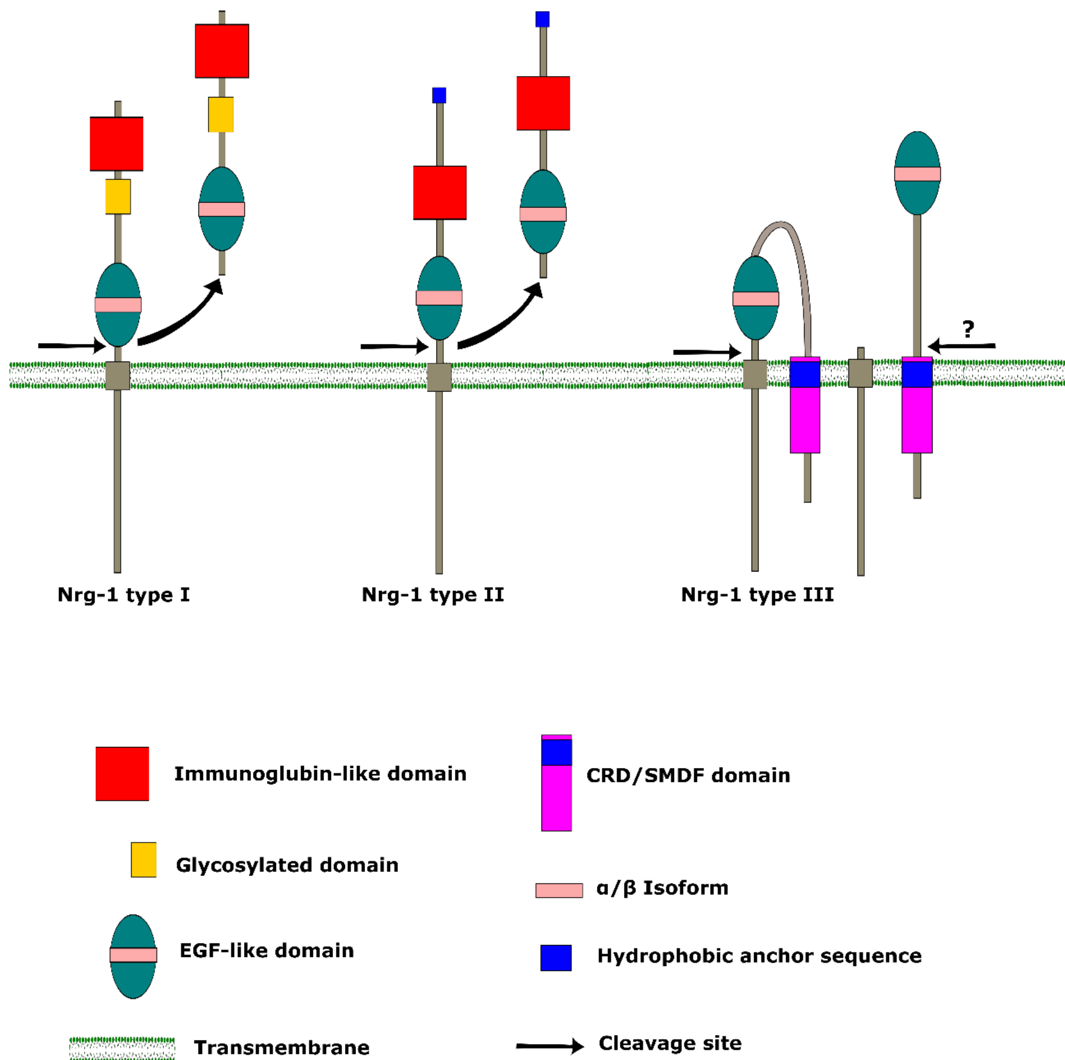


Figure 5. Nrg-1 isoforms and their structures. The gene encoding Nrg-1 undergoes alternative splicing to generate different isoforms for an isolated protein (Falls, 2003). All the Nrg-1 isoforms share an extracellular EGF-like domain for activation of ErbB receptors. EGF-like domain variants include α and β (Burden and Yarden, 1997). The extracellular motifs in Nrg-1 proteins consist of a single peptide, N-terminal domain variants including cysteine-rich domain (CRD) or kringle motif-related domain, transmembrane and glycosylation domains and an Ig-like loop (Burden and Yarden, 1997).

Nrg-1 isoforms are initially produced as precursor proteins that are anchored to the cell membrane. Nrg-1 precursor protein, also known as pro-Nrg-1 contains a functional EGF-like domain extracellularly. The juxta-membrane region of pro-Nrg-1 is then proteolytically cleaved on the C-terminus of EGF-like domain to release Nrg-1 ligand by TNF-α converting enzymes (TACE or ADAM17) (Loeb et al., 1998; Montero et al., 2007), β-site of amyloid precursor protein cleaving enzyme (BACE) (Hu et al., 2006; Willem et al., 2006), and meltrin beta (ADAM19). All Nrg-1 isoforms, except type III, are released to the extracellular space as a diffusible and mature protein (Yokozeki et al., 2007).

Nrg-1 ligand signals through activation of ErbB receptors. ErbB family of receptors comprises four structurally-homologous transmembrane protein tyrosine kinases, including ErbB1 (EGFR, HER1), ErbB2 (Neu or HER2), ErbB3 (HER3) and ErbB4 (HER4) (Bublil and Yarden, 2007). Among these ErbB receptors, ErbB3 and ErbB4 contain Nrg-1 binding domain. Binding of Nrg-1 EGF-like domain stimulates ErbB3 or ErbB4 receptors, which leads to ErbB4 homodimer or their heterodimers with ErbB2 and thereby tyrosine phosphorylation (Yarden and Sliwkowski, 2001; Esper et al., 2006; Talmage, 2008). Since ErbB4 has both Nrg-1 binding site and intracellular tyrosine kinase domain, it can both interact with Nrg-1 and become phosphorylated. However, ErbB2 lacks the binding site for the ligand, thus it only acts as a co-receptor for ErbB3 and ErbB4 to form heterodimers (Tzahar et al., 1996). On the other hand, ErbB3 possesses an impaired kinase domain and therefore its homodimers are catalytically inactivated (Figure 6) (Guy et al., 1994).

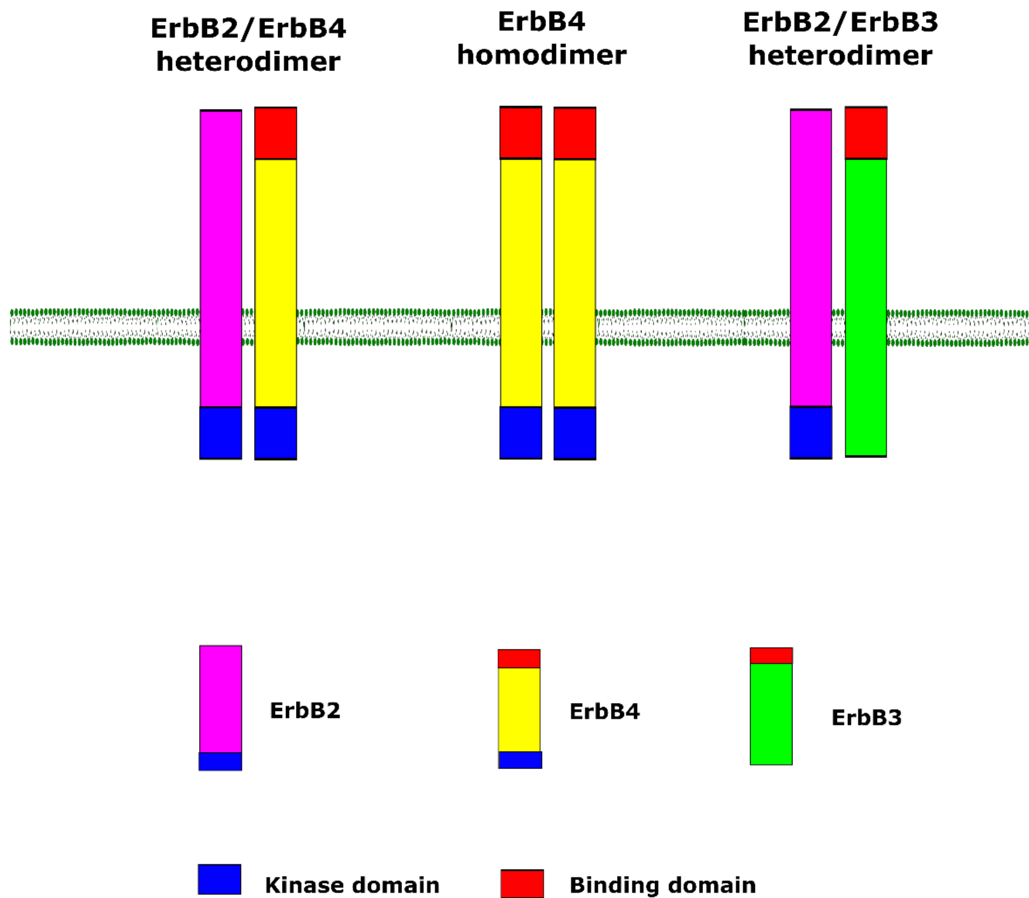


Figure 6. Nrg-1 signaling through ErbB receptors. Nrg-1 binding to the functional EGF-like domain of ErbB receptors stimulates ErbB3 or ErbB4 receptors which leads to ErbB4 homodimer or their heterodimers with ErbB2 and thereby tyrosine phosphorylation (Yarden and Sliwkowski, 2001; Esper et al., 2006; Talmage, 2008). ErbB2 lacks the binding site for the ligand, thus it only acts as a co-receptor for ErbB3, which possesses an impaired kinase domain, or ErbB4 to form heterodimers (Guy et al., 1994; Tzahar et al., 1996).

1.5. Role of Nrg-1 in the normal CNS

Nrg-1 is an important growth factor for multiple organs. The studies in mice show that Nrg-1-null mutant mice die at mid-gestation stage due to cardiac defects indicating that Nrg-1 is essential for cardiac development and life (Gassmann et al., 1995; Lee et al., 1995; Kramer et al., 1996). In the normal CNS, Nrg-1 and its receptors are ubiquitously expressed in neurons and glial cells in regions where gliogenesis or neurogenesis occurs during development and adulthood (Corfas et al., 1995; Steiner et al., 1999).

In the developing CNS, Nrg-1 signaling plays an important role in neuronal migration (Anton et al., 1997; Rio et al., 1997). Nrg-1, which is expressed by migrating neurons, promotes radial glial cell formation and extension through ErbB2 and ErbB3 receptors (Rio et al., 1997). Furthermore, Nrg-1-ErbB4 signaling promotes NPCs migration from the SVZ into the olfactory bulbs through the RMS to replace interneurons in the adult brain of rodents (Luskin, 1993). A subsequent study on ErbB4-deficient mice indicated a regulatory role for ErbB4 in migration and organization of neuroblast chains and differentiation of new interneurons in olfactory bulbs (Anton et al., 2004). Nrg-1 Type III-ErbB4 signaling also mediates migration of GABAergic interneurons from the medial ganglionic eminence to postnatal cortex (Flames et al., 2004). Nrg-1 is also implicated in the formation of neuronal networks by participating in axon guidance. Studies using recombinant Nrg-1 have shown induction of neurite outgrowth in hippocampal, thalamic, retinal and cerebellar neurons (Bermingham-McDonogh et al., 1996; Rieff et al., 1999; Gerecke et al., 2004; Lopez-Bendito et al., 2006).

Nrg-1-ErbB signaling controls the development of oligodendrocytes in the CNS (Barres and Raff, 1999). Nrg-1 promotes the proliferation, survival, and maturation of oligodendrocytes in the CNS (Vartanian et al., 1994; Fernandez et al., 2000; Miller, 2002; Makinodan et al., 2012).

Nrg-1 expression in the SVZ coincides with oligodendrocyte differentiation in rats (Vartanian et al., 1994). Nrg-1 promotes differentiation of NPCs and bipotential glial progenitors, oligodendrocyte-type-2 astrocyte (O2A), into oligodendrocytes (Vartanian et al., 1994). Our group and others have shown that addition of recombinant Nrg-1 to NPC cultures significantly induces oligodendrocyte differentiation (Calaora et al., 2001; Gauthier et al., 2013). Importantly, *In vitro* studies have unraveled an essential role for Nrg-1 in oligodendrocyte differentiation as oligodendrogenesis failed in the Nrg-1-null mutant embryos (Vartanian et al., 1999). Interestingly, exogenous Nrg-1 treatment was able to rescue oligodendrogenesis in these experiments (Vartanian et al., 1999). The knock-down of ErbB2 results in oligodendrocyte apoptosis and widespread hypomyelination in mice (Kim et al., 2003). These studies suggest that Nrg-1-ErbB2 signaling is a key player in regulating the timing that OPCs exit their proliferation state and differentiate into myelinating oligodendrocytes (Kim et al., 2003). This notion was further supported by studies using ErbB2-null mutant mice in which development of oligodendrocytes was stopped at a pre-oligodendroblast stage (Park et al., 2001). This study showed that disruption of Nrg-1-ErbB has no effect on the number of myelinated vs unmyelinated axons in the CNS, supporting the idea that Nrg-1 is necessary for the final stage of oligodendrocytes differentiation (Park et al., 2001). Nrg-1-ErbB signaling has also been reported to support differentiation, migration, and expansion of oligodendrocytes precursors (Miller, 2002; Azim et al., 2012; Ortega et al., 2012). For example, Nrg-1-ErbB4 interactions regulate OPC migration from the neural tube to the optic nerve and OPC colonization during early CNS development (Ortega et al., 2012). Nrg-1 is important for the quality of myelination. Studies on dominant-negative (DN) transgenic animals have shown a reduction in myelin thickness associated with reduced axonal conduction velocity in DN-ErbB4 mice compared to wide-type animals (Roy et al., 2007). In spinal cord injury, combination of glial growth factor

2 (GGF2, Nrg-1β3) and fibroblast growth factor 2 (FGF2) induces remyelination processes through OPC and Schwann cell recruitment (Lytle et al., 2009; Whittaker et al., 2012). In focal demyelinating lesions, our groups has also demonstrated that Nrg-1 therapy accelerates remyelination and promotes myelin thickness (Kataria et al., 2018).

Nrg-1 is implicated in synapse formation during the development and adulthood (Jessell et al., 1979; Falls et al., 1993). In the developing CNS, acetylcholine receptor (AChR) synthesis is influenced by Nrg-1 type III, while $GABA_A$ receptors expression are altered by Nrg-1 type I and II isoforms (Rieff et al., 1999; Liu et al., 2001; Okada and Corfas, 2004). However, glutamate receptors such as N-methyl-D aspartate (NMDA) and α-amino-3-hydroxyl-5-methyl-4-isoxazole propionic acid (AMPA) are altered by three isoforms of Nrg-1 (Ozaki et al., 1997; Bjarnadottir et al., 2007). Studies on $Nrg\text{-}1^{+/-}$ mice have shown a considerable reduction in the number of functional NMDA receptors in forebrain. In the adult brain, Nrg-1/ErbB4 signaling is also critical for controlling excitatory and inhibitory synaptic transmissions such as glutamatergic, GABAergic and dopaminergic neurotransmissions (Harrison, 2004; Moghaddam, 2004; Lewis and Moghaddam, 2006; Neddens et al., 2009; Balu and Coyle, 2011). Altogether, depending on the type of Nrg-1 expressed in the CNS regions, glutamate receptors, which are involved in regulation of synaptic transmissions, are differentially modulated (Stefansson et al., 2002; Niswender and Conn, 2010).

1.6. Role of Nrg-1 in pathologic CNS

While the role of Nrg-1 has been studied for decades in the normal developing and adult CNS, its role in CNS pathology has been explored in recent years by our group and others. Our group has performed extensive investigations and provided the first evidence that Nrg-1 is dysregulated in

traumatic SCI and focal demyelinating lesions of the spinal cord (Gauthier et al., 2013; Kataria et al., 2018). Outcomes of these studies have identified a significant role for Nrg-1 in pathophysiology of SCI and demyelination, which will be discussed in the following sections.

1.6.1. Nrg-1 and endogenous cell response after injury and demyelinating disease

As discussed earlier, spinal cord and brain precursor cells show a restricted capacity to replace oligodendrocytes and remyelinate injured axons in the hostile dysregulated microenvironment of demyelinating lesions (Lau et al., 2012; Alizadeh et al., 2015). This is partly attributed to injury-induced downregulation of essential growth factors for oligodendrogenesis, oligodendrocytes maturation and axon-oligodendrocytes signaling for myelination (Alizadeh et al., 2015). Our group has shown for the first time that Nrg-1 expression is dysregulated in lesions of SCI and focal demyelination (Gauthier et al., 2013; Kataria et al., 2018). In rat SCI, Nrg-1 protein and transcript is rapidly and persistently reduced in the lesion with no restoration in its levels at later stages of injury (Gauthier et al., 2013). We have made similar observations in focal demyelinating lesions induced by administration of lysophosphatidyl-choline (lysolecithin, LPC) into the spinal cord of rats (Kataria et al., 2018). In these studies, we have established a strong correlation between dysregulation of Nrg-1-ErbB signaling and inadequate oligodendrocytes replacement in these lesions. Additionally, these studies have shown a strong association between reduced Nrg-1 levels and induced astrogliosis, which leads to glial scar formation and inhibition of remyelination following injury (Kataria et al., 2018). Interestingly, the delivery of recombinant human Nrg-1 consisting of the EGF-like functional domain to the injured spinal cord enhances oligodendrocyte differentiation and preservation, and reduces astrocyte differentiation in a compressive/contusive model of SCI in the rat (Gauthier et al., 2013). Nrg-1 restoration also mitigates SCI-induced axonal

degeneration, which results in tissue preservation and functional recovery in rats with SCI (Gauthier et al., 2013). Another group has also shown that Nrg-1 signaling induces endogenous remyelination and functional recovery in mice with SCI by trans-differentiation of OPCs into PNS-like Schwan cells (Bartus et al., 2016).

In LPC-induced focal demyelinating lesions of spinal cord, we have demonstrated that a sustained release of human recombinant Nrg-1b1 (rhNrg-1ß1) into the spinal cord for 28 days using poly lactic-co-glycolic acid microcarriers promotes oligodendrogenesis, oligodendrocytes maturation, and remyelination in focal demyelinating lesions (Kataria et al., 2018). In addition, intraspinal delivery of Nrg-1 accelerates oligodendrocyte and Schwann cell-mediated remyelination and induces the thickness of new myelin sheath around the remyelinated axons (Kataria et al., 2018). This is particularly important as remyelinated axons show myelin thinning and suboptimal function compared to normally myelinated axons. Our *in vitro* results have also revealed a significant role for Nrg-1 in promoting morphological complexity of oligodendrocytes and their maturation into myelinating cells (Kataria et al., 2018). Collectively, these studies have established an important role for Nrg-1 in supporting oligodendrogenesis and remyelination after CNS injury.

1.6.2. Nrg-1 and neuroinflammation

There is also an increasing body of evidence indicating a neuroprotective role for Nrg-1 in ischemic stroke models that it is attributed to its ability in reducing the release of pro-inflammatory cytokines (Xu et al., 2006; Mencel et al., 2013; Li et al., 2015). Nrg-1 pre-treatment of animals with cerebral ischemia considerably reduces the transcript expression of pro-inflammatory cytokines, IL-1β and IL-6 but not TNF-α compared to non-treated animals (Li et al., 2015). *In*

vitro studies by our group and others have also shown the ability of Nrg-1 in reducing the expression of pro-inflammatory cytokines in rat SCI and in LPS-activated microglia *in vitro* (Mencel et al., 2013; Alizadeh et al., 2017). Another study on N9 microglia cell line has shown anti-inflammatory effects of GGF2 on production of free radicals in activated microglia cultures (Dimayuga et al., 2003). Immunomodulatory effects of Nrg-1 have also been demonstrated in other inflammatory models such as cerebral malaria model (Solomon et al., 2014).

Our group has recently provided strong evidence that Nrg-1 can induce the expression of IL-10 by activated microglia/macrophages and regulatory T cells in SCI and LPC-induced focal demyelination models (Alizadeh et al., 2017; Alizadeh et al., 2018; Kataria et al., 2018). IL-10 is highly expressed by alternatively-activated M2 microglia and plays positive roles in oligodendrogenesis and remyelination (Alizadeh and Karimi-Abdolrezaee, 2016). In primary NPC cultures, IL-10 directly drives their fate toward oligodendrocyte lineage (Dyck et al., 2018). Interestingly, IL-10 expression is dramatically declined in MS-affected patients as compared to healthy individuals (Salmaggi et al., 1996; Ozenci et al., 1999). Injection of adult NPCs expressing IL-10 in EAE mice attenuates inflammatory responses and disease severity (Yang et al., 2009). Likewise, direct administration of IL-10 prevents EAE development (Rott et al., 1994). Immunomodulatory effects of IL-10 are shown to be mediated through down-regulation of MHC class II molecules in monocytes and suppressing the production of NO, IFN-γ, TNFα, IL-1, IL-6 and GM-CSF by activated microglia/macrophages (Howard and O'Garra, 1992; Oswald et al., 1992; Conti et al., 2003).

In SCI, our group has identified that intrathecal or systemic delivery of Nrg-1 fosters an M2 phenotype in microglia/macrophages characterized by increased expression of IL-10 and arginase-1, while reduces expression of pro-inflammatory mediators including NO, IL-1β and

TNF-α, matrix metalloproteinase (MMP)-2 and -9 in both primary cultures of microglia and severe compressive SCI in rats. Additionally, our studies have identified that Nrg-1 treatment promotes the populations of regulatory T cells (T_{regs}) and regulatory B cells (B_{regs}) in the blood and spinal cord following SCI (Alizadeh et al., 2018). These cell populations are known to exert beneficial roles in the injured spinal cords by increasing the levels of IL-10 (Chung et al., 2007; Walsh et al., 2014; Puntambekar et al., 2015). Interestingly, Nrg-1 treatment induces the expression of CCL11 which is known to induce NPC migration and proliferation after cerebral ischemic injury (Wang et al., 2017; Alizadeh et al., 2018). On the other hand, Nrg-1 mitigates the expression of pro-inflammatory cytokines, IL-6, IFN-γ and chemokines, CXCL1, CXCL2, and CXCL3 in rat SCI (Alizadeh et al., 2018). Other groups have shown that Nrg-1 attenuates inflammatory responses and induces neuroprotection in ischemic models of stroke and neuroinflammation (Xu et al., 2005; Li et al., 2007; Li et al., 2015). Immunomodulatory effects of Nrg-1 on microglia are shown to be mediated through mitigating NF-κB pathway, which is a key regulatory pathway in neuroinflammation (Simmons et al., 2016).

Our studies has also unraveled a positive role for Nrg-1 in regulating astrocyte activity in response to injury (Alizadeh et al., 2017). We have shown that increased bio-availability of Nrg-1 in rat SCI or cultures of activated rat astrocytes moderates astrogliosis (Alizadeh et al., 2017). More importantly, Nrg-1 treatment results in a remarkable decrease in production of inhibitory CSPGs by activated astrocytes. Of note, CSPGs are a negative regulator of inflammation after SCI and EAE (Dyck et al., 2018; Stephenson et al., 2018). Moreover, studies in our laboratory has identified that CSPGs inhibit several properties of NPCs including their potential for oligodendrogenesis (Dyck et al., 2015). Mechanistically, our earlier studies showed that Nrg-1 binding at the cell surface of rat astro-microglia causes ErbB3/ErbB2 heterodimerization and

ErbB2 tyrosine phosphorylation. Subsequently, activation of Nrg-1/ErbB signaling leads to downregulation of MyD88, an adaptor protein in TLR/NF-κB pathways while promoting ErK1/2 and STAT3 phosphorylation (Alizadeh et al., 2017).

In summary, evidence from our group and others suggest a positive role for Nrg-1 in regulating glial cells as well as infiltrating leukocytes after SCI and demyelinating lesions. Our laboratory has demonstrated that intrathecal and systemic delivery of Nrg-1 into the injured spinal cord of rats positively modulates post-injury microenvironment and improves neurological recovery from SCI. Therefore, my thesis is focused on understanding how Nrg-1 influences the activity of microglia under normal and injury states, and determine whether the presence of Nrg-1 can harness the potential of microglia in promoting a regenerative response by NPCs.

Chapter II: Study rationale, hypothesis and research objectives

2.1. Study rationale

Neuroinflammation is a pathological hallmark of several neurological conditions of the CNS, including neurotrauma, stroke and MS (Alexander and Popovich, 2009a; David and Kroner, 2011; Alizadeh et al., 2015). Microglia are the main innate immune cells of the CNS that in concert with other infiltrating cells initiate neuroinflammatory responses leading to oligodendroglial death and myelin damage in demyelinating conditions (Zimmerman, 1956). Demyelination results in permanent degeneration of axons and functional deficit (Zimmerman, 1956; Caprariello et al., 2012). Thereby, replacement of damaged oligodendrocytes is a vital repair strategy for improving neurological functions after demyelinating conditions.

Adult brain and spinal cord contain precursor cells with the capacity to replace oligodendrocytes and remyelinate injured axons; however the efficacy of this process is limited in the hostile inflammatory environment of demyelinating lesions (Lau et al., 2012; Alizadeh et al., 2015). Our lab has demonstrated that injury-induced downregulation of Nrg-1 underlies the inadequate replacement of oligodendrocytes in demyelinating lesions of the spinal cord (Gauthier et al., 2013). Nrg-1 is a neuronally-derived growth factor that plays essential roles in oligodendrocyte differentiation, survival, maturation, and myelination during the development and in the adulthood (Vartanian et al., 1999; Mei and Xiong, 2008). Our evidence from preclinical models of SCI and focal demyelinating-MS like lesions indicate that Nrg-1 level is robustly and persistently decreased in demyelinating lesions (Gauthier et al., 2013; Kataria et al., 2018). We also observed the same results in the animal model of MS, EAE, as well as human brain MS tissues (unpublished data). In our previous studies, we also showed that Nrg-1 therapy can promote

oligodendrocyte replacement following SCI (Gauthier et al., 2013). In LPC-induced demyelination model, we also have demonstrated that a sustained release of rhNrg-1b1 using poly lactic-co-glycolic acid microcarriers to the spinal cord promotes oligodendrogenesis, oligodendrocytes maturation, and remyelination in focal demyelinating lesions (Kataria et al., 2018). Importantly, our lab has also identified a positive immunomodulatory role for Nrg-1, which induces a switch from a pro-inflammatory to an anti-inflammatory immune response (Alizadeh et al., 2017; Alizadeh et al., 2018). Altogether, this evidence suggests an immunomodulatory role for Nrg-1 in CNS injury. The present study will provide us with further insight into the direct effects of Nrg-1 on the activity of microglia using primary *in vitro* systems.

2.2. Hypothesis: Availability of Nrg-1 promotes a supportive phenotype in microglia that can enhance the regenerative response of neural precursor cells.

2.3. Research objectives

1. To investigate the role of Nrg-1 treatment in regulating the activity of microglia under normal and injury states using *in vitro systems.*
2. To investigate the paracrine effects of Nrg-1 treated microglia on the properties of neural precursor cells (NPCs) *in vitro.*

Chapter III: Material and methods

3.1. Materials and animals

3.1.1. Animals

All experimental procedures in the present study were approved by the University of Manitoba Animal Ethics Committee in accordance with the Canadian Council of Animal Care guidelines and policies. We used a total of 137 enhanced yellow fluorescent protein-tagged (EYFP Tg (strain 129-Tg (ACTB-EYFP) 2Nagy/J) mice pups (1–3 days old) for microglia and NPC cultures from a colony (CAG-EYFP tg) maintained at local facility of the University of Manitoba, Winnipeg, Canada. Adult mice were housed in standard plastic cages for their newborn pups at 22°C with a 12 hour light/dark cycle. Pelleted food and drinking water were available *ad libitum*.

3.1.2. Materials

Table 1. Reagents

Product	Source
Acrylamide (30% Ac/Bis sol. 29:1)	Bio-Rad
APS powder	Sigma
Agarose	Invitrogen
B-mercaptoethanol	Sigma
BrdU	Sigma
BSA (albumin from bovine serum)	Sigma
BSA protein standart (2mg)	Sigma
Calcium chloride dihydrate	Sigma
cDNA synthesis kit	Abm
Chloroform	Sigma
DAPI	Sigma
D-Glucose	Sigma
DMEM (powder) NPC	Invitrogen
DNA ladder 100bp	NEB
Dulbecco's PBS	GIBCO
ECL kit (Supersignal West Pico)	Thermo Scientific Fisher
ECL-Frogga Bio	FroggaBio
Fetal bovine serum	Invitrogen
F12 nutrient (powder)	Invitrogen
Folin & Ciocalteu's Phenol Reagent	Sigma
100% Ethanol	Commercial Alcohol
Gibco fetal bovine serum (FBS)	Fisher scientific
Glycine	Amresco
Greiss nitrite kit	Fisher (Promega)
HEPES (powder)	Sigma
HEPES (1M solution)	Invitrogen
Horse serum	Sigma
Latex beads, amine-modified polystyrene, fluorescent red	Sigma
L-Glutamin (100x)	Invitrogen
Lowry reagent powder (2gr)	Sigma
Magnesium chloride hyxahydrate	Sigma
Matrigel	BD-VWR
Methanol	Fisher
Milk powder (Blocking grade)	BioRad
Mowiol	Sigma
NaHCO3	Sigma
Paraformaldehyde	Sigma

Penicilin/streptomycin/neomycin	Invitrogen
Phenol red	Sigma
Poly (D,L-lactide-co-glycolide)	Sigma
Polyethylenimine (PEI)	Sigma
Ponceau powder - 50g -	MP Biomedicals
Potassium chloride	Sigma
Recombinant human Nrg-1	Shenandoah Biotechnology
Recombinant mouse IFN-γ	Shenandoah Biotechnology
Recombinant mouse TNF-α	Shenandoah Biotechnology
Rnase free water (Ultrapur)	Invitrogen
SDS	Sigma
sterile BSA 7.5% (gradient)	Sigma
Stripping Buffer Gentle review	VWR
Sodium Chloride	Sigma
Sodium Pyruvate	Sigma
Sodium Tetraborate Decahydrate	Fisher scientific
SYBER GREEN	Invitrogen
TEMED	Sigma
Triton® X-100X	Sigma
Trizma Base Cell culture Tested	Sigma
TRIzol® reagent	Invitrogen
Tween	Fisher

Table 2. Antibodies

Antibody	Source	Application	Dilution factor
Alexa Fluor® 568 goat anti-Chicken	Invitrogen (Chicken)	ICC	1:600
Alexa Fluor® 568 goat anti-mouse	Invitrogen (Mouse)	ICC	1:600
Alexa Fluor® 568 goat anti-Rabbit	Invitrogen (Rabbit)	ICC	1:600
Alexa Fluor® 647 goat anti-Mouse	Invitrogen (Mouse)	ICC	1:600
Alexa Fluor® 647 goat anti-Rabbit	Invitrogen (Rabbit)	ICC	1:600
BrdU	BD (Mouse)	ICC	1:400
ErbB2	Santa Cruz (Rabbit)	ICC	1:100
ErbB3	Santa Cruz (Rabbit)	ICC	1:100
ErbB4	Santa Cruz (Rabbit)	ICC	1:100
GAPDH	Santa Cruz (Rabbit)	WB	1:1000
GFAP	DAKO (Rabbit)	ICC	1:800
Iba-1	Wako (Rabbit)	ICC	1:500
IFN-γ	R&D (Rat)	WB	1:1000
Nestin	AVES (Chicken)	ICC	1:500
NG2	Chemicon (Rabbit)	ICC	1:300
OX42 (CD11b)	Cederlane (Mouse)	ICC	1:100
TNF-α	Serotec (Rabbit)	WB	1:1000

Table 3. Primers

Genes	Primer	Size
CD86	F: GAAGCCGAATCAGCCTAGCAG R: CACTCTGCATTTGGTTTTGCTGAAG	132
ErbB2	F: CTTTGGGGCCAAACCTTACG R: GGAGTCAATCATCCAACATTTGACC	139
ErbB3	F: GACGAGAATATTCGCCCAACCT R: CCCACTCGCTCTCTTGATGAC	99
ErbB4	F: CTGATGGTGGCAAGATGCCAAT R: CATAGGGCTTTCCTCCAAAGGT	135
H2afz	F: CTCACCGCAGAGGTACTTGA R: CCACGTATAGCAAGCTGCAAG	95
IL-6	F: TCCTCTCTGCAAGAGACTTCC R: AGTCTCCTCTCCGGACTTGT	96
IL10	F: ACTTGGGTTGCCAAGCCTTA R: AGAAATCGATGACAGCGCCT	160
TNF-a	F: GTAGCCCACGTCGTAGCAAAC R: GTCTTTGAGATCCATGCCGTTG	99

3.2. Methods

3.2.1. Microglia isolation and culture

Primary microglia cultures were prepared from the cerebral cortex of postnatal EYFP Tg mice (1-3 days of age) as we described previously (Alizadeh et al., 2017). Briefly, meninges were stripped away from cerebral cortices. Tissues were mechanically dissociated in artificial cerebrospinal fluid solution (aCFS) (containing 124 mM NaCl, 3 mM KCl, 1 mM NaHPO4, 26 mM NaHCO3, 1.5 mM MgSO4, 1.5 mM CaCl2, and 10 mM glucose) with 1% PSN by 50–60 pipetting strokes. Cells were isolated by centrifugation at 1,000 rpm for 5 min, and the pellet was dissolved in complete DMEM supplemented with 10% heat inactivated fetal bovine serum, 1% penicillin–streptomycin–neomycin PSN. Dissociated cells were plated into 75 cm^2 flasks coated with 1mg/ml polyethylenimine (PEI) diluted in borate buffer (pH=8.3) (Michel et al., 1997; Rousseau et al., 2013). Then, flasks were washed 4 times with PBS before cell seeding. After 48h, eight milliliters of medium was removed to eliminate floating debris and 12 ml of fresh medium was added. No additional medium change was done until the cultures were shaken to separate microglia from any remaining astrocytes (10-14 days). In this method, PEI coating limits the growth of astrocytes and thereby increases the purity and the number of microglia in primary cultures. Of note, we isolated microglia from EYFP mice to be consistent with the strain of the mice that were utilized in our NPC culture.

3.2.2. Microglia polarization

Microglia polarization was performed as described in our previous studies (Dyck et al., 2018). Primary mouse microglia were plated in 24 well plates ($1.25x10^5$ cells per well) in 50% glia

conditioned media (CM) (collected and filter sterilized after shaking) and 50% complete DMEM with 10% FBS media. Cells were allowed one day to attach and spread their processes. Media was then changed to serum free media (SFM) to rule out the influence of serum compositions. One day following serum deprivation, microglia were treated with the combination of IFN-γ and TNF-α. To determine the effective concentration of IFN-γ for microglia polarization into M1 phenotype, pure microglia culture were treated with the combination of 50 ng/ml of TNF-α and 10, 20, 40 ng/ml of IFN-γ, representing as low, mid, and high dosages. Microglia condition media (MCM) was collected at 24 h and 72 h after polarization and assessed for its nitrite content using Griess assay. The most effective combination of IFN-γ and TNF-α for microglia polarization into M1 phenotype was determined to be 50 ng/ml of TNF-α and 40ng/ml of IFN-γ. M1 polarization was confirmed using Griess assay, real-time qPCR, and immunocytochemical analyses for M1 markers.

3.2.3. Optimization of Nrg-1 concentration in microglia cultures

We performed an *in vitro* assay to determine an optimal concentration for Nrg-1 in our microglia study. Our dosing assay included 10, 25, 50, 200 ng/ml of Nrg-1 that is representative of low (10 and 25 ng/ml), medium (50 ng/ml), and high (200 ng/ml) concentration *in vitro*. The effects of Nrg-1 on microglia polarization were then assessed by Griess assay for nitrite levels in MCM at 24 h and 72 h after Nrg-1 treatment as well as and real-time PCR on microglia cell lysate at 24 h time point. Our dose-response assay identified the efficacy of both 50 ng/ml (low dose) and 200 ng/ml (high dose) concentration of Nrg-1 in various assessments (Figure 4A,B). Our experimental conditions included **(1)** non-activated resting microglia termed as M0, **(2)** Nrg-1 50 ng/ml-treated M0 microglia termed as "M0+Nrg-1 50 ng/ml", **(3)** Nrg-1 200 ng/ml-treated M0 microglia as

"M0+Nrg-1 200 ng/ml", **(4)** IFN-γ+ TNF-α-activated microglia termed as "M1", **(5)** Nrg-1 50 ng/ml-treated M1 microglia as "M1+ Nrg-1 50 ng/ml", **(6)** Nrg-1 200 ng/ml-treated M1 microglia as "M1+Nrg-1 200 ng/ml". At 72 h following treatment, MCM were collected and stored in -80 for future experiments.

3.2.4. Griess assay for detection of nitrite

Nitrite level was measured as a representative of iNOS activity in MCM collected at 24 h and 72 h following M1 polarization and Nrg-1 treatment using an Griess assay kit (Promega corp. USA) according to the manufacturer instructions (Hu J, 1996). These experiments were performed in phenol red free media to remove possible color interference in measurement of nitrite levels.

3.2.5. RNA extraction and real time PCR

One day following microglia polarization and Nrg-1 (50, 200 ng/ml) treatment, microglia RNA was extracted using Trizol-based manual method and first strand cDNA was synthesized using Reverse Transcriptase (Applied Biological Materials Co.). Quantitative analysis of mRNA expression was performed using quantitative PCR (ABI, Perkin-Elmer PE Biosystems, USA), fluorophore SYBR Green I kit (Invitrogen, Canada) and delta-delta Ct method as we previously described (Gauthier et al., 2013). List of the designed primers in our studies is illustrated in Table 1.

3.2.6. Western blot analysis for cytokine expression in microglia conditioned media

For Western analysis, microglia conditioned media (MCM) was concentrated 30 times using centrifugal column with molecular weight cutoff at 3 kDa (VWR). The concentration of each

sample was measured using the modified Lowry assay. The blocking and antibody solution contained 5% nonfat milk in Tris-buffered saline with 0.5% Tween-20 (TBST). A list of the antibodies used in immunoblotting is provided in Table 2. Specificity of the used anti-bodies in detecting the protein of interest was verified by its specified molecular weight. Fifty µg of MCM, 100 ng/ml and 1µg/ml of recombinant TNF-α and 50 ng/ml of recombinant IFN-γ were loaded onto SDS-PAGE gels and transferred onto nitrocellulose membranes (Bio-Rad). To control for equal protein loading, the membranes were then stained with Ponceau S staining. After washing the membrane, it was blocked in 5% non-fat milk in Tween Tris buffered saline (TTBS) for 1h at room temperature and then incubated with primary antibodies overnight at 4°C (Table 2). After one day, the membrane was washed and incubated with horseradish peroxidase-conjugated goat anti-mouse or anti-rabbit secondary antibodies (1:4000; BioRad) for 1h at room temperature. For developing the membrane, it was incubated in enhanced chemiluminescent immunoblotting detection reagents (FroggaBio, Canada) according to kit's instructions.

3.2.7. Microglia phagocytosis assay

Microglia phagocytosis assay was performed as described in previous studies (Lian et al., 2016; Dyck et al., 2018). One one µl of green fluorescent latex beads with 1µm diameter (Sigma, L1030) was added to 5µl of FBS and kept in incubator for 1hour. The mixture of FBS and beads was then diluted in microglia SFM and added to cell culture at the final concentration of 0.01% (*v/v*). After 1 hour, media was removed and cells were washed one time with PBS (1x). The cells were fixed with paraformaldehyde (PFA) (3%) and then stained with DAPI and imaged. For quantitative analysis, the number of DAPI+ cells containing fluorescent beads was counted. Fluorescent bead

engulfment was confirmed by Z-stacking and co-localization of cells positive for Iba-1 with green fluorescent signal emitted from beads.

3.2.8. Microglia mobility assay

Microglia mobilization was performed as described in previous studies (Miron et al., 2013; Dyck et al., 2018). Microglia were plated onto a non-coated (0.1mg/ml) 24-well plate in SFM. One day after plating, microglia were either polarized into M1 phenotype by IFNγ+TNF-α or remained as non-activated M0. At 24h after polarization, M0 and M1 microglia were collected, centrifuged and plated onto a PDL-coated poly-carbonate transwell culture inserts (Corning, 30,000 cells per transwell) in SFM in a 24-well plate. Nrg-1 (50, 200 ng/ml) was added to the bottom chamber. Cells were then incubated for 16 hours at 37°C to allow for their mobilization to the bottom chamber. After 16h mobilization, cells were fixed with 3% PFA for 20 minutes and stained for DAPI (1:5000). The remaining cells on the upper side of the transwell were gently scraped off with a cotton swab. Cells mobilizing to the bottom side of the transwell membrane were then visualized by DAPI as described previously. The total number of DAPI+ mobilized microglia were determined by taking eight images at 40x magnification.

3.2.9. Immunocytochemistry, imaging and analysis

For immunocytochemistry, cultures were fixed with 3% PFA for 20 min at room temperature and washed with PBS. Cells were incubated in a blocking solution containing 5% non-fat milk, 1% BSA, and 0.5% Triton X-100 in 0.1 M PBS for 1 h. Cultures then underwent an immunostaining procedure as described in our previous studies (Dyck et al., 2018). For BrdU immunodetection, prior to blocking, the slides were incubated in 2 N HCl and 0.5% Triton for 30 min at 37 °C, and

then washed with 0.1 M sodium borate in PBS for 10 min. During this step (referred as DNA denaturing step), DNA is hydrolyzed to make the anti-BrdU antibody accessible to the BrdU within the DNA. After blocking, the slides were incubated with primary antibodies overnight and secondary antibodies were added as previously described (Table 2) (Dyck et al., 2018). For immunocytochemistry quantification, 5-6 separate fields (under $\times$ 20 objective) containing an average of 200-250 cells for each condition were randomly imaged using a Zeiss Imager 2 epi-fluorescence microscope. For each condition, first the total number of DAPI-positive cells was assessed, and then the number of cells which were positive for Nestin, GFAP, or BrdU and DAPI were counted. Under each experimental condition, the percentage of abundance of each cell type was calculated by dividing the number of positive cells for a specific marker by the total number of DAPI+ cells. For relative comparison, values were then normalized to control condition.

3.2.10. Isolation and culture of adult neural precursor cells

Isolation of adult NPCs was performed from SVZ of Sprague Dawley (SD) male rats (6–8 weeks old) as we described previously (Karimi-Abdolrezaee et al., 2006; Kataria et al., 2018). Briefly, mice were euthanized by decapitation after being deeply anesthetized with 40% isoflurane and 60% propylene glycol in a bell jar. Under sterile conditions, the brain was then excised and transferred to aCSF solution. SVZs of each brain were dissected and digested with 1 ml of enzyme mix (1.25 mg/ml trypsin, 0.6 mg/ml hyaluronidase, 0.13 mg/ml kynurenic acid; Sigma) at 37°C for 45 min. Quenching of trypsin activity was performed using three volumes of inhibitor solution (1 mg/ml; Sigma). Cellular components were then spun for 5 min at 1,500g and the pellet was resuspended in growth medium. Resuspended cells were then plated onto uncoated tissue culture flasks (Biolite, Fisher Scientific) in final volume of 10 ml of serum free media (SFM) containing:

(Neurobasal-A medium (Invitrogen), 30% glucose, 7.5% $NaHCO3$, 1 M HEPES, 10 mg of transferrin, 2.5 mg of insulin, 0.96 mg of putrescine, 1 µl of selenium, 1 µl of progesterone, 1% L-glutamine, 1% penicillin/streptomycin/neomycin), and growth factors (I µg of FGF2, 2 µg of EGF, and 200 µg of heparin). The neurospheres generated were passaged weekly by mechanical dissociation in growth media. SFM plus growth factors will be referred to as "growth media" in the text.

3.2.11. Assessment of the effects of microglia conditioned media on NPCs proliferation

NPC proliferation assay was performed as we described previously (Dyck et al., 2018). NPC neurospheres were dissociated into single cells and plated onto matrigel coated multi-chamber glass slides (25,000 cells per chamber) (LabTek II) in growth media. At 24h following cell seeding, media was changed to 50% fresh NPC SFM and 50% microglia CM (MCM collected at 72 h time-point). For BrdU assay, microglia were polarized and treated with Nrg-1 in SFM to rule out the effect of serum on NPCs proliferation. The experimental conditions were as follows: **(1)** control media (50% NPC SFM+ 50% incubated microglia SFM); **(2)** M0 MCM (50% NPC SFM+ 50% M0 MCM); **(3)** M0+Nrg-1 50 ng/ml MCM (50% NPC SFM+ 50% Nrg-1 50 ng/ml-treated M0 MCM); **(4)** M0+Nrg-1 200 ng/ml MCM (50% NPC SFM+ 50% Nrg-1 200 ng/ml-treated M0 MCM); **(5)** M1 MCM (50% NPC SFM+ 50% M1 MCM); **(6)** M1+ Nrg-1 50 ng/ml MCM (50% NPC SFM+ 50% Nrg-1 50 ng/ml-treated M1 MCM) and **(7)** M1+ Nrg-1 200 ng/ml MCM (50% NPC SFM+ 50% Nrg-1 200 ng/ml-treated M1 MCM). Fresh and 72 h pre-incubated Nrg-1 were also used as controls at their defined concentrations. For assessing NPCs proliferation, BrdU (20µM, Sigma) was added to the cultures 4h before NPCs processing for immunocytochemistry as described above.

3.2.12. Assessment of the effects of microglia conditioned media on NPCs differentiation

NPC differentiation assay was performed as described in our previous studies (Dyck et al., 2018). Dissociated NPCs were plated onto matrigel coated multi-chamber glass slides (15,000 cells per chamber) (LabTek II) in growth media. At 24h following cell plating, media was changed to 50% fresh NPC SFM and 50% MCM. For differentiation assay, microglia were polarized and treated with Nrg-1 in microglia regular media. The experimental conditions were as described above. NPCs were then kept in incubator at 37ºC for 7 days to induce differentiation and media was refreshed after 3 days. NPCs differentiation was assessed using immunocytochemistry against the NPCs specific marker, Nestin, the astrocyte specific marker, glial fibrillary acidic protein (GFAP), and the OPC specific marker, NG2.

3.2.13. Assessment of the effects of microglia conditioned media on NPCs migration

Dissociated NPCs were seeded over the right-sided well in upper compartment of each micro-device (Ananda), which was placed on a PDL-coated dish (5000 cells per well) (Corning) in NPCs SFM. Cells were allowed to migrate to lower compartment through capillaries for 20 h. Lower compartments contained **(1)** microglia SFM as the baseline control (control media), **(2,3)** fresh and 72 h pre-incubated Nrg-1 50 ng/ml, **(4,5)** fresh and 72 h pre-incubated Nrg-1 200 ng/ml, **(6)** M0 MCM, **(7)** M0+ Nrg-1 50 ng/ml MCM, **(8)** M0+ Nrg-1 200 ng/ml MCM, **(9)** M1, **(10)** M1+ Nrg-1 50 ng/ml MCM, or **(11)** M1+ Nrg-1 200 ng/ml MCM. Incubated Nrg-1 (50 and 200 ng/ml) were added to the lower compartment to eliminate the possibility of Nrg-1 effects per se on NPCs migration. Addition of more medium to the wells of upper compartment makes a gradient slope that directs movement of NPCs downward the lower compartment through capillaries. Migrated

cells were imaged and counted in capillaries and lower compartment which connects two wells in each micro-device (Figure 7).

Ananda micro-device

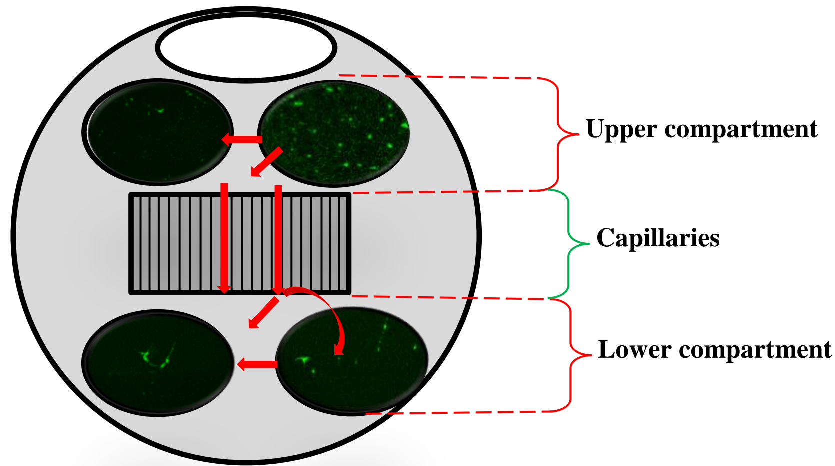


Figure 7. NPC migration path in Ananda migration micro-device. A few NPCs migrate from right-sided well to left-sided well in upper compartment of each Ananda micro-device. However, majority of cells move from right-sided well in upper compartment (lower gradient slope) toward lower compartment (higher gradient slop) through capillaries. NPCs passing through capillaries can enter right-sited well or the area between two wells in lower compartment. NPCs also move from right sided well toward left-sided well in lower compartment.

3.2.14. Statistical Analysis

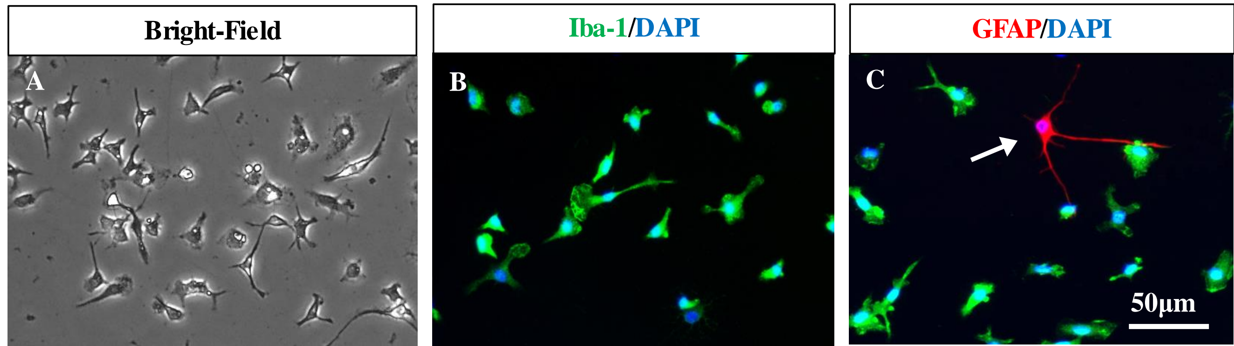
Using SigmaStat Software (4.0), we performed one-way analysis of variance (ANOVA) followed by Holm-Sidak post-hoc test in all immunocytochemistry. Student t-test was used when two groups were compared. The data was reported as means ± standard error of the mean (SEM). $P \leq 0.05$ was considered statistically significant.

Chapter IV: Results

4.1. Defining the role of neuregulin-1 in regulation of primary mouse microglia

4.1.1. Characterization of primary microglia cultures

In this study, we utilized primary cultures of microglia harvested from the cortex of postnatal EYFP Tg mice as described in the method section. We assessed the purity and quality of these cultures. Assessment of microglia culture with bright-field microscopy indicated the presence of resting microglia with ramified morphology (Figure 8A). To determine the purity of microglia cultures, we performed double labeling immunocytochemistry for the microglia specific marker, Iba-1 and the astrocyte specific marker, GFAP (Figure 8B, C). DAPI counterstaining was also used to identify nuclei. Quantitative analysis of Iba-1+/DAPI+ cells verified the presence of 98% microglia in these cultures with only 2% of GFAP+/DAPI+ astrocytes (Figure 8B-D).



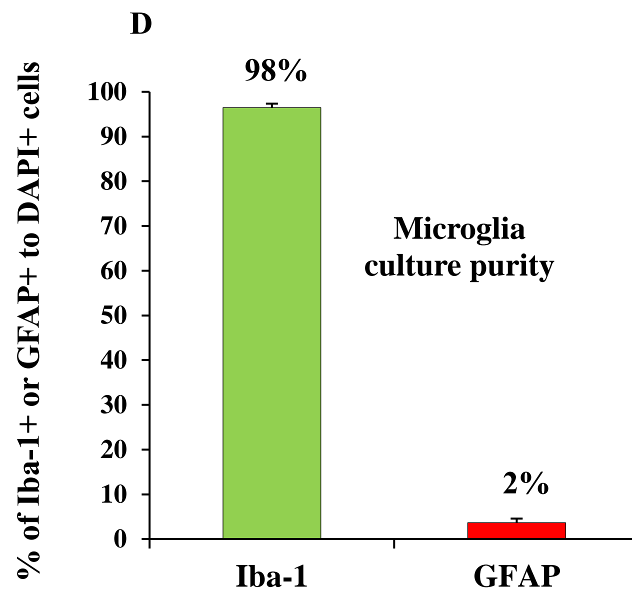


Figure 8. Characterization of primary microglia cultures from postnatal CAG-EYFP Tg mouse. (A) The bright-field microscopy shows resting microglia with ramified morphology and rare presence of astrocytes. (B-D) Quantitative analysis of Iba-1+/DAPI+ microglia and GFAP+/DAPI+ astrocytes revealed that the number of microglia exceeds over 98% with less than 2% contaminating astrocytes confirming the high purity of our microglia cultures. The data represent mean± SEM, N=4 independent cultures.

4.1.2. Mouse microglia express ErbB receptors to respond to Nrg-1 bioavailability

For studying the effects of Nrg-1, we next investigated whether mouse microglia express Nrg-1 receptors using co-immunostaining of OX42 (a known marker for microglia) with ErbB2, ErbB3 and ErbB4. These immunohistochemical assessments confirmed that OX42+/DAPI+ microglia express all three Nrg-1 receptors indicating their responsiveness to Nrg-1 treatment (Figure 9A-C).

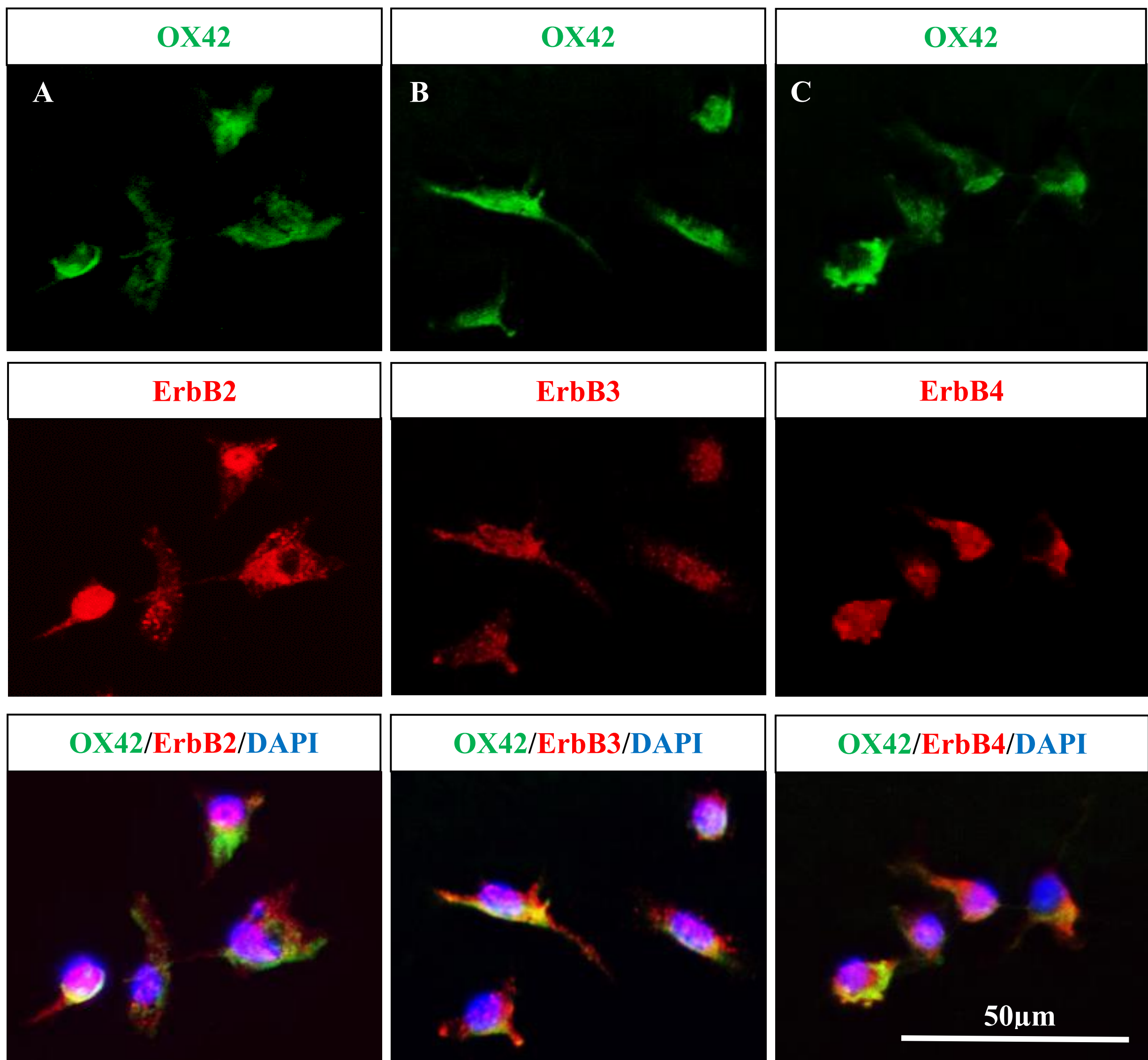


Figure 9. Primary mouse microglia express ErbB receptors (A-C) Co-immunostaining of microglia (OX42) with ErbB 2, 3, 4 receptors confirmed the expression of all three Nrg-1 receptors in microglia.

4.1.3. M1 microglia polarization induces nitrite production in a dose-dependent manner

To study the effects of Nrg-1 on the pro-inflammatory response of microglia, we polarized primary microglia to an M1 phenotype by a combination of IFN-γ and TNF-α, two relevant cytokines involved in activation of microglia in CNS neuroinflammation (Barcia et al., 2012; Goldmann and Prinz, 2013). We optimized and verified M1 polarization by assessment of nitrite levels in microglia conditioned media (MCM) of cultures treated with IFN-γ and TNF-α. Induced nitrite levels is a known property of M1 microglia that implicate them in oxidative stress and neurotoxicity (Wang et al., 2000; Bal-Price and Brown, 2001). Our dosing assay with various concentration of IFN-γ (10ng/ml, 20ng/ml or 40 ng/ml) identified that a concentration of 40 ng/ml of IFNγ in conjunction with 50ng/ml of TNFα was the most optimal concentration to induce nitrite production in M1 MCM at both 24 h and 72 h time-points compared to other concentrations of IFNγ (10 and 20 ng/ml) and to non-treated (M0) condition. The optimal concentration of IFNγ resulted in a significant 2.6- and 7-fold increase in the nitrite content of MCM at 24 h and 72 h time-points, respectively ($p<0.01$, one-way ANOVA, N=3) (Figure 10A,B). We verified M1 polarization by assessing microglia morphology using bright-field microscopy. Our observations confirmed M1 polarization by transitioning from resting microglia morphology with branches in M0 primary cultures (non-activated microglia) to a round-shaped microglia in activated M1 cultures (Figure 10C-D). Moreover, we performed co-immunostaining for OX42 and the M1 microglia specific marker, CD86 which confirmed M1 polarization of mouse microglia through induction of CD86 expression in IFNγ and TNFα treated M1 microglia (Figure 10E-L). We employed this culture system to examine the effects of Nrg-1 on M1 microglia polarization. We will refer to IFNγ and TNFα co-treated microglia as M1-polarized microglia and to non-treated (resting) microglia as M0-polarized microglia in the rest of this study.

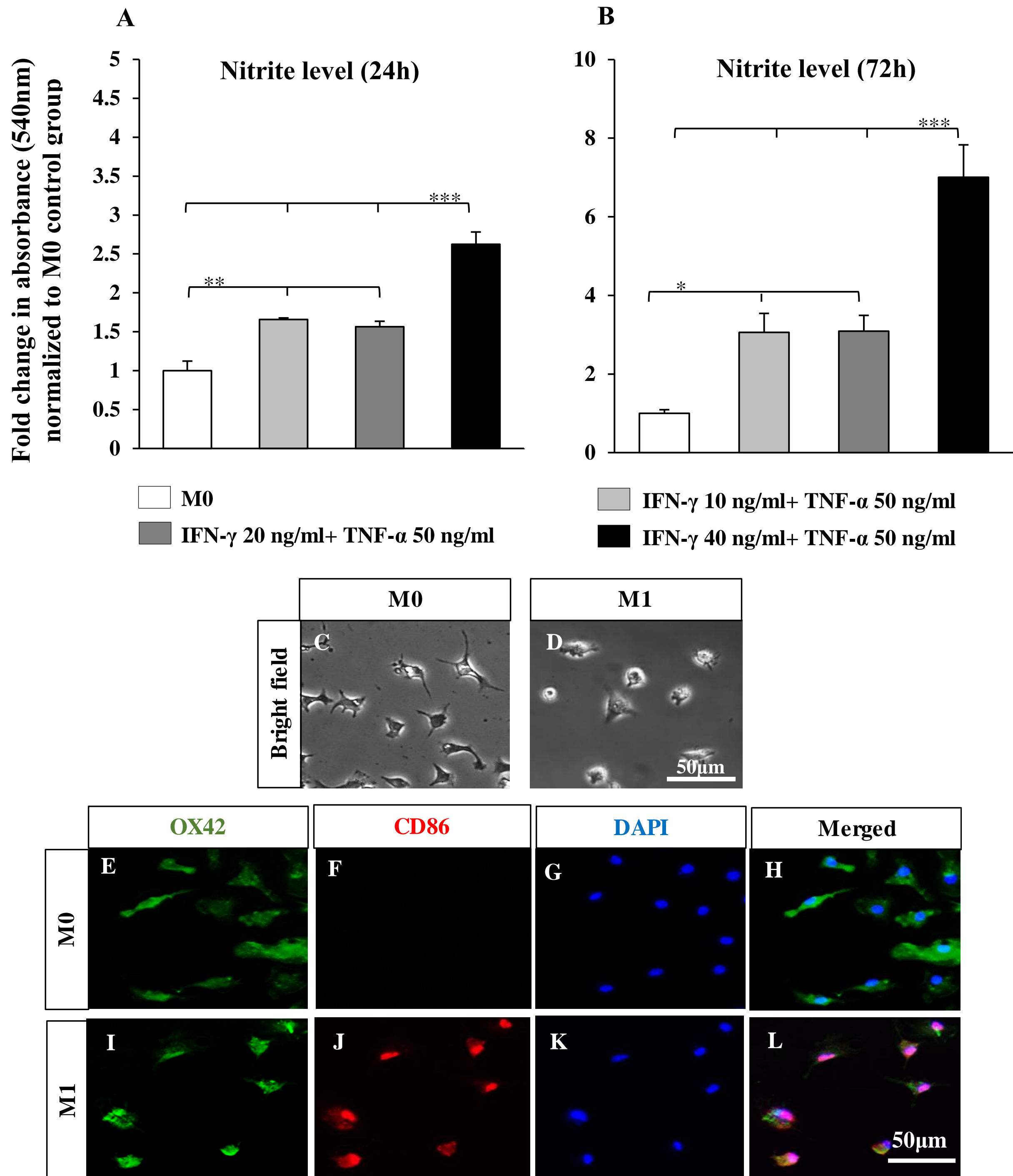


Figure 10. M1 microglia polarization significantly increases nitrite production in a dose-dependent manner. (A-B) Griess nitrite assay determined the combination of 40 ng/ml of IFN-γ and 50 ng/ml of TNF-α as an effective concentration for M1 polarization of mouse microglia

when compared to M0 condition at 24 h and 72 h time-points. (C-D) The bright-field microscopy indicated resting microglia with branches in M0 condition and round-shaped microglia in M1 cultures. (E-L) Co-immunostaining of microglia (OX42) with CD86 antibody showed the induced expression of CD86 in M1-polarized microglia as compared to M0-polarized microglia with no detectable expression of CD86. The data represent mean± SEM, ***$p<0.001$, **$p<0.01$, *$p<0.05$, N=3 independent cultures, one-way ANOVA, followed by Holm–Sidak *post hoc* test.

4.1.4. Nrg-1 attenuates nitrite production in M1-polarized microglia cultures

Modulating microglia polarization has been demonstrated to be a promising therapy for CNS neuroinflammatory disorders (Carta and Pisanu, 2013; Alizadeh et al., 2017; Song and Suk, 2017; Dyck et al., 2018). Here, we investigated whether bio-availability of Nrg-1 at the time of microglia activation can influence their phenotype. First, to determine the best concentration of human recombinant Nrg-1β1 in our purified mouse microglia cultures, we treated M0 and M1-polarized microglia with various concentrations including 10, 25, 50, and 200 ng/ml representing low, medium, and high concentrations. Nrg-1 treatment was added at the time of M1 polarization with IFNγ and TNFα. At 24 h post-Nrg-1 200ng/ml treatment there was a significant decrease in nitrite levels in the MCM of Nrg-1-treated M0 microglia in relation to non-treated M0 condition indicating that Nrg-1 200 ng/ml reduced the baseline level of nitrite in M0 microglia ($p<0.01$, one-way ANOVA, N=3 cultures) (Figure 11A). However, there was a trend towards a reduction in nitrite production in the MCM of Nrg-1-treated M0 cultures ($p>0.05$, N=3) (Figure 11A). M1 polarization of microglia induced nitrite levels significantly by 2 folds compared to M0 microglia at 24 h ($p<0.001$, one-way ANOVA, N=3) (Figure 11A). Treatment with 200 ng/ml of Nrg-1 resulted in a significant 1.7-fold reduction in nitrite levels in M1 MCM. We found a decreasing trend in nitrite levels in M1 MCM with Nrg-1 50ng/ml, however the difference was not statistically significant ($p>0.05$, N=3) (Figure 11A). We also found no change in nitrite level in MCM of M1-polarized microglia treated with 10 ng/ml and 25 ng/ml when compared to non-treated M1 cells ($p<0.001$, one-way ANOVA, N=3) (Figure 11A). At 72 h time-point, M1 polarization resulted in a significant 15.5-fold induction in nitrite production when compared with non-activated M0 condition ($p<0.001$, one-way ANOVA, N=3) (Figure 11B). Treatment of M1-polarized microglia cultures with 50 ng/ml (1.2 folds) and 200 ng/ml (1.4 folds) of Nrg-1 significantly attenuated nitrite

production at 72 h time-point ($p<0.001$, one-way ANOVA, N=3) (Figure 11B). However, there was a significant increase in the production of nitrite when M1-polarized microglia were treated with 25 ng/ml of Nrg-1($p<0.01$, one-way ANOVA, N=3) (Figure 11B). Considering the outcomes of our dosing study for Nrg-1 effect at both 24 h and 72 h time-points, the best dosing for Nrg-1 was determined to be 50 ng/ml (low dose) and 200 ng/ml (high dose) for the subsequent experiments.

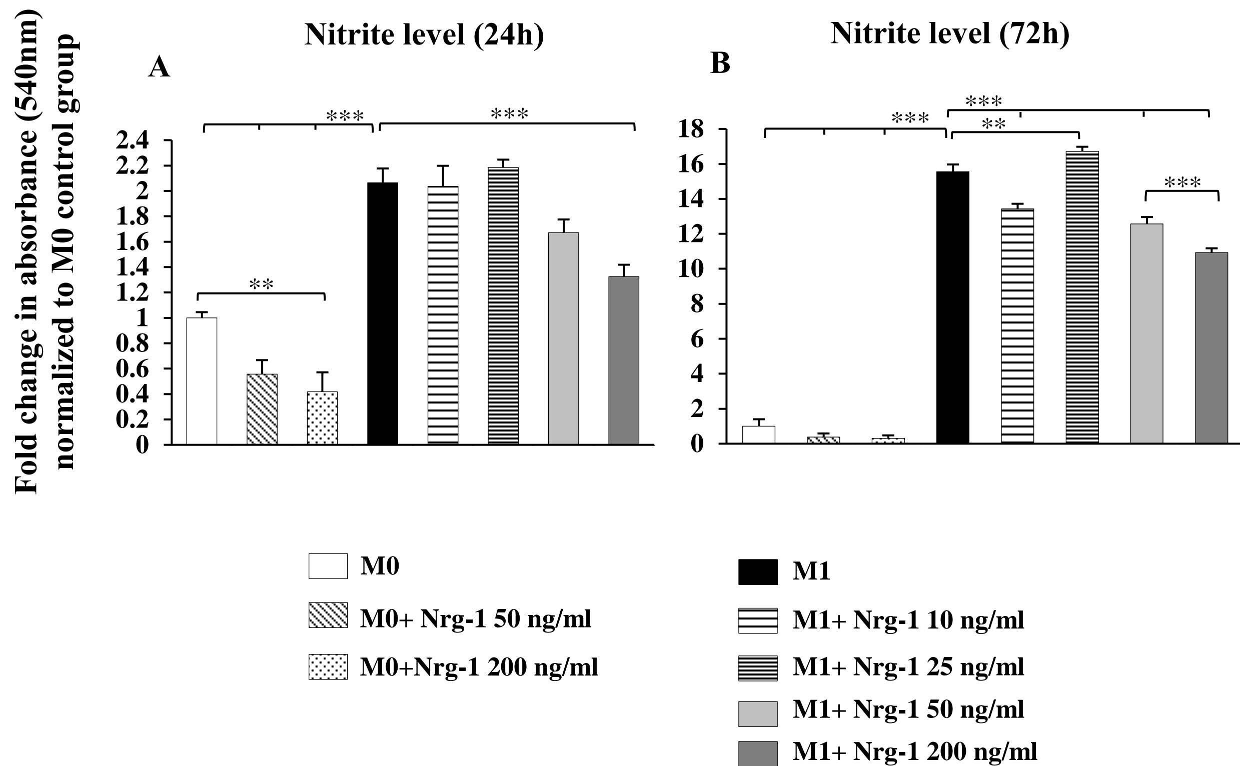


Figure 11. Effects of M1 polarization and Nrg-1 treatment on pure microglia cultures. (A, B) M1 polarization significantly increased nitrite production in MCM as compared to M0 microglia cultures. Four concentrations of Nrg-1 were assessed at 24 h and 72 h time-points. Greiss assay identified that the most effective concentrations of Nrg-1 in attenuating nitrite production by M1-polarized microglia were 50 ng/ml and 200 ng/ml. The data represent mean± SEM, ***p<0.001, **p<0.01, *p<0.05, N=3 independent cultures, one-way ANOVA, followed by Holm–Sidak *post hoc* test.

4.1.5. Nrg-1 attenuates the induced expression of pro-inflammatory mediators in M1-polarized microglia

Activated microglia are involved in neuronal injury and death by producing pro-inflammatory cytokines and mediators (Ramesh et al., 2013). Microglia also act as APC through the expression of co-stimulatory molecules (CD86 receptors), which activate other immune cells (Chastain et al., 2011). In our pure microglia cultures, we sought to determine whether Nrg-1 at low and high concentrations can modulate microglia transcript expression of pro-inflammatory cytokines, TNF-α and IL-6, anti-inflammatory cytokine, IL-10, and CD86 receptor at 24 h after M1 polarization and Nrg-1 treatment. Quantitative real-time PCR revealed a significant increase in mRNA expression of TNF-α (9 folds) and IL-6 (7.2 folds) in M1-polarized microglia compared to rather low basal levels of these cytokines in M0 cells ($p<0.001$, one-way ANOVA, N=4) (Figure 12A-B). Interestingly, Nrg-1 at 200 ng/ml significantly attenuated the transcript levels of TNF-α (2.6 folds) and IL-6 (3 folds) in M1-polarized microglia in relation to non-treated M1 cells ($p<0.001$, one-way ANOVA, N=4) (Figure 12A-B). We also found a significant 7.2-fold increase in the mRNA level of CD86 in M1-polarized microglia, which was significantly reduced by 2.8 folds after treatment with Nrg-1 200 ng/ml (Figure 5C, $p<0.001$, one-way ANOVA, N=4). Analysis of IL-10 transcript levels indicated that M1 polarization significantly reduces (4.5 folds) IL-10 gene expression in pure microglia cultures ($p<0.001$, one-way ANOVA, N=4) (Figure 12D). Interestingly, Nrg-1 50 ng/ml partially recovered the suppressed gene expression of IL-10 ($p<0.01$, Student t-test, N=4) (Figure 12D). However, Nrg-1 200 ng/ml did not change IL-10 gene expression in M1-polarized microglia ($p>0.05$, N=4) (Figure 12D).

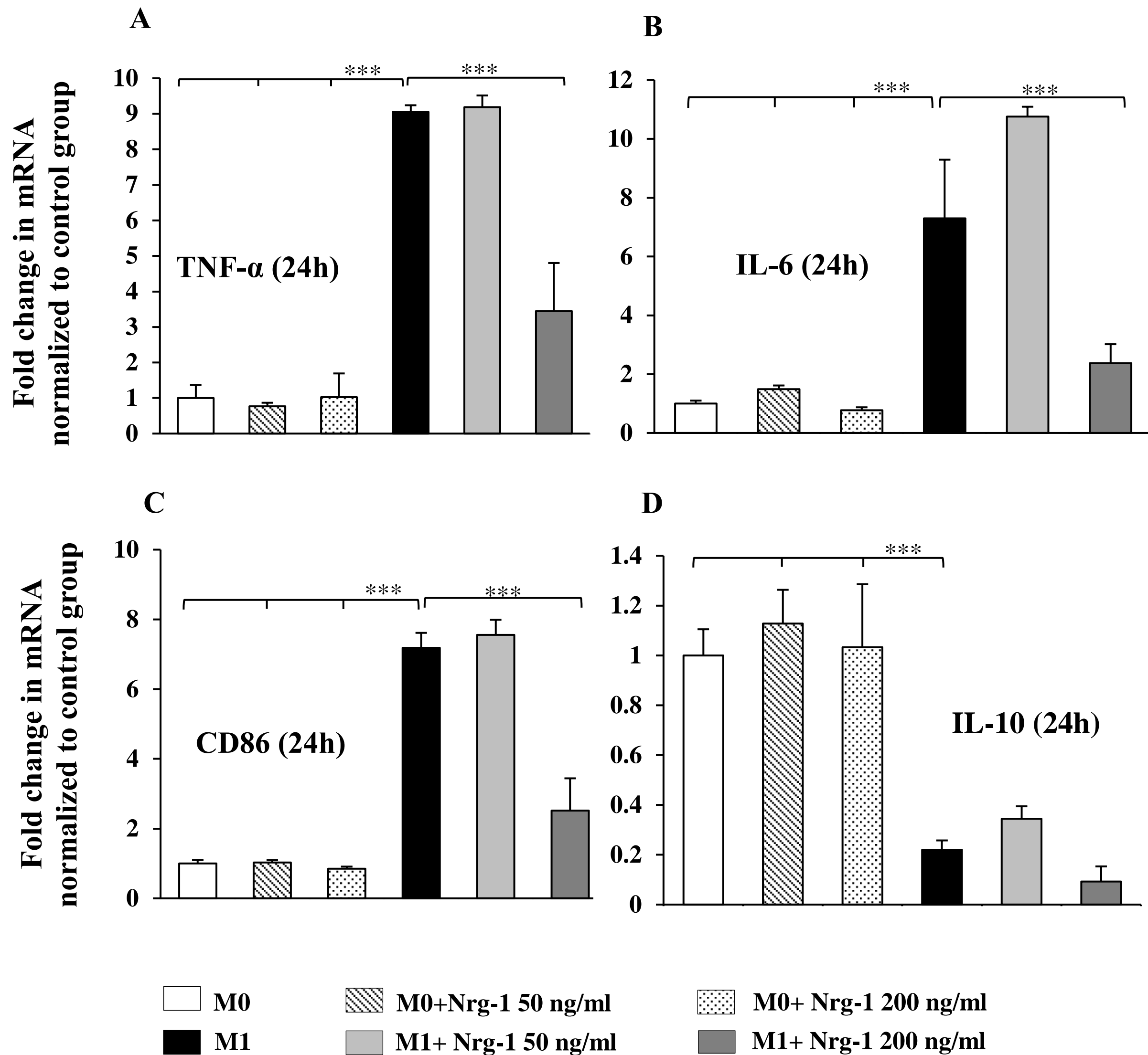


Figure 12. Nrg-1 treatment positively modulates transcript expression of cytokines and CD86 receptor in microglia. (A-D) M1 polarization significantly increased the mRNA expression of TNF-α, IL-6 and CD86 at 24 h time-point. Nrg-1 treatment at 200 ng/ml significantly attenuated the induced expression of TNF-α, IL-6 and CD86, while Nrg-1 50ng/ml had no effect on the transcript levels. Analysis of IL-10 transcript levels indicated that M1 polarization caused a significant reduction in gene expression. Nrg-1 at 50 ng/ml partially recovered the suppressed mRNA level of IL-10 ($p<0.01$, Student t-test). Results were normalized to the mRNA level of

H2afz as a housekeeping gene prior to the subsequent normalization to the control values. The data represent mean± SEM, ***$p<0.001$, **$p<0.01$, *$p<.05$, N=4 independent cultures, t-test and one-way ANOVA, followed by Holm–Sidak *post hoc* test.

4.1.6. The effects of M1 polarization and Nrg-1 treatment on the expression of ErbB receptors

We next investigated the effects of M1 polarization and Nrg-1 treatment on transcript levels of Nrg-1 receptors (ErbB2, ErbB3 and ErbB4) in mouse microglia cultures. At 24 h time-point, real-time PCR analysis of ErbB2 receptor showed that Nrg-1 treatment at 200 ng/ml resulted in a significant 1.4-fold reduction in mRNA expression of ErbB2 in M0 microglia cultures, while Nrg-1 50 ng/ml treatment had no effect on ErbB2 transcript levels (Figure 13A) ($p<0.01$, one-way ANOVA, N=3). Following M1 polarization, there was a significant 2.7-fold reduction in ErbB2 receptor expression ($p<0.001$) in microglia, which was not altered after Nrg-1 treatment (50 and 200 ng/ml) ($p>0.05$, N=3) (Figure 13A). Our quantitative analysis showed that Nrg-1 at 200 ng/ml significantly induced the gene expression of ErbB3 receptor by 2.4 folds in M0 microglia ($p>0.05$, N=3) (Figure 13B). In contrast, M1 polarization had no effect on mRNA level of ErbB3 ($p>0.05$, N=3) (Figure 13B). Of note, we found a significant 1.9-fold increase in transcript level of ErbB3 in Nrg-1 200 ng/ml treated M1-polarized microglia as compared to non-treated M1 cells ($p<0.05$, N=3) (Figure 13B). We also found that the gene expression of ErbB4 was significantly down-regulated (1.3 folds) in Nrg-1 200 ng/ml treated M0 microglia compared to M0 condition ($p<0.001$, one-way ANOVA, N=3), while Nrg-1 50 ng/ml had no effect on mRNA level of ErbB4 receptors in control M0 microglia cultures ($p>0.05$, N=3) (Figure 13C). Quantitative analysis of ErbB4 indicated a significant 1.2-fold reduction in mRNA expression in M1-polarized microglia ($p<0.01$, one-way ANOVA, N=3) (Figure 13C). Nrg-1 treatment at 200 ng/ml further reduced ErbB4 mRNA levels by 4.6 folds in M1-polarized microglia cultures ($p<0.001$, one-way ANOVA, N=3); however, there was no change in transcript level of ErbB4 with N rg-1 50 ng/ml treatment ($p>0.05$, N=3) (Figure 13C).

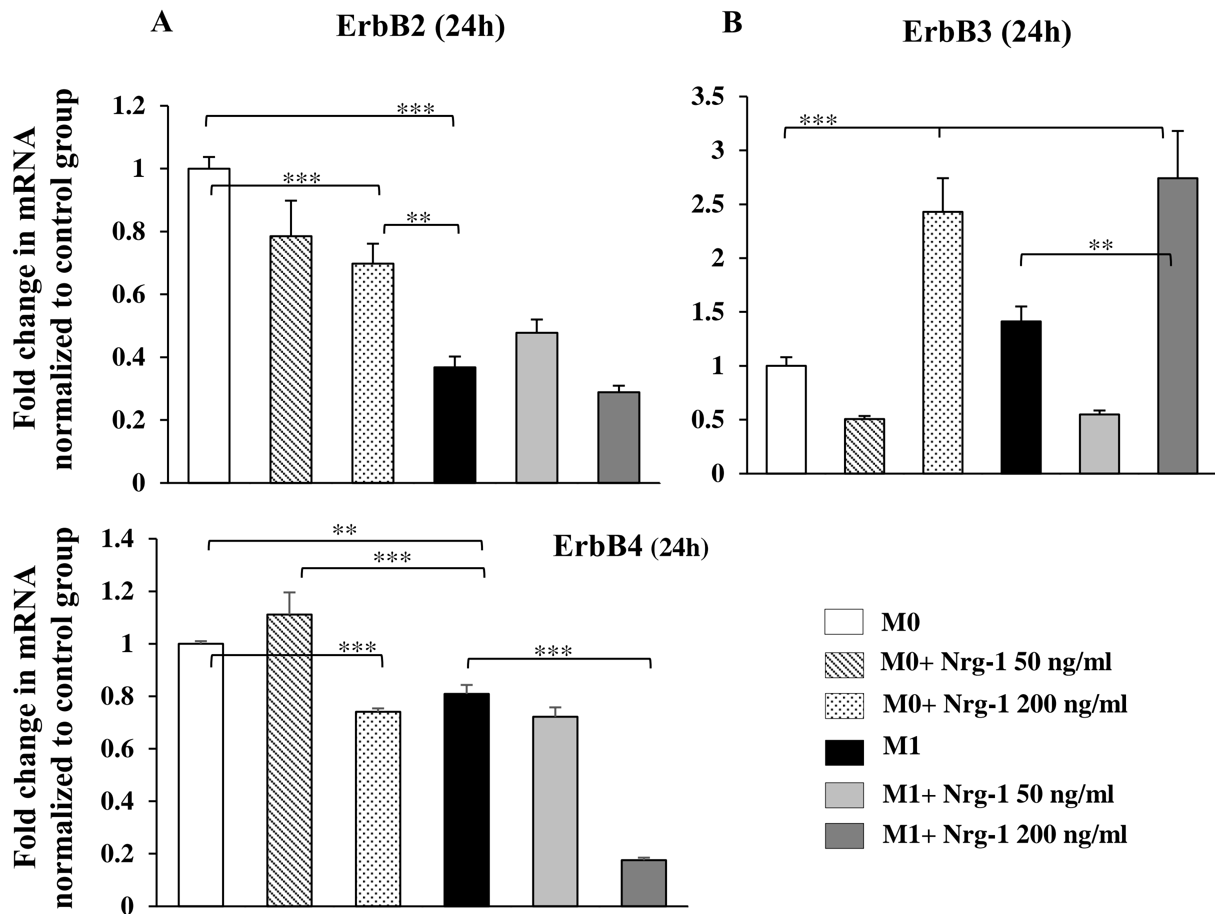


Figure 13. Effects of M1 polarization and Nrg-1 treatment on mRNA levels of ErbB receptors. (A-C) M1-polarization in microglia significantly decrease mRNA levels of ErbB2 and ErbB4 compared to M0 cultures, while had no effect on the transcript level of ErbB3. However, we found that Nrg-1 (200 ng/ml) significantly increased the gene expression of ErbB3 in M0 and M1-polarized microglia, while resulted in a significant reduction in mRNA level of ErbB2 in M0 microglia and ErbB4 in M0 and M1-polarized microglia. The data represent mean± SEM, ***p<0.001, **p<0.01, *p<0.05, N=3 independent cultures, one-way ANOVA, followed by Holm–Sidak *post hoc* test.

4.1.7. Nrg-1 treatment significantly induces proliferative activity of M0 microglia

Following injury to the CNS, activated microglia proliferate and migrate toward the injury site contributing to neuroinflammation (Loane and Byrnes, 2010; Li and Zhang, 2016). In our pure microglia culture, we showed that Nrg-1 200 ng/ml significantly increases proliferation of M0 microglia, while there was no effect on M1-polarized microglia (14A-G). Our quantitative immunocytochemical analysis of microglia proliferation using BrdU assay revealed a significant 2.4-fold increase in the percentage of proliferating M0 microglia (BrdU+/DAPI+ cells) after Nrg-1 (200 ng/ml) treatment compared to non-treated M0 microglia ($p<0.001$, one-way ANOVA, N=4). It is noteworthy that the Nrg-1 induced increase in microglia proliferation was observed in 4.3% of M0 microglia as compared to 1.7% of baseline proliferation in these cultures indicating a relatively low proliferative activity in these cells. At 24 h following M1 polarization, there was no difference in the number of BrdU+/DAPI+ microglia between M1 and M0 conditions. We also found that Nrg-1 treatment (50 and 200 ng/ml) had no effect on microglia proliferation in M1-polarized cultures.

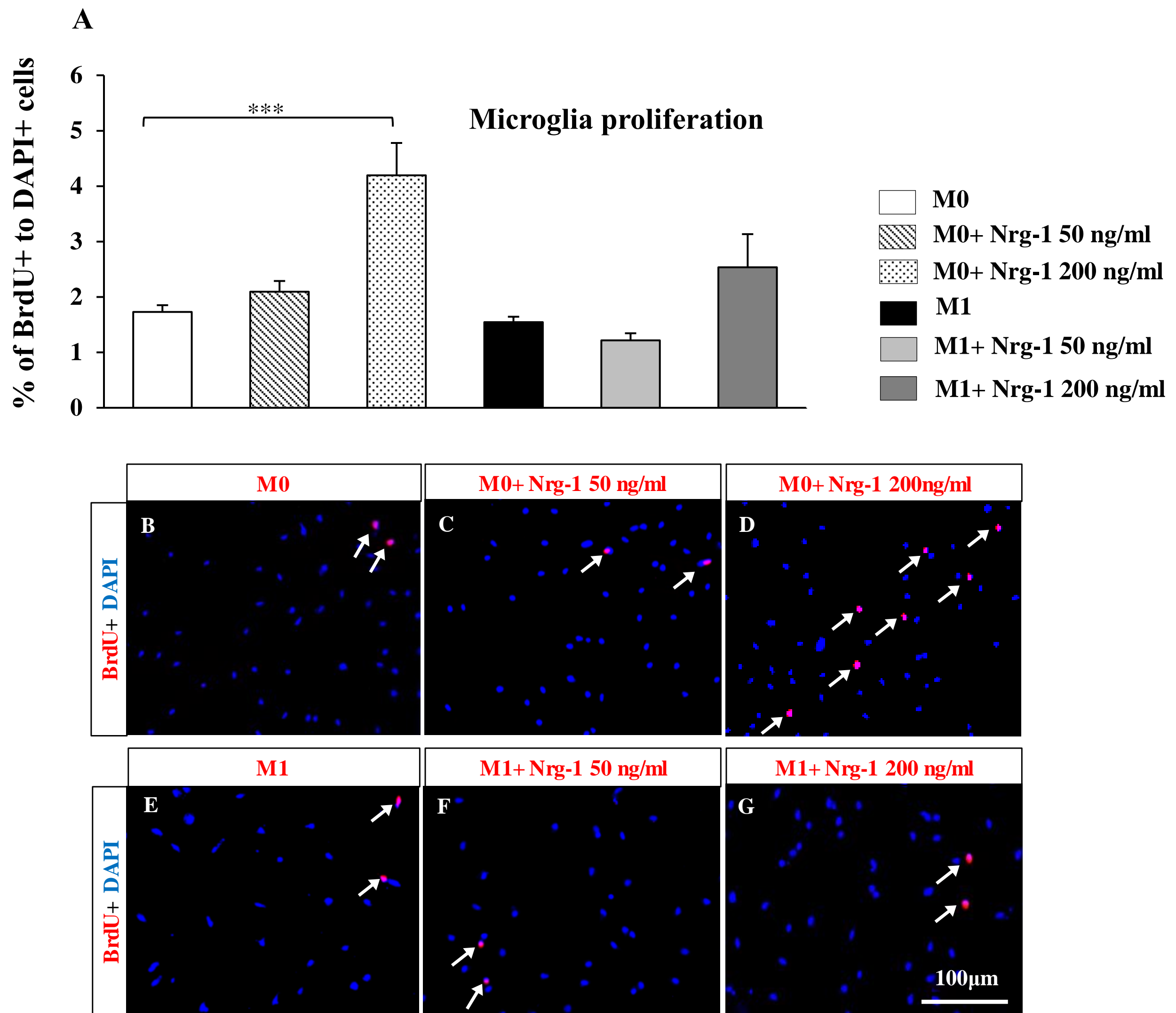


Figure 14. Nrg-1 200 ng/ml significantly increases proliferation of M0 microglia. (A-G) Immunocytochemical analysis of BrdU in microglia after 24h exposure to Nrg-1 200 ng/ml showed a small but significant increase in microglia proliferation in M0 condition, while there was no significant effects on the percentage of BrdU+/DAPI+ cells under Nrg-1 50 ng/ml treatment. Quantitative analysis of BrdU+/DAPI+ microglia demonstrated no apparent difference in proliferation between M0 and M1 conditions with and without Nrg-1 treatment. The data represent mean± SEM, ***$p<0.001$, **$p<0.01$, *$p<0.05$, N=4 independent cultures, one-way ANOVA, followed by Holm–Sidak *post hoc* test.

4.1.8. Nrg-1 treatment significantly restores the suppressed phagocytic activity of M1-polarized microglia

In CNS inflammatory conditions, TNF-α and IFN-γ drive microglia polarization into M1 phenotype that is associated with their reduced phagocytic activity (Mills et al., 2000; Kettenmann et al., 2011; Dyck et al., 2018). In contrast, M2 microglia represent increased ability for phagocytosis of cellular debris which promotes wound healing and tissue repair (Peress et al., 1993; Hu et al., 2012). Here, we examined whether Nrg-1 can promote the phagocytic activity of M1-polarized microglia. Phagocytosis was assessed in cultures of M0 and M1-polarized microglia using serum pre-opsonized red-fluorescent beads. Extent of phagocytosis was determined by quantifying the number of Iba-1+/DAPI+ cells containing beads normalized to the total number of Iba-1+/DAPI+ microglia. Our quantitative analysis of phagocytosis in M1-polarized microglia at 24 h after polarization indicated a significant 1.9-fold reduction in their phagocytosis. Interestingly, Nrg-1 50 ng/ml (2.7 folds) and Nrg-1 200 ng/ml (1.8 folds) was able to significantly restore the reduced ability of M1-polarized microglia for phagocytosis ($p<0.001$, one-way ANOVA, N=4) (Figure 15A). Likewise, M1-polarized microglia activated for 72 h showed significantly diminished phagocytic activity by 2.4 folds compared to their M0 counterparts in which was significantly restored with Nrg-1 50 ng/ml (4 folds) and 200 ng/ml (2.1 folds) ($p<0.001$, one-way ANOVA, N=4) (Figure 15B). Our studies showed no apparent effect of Nrg-1 50 ng/ml treatment on the ability of M0 microglia for phagocytosis. However, there was a significant reduction in phagocytosis in Nrg-1 200 ng/ml-treated M0 microglia cultures at 72 h time-point ($p<0.01$, one-way ANOVA, N=4) (Figure 15B). We verified the specificity of phagocytosis through the detection of intracellular red fluorescent beads in Iba-1+/DAPI+ microglia using z-stack imaging (Figure 15C,D).

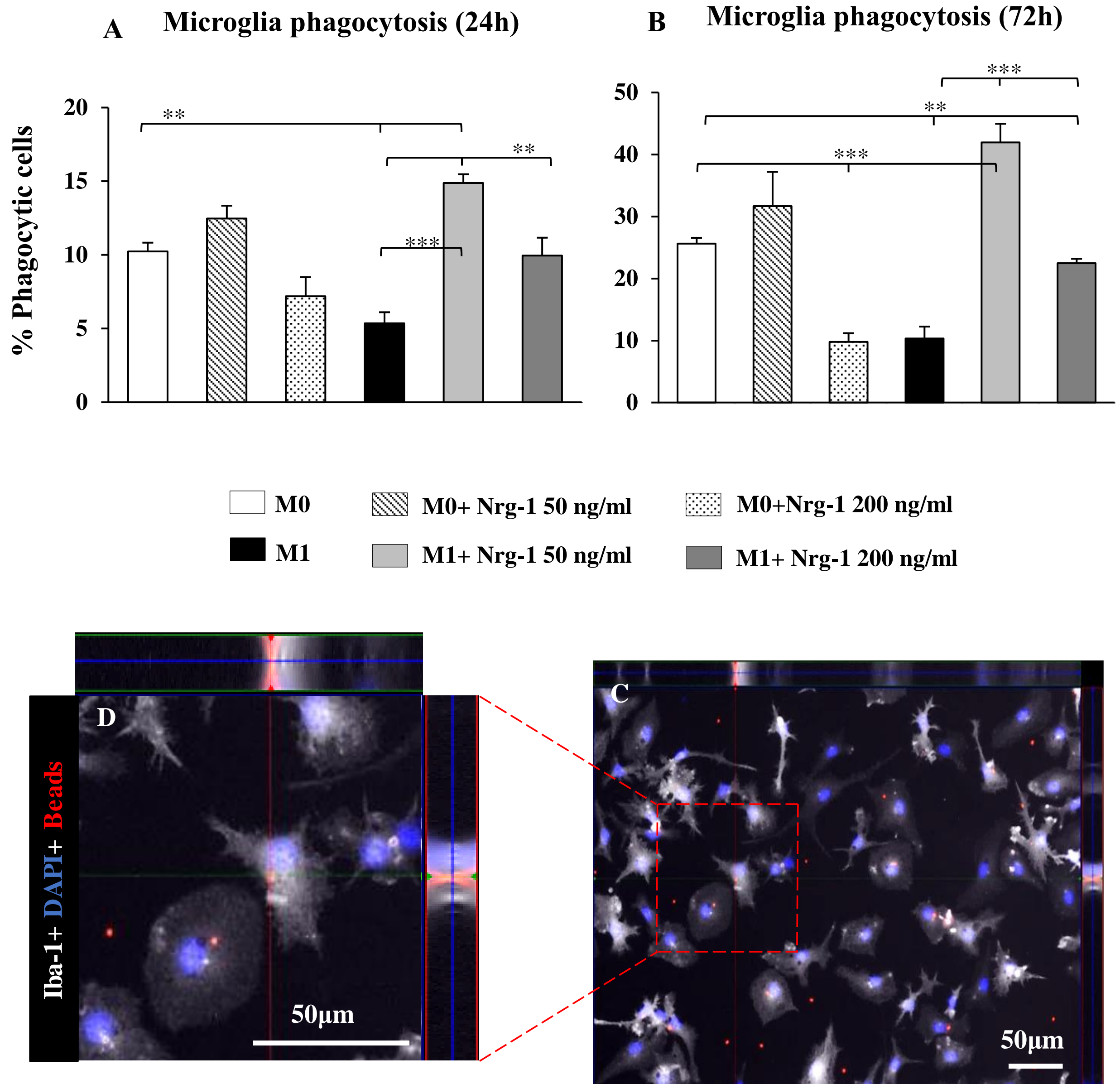


Figure 15. Nrg-1 treatment significantly restored the suppressed phagocytosis of M1-polarized microglia. (A-B) Immunocytochemical analysis of Iba-1+/DAPI+ microglia containing red-fluorescent beads indicated a significant reduction in phagocytic ability of M1-polarized microglia at 24 h and 72 h after M1 polarization. Co-treatment of M1-polarized microglia with Nrg-1 significantly restored the percentage of phagocytic cells. (C-D) Representing images

showing our phagocytosis assay. To verify phagocytosis success, intracellular red fluorescent beads in Iba-1+/DAPI+ microglia was detected using z-stack imaging. The data represent mean± SEM, ***$p<0.001$, **$p<0.01$, *$p<0.05$, N=4 independent cultures, one-way ANOVA, followed by Holm–Sidak *post hoc* test.

4.1.9. Nrg-1 treatment does not influence microglia mobility

Injury to the CNS elicits microglia mobilization to the site of injury, where they contribute to the neuroinflammatory process through production of inflammatory mediators and phagocytosis of cellular debris (Hu et al., 2014). Thus, we investigated whether Nrg-1 treatment plays a role in regulation of microglial cell mobility using transwell cell mobility assay. We quantified the total number of DAPI+ microglia which had mobilized to the bottom chamber of culture inserts. We found no apparent difference in the mobility of M0 and M1-polarized microglia. We also found no change in microglia mobilization under Nrg-1 treatment in M0 or M1-polarized microglia (One-way ANOVA, N=4) (Figure 16).

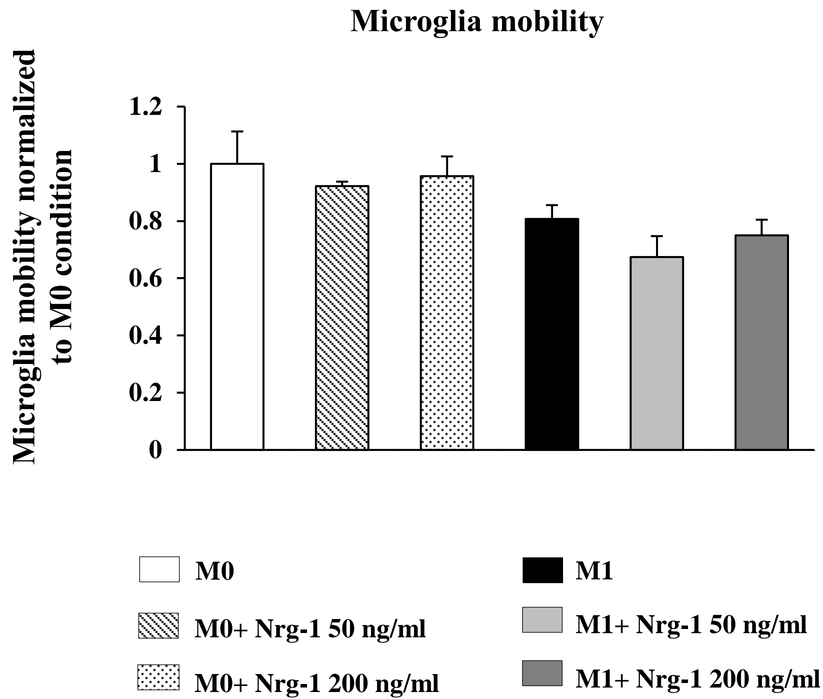


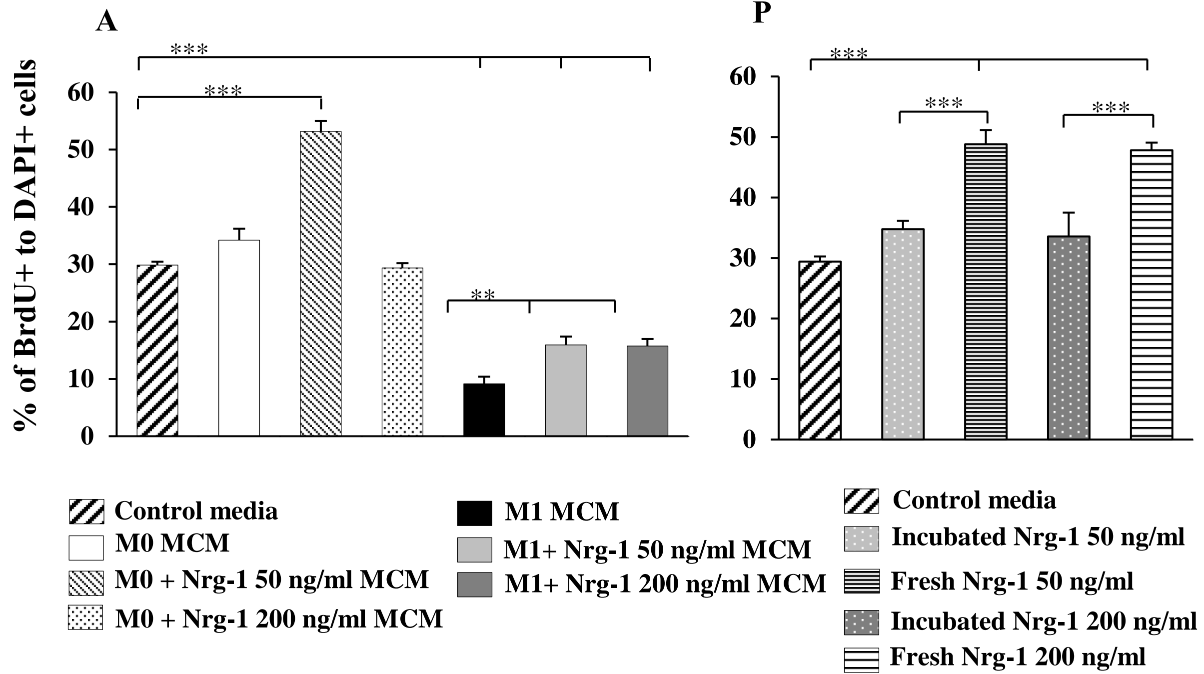
Figure 16. Nrg-1 treatment had no effect on the mobility of M0 and M1-polarized microglia. Quantitative analysis of DAPI+ microglia mobilizing to the bottom chamber of poly-carbonate transwell culture inserts indicated no apparent difference in the mobility of M0 and M1-polarized microglia cultures with and without Nrg-1 (50 and 200 ng/ml) treatment. The data represent mean± SEM, N=3 independent cultures, one-way ANOVA.

4.2. The effects of microglia conditioned media on the properties of neural precursor cells

4.2.1. Nrg-1 promotes a supportive phenotype in microglia, which positively modulates NPC proliferation

We sought to investigate whether Nrg-1-modulation of microglia may influence the properties of adult NPCs including their capacity for proliferation, differentiation and migration. Three days following microglial polarization and Nrg-1 treatment, microglia conditioned media (MCM) was collected and added to brain-derived NPCs in culture for assessment of proliferation, differentiation and migration. We utilized defined NPC serum free media in these experiments as described in the method section. Dissociated NPCs were treated with MCM collected from the following conditions: M0, M0+ Nrg-1 50 ng/ml, M0+ Nrg-1 200 ng/ml, M1, M1+ Nrg-1 50 ng/ml, and M1+ Nrg-1 200 ng/ml. Our BrdU proliferation assays indicated that microglia treated with Nrg-1 showed the ability to increase NPC proliferation (Figure 17A-O). Nrg-1 50ng/ml-treated M0 MCM induced a significant 1.7-fold and 1.5-fold increase in the percentage of proliferating NPCs, marked as BrdU+/DAPI+, compared to non-treated control media and M0 MCM treated cultures, respectively ($p<0.001$, one-way ANOVA, N=4). Following treatment with M1 MCM, we found a significant 3.2-fold and 3.7-fold reduction in the percentage of BrdU+ NPCs as compared to control media and M0 MCM conditions, respectively ($p<0.001$, one-way ANOVA, N=4). Our quantitative analysis showed that M1 treated with Nrg-1 50 ng/ml and 200 ng/ml significantly recovered (1.7 folds) NPCs proliferative activity evident by increased number of BrdU+/DAPI+ cells normalized to the total DAPI+ NPCs ($p<0.01$, one-way ANOVA, N=4). To rule out the possibility that the effects of MCM from Nrg-1 treated M0 and M1-polarized microglia on NPCs proliferation is not due to Nrg-1 peptide *per se*, we treated NPCs with fresh and 72 h incubated Nrg-1 at 50 ng/ml and 200 ng/ml concentrations. Previous studies by our group and others have

shown that Nrg-1 stimulates NPCs proliferation (Lai and Feng, 2004; Gauthier et al., 2013). Here, as anticipated, we confirmed that fresh Nrg-1 (50 and 200 ng/ml) significantly increased the percentage of proliferating BrdU+ NPCs as compared to control media ($p<0.001$, one-way ANOVA, N=4). We also tested Nrg-1 incubated for 72 h prior to its addition to NPC culture to more closely determine the effects and potency of any residual Nrg-1 treatment in MCM. Our quantitative analysis showed that treating NPCs directly with 72 h incubated Nrg-1 (50 and 200 ng/ml) had no apparent effects on NPCs proliferative activity ($p>0.05$, one-way ANOVA, N=4) (Figure 17P-X). This evidence confirmed that the effect of MCM that we observed in NPC cultures was due to the Nrg-1-modulated microglia secretion and not the original Nrg-1 treatment *per se*.


A
% of BrdU+ to DAPI+ cells
***
***
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P
***
***
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Control media
M0 MCM
M0 + Nrg-1 50 ng/ml MCM
M0 + Nrg-1 200 ng/ml MCM
M1 MCM
M1+ Nrg-1 50 ng/ml MCM
M1+ Nrg-1 200 ng/ml MCM
Control media
Incubated Nrg-1 50 ng/ml
Fresh Nrg-1 50 ng/ml
Incubated Nrg-1 200 ng/ml
Fresh Nrg-1 200 ng/ml

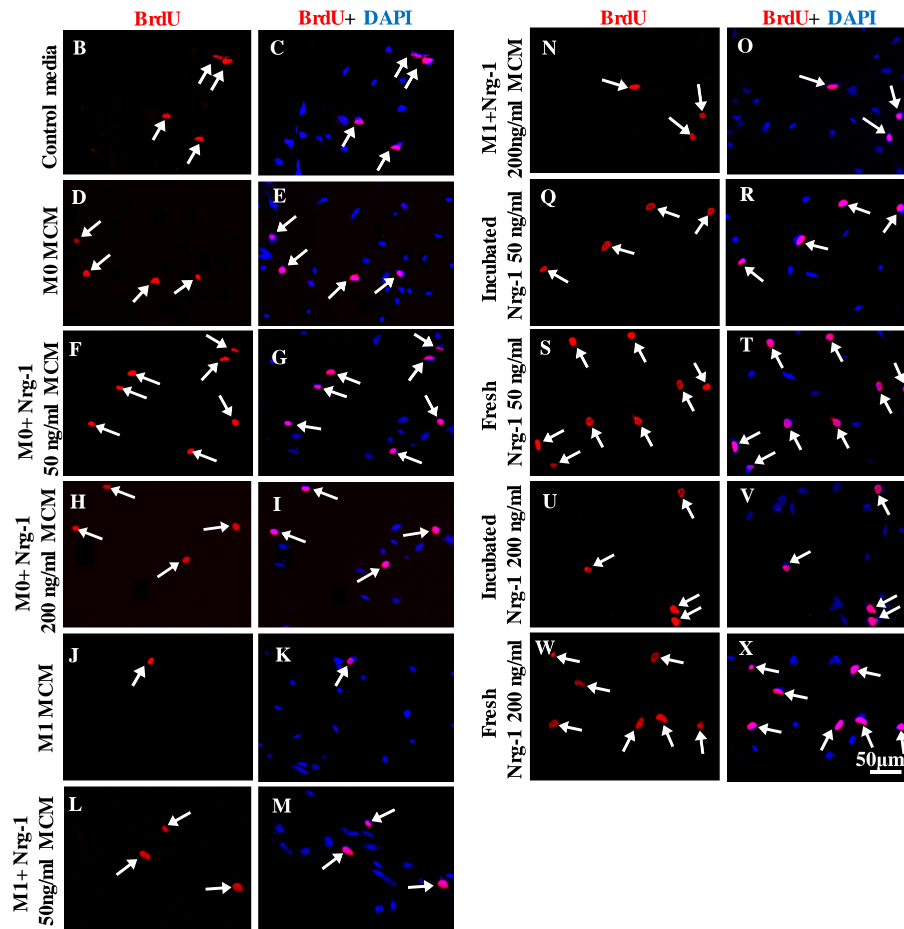

BrdU
BrdU+ DAPI
Control media
M0 MCM
M0+ Nrg-1 50 ng/ml MCM
M0+ Nrg-1 200 ng/ml MCM
M1 MCM
M1+ Nrg-1 50ng/ml MCM
M1+Nrg-1 200ng/ml MCM
Incubated Nrg-1 50 ng/ml
Fresh Nrg-1 50 ng/ml
Incubated Nrg-1 200 ng/ml
Fresh Nrg-1 200 ng/ml
50µm

Figure 17. Availability of Nrg-1 fosters a phenotype in microglia that promotes NPC proliferation. (A-O) Exposure of NPCs to M1 MCM significantly decreased the proliferative activity of brain-derived NPCs. MCM from M1-polarized cells treated with Nrg-1 (50 and 200 ng/ml) significantly increased the reduced proliferative ability of NPCs. (P-X) Addition of fresh Nrg-1 significantly increased NPCs proliferation (BrdU+/DAPI+) as anticipated. However, 72-pre incubated Nrg-1 had no apparent effect on the proliferative ability of NPCs cultures. The data represent mean± SEM, ***$p<0.001$, **$p<0.01$, *$p<0.05$, N=4 independent cultures, one-way ANOVA, followed by Holm–Sidak *post hoc* test.

4.2.2. M1-polarized microglia conditioned media does not contain the original IFN-γ and TNF-α peptides

Next, we determined whether M1 MCM contained any residual of the original IFN-γ and TNF-α peptides, which were used for M1 polarization. We assessed the presence of IFN-γ and TNF-α recombinant peptides as well as the endogenous expression of the cytokines in MCM using Western blotting (Figure 18A,B). This analysis was performed to rule out the potential effects of IFN-γ and TNF-α recombinant peptides in MCM on NPCs activities. In addition to M0 and M1 MCM, in our Western blotting we included IFN-γ and TNF-α recombinant peptides (100 ng/ml and 1 μg/ml) as well as spinal cord tissue samples from EAE (experimental autoimmune encephalomyelitis) mouse tissue as positive controls. Our analysis showed the expression of pro and active TNF-α in M1 MCM as compared to undetectable levels of both TNF-α isoforms in M0 MCM. We also detected TNF-α recombinant peptide with the same molecular weight as active TNF-α. However, the detected active TNF-α in M1 MCM was much more prominent than 100ng/ml TNF-α peptide. Considering we used 50ng/ml of TNF-α peptide in our microglia polarization, which is less than 100ng/ml, and the presence of pro TNF-α only in M1-polarized microglia CM, this data indicates the endogenous expression of TNF-α in M1 cultures. Conversely, we found no detectable expression of IFN-γ in M0 and M1 MCM, while there was considerable levels of IFN-γ in the spinal cord tissue samples of EAE mice as positive controls. Our Western blot also unraveled that IFN-γ peptide was not present in M1 MCM at 24 h and 72 h time-points. Specificity of IFN-γ antibody was confirmed by detection of IFN-γ recombinant peptide (50 ng/ml) and IFN-γ cytokine in EAE tissue as positive controls. Altogether, these analyses confirmed that M1 MCM cultures do not contain the original IFN-γ and TNF-α recombinant peptides. Moreover,

these experiments revealed that our M1-polarized microglia endogenously release TNF-α but not IFN-γ in the conditioned media.

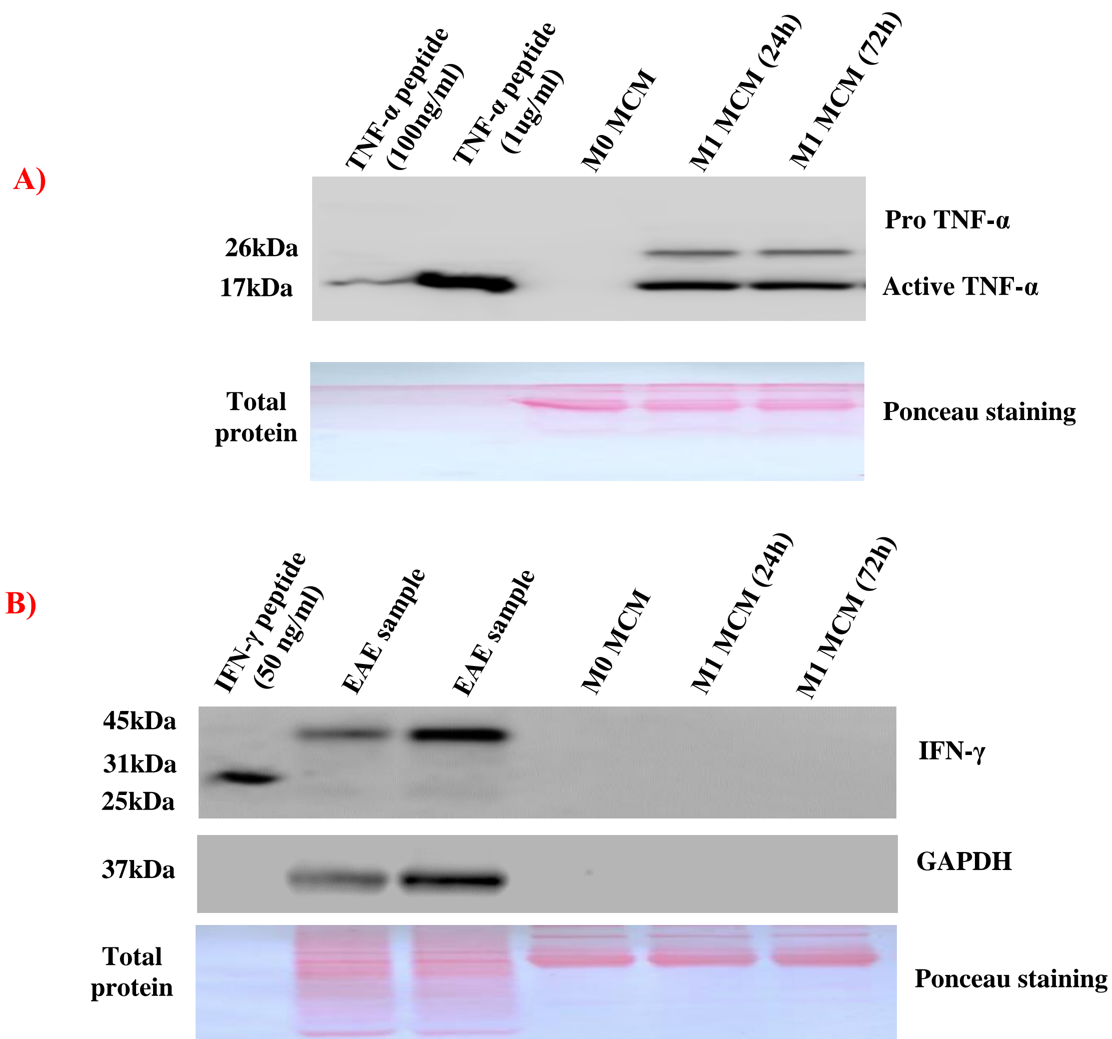
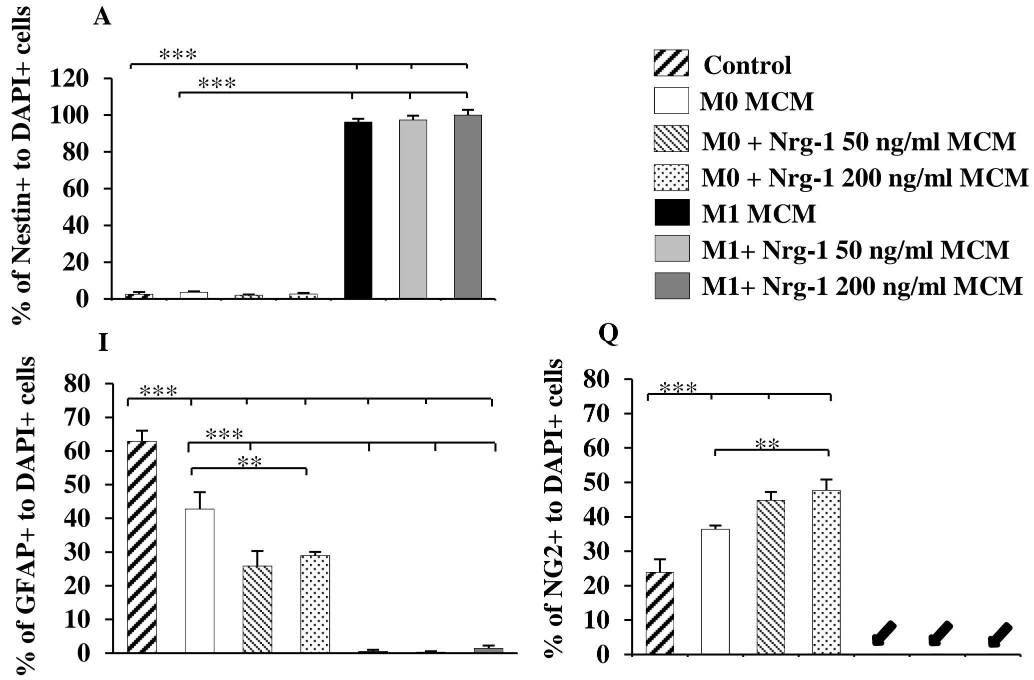


Figure 18. M1-polarized microglia conditioned media does not contain the original IFN-γ and TNF-α peptides. (A,B) Western blot analysis of MCM at 24 h and 72 h after M1 polarization showed endogenous expression of pro and active forms of TNF-α, but not IFN-γ in M1 MCM. We also found that TNF-α and IFN-γ recombinant peptides used for M1 polarization were not present in M1 MCM as compared to the detected peptides in our positive controls. Representative blots are illustrated for each experimental condition. GAPDH and Ponceau S were used as loading controls for cell lysate and conditioned media, respectively.

4.2.3. M1-polarized microglia suppress the differentiation of neural precursor cells

Next, we investigated whether M0 and M1 MCM with or without Nrg-1 treatment influence NPC differentiation. To allow differentiation, NPCs were grown in a medium composed of NPC medium (growth factor and serum free) and microglia medium containing fetal bovine serum (1:1 ratio) for 7 days. We treated NPCs with M0, M0+ Nrg-1 50 ng/ml, M0+ Nrg-1 200 ng/ml, M1, M1+ Nrg-1 50 ng/ml, and M1+ Nrg-1 200 ng/ml MCM. NPCs were plated in control media served as the baseline control for quantitative analysis of differentiation. We performed immunocytochemical analysis using Nestin (NPCs lineage marker), GFAP (astrocyte specific marker), and NG2 (OPC specific marker) (Figure 19A-W). After 7 days of differentiation, our quantification for Nestin showed that NPCs differentiated in the control media and M0 MCM with and without Nrg-1 at both concentrations we studied. Under control condition, 62% of NPCs differentiated into astrocytes while 23% were NG2 expressing OPCs as anticipated. Addition of M0 MCM into NPC cultures significantly increased the percentage of NG2+/DAPI+ OPCs at the expense of GFAP+/DAPI+ astrocytes as compared to control media. Moreover, Nrg-1 (50 and 200 ng/ml)-treated M0 MCM significantly increased the percentage of NG2+/DAPI+ OPCs while reducing the percentage of GFAP+/DAPI+ astrocytes as compared to M0 MCM. However, interestingly, M1 MCM entirely suppressed NPC differentiation as evident by Nestin expression in NPCs compared to all other conditions. Treatment of M1-polarized microglia with Nrg-1 (50 and 200 ng/ml) did not change their inhibitory effects on NPC differentiation. Of note, Nestin is expressed by undifferentiated multipotent NPCs (N=4, ***$p<0.001$, **$p<0.01$, one-way ANOVA. We rarely found GFAP+ astrocytes or NG2+ OPCs in M1 and M1+ Nrg-1 MCM-treated NPC cultures.


A
% of Nestin+ to DAPI+ cells
***
***
Control
M0 MCM
M0 + Nrg-1 50 ng/ml MCM
M0 + Nrg-1 200 ng/ml MCM
M1 MCM
M1+ Nrg-1 50 ng/ml MCM
M1+ Nrg-1 200 ng/ml MCM
I
% of GFAP+ to DAPI+ cells
Q
% of NG2+ to DAPI+ cells
**

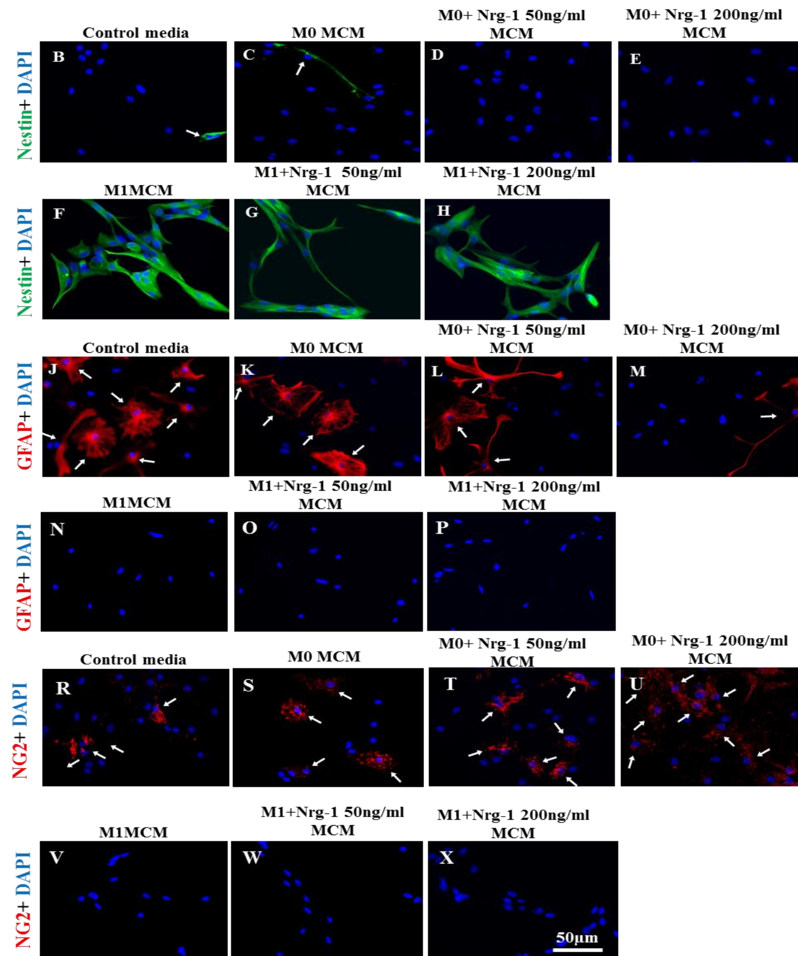
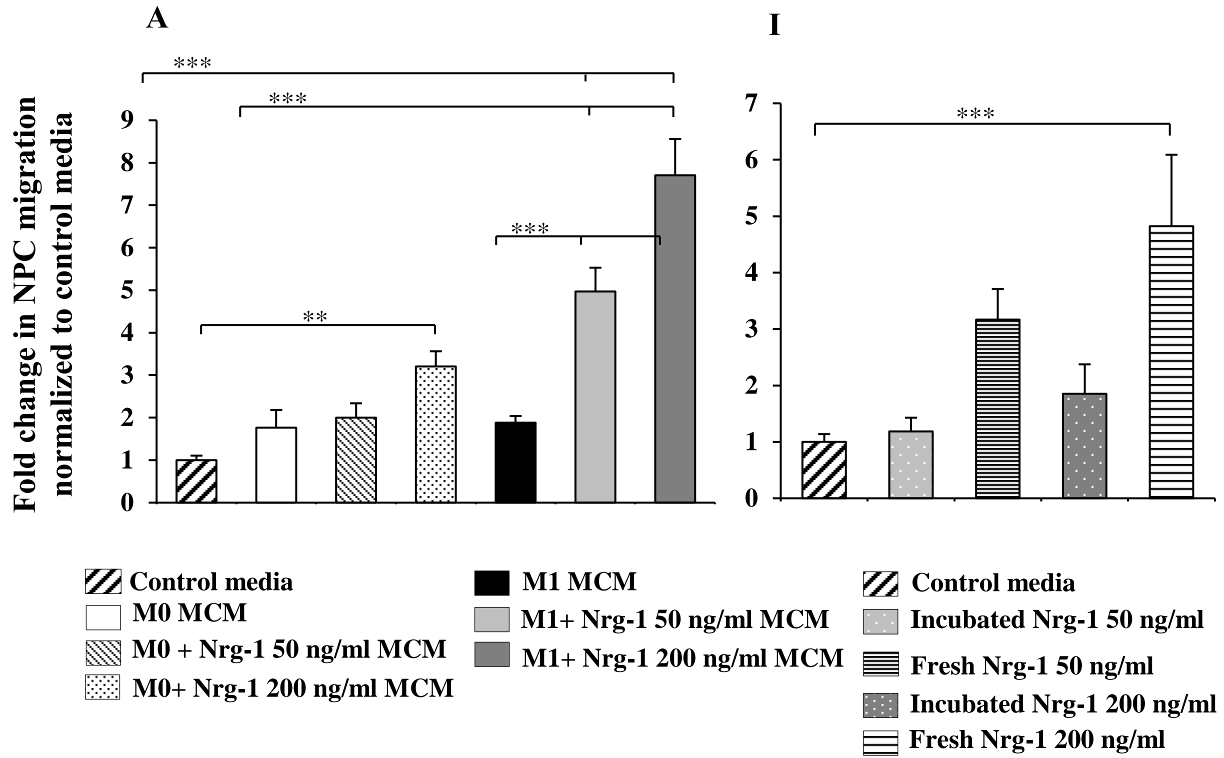

Control media
M0 MCM
M0+ Nrg-1 50ng/ml MCM
M0+ Nrg-1 200ng/ml MCM
Nestin+ DAPI
M1MCM
M1+Nrg-1 50ng/ml MCM
M1+Nrg-1 200ng/ml MCM
GFAP+ DAPI
NG2+ DAPI
50μm

Figure 19. M1-polarized microglia blocks differentiation of adult NPCs. (A-W) Immunocytochemistry for NPCs specific marker, Nestin, the astrocyte specific marker, GFAP, and the OPC specific marker, NG2, indicated that NPCs differentiated in control media, M0 and M0+ Nrg-1 MCM conditions. However, nearly all NPCs remained Nestin+ undifferentiated cells under M1 and M1+ Nrg-1 MCM treatment. Black arrows show M1 MCM with and without Nrg-1 (50 and 200 ng/ml). The data represent mean±SEM, ***$p<0.001$, **$p<0.01$, N=4 independent cultures, one-way ANOVA, followed by Holm–Sidak *post hoc* test.

4.2.4. Nrg-1 promotes a supportive phenotype in microglia, which fosters the migration of NPCs

Evidence shows that Nrg-1-ErbB signaling induces the migration of OPCs during development (Ortega et al., 2012). Nrg-1 has also shown to play an important role in NPC migration from the SVZ into the olfactory bulbs through the RMS to replace interneurons in the adult brain of rodents (Luskin, 1993). Here, we sought to investigate whether MCM of Nrg-1-treated microglia may influence the migration of adult NPCs. Three days following microglia polarization, MCM was collected from the following conditions: **(1)** M0, **(2)** M0+ Nrg-1 50 ng/ml, **(3)** M0+ Nrg-1 200 ng/ml, **(4)** M1, **(5)** M1+ Nrg-1 50 ng/ml, and **(6)** M1+ Nrg-1 200 ng/ml. MCM was added to lower compartment of each micro-device for migration assessment (as illustrated in Figure 7). Dissociated NPCs were plated onto the right-sided well in upper compartment of micro-device and cells were allowed to migrate from upper compartment through capillaries toward lower compartment for 20 h. Our quantification showed that MCM of Nrg-1 treated microglia stimulates the migration of NPCs in a concentration dependent manner (Figure 20A-H). MCM of Nrg-1 200ng/ml treated M0 microglia induced a significant 3.2-fold increase in the proportion of NPCs migrated toward lower compartments compared to control media ($p<0.01$, one-way ANOVA, N=3). We also found that the MCM of Nrg-1 (50 and 200 ng/ml) treated M1 microglia resulted in a significant increase in the number of migrated NPCs as compared to control media, M0 and M1 MCM conditions ($p<0.001$, one-way ANOVA, N=3). Our quantitative analysis showed a significant 1.5-fold increase in the number of migrated NPCs in M1+ 200ng/ml MCM condition as compared to M1+ Nrg-1 50 ng/ml MCM condition ($p<0.001$, one-way ANOVA, N=3). To rule out the possibility that residual of Nrg-1 recombinant peptide in MCM may affect NPC migration, we also added fresh and 72 h pre-incubated Nrg-1 (50 and 200 ng/ml) to wells in lower

compartment of micro-devices. We found that fresh Nrg-1 200 ng/ml significantly induced the proportion of migrated NPCs as compared to control media ($p<0.001$, one-way ANOVA, N=3). However, there was no difference between 72 h incubated Nrg-1 (at two concentrations) and control media in terms of NPC migration ($p>0.05$, one-way ANOVA, N=3) (Figure 20I-M). This evidence confirmed that the effect of MCM that we observed in NPC cultures was due to the Nrg-1-modulated microglia secretion and not the original Nrg-1 treatment *per se*.


A
I
Fold change in NPC migration normalized to control media
***
**
0
1
2
3
4
5
6
7
8
9
Control media
M0 MCM
M0 + Nrg-1 50 ng/ml MCM
M0+ Nrg-1 200 ng/ml MCM
M1 MCM
M1+ Nrg-1 50 ng/ml MCM
M1+ Nrg-1 200 ng/ml MCM
Incubated Nrg-1 50 ng/ml
Fresh Nrg-1 50 ng/ml
Incubated Nrg-1 200 ng/ml
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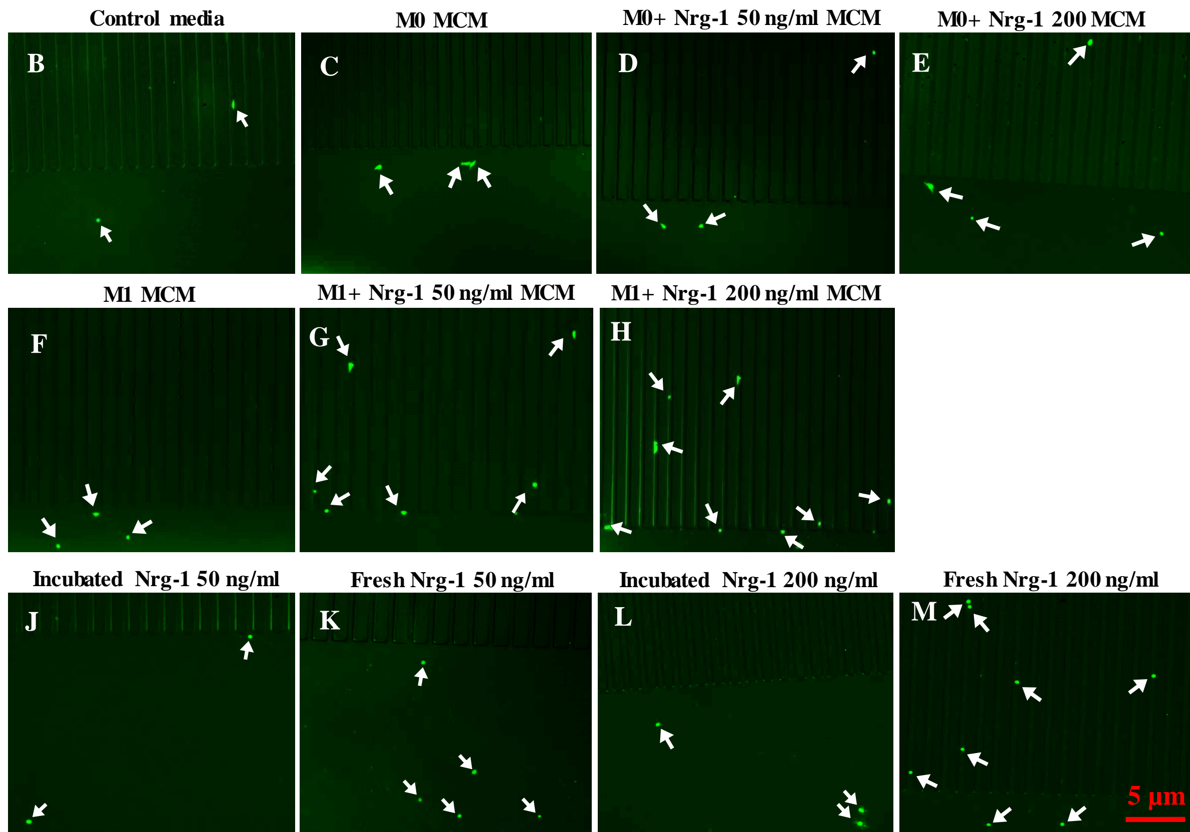
Control media
M0 MCM
M0+ Nrg-1 50 ng/ml MCM
M0+ Nrg-1 200 MCM
B
C
D
E
M1 MCM
M1+ Nrg-1 50 ng/ml MCM
M1+ Nrg-1 200 ng/ml MCM
F
G
H
Incubated Nrg-1 50 ng/ml
Fresh Nrg-1 50 ng/ml
Incubated Nrg-1 200 ng/ml
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5 µm

Figure 20. Nrg-1-modulated microglia promotes NPC migration. (A-H) MCM from M0 microglia treated with Nrg-1 200 ng/ml significantly increased the number of migrated NPCs. Addition of Nrg-1 (50, 200 ng/ml) treated M1 MCM to the lower compartment significantly induced migration of NPCs. (I-M) In response to fresh Nrg-1 200 ng/ml, NPC migration was significantly increased. However, fresh Nrg-1 50 ng/ml and 72-pre incubated Nrg-1 had no apparent effect on migration of NPCs. White arrows indicate migrated cells. The data represent mean± SEM, ***$p<0.001$, **$p<0.01$, *$p<0.05$, N=3 independent cultures, one-way ANOVA, followed by Holm–Sidak *post hoc* test.

Chapter V: Discussion

5.1. General Overview of findings and discussion

In the present study, we demonstrate a beneficial role for Nrg-1 in regulating microglia response. Using relevant primary culture systems and microglia polarization strategy, we found that availability of Nrg-1 in the microenvironment of M1-polarized microglia moderates their pro-inflammatory phenotype. Nrg-1 was able to positively modulate cytokine and receptor expression profile of M1-polarized microglia as well as their ability for proliferation and phagocytosis. Importantly, our findings unravel that microglia treated with Nrg-1 can promote the regenerative abilities of NPCs in primary cultures. Collectively, our study suggests that presence of Nrg-1 can harness the potential of microglia in facilitating cellular repair process in injury conditions.

Following injury and demyelinating conditions, resident NPCs and OPCs have restricted regenerative capacity to replenish endogenous oligodendrocytes, which limits spontaneous remyelination (Cao et al., 2002; Mason et al., 2004; Zai and Wrathall, 2005; Horky et al., 2006; Almad et al., 2011). This limitation has been attributed to the hostile milieu of the injured CNS including the pro-inflammatory response of microglia and astrocytes (Alizadeh and Karimi-Abdolrezaee, 2016). Previous studies in our laboratory identified that dysregulation of Nrg-1/ErbB signaling within the lesions of SCI and focal demyelinating conditions contributes to the imbalanced microenvironment of injury and underlies the inadequate oligodendrocyte replacement (Gauthier et al., 2013; Kataria et al., 2018). Neuregulin-1 is an axonally-localized growth factor that with its signaling receptors, ErbB2, 3 and 4, plays critical roles in oligodendrocyte development, survival, maturation, and myelination (Vartanian et al., 1999; Flores et al., 2000; Gauthier et al., 2013; Kataria et al., 2018). Previously, our group reported that the protein and

mRNA levels of Nrg-1 are down-regulated acutely within one day after compressive SCI and in LPC-induced focal demyelinating lesions in rats without any recovery to its basal levels at later time-points (Gauthier et al., 2013; Kataria et al., 2018). These studies identified that downregulation of Nrg-1 is likely attributed to degeneration of neurons, axons and oligodendrocytes, which are the endogenous source of Nrg-1 expression. Interestingly, restoring the deficient levels of Nrg-1 through recombinant peptide therapy was able to enhance oligodendrogenesis and remyelination in both SCI and focal demyelinating lesions (Gauthier et al., 2013; Kataria et al., 2018).

Parallel *in vitro* studies also identified that exogenous Nrg-1 enhances the capacity of spinal cord-derived NPCs for proliferation and oligodendrocyte differentiation while reducing the number of NPC-derived astrocytes (Gauthier et al., 2013). Moreover, our recent studies showed that Nrg-1 increases the morphological complexity of OPCs and supports their differentiation into mature myelinating oligodendrocytes through an ErbB2/ErbB4 dependent mechanism (Kataria et al., 2018). These studies have collectively uncovered an important role for Nrg-1 in regulating the activity of endogenous precursor cells and their ability for oligodendrocyte replacement in the spinal cord.

Additionally, recent work from our group has identified a role for Nrg-1 in regulating immune response as well as astrocyte activity and scar formation after SCI (Alizadeh et al., 2017; Alizadeh et al., 2018). In rat SCI and primary cultures, we showed that activated astrocytes can respond to Nrg-1 and change their otherwise scar forming phenotype to a more supportive one in an ErbB2/3 dependent mechanism. It is known that microglia also respond to injury. Our studies and that of others have shown that microglia also express ErbB 2, 3, and 4 receptors and thereby are responsive to Nrg-1 bioavailability (Calvo et al., 2010; Alizadeh et al., 2017). The goal of my

thesis was to understand how Nrg-1 influences microglia activity and their effects on neural precursor cells.

Effects of Nrg-1 on cytokine expression of M1-polarized microglia: Classically activated microglia (pro-inflammatory M1 phenotype) are known to contribute to demyelination and the inadequate spontaneous remyelination through their expression of proinflammatory cytokines, IL-β, IL-6 and TNF-α, promoting oxidative stress, antigen presenting ability and ineffective clearance of myelin debris (Mack et al., 2003; Voss et al., 2012; Miron et al., 2013; Fenn et al., 2014; Lampron et al., 2015). LPS-induced M1 microglia block NPC differentiation through the expression of TNF-α (Butovsky et al., 2006). TNF-α is also known to contribute to the hostile post-injury microenvironment by altering microglia from pro-regenerative to pro-inflammatory through upregulation of microglia iron levels (Kroner et al., 2014). Upregulation of TNF-α by LPS-activated microglia induces apoptosis in mouse NPCs via NF-κB signaling pathway (Guadagno et al., 2013). TNF-α also triggers the production of glutamate in primary rat and human neurons, which leads to neurotoxicity (Ye et al., 2013). Another study demonstrated that IL-6 inhibits hippocampal neurogenesis (Monje et al., 2003). Following SCI, upregulation of IL-6 is involved in secondary injury mechanisms and promotes tissue damage (Nakamura et al., 2005). In contrast to M1 pro-inflammatory cells, microglia polarization into a pro-regenerative M2 phenotype fosters repair and recovery after CNS injuries. M2 polarization of microglia exerts positive neuroprotective effects on remyelination through phagocytosis of myelin debris and secretion of IGF-I and TGF-β1 which promote oligodendrocytes differentiation (Hinks and Franklin, 1999; Ousman and David, 2000). Microglia exposure to IL-4 can favor NPC oligodendrogenesis or neurogenesis through the expression of IGF-I (Butovsky et al., 2006).

Accordingly, a balance between M1/M2 microglia phenotypes has been considered as a determining factor for tissue degeneration/repair following CNS injuries or diseases.

Our studies on SCI and LPC-induced focal demyelination models demonstrated that Nrg-1 treatment provides a pro-regenerative microenvironment by attenuating the pro-inflammatory response of activated astrocytes and microglia as well as peripherally recruited immune cells (Gauthier et al., 2013; Alizadeh et al., 2017; Alizadeh et al., 2018; Kataria et al., 2018). Previously, we showed that Nrg-1 bioavailability efficiently mitigates the tissue level of TNF-α, IL-β and IL-6 after compressive SCI (Gauthier et al., 2013; Alizadeh et al., 2017; Alizadeh et al., 2018). Moreover, Nrg-1 treatment considerably reduces the expression and activity of MMP-2 and MMP-9 following SCI (Alizadeh et al., 2017). Here, we demonstrate that 200 ng/ml of Nrg-1 directly affects mouse M1-polarized microglia in culture and mitigates the transcript levels of pro-inflammatory markers, TNF-α, IL-6 and CD86. Interestingly, evidence shows that the basal levels of Nrg-1β in the serum of healthy individuals are ranged from 30 ng/ml to 473 ng/ml (mean = 217 ±170 ng/mL) suggesting that the 200 ng/ml dose of Nrg-1 used in our *in vitro* studies could be a physiologically achievable dose (Moondra et al., 2009). Importantly, our recent studies in rat spinal cord injury have shown that systemic Nrg-1 treatment (up to 2 μg per day) can effectively modulate microglia/macrophages response without any detectable side effects during a 42-day treatment period (Alizadeh et al., 2017; Alizadeh et al., 2018).

M1 microglia upregulate CD86 receptors to act as APCs and to communicate with other immune cells (Taylor et al., 2005). During EAE progression, the expression of CD86 correlates with an increase in clinical scores (Issazadeh et al., 1998). Moreover, CD86 is upregulated in activated microglia following brain injury (Bechmann et al., 2001). Following nerve axotomy in transgenic mice with IL-6-specific astrocytes, upregulation of IL-6 triggers the expression of CD86

in activated microglia/macrophages, which leads to increased recruitment of pro-inflammatory T cells and neuronal death (Almolda et al., 2014). Thus, the ability of Nrg-1 to moderate the pro-inflammatory activity of M1 microglia is potentially beneficial for the repair process.

Previously, we identified that Nrg-1 treatment remarkably increases the protein levels of IL-10 after SCI and LPC-induced focal demyelination (Alizadeh et al., 2017; Kataria et al., 2018). We further showed that Nrg-1 induced release of IL-10 in the injured spinal cord is attributed to an increase in the populations of M2 microglia/macrophages as well as T and B regulatory cells (Alizadeh et al., 2018; Kataria et al., 2018). IL-10 is a pro-regenerative cytokine essential for oligodendrocyte differentiation and remyelination (Yang et al., 2009). Evidence shows that IL-10 expressed by microglia/macrophages and T regulatory cells prevents the expression of IFN-γ, TNF-α IL-1, and IL-6 by T helper cells (Conti et al., 2003; Saraiva and O'Garra, 2010). IL-10 expression is also decreased in the MS-afflicted patients compared to healthy individuals (Salmaggi et al., 1996; Ozenci et al., 1999). Moreover, intraperitoneal injection of adult neural stem cells engineered to secrete IL-10 during EAE attenuates demyelination and enhances repair and regeneration (Yang et al., 2009). Administration of exogenous IL-10 into animals inhibits EAE development (Rott et al., 1994). IL-10 suppresses cytotoxic function of activated microglia/macrophages by reducing the production of NO (Howard and O'Garra, 1992; Oswald et al., 1992). IL-10 further supports would healing and regeneration by improving phagocytosis of myelin debris by activated microglia/macrophages (Puntambekar et al., 2015). Thus, the induced expression of IL-10 by Nrg-1 treatment in our previous SCI and LPC-induced demyelination studies suggest a beneficial role for Nrg-1 in promoting tissue repair and regeneration following injury (Yang et al., 2009; Mantovani et al., 2013). In the present study, we demonstrate that M1 polarization of mouse microglia cultures under IFN-γ and TNF-α treatment reduces IL-10

expression compared to M0 microglia. Although Nrg-1 treatment significantly reduced the transcript levels of pro-inflammatory cytokines, it was not able to significantly restore reduced IL-10 expression in M1-polarized microglia. This observation suggests that the increased IL-10 expression after Nrg-1 treatment in SCI may be largely attributed to the increase in monocyte derived macrophages as well as T and B regulatory cells. Furthermore, the results of this study show that the effects of Nrg-1 on pure microglia cultures seems to be primarily mediated through modulation of transcript expression of pro-inflammatory cytokines, rather than the anti-inflammatory cytokine, IL-10. It is also plausible that Nrg-1 modulates other anti-inflammatory/pro-regenerative mediators expressed by microglia that were not studied in our experiments.

Microglia Phagocytosis: For the first time, we provide evidence that Nrg-1 fosters a pro-regenerative phenotype in microglia through improvement of phagocytosis. We demonstrate that M1 polarization suppresses phagocytosis in microglia cultures. Interestingly, a lower concentration of Nrg-1 treatment was not only able to recover the impaired ability of M1-polarized microglia for phagocytosis, but also to make it better than M0 microglia. It is known that efficient microglia phagocytosis of cell and myelin debris is required for repair and regeneration (Boekhoff et al., 2012; Redondo-Castro et al., 2013). Thus, the impaired ability of microglia for phagocytosis has been suggested as an underlying cause for the limited tissue regeneration and remyelination after CNS injury (Neumann et al., 2009). In AD brain, pro-inflammatory environment impairs microglia phagocytosis of Aβ peptide causing neuronal death, while the expression of anti-inflammatory factors improves the clearance of Aβ (Koenigsknecht-Talboo and Landreth, 2005). Additionally, direct *in vitro* studies show that LPS, IL-1β, TNF-α, or IFN-γ treatment suppress the

capacity of microglia to phagocytose Aβ peptides (Koenigsknecht-Talboo and Landreth, 2005). In contrast, BV2 cell lines exhibit stronger phagocytic ability under the treatment of anti-inflammatory cytokines, IL-4 and IL-10 (Koenigsknecht-Talboo and Landreth, 2005). Moreover, anti/pro-inflammatory environment positively/negatively influences microglia ability for phagocytosis of degenerated myelin in demyelinating conditions (Smith, 1999). Given that clearance of myelin debris is essential to foster a beneficial microenvironment for repair and axonal regeneration following demyelination, restoration of microglia ability for phagocytosis can be critical for recovery from CNS injury (Smith, 1999). Of note, our findings suggest that Nrg-1 reduced phagocytosis of M0 microglia particularly at higher concentration. This observation indicates that Nrg-1 may exert differential effects on microglia phagocytosis depending on their state of activation at least under our *in vitro* conditions. Further elucidation is required to understand the underlying mechanism(s) by which Nrg-1 regulates microglia phagocytosis under different microglia polarization.

Microglia proliferation and mobilization: Following CNS injuries/diseases, resident microglia rapidly proliferate and mobilize to the site of injury at early stages, which leads to their polarization and morphological changes (Kawabori and Yenari, 2015; Mosser et al., 2017). Here, we demonstrate that M1 polarization of microglia cultures with the combination of IFN-γ and TNF-α does not induce microglia proliferation and Nrg-1 does not change this response. However, our data also indicate that Nrg-1 stimulates the proliferative activity of M0 microglia at higher concentration. Our results are in agreement with previous findings that showed Nrg-1 stimulates proliferation of M0 microglia (Calvo et al., 2010). We also demonstrate that both M1 polarization and Nrg-1 treatment do not influence mobilization of mouse microglia cultures. Evidence shows that microglial mobilization is induced by adenosine triphosphate (ATP), complement component

5a (C5a), and fraktalkine in post-injury microenvironment (Harrison et al., 1998; Miller and Stella, 2009).

ErbB receptors: Recently, our lab showed that Nrg-1 modulates the activity of glial cells through hetero-dimerization of ErbB2 with ErbB3 receptors in mixed rat astro-microglia cultures (Alizadeh et al., 2017). ErbB2 is known to lack the ligand-binding domain, while ErbB3 does not have the kinase domain (Guy et al., 1994; Tzahar et al., 1996). Thus, ErbB2 and ErbB3 need to form hetero-dimers to transduce Nrg-1 signals. In the present study, we show the effects of M1 polarization and Nrg-1 treatment on mRNA levels of ErbB2, ErbB3 and ErbB4 receptors in pure mouse microglia cultures. We demonstrate that ErbB2 and ErbB4 receptors are significantly downregulated in microglia cultures upon M1 polarization, while there is no change in ErbB3 receptor mRNA expression. Interestingly, Nrg-1 treatment of M1-polarized microglia further reduces mRNA levels of ErbB4, which has both ligand binding and kinase domains. This suggests either ErbB4 receptor is not involved in Nrg-1 signal transduction in microglia or Nrg-1 modulation of ErbB4 receptor is a more energy-consuming pathway for microglia. We also show that Nrg-1 treatment in both M0 and M1 microglia cultures stimulates mRNA expression of ErbB3 receptor. However, we found no change in mRNA levels of ErbB2 in Nrg-1-treated compared to non-treated M1-polaried cells. Our findings collectively show that Nrg-1 treatment upregulates transcript expression of ErbB3 receptor in microglia. However, further genetic and pharmacological studies are required to identify ErbB receptor(s) involved in Nrg-1 regulation of microglia.

Paracrine effects of Nrg-1 treated microglia on the properties of NPCs: We recently showed that Nrg-1 directly exerts positive effects on remyelination and repair processes by promoting NPC proliferation and their differentiation into oligodendrocytes *in vitro* and following

SCI (Gauthier et al., 2013). Moreover, we unraveled that Nrg-1 treatment enhances maturation of NPCs and OPCs-derived oligodendrocytes and promotes axonal myelination *in vitro* and following LPC-induced focal demyelination (Kataria et al., 2018). Our lab also provided strong evidence that Nrg-1 positively modulates the response of glial and peripheral immune cells following SCI and LPC-induced focal demyelination (Alizadeh et al., 2017; Alizadeh et al., 2018; Kataria et al., 2018). It is known that pro-inflammatory M1 and pro-regenerative M2 microglia regulate the ability of NPCs for repair and regeneration following CNS injuries or diseases (Wang et al., 2007; Kokaia et al., 2012). A recent work in our lab indicated that M1-polaried microglia reduces the proliferation of spinal cord-derived NPCs (Dyck et al., 2018). Others showed that pro-inflammatory cytokine, IL-1β prevents NPC proliferation in a dose-dependent manner (Wang et al., 2007). Moreover, TNF-α binding to TNF receptor I (TNFRI) inhibits the proliferative ability of adult NPCs (Iosif et al., 2006). On the other hand, M2 microglia-derived growth factor, TGF-β induces proliferation of NPCs following ischemic damage to the SVZ (Ma et al., 2008).

Here, we provide direct evidence that Nrg-1 treatment fosters a pro-regenerative phenotype in microglia, which is potentially beneficial for proliferation and mobilization of NPCs. Our *in vitro* studies on NPCs show that Nrg-1 treated M0 microglia stimulate the proliferative ability of NPCs in a paracrine manner. These findings suggest a role for Nrg-1 in regulating the regenerative activities of microglia under homeostasis condition. We also demonstrate that under activation state Nrg-1 treatment can mitigate the repressive effects of M1-polarized microglia on NPC proliferation and harness their potential for promoting stem cell activation. This observation provides compelling evidence to suggest that dysregulation of Nrg-1 in the injured spinal cord may underlie the detrimental effects of microglia on NPCs following SCI.

We have confirmed that the beneficial effects of Nrg-1 treated M1 MCM on NPC proliferation is through the factors released by microglia and not the possible availability of Nrg-1 recombinant peptide in the MCM. We verified this by addition of 72 h-pre incubated Nrg-1, mimicking Nrg-1 treatment in MCM, into NPCs cultures in which showed no effect on cell proliferation. However, treating NPCs with fresh Nrg-1 stimulates NPC proliferation, which is in agreement with our previous findings (Gauthier et al., 2013). Altogether, this evidence confirms that Nrg-1 treatment leads to changes in microglia secreted factors that are beneficial for NPC proliferation.

Similarly, we also verified that the effects of M1 MCM is not due to the retention of original IFN-γ and TNF-α peptides, which were used for M1 polarization. These verification studies indicated that M1 mouse microglia endogenously express pro and active TNF-α, but not IFN-γ. IFN-γ is a glycoprotein, which functions as a homodimer of approximately 45 kDa. However, it can also be detected as a combination of 25, 20 and 15.5 kDa bands on SDS-PAGE as a result of different glycosylation. In addition, the purified recombinant IFN-γ peptide has been reported to have the molecular weight of 31 kDa as a homodimer (Kelker et al., 1984). Interestingly, in the our study, we detected two bands for IFN-γ with the molecular weight of 45 and 25 kDa in EAE samples and recombinant IFN-γ with the molecular weight of 31 kDa as positive controls by western blot. We previously demonstrated that rat microglia cultures highly express TNF-α in response to LPS, which is in agreement with other studies (Welser-Alves and Milner, 2013; Alizadeh et al., 2017; Yu et al., 2017). However, IFN-γ is principally released by lymphocytes such as CD8+ or gamma delta T cells (Gao et al., 2003; Kambayashi et al., 2003). In addition to peripheral immune cells, microglia were reported to express IFN-γ under specific conditions, such as IL-12 and/or IL-18 exposure or following acute toxoplasmosis in the CNS (Kawanokuchi et al.,

2006; Wang and Suzuki, 2007). Collectively, our control studies have ruled out the possibility that the effects of MCM on NPCs are due to the original TNF-α, IFN-γ or Nrg-1 recombinant peptides.

Following CNS injury or disease, endogenous cell renewal is restricted or blocked in the hostile microenvironment. Microglia have been reported to critically influence this process in beneficial or detrimental way depending on their activation and phenotype (Butovsky et al., 2006). Under IL-4/low IFN-γ exposure, microglia support NPC differentiation into oligodendrocytes and neurons through an IGF-I dependent mechanism (Butovsky et al., 2006). However, exposure of microglia to LPS results in high level of TNF-α expression, which blocks differentiation and cell renewal of mouse brain-derived NPCs (Butovsky et al., 2006). Other studies have shown that CM of LPS-activated microglia stimulates differentiation of fetal rat spinal cord-derived NPCs into astrocytes (Liu et al., 2013b). TNF-α expression in LPS-activated microglia was shown to reduce neurogenesis from fetal rat brain-derived NPCs. The recent study in our laboratory also showed that CM of rat microglia, which were activated with combination of IFN-γ and TNF-α, induces astrocytes differentiation of adult mouse spinal cord-derived NPCs (Dyck et al., 2018). In the present study, we demonstrate that mouse M1 MCM completely blocks NPC differentiation and maintain them as Nestin expressing multipotent NPCs, while M0 MCM promotes NPC differentiation. Interestingly, Nrg-1 had no effects on M1 mediated suppression of NPC differentiation. However, the MCM of Nrg-1 treated M0 microglia promotes the differentiation of NPCs into NG2 positive OPCs while decreasing the fate specification to astrocytes. These studies suggest that Nrg-1 has differential roles in microglia mediated effects on NPC proliferation and differentiation.

In response to an injury, resident precursor cells of the brain and spinal cord migrate toward the injury site during the repair process (Mao et al., 2016). Several inhibitory and promoting factors

have been identified that regulate the migration of precursor cells following CNS injury or disease. For example, reactive astrocytes and glial scar-associated CSPGs exert inhibitory effects on NPC and OPC migration in SCI or other CNS disorders (Dyck and Karimi-Abdolrezaee, 2015). On the other hand, local astrocytes and endothelial cells express stromal cell-derived factor 1 alpha (SDF-1α) as an inflammatory chemoattractant, which induces NPC migration from contralateral hemisphere toward the site of ischemic injury (Imitola et al., 2004). Nrg-1 is another promoting factor which fosters migration and survival of OPCs and their differentiation into myelinating oligodendrocytes during the development (Vartanian et al., 1999; Miller, 2002; Ortega et al., 2012). Here, we show that Nrg-1 treated M0 MCM stimulates NPC migration. We also demonstrate that M1 MCM had no effect on NPC migration, while Nrg-1 treated M1 MCM significantly induces migration of NPCs in a dose-independent manner.

In these studies, we have verified that the effects of MCM on NPC migration is through the factors released by Nrg-1 treated microglia cultures, as addition of 72 h-pre incubated Nrg-1 into the lower compartment of micro-devices had no effects on NPC migration. This evidence confirms that availability of Nrg-1 promotes a supportive phenotype in microglia that indirectly enhances migration of NPCs.

5.2. Study limitations

Although our studies provide new findings on the direct role of Nrg-1 in regulating microglia phenotype and their effects on NPCs, they must be interpreted with caution. Similar to any other *in vitro* study, our study has its own limitations. While our goal was to understand the impact of Nrg-1 on microglia directly in isolated cultures, microglia are not the only cells involved in CNS neuroinflammation. Astrocytes and peripheral immune cells, which enter the CNS through the compromised BBB, also play critical roles in trauma and demyelinating conditions. In addition, these cell populations have regulatory effects on one another. Thus, future co-culture studies can provide a better platform to simulate the cellular network that microglia form with other populations in the CNS.

In this study, we focused on paracrine effects of Nrg-1 treated microglia on NPCs. However, there are also cell-cell communications between microglia and NPCs, which were not pursued in this study due to time limitation associated with an MSc project. These cellular interactions need to be further studied to delineate the role of Nrg-1 in regulating microglia/NPC cross-talk.

Another limitation of the present study is that primary microglia were isolated from both sexes. Future sex specific investigations are needed to study the effects of gender on microglia response under Nrg-1 treatment.

5.3. Conclusions

In conclusion, our findings provide strong evidence that Nrg-1 fosters a pro-regenerative phenotype in primary mouse microglia cultures, which could support repair and regeneration. We have identified that Nrg-1 positively modulates microglia response to the combination of IFN-γ and TNF-α. Nrg-1 treated microglia express lower levels of pro-inflammatory mediators, TNF-α and IL-6. Nrg-1 treatment also reduces the mRNA expression of CD86 on microglia, indicating a reduction in antigen presenting ability of these cells. We further demonstrate a supportive role for Nrg-1 in the repair process by showing that Nrg-1 stimulates phagocytic ability of microglia. Importantly, our findings indicate that availability of Nrg-1 in the microenvironment of M1-polarized microglia improves the reduced proliferative ability of NPCs and increases their migration.

Given the fact that Nrg-1 is persistently depleted in the spinal cord following SCI and demyelinating conditions, we propose that increasing the deficient levels of Nrg-1 in the spinal cord can promote the response of microglia and NPCs, which are important for repair and regeneration of the injured and diseased CNS.

5.4. Future Directions

This study has identified a role for Nrg-1 in modulating microglia activity, and their effects on NPC properties. However, further investigations are required to provide new insights into the molecular mechanisms by which Nrg-1 regulates microglia activity. For example, one of the essential steps for tissue repair and regeneration is phagocytosis of cell and myelin debris, which may be mediated by different receptors expressed in microglia (Fu et al., 2014). Here, we showed

that Nrg-1 improves the reduced phagocytic ability of M1-polarized microglia. It is interesting to elucidate the involved receptor(s) and pathways by which Nrg-1 treatment modulate microglia phagocytosis.

This work showed that Nrg-1 induces a pro-regenerative phenotype in M1-polarized microglia, which fosters the proliferation of NPCs. Previous *in vitro* studies in our laboratory unraveled that addition of Nrg-1 into spinal cord-NPC cultures promotes NPCs proliferation, which is mediated through ErbB2/ErbB4 receptors (Gauthier et al., 2013). Future experiments could elucidate whether microglia-mediated Nrg-1 effects on NPCs proliferation is also through ErbB2/ErbB4-dependent mechanisms.

We also found that M1 MCM prevents NPC differentiation. Other groups have shown that LPS-induced M1 microglia block NPC differentiation via TNF-α expression (Butovsky et al., 2006). A future experiment could investigate the factor(s) in M1 MCM, which play a role in the inhibition of NPC differentiation. Since this study has unraveled high expression of TNF-α in M1-polarized microglia cultures, it would be interesting to study whether M1-polarized microglia prevent NPCs differentiation via TNF-α-dependent mechanism, although a combination of factors may have been involved.

5.5. Implications of this study

This project utilized primary microglial systems that were essential to examine the direct role for Nrg-1 in modulating Microglia. Outcomes of this study together with our previous finding suggest that Nrg-1 can potentially target both inflammation and demyelination; two hallmarks of SCI and demyelinating conditions. Therapeutically, Nrg-1 offers high translational feasibility as a new target for these disorders, owing to its safety approval by the Food and Drug Administration (FDA)

and its ideal pharmacokinetics that facilitates its entry to the CNS tissue through blood-brain-barrier (Kastin et al., 2004; Gao et al., 2010).

References

Aarum J, Sandberg K, Haeberlein SL, Persson MA (2003) Migration and differentiation of neural precursor cells can be directed by microglia. Proceedings of the National Academy of Sciences of the United States of America 100:15983-15988.

Aberg MA, Aberg ND, Palmer TD, Alborn AM, Carlsson-Skwirut C, Bang P, Rosengren LE, Olsson T, Gage FH, Eriksson PS (2003) IGF-I has a direct proliferative effect in adult hippocampal progenitor cells. Molecular and cellular neurosciences 24:23-40.

Adlkofer K, Lai C (2000) Role of neuregulins in glial cell development. Glia 29:104-111.

Ajami B, Bennett JL, Krieger C, Tetzlaff W, Rossi FM (2007) Local self-renewal can sustain CNS microglia maintenance and function throughout adult life. Nature neuroscience 10:1538-1543.

Ajami B, Bennett JL, Krieger C, McNagny KM, Rossi FM (2011) Infiltrating monocytes trigger EAE progression, but do not contribute to the resident microglia pool. Nature neuroscience 14:1142-1149.

Alexander JK, Popovich PG (2009a) Neuroinflammation in spinal cord injury: therapeutic targets for neuroprotection and regeneration. 175:125-137.

Alexander JK, Popovich PG (2009b) Neuroinflammation in spinal cord injury: therapeutic targets for neuroprotection and regeneration. Progress in brain research 175:125-137.

Alizadeh A, Karimi-Abdolrezaee S (2016) Microenvironmental regulation of oligodendrocyte replacement and remyelination in spinal cord injury. The Journal of physiology 594:3539-3552.

Alizadeh A, Dyck SM, Karimi-Abdolrezaee S (2015) Myelin damage and repair in pathologic CNS: challenges and prospects. Frontiers in molecular neuroscience 8:35.

Alizadeh A, Santhosh KT, Kataria H, Gounni AS, Karimi-Abdolrezaee S (2018) Neuregulin-1 elicits a regulatory immune response following traumatic spinal cord injury. Journal of neuroinflammation 15:53.

Alizadeh A, Dyck SM, Kataria H, Shahriary GM, Nguyen DH, Santhosh KT, Karimi-Abdolrezaee S (2017) Neuregulin-1 positively modulates glial response and improves neurological recovery following traumatic spinal cord injury. Glia 65:1152-1175.

Alliot F, Godin I, Pessac B (1999) Microglia derive from progenitors, originating from the yolk sac, and which proliferate in the brain. Brain research Developmental brain research 117:145-152.

Almad A, Sahinkaya FR, McTigue DM (2011) Oligodendrocyte fate after spinal cord injury. Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics 8:262-273.

Almolda B, Villacampa N, Manders P, Hidalgo J, Campbell IL, Gonzalez B, Castellano B (2014) Effects of astrocyte-targeted production of interleukin-6 in the mouse on the host response to nerve injury. Glia 62:1142-1161.

Altman J, Das GD (1965) Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. The Journal of comparative neurology 124:319-335.

Amor S, Puentes F, Baker D, van der Valk P (2010) Inflammation in neurodegenerative diseases. Immunology 129:154-169.

Anton ES, Marchionni MA, Lee KF, Rakic P (1997) Role of GGF/neuregulin signaling in interactions between migrating neurons and radial glia in the developing cerebral cortex. Development 124:3501-3510.

Anton ES, Ghashghaei HT, Weber JL, McCann C, Fischer TM, Cheung ID, Gassmann M, Messing A, Klein R, Schwab MH, Lloyd KC, Lai C (2004) Receptor tyrosine kinase ErbB4 modulates neuroblast migration and placement in the adult forebrain. Nature neuroscience 7:1319-1328.

Antony JM, Paquin A, Nutt SL, Kaplan DR, Miller FD (2011) Endogenous microglia regulate development of embryonic cortical precursor cells. Journal of neuroscience research 89:286-298.

Armulik A, Genove G, Mae M, Nisancioglu MH, Wallgard E, Niaudet C, He L, Norlin J, Lindblom P, Strittmatter K, Johansson BR, Betsholtz C (2010) Pericytes regulate the blood-brain barrier. Nature 468:557-561.

Arvidsson A, Collin T, Kirik D, Kokaia Z, Lindvall O (2002) Neuronal replacement from endogenous precursors in the adult brain after stroke. Nature medicine 8:963-970.

Ashwell K (1990) Microglia and cell death in the developing mouse cerebellum. Brain research Developmental brain research 55:219-230.

Azim K, Raineteau O, Butt AM (2012) Intraventricular injection of FGF-2 promotes generation of oligodendrocyte-lineage cells in the postnatal and adult forebrain. Glia 60:1977-1990.

Bahrini I, Song JH, Diez D, Hanayama R (2015) Neuronal exosomes facilitate synaptic pruning by up-regulating complement factors in microglia. Scientific reports 5:7989.

Bal-Price A, Brown GC (2001) Inflammatory neurodegeneration mediated by nitric oxide from activated glia-inhibiting neuronal respiration, causing glutamate release and excitotoxicity. The Journal of neuroscience : the official journal of the Society for Neuroscience 21:6480-6491.

Balabanov R, Strand K, Goswami R, McMahon E, Begolka W, Miller SD, Popko B (2007) Interferon-gamma-oligodendrocyte interactions in the regulation of experimental autoimmune encephalomyelitis. The Journal of neuroscience : the official journal of the Society for Neuroscience 27:2013-2024.

Balu DT, Coyle JT (2011) Neuroplasticity signaling pathways linked to the pathophysiology of schizophrenia. Neuroscience and biobehavioral reviews 35:848-870.

Bao J, Wolpowitz D, Role LW, Talmage DA (2003) Back signaling by the Nrg-1 intracellular domain. The Journal of cell biology 161:1133-1141.

Barcia C, Ros CM, Annese V, Gomez A, Ros-Bernal F, Aguado-Llera D, Martinez-Pagan ME, de Pablos V, Fernandez-Villalba E, Herrero MT (2012) IFN-gamma signaling, with the synergistic contribution of TNF-alpha, mediates cell specific microglial and astroglial activation in experimental models of Parkinson's disease. Cell death & disease 3:e379.

Barnabe-Heider F, Goritz C, Sabelstrom H, Takebayashi H, Pfrieger FW, Meletis K, Frisen J (2010) Origin of new glial cells in intact and injured adult spinal cord. Cell stem cell 7:470-482.

Barres BA, Raff MC (1999) Axonal control of oligodendrocyte development. The Journal of cell biology 147:1123-1128.

Bartus K, Galino J, James ND, Hernandez-Miranda LR, Dawes JM, Fricker FR, Garratt AN, McMahon SB, Ramer MS, Birchmeier C, Bennett DL, Bradbury EJ (2016) Neuregulin-1 controls an endogenous repair mechanism after spinal cord injury. Brain : a journal of neurology 139:1394-1416.

Beattie MS, Bresnahan JC, Komon J, Tovar CA, Van Meter M, Anderson DK, Faden AI, Hsu CY, Noble LJ, Salzman S, Young W (1997) Endogenous repair after spinal cord contusion injuries in the rat. Experimental neurology 148:453-463.

Becher B, Spath S, Goverman J (2017) Cytokine networks in neuroinflammation. Nature reviews Immunology 17:49-59.

Bechmann I, Peter S, Beyer M, Gimsa U, Nitsch R (2001) Presence of B7--2 (CD86) and lack of B7--1 (CD(80) on myelin phagocytosing MHC-II-positive rat microglia is associated with nondestructive immunity in vivo. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 15:1086-1088.

Beck KD, Powell-Braxton L, Widmer HR, Valverde J, Hefti F (1995) Igf1 gene disruption results in reduced brain size, CNS hypomyelination, and loss of hippocampal granule and striatal parvalbumin-containing neurons. Neuron 14:717-730.

Beers DR, Henkel JS, Xiao Q, Zhao W, Wang J, Yen AA, Siklos L, McKercher SR, Appel SH (2006) Wild-type microglia extend survival in PU.1 knockout mice with familial amyotrophic lateral sclerosis. Proceedings of the National Academy of Sciences of the United States of America 103:16021-16026.

Benveniste EN (1997) Role of macrophages/microglia in multiple sclerosis and experimental allergic encephalomyelitis. Journal of molecular medicine 75:165-173.

Berbel P, Innocenti GM (1988) The development of the corpus callosum in cats: a light- and electron-microscopic study. The Journal of comparative neurology 276:132-156.

Bermingham-McDonogh O, McCabe KL, Reh TA (1996) Effects of GGF/neuregulins on neuronal survival and neurite outgrowth correlate with erbB2/neu expression in developing rat retina. Development 122:1427-1438.

Bessis A, Bechade C, Bernard D, Roumier A (2007) Microglial control of neuronal death and synaptic properties. Glia 55:233-238.

Biber K, Vinet J, Boddeke HW (2008) Neuron-microglia signaling: chemokines as versatile messengers. Journal of neuroimmunology 198:69-74.

Bilbo SD, Levkoff LH, Mahoney JH, Watkins LR, Rudy JW, Maier SF (2005) Neonatal infection induces memory impairments following an immune challenge in adulthood. Behavioral neuroscience 119:293-301.

Bilbo SD, Newsum NJ, Sprunger DB, Watkins LR, Rudy JW, Maier SF (2007) Differential effects of neonatal handling on early life infection-induced alterations in cognition in adulthood. Brain, behavior, and immunity 21:332-342.

Biswas SK, Mantovani A (2010) Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm. Nature immunology 11:889-896.

Bjarnadottir M, Misner DL, Haverfield-Gross S, Bruun S, Helgason VG, Stefansson H, Sigmundsson A, Firth DR, Nielsen B, Stefansdottir R, Novak TJ, Stefansson K, Gurney ME, Andresson T (2007) Neuregulin1 (NRG1) signaling through Fyn modulates NMDA receptor phosphorylation: differential synaptic function in NRG1+/- knock-outs compared with wild-type mice. The Journal of neuroscience : the official journal of the Society for Neuroscience 27:4519-4529.

Bjurstom H, Wang J, Ericsson I, Bengtsson M, Liu Y, Kumar-Mendu S, Issazadeh-Navikas S, Birnir B (2008) GABA, a natural immunomodulator of T lymphocytes. Journal of neuroimmunology 205:44-50.

Blinzinger K, Kreutzberg G (1968) Displacement of synaptic terminals from regenerating motoneurons by microglial cells. Zeitschrift fur Zellforschung und mikroskopische Anatomie 85:145-157.

Block ML, Hong JS (2007) Chronic microglial activation and progressive dopaminergic neurotoxicity. Biochemical Society transactions 35:1127-1132.

Block ML, Zecca L, Hong JS (2007) Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. Nature reviews Neuroscience 8:57-69.

Boche D, Perry VH, Nicoll JA (2013) Review: activation patterns of microglia and their identification in the human brain. Neuropathology and applied neurobiology 39:3-18.

Boekhoff TM, Ensinger EM, Carlson R, Bock P, Baumgartner W, Rohn K, Tipold A, Stein VM (2012) Microglial contribution to secondary injury evaluated in a large animal model of human spinal cord trauma. Journal of neurotrauma 29:1000-1011.

Brionne TC, Tesseur I, Masliah E, Wyss-Coray T (2003) Loss of TGF-beta 1 leads to increased neuronal cell death and microgliosis in mouse brain. Neuron 40:1133-1145.

Brown GC (2010) Nitric oxide and neuronal death. Nitric oxide : biology and chemistry 23:153-165.

Brown GC, Neher JJ (2010) Inflammatory neurodegeneration and mechanisms of microglial killing of neurons. Molecular neurobiology 41:242-247.

Bublil EM, Yarden Y (2007) The EGF receptor family: spearheading a merger of signaling and therapeutics. Current opinion in cell biology 19:124-134.

Buntinx M, Gielen E, Van Hummelen P, Raus J, Ameloot M, Steels P, Stinissen P (2004) Cytokine-induced cell death in human oligodendroglial cell lines. II: Alterations in gene

expression induced by interferon-gamma and tumor necrosis factor-alpha. Journal of neuroscience research 76:846-861.

Burden S, Yarden Y (1997) Neuregulins and their receptors: a versatile signaling module in organogenesis and oncogenesis. Neuron 18:847-855.

Butovsky O, Talpalar AE, Ben-Yaakov K, Schwartz M (2005) Activation of microglia by aggregated beta-amyloid or lipopolysaccharide impairs MHC-II expression and renders them cytotoxic whereas IFN-gamma and IL-4 render them protective. Molecular and cellular neurosciences 29:381-393.

Butovsky O, Ziv Y, Schwartz A, Landa G, Talpalar AE, Pluchino S, Martino G, Schwartz M (2006) Microglia activated by IL-4 or IFN-gamma differentially induce neurogenesis and oligodendrogenesis from adult stem/progenitor cells. Molecular and cellular neurosciences 31:149-160.

Cacci E, Ajmone-Cat MA, Anelli T, Biagioni S, Minghetti L (2008) In vitro neuronal and glial differentiation from embryonic or adult neural precursor cells are differently affected by chronic or acute activation of microglia. Glia 56:412-425.

Calaora V, Rogister B, Bismuth K, Murray K, Brandt H, Leprince P, Marchionni M, Dubois-Dalcq M (2001) Neuregulin signaling regulates neural precursor growth and the generation of oligodendrocytes in vitro. The Journal of neuroscience : the official journal of the Society for Neuroscience 21:4740-4751.

Calvo M, Zhu N, Tsantoulas C, Ma Z, Grist J, Loeb JA, Bennett DL (2010) Neuregulin-ErbB signaling promotes microglial proliferation and chemotaxis contributing to microgliosis and pain after peripheral nerve injury. The Journal of neuroscience : the official journal of the Society for Neuroscience 30:5437-5450.

Cameron B, Landreth GE (2010) Inflammation, microglia, and Alzheimer's disease. Neurobiology of disease 37:503-509.

Cannella B, Raine CS (2004) Multiple sclerosis: cytokine receptors on oligodendrocytes predict innate regulation. Annals of neurology 55:46-57.

Cao QL, Howard RM, Dennison JB, Whittemore SR (2002) Differentiation of engrafted neuronal-restricted precursor cells is inhibited in the traumatically injured spinal cord. Experimental neurology 177:349-359.

Caprariello AV, Mangla S, Miller RH, Selkirk SM (2012) Apoptosis of oligodendrocytes in the central nervous system results in rapid focal demyelination. Annals of neurology 72:395-405.

Cardona AE, Pioro EP, Sasse ME, Kostenko V, Cardona SM, Dijkstra IM, Huang D, Kidd G, Dombrowski S, Dutta R, Lee JC, Cook DN, Jung S, Lira SA, Littman DR, Ransohoff RM (2006) Control of microglial neurotoxicity by the fractalkine receptor. Nature neuroscience 9:917-924.

Carniglia L, Ramirez D, Durand D, Saba J, Turati J, Caruso C, Scimonelli TN, Lasaga M (2017) Neuropeptides and Microglial Activation in Inflammation, Pain, and Neurodegenerative Diseases. Mediators of inflammation 2017:5048616.

Carson MJ, Doose JM, Melchior B, Schmid CD, Ploix CC (2006) CNS immune privilege: hiding in plain sight. Immunological reviews 213:48-65.

Carta AR, Pisanu A (2013) Modulating microglia activity with PPAR-gamma agonists: a promising therapy for Parkinson's disease? Neurotoxicity research 23:112-123.

Chamak B, Morandi V, Mallat M (1994) Brain macrophages stimulate neurite growth and regeneration by secreting thrombospondin. Journal of neuroscience research 38:221-233.

Chastain EM, Duncan DS, Rodgers JM, Miller SD (2011) The role of antigen presenting cells in multiple sclerosis. Biochimica et biophysica acta 1812:265-274.

Cheepsunthorn P, Palmer C, Connor JR (1998) Cellular distribution of ferritin subunits in postnatal rat brain. The Journal of comparative neurology 400:73-86.

Chen WW, Zhang X, Huang WJ (2016) Role of neuroinflammation in neurodegenerative diseases (Review). Molecular medicine reports 13:3391-3396.

Chen Z, Jalabi W, Shpargel KB, Farabaugh KT, Dutta R, Yin X, Kidd GJ, Bergmann CC, Stohlman SA, Trapp BD (2012) Lipopolysaccharide-induced microglial activation and neuroprotection against experimental brain injury is independent of hematogenous TLR4. The Journal of neuroscience : the official journal of the Society for Neuroscience 32:11706-11715.

Chen Z, Jalabi W, Hu W, Park HJ, Gale JT, Kidd GJ, Bernatowicz R, Gossman ZC, Chen JT, Dutta R, Trapp BD (2014) Microglial displacement of inhibitory synapses provides neuroprotection in the adult brain. Nature communications 5:4486.

Cherry JD, Olschowka JA, O'Banion MK (2014) Neuroinflammation and M2 microglia: the good, the bad, and the inflamed. Journal of neuroinflammation 11:98.

Chew LJ, Fusar-Poli P, Schmitz T (2013) Oligodendroglial alterations and the role of microglia in white matter injury: relevance to schizophrenia. Developmental neuroscience 35:102-129.

Chirumamilla S, Sun D, Bullock MR, Colello RJ (2002) Traumatic brain injury induced cell proliferation in the adult mammalian central nervous system. Journal of neurotrauma 19:693-703.

Chitnis T, Imitola J, Wang Y, Elyaman W, Chawla P, Sharuk M, Raddassi K, Bronson RT, Khoury SJ (2007) Elevated neuronal expression of CD200 protects Wlds mice from inflammation-mediated neurodegeneration. The American journal of pathology 170:1695-1712.

Choi SH, Veeraraghavalu K, Lazarov O, Marler S, Ransohoff RM, Ramirez JM, Sisodia SS (2008) Non-cell-autonomous effects of presenilin 1 variants on enrichment-mediated hippocampal progenitor cell proliferation and differentiation. Neuron 59:568-580.

Chung EY, Liu J, Homma Y, Zhang Y, Brendolan A, Saggese M, Han J, Silverstein R, Selleri L, Ma X (2007) Interleukin-10 expression in macrophages during phagocytosis of apoptotic cells is mediated by homeodomain proteins Pbx1 and Prep-1. Immunity 27:952-964.

Clark DA, Gorczynski RM, Blajchman MA (2008) Transfusion-related immunomodulation due to peripheral blood dendritic cells expressing the CD200 tolerance signaling molecule and alloantigen. Transfusion 48:814-821.

Clemente D, Ortega MC, Melero-Jerez C, de Castro F (2013) The effect of glia-glia interactions on oligodendrocyte precursor cell biology during development and in demyelinating diseases. Frontiers in cellular neuroscience 7:268.

Conti P, Kempuraj D, Kandere K, Di Gioacchino M, Barbacane RC, Castellani ML, Felaco M, Boucher W, Letourneau R, Theoharides TC (2003) IL-10, an inflammatory/inhibitory cytokine, but not always. Immunology letters 86:123-129.

Corfas G, Rosen KM, Aratake H, Krauss R, Fischbach GD (1995) Differential expression of ARIA isoforms in the rat brain. Neuron 14:103-115.

Cox FF, Carney D, Miller AM, Lynch MA (2012) CD200 fusion protein decreases microglial activation in the hippocampus of aged rats. Brain, behavior, and immunity 26:789-796.

Cuadros MA, Martin C, Coltey P, Almendros A, Navascues J (1993) First appearance, distribution, and origin of macrophages in the early development of the avian central nervous system. The Journal of comparative neurology 330:113-129.

Cunningham CL, Martinez-Cerdeno V, Noctor SC (2013) Microglia regulate the number of neural precursor cells in the developing cerebral cortex. The Journal of neuroscience : the official journal of the Society for Neuroscience 33:4216-4233.

da Fonseca AC, Matias D, Garcia C, Amaral R, Geraldo LH, Freitas C, Lima FR (2014) The impact of microglial activation on blood-brain barrier in brain diseases. Frontiers in cellular neuroscience 8:362.

Davalos D, Grutzendler J, Yang G, Kim JV, Zuo Y, Jung S, Littman DR, Dustin ML, Gan WB (2005) ATP mediates rapid microglial response to local brain injury in vivo. Nature neuroscience 8:752-758.

David S, Kroner A (2011) Repertoire of microglial and macrophage responses after spinal cord injury. Nature reviews Neuroscience 12:388-399.

de Haas AH, van Weering HR, de Jong EK, Boddeke HW, Biber KP (2007) Neuronal chemokines: versatile messengers in central nervous system cell interaction. Molecular neurobiology 36:137-151.

Del Rio-Hortega P (1920) La microglia y su transformacion encélulas en bastoncito y cuerpos granulo-adiposos. Trabajos del laboratorio de investigaciones biologicas 18.

Del Rio-Hortega P (1932) Microglia. In: Penfield W, editor. Cytology and cellular pathology of the nervous system. New York, NY: Hoeber:481–534.

Del Rio-Hortega P, Penfield W (1927) Cerebral cicatrix. The reaction of neuroglia and microglia to brain wounds. . Bulletin of the Johns Hopkins Hospital 41:278–282.

Delgado M, Varela N, Gonzalez-Rey E (2008) Vasoactive intestinal peptide protects against beta-amyloid-induced neurodegeneration by inhibiting microglia activation at multiple levels. Glia 56:1091-1103.

Deng W, Aimone JB, Gage FH (2010) New neurons and new memories: how does adult hippocampal neurogenesis affect learning and memory? Nature reviews Neuroscience 11:339-350.

Deng Y, Lu J, Sivakumar V, Ling EA, Kaur C (2008) Amoeboid microglia in the periventricular white matter induce oligodendrocyte damage through expression of proinflammatory cytokines via MAP kinase signaling pathway in hypoxic neonatal rats. Brain pathology 18:387-400.

Denieffe S, Kelly RJ, McDonald C, Lyons A, Lynch MA (2013) Classical activation of microglia in CD200-deficient mice is a consequence of blood brain barrier permeability and infiltration of peripheral cells. Brain, behavior, and immunity 34:86-97.

Dimayuga FO, Ding Q, Keller JN, Marchionni MA, Seroogy KB, Bruce-Keller AJ (2003) The neuregulin GGF2 attenuates free radical release from activated microglial cells. Journal of neuroimmunology 136:67-74.

DiSabato DJ, Quan N, Godbout JP (2016) Neuroinflammation: the devil is in the details. Journal of neurochemistry 139 Suppl 2:136-153.

Dissing-Olesen L, LeDue JM, Rungta RL, Hefendehl JK, Choi HB, MacVicar BA (2014) Activation of neuronal NMDA receptors triggers transient ATP-mediated microglial process outgrowth. The Journal of neuroscience : the official journal of the Society for Neuroscience 34:10511-10527.

Du RH, Sun HB, Hu ZL, Lu M, Ding JH, Hu G (2018) Kir6.1/K-ATP channel modulates microglia phenotypes: implication in Parkinson's disease. Cell death & disease 9:404.

Dyck S, Kataria H, Alizadeh A, Santhosh KT, Lang B, Silver J, Karimi-Abdolrezaee S (2018) Perturbing chondroitin sulfate proteoglycan signaling through LAR and PTPsigma receptors promotes a beneficial inflammatory response following spinal cord injury. Journal of neuroinflammation 15:90.

Dyck SM, Karimi-Abdolrezaee S (2015) Chondroitin sulfate proteoglycans: Key modulators in the developing and pathologic central nervous system. Experimental neurology 269:169-187.

Dyck SM, Alizadeh A, Santhosh KT, Proulx EH, Wu CL, Karimi-Abdolrezaee S (2015) Chondroitin Sulfate Proteoglycans Negatively Modulate Spinal Cord Neural Precursor Cells by Signaling Through LAR and RPTPsigma and Modulation of the Rho/ROCK Pathway. Stem cells 33:2550-2563.

Edwards JP, Zhang X, Frauwirth KA, Mosser DM (2006) Biochemical and functional characterization of three activated macrophage populations. Journal of leukocyte biology 80:1298-1307.

Eftekharpour E, Karimi-Abdolrezaee S, Fehlings MG (2008) Current status of experimental cell replacement approaches to spinal cord injury. Neurosurgical focus 24:E19.

Eglitis MA, Mezey E (1997) Hematopoietic cells differentiate into both microglia and macroglia in the brains of adult mice. Proceedings of the National Academy of Sciences of the United States of America 94:4080-4085.

Ekdahl CT, Claasen JH, Bonde S, Kokaia Z, Lindvall O (2003) Inflammation is detrimental for neurogenesis in adult brain. Proceedings of the National Academy of Sciences of the United States of America 100:13632-13637.

Eriksson PS, Perfilieva E, Bjork-Eriksson T, Alborn AM, Nordborg C, Peterson DA, Gage FH (1998) Neurogenesis in the adult human hippocampus. Nature medicine 4:1313-1317.

Esper RM, Pankonin MS, Loeb JA (2006) Neuregulins: versatile growth and differentiation factors in nervous system development and human disease. Brain research reviews 51:161-175.

Eugenin EA, Eckardt D, Theis M, Willecke K, Bennett MV, Saez JC (2001) Microglia at brain stab wounds express connexin 43 and in vitro form functional gap junctions after treatment with interferon-gamma and tumor necrosis factor-alpha. Proceedings of the National Academy of Sciences of the United States of America 98:4190-4195.

Falls DL (2003) Neuregulins: functions, forms, and signaling strategies. Experimental cell research 284:14-30.

Falls DL, Rosen KM, Corfas G, Lane WS, Fischbach GD (1993) ARIA, a protein that stimulates acetylcholine receptor synthesis, is a member of the neu ligand family. Cell 72:801-815.

Fenn AM, Hall JC, Gensel JC, Popovich PG, Godbout JP (2014) IL-4 signaling drives a unique arginase+/IL-1beta+ microglia phenotype and recruits macrophages to the inflammatory CNS: consequences of age-related deficits in IL-4Ralpha after traumatic spinal cord injury. The Journal of neuroscience : the official journal of the Society for Neuroscience 34:8904-8917.

Fernandez PA, Tang DG, Cheng L, Prochiantz A, Mudge AW, Raff MC (2000) Evidence that axon-derived neuregulin promotes oligodendrocyte survival in the developing rat optic nerve. Neuron 28:81-90.

Ferrer I, Bernet E, Soriano E, del Rio T, Fonseca M (1990) Naturally occurring cell death in the cerebral cortex of the rat and removal of dead cells by transitory phagocytes. Neuroscience 39:451-458.

Filardy AA, Pires DR, Nunes MP, Takiya CM, Freire-de-Lima CG, Ribeiro-Gomes FL, DosReis GA (2010) Proinflammatory clearance of apoptotic neutrophils induces an IL-12(low)IL-10(high) regulatory phenotype in macrophages. Journal of immunology 185:2044-2050.

Flames N, Long JE, Garratt AN, Fischer TM, Gassmann M, Birchmeier C, Lai C, Rubenstein JL, Marin O (2004) Short- and long-range attraction of cortical GABAergic interneurons by neuregulin-1. Neuron 44:251-261.

Flavin MP, Zhao G, Ho LT (2000) Microglial tissue plasminogen activator (tPA) triggers neuronal apoptosis in vitro. Glia 29:347-354.

Flores AI, Mallon BS, Matsui T, Ogawa W, Rosenzweig A, Okamoto T, Macklin WB (2000) Akt-mediated survival of oligodendrocytes induced by neuregulins. The Journal of neuroscience : the official journal of the Society for Neuroscience 20:7622-7630.

Frade JM, Barde YA (1998) Microglia-derived nerve growth factor causes cell death in the developing retina. Neuron 20:35-41.

Franco R, Fernandez-Suarez D (2015) Alternatively activated microglia and macrophages in the central nervous system. Progress in neurobiology 131:65-86.

Frank-Cannon TC, Alto LT, McAlpine FE, Tansey MG (2009) Does neuroinflammation fan the flame in neurodegenerative diseases? Molecular neurodegeneration 4:47.

Franklin RJ, Goldman SA (2015) Glia Disease and Repair-Remyelination. Cold Spring Harbor perspectives in biology 7:a020594.

Frost JL, Schafer DP (2016) Microglia: Architects of the Developing Nervous System. Trends in cell biology 26:587-597.

Fu R, Shen Q, Xu P, Luo JJ, Tang Y (2014) Phagocytosis of microglia in the central nervous system diseases. Molecular neurobiology 49:1422-1434.

Gadani SP, Cronk JC, Norris GT, Kipnis J (2012) IL-4 in the brain: a cytokine to remember. Journal of immunology 189:4213-4219.

Gao HM, Zhou H, Zhang F, Wilson BC, Kam W, Hong JS (2011) HMGB1 acts on microglia Mac1 to mediate chronic neuroinflammation that drives progressive neurodegeneration. The Journal of neuroscience : the official journal of the Society for Neuroscience 31:1081-1092.

Gao R, Zhang J, Cheng L, Wu X, Dong W, Yang X, Li T, Liu X, Xu Y, Li X, Zhou M (2010) A Phase II, randomized, double-blind, multicenter, based on standard therapy, placebo-controlled study of the efficacy and safety of recombinant human neuregulin-1 in patients with chronic heart failure. Journal of the American College of Cardiology 55:1907-1914.

Gao X, Enikolopov G, Chen J (2009) Moderate traumatic brain injury promotes proliferation of quiescent neural progenitors in the adult hippocampus. Experimental neurology 219:516-523.

Gao Y, Yang W, Pan M, Scully E, Girardi M, Augenlicht LH, Craft J, Yin Z (2003) Gamma delta T cells provide an early source of interferon gamma in tumor immunity. The Journal of experimental medicine 198:433-442.

Garratt AN (2006) "To erb-B or not to erb-B..." Neuregulin-1/ErbB signaling in heart development and function. Journal of molecular and cellular cardiology 41:215-218.

Garratt AN, Britsch S, Birchmeier C (2000) Neuregulin, a factor with many functions in the life of a schwann cell. BioEssays : news and reviews in molecular, cellular and developmental biology 22:987-996.

Gassmann M, Casagranda F, Orioli D, Simon H, Lai C, Klein R, Lemke G (1995) Aberrant neural and cardiac development in mice lacking the ErbB4 neuregulin receptor. Nature 378:390-394.

Gauthier MK, Kosciuczyk K, Tapley L, Karimi-Abdolrezaee S (2013) Dysregulation of the neuregulin-1-ErbB network modulates endogenous oligodendrocyte differentiation and preservation after spinal cord injury. The European journal of neuroscience 38:2693-2715.

Gehrmann J, Matsumoto Y, Kreutzberg GW (1995) Microglia: intrinsic immuneffector cell of the brain. Brain research Brain research reviews 20:269-287.

Gensert JM, Goldman JE (1997) Endogenous progenitors remyelinate demyelinated axons in the adult CNS. Neuron 19:197-203.

Gerecke KM, Wyss JM, Carroll SL (2004) Neuregulin-1beta induces neurite extension and arborization in cultured hippocampal neurons. Molecular and cellular neurosciences 27:379-393.

Ginhoux F, Lim S, Hoeffel G, Low D, Huber T (2013) Origin and differentiation of microglia. Frontiers in cellular neuroscience 7:45.

Ginhoux F, Greter M, Leboeuf M, Nandi S, See P, Gokhan S, Mehler MF, Conway SJ, Ng LG, Stanley ER, Samokhvalov IM, Merad M (2010) Fate mapping analysis reveals that adult microglia derive from primitive macrophages. Science 330:841-845.

Giovanoli S, Engler H, Engler A, Richetto J, Voget M, Willi R, Winter C, Riva MA, Mortensen PB, Feldon J, Schedlowski M, Meyer U (2013) Stress in puberty unmasks latent

neuropathological consequences of prenatal immune activation in mice. Science 339:1095-1099.

Goldmann T, Prinz M (2013) Role of microglia in CNS autoimmunity. Clinical & developmental immunology 2013:208093.

Gosselin D, Rivest S (2007) Role of IL-1 and TNF in the brain: twenty years of progress on a Dr. Jekyll/Mr. Hyde duality of the innate immune system. Brain, behavior, and immunity 21:281-289.

Gottfried-Blackmore A, Kaunzner UW, Idoyaga J, Felger JC, McEwen BS, Bulloch K (2009) Acute in vivo exposure to interferon-gamma enables resident brain dendritic cells to become effective antigen presenting cells. Proceedings of the National Academy of Sciences of the United States of America 106:20918-20923.

Guadagno J, Xu X, Karajgikar M, Brown A, Cregan SP (2013) Microglia-derived TNFalpha induces apoptosis in neural precursor cells via transcriptional activation of the Bcl-2 family member Puma. Cell death & disease 4:e538.

Guan Y, Jiang Z, Ciric B, Rostami AM, Zhang GX (2008) Upregulation of chemokine receptor expression by IL-10/IL-4 in adult neural stem cells. Experimental and molecular pathology 85:232-236.

Guy PM, Platko JV, Cantley LC, Cerione RA, Carraway KL, 3rd (1994) Insect cell-expressed p180erbB3 possesses an impaired tyrosine kinase activity. Proceedings of the National Academy of Sciences of the United States of America 91:8132-8136.

Han Q, Lin Q, Huang P, Chen M, Hu X, Fu H, He S, Shen F, Zeng H, Deng Y (2017) Microglia-derived IL-1beta contributes to axon development disorders and synaptic deficit through p38-MAPK signal pathway in septic neonatal rats. Journal of neuroinflammation 14:52.

Hanisch UK (2002) Microglia as a source and target of cytokines. Glia 40:140-155.

Hanisch UK, Kettenmann H (2007) Microglia: active sensor and versatile effector cells in the normal and pathologic brain. Nature neuroscience 10:1387-1394.

Harrison JK, Jiang Y, Chen S, Xia Y, Maciejewski D, McNamara RK, Streit WJ, Salafranca MN, Adhikari S, Thompson DA, Botti P, Bacon KB, Feng L (1998) Role for neuronally derived fractalkine in mediating interactions between neurons and CX3CR1-expressing microglia. Proceedings of the National Academy of Sciences of the United States of America 95:10896-10901.

Harrison PJ (2004) The hippocampus in schizophrenia: a review of the neuropathological evidence and its pathophysiological implications. Psychopharmacology 174:151-162.

Harry GJ, Kraft AD (2012) Microglia in the developing brain: A potential target with lifetime effects. Neurotoxicology 33:191-206.

Hashimoto D et al. (2013) Tissue-resident macrophages self-maintain locally throughout adult life with minimal contribution from circulating monocytes. Immunity 38:792-804.

Hauben E, Schwartz M (2003) Therapeutic vaccination for spinal cord injury: helping the body to cure itself. Trends in pharmacological sciences 24:7-12.

Henry CJ, Huang Y, Wynne AM, Godbout JP (2009) Peripheral lipopolysaccharide (LPS) challenge promotes microglial hyperactivity in aged mice that is associated with exaggerated induction of both pro-inflammatory IL-1beta and anti-inflammatory IL-10 cytokines. Brain, behavior, and immunity 23:309-317.

Heppner FL, Ransohoff RM, Becher B (2015) Immune attack: the role of inflammation in Alzheimer disease. Nature reviews Neuroscience 16:358-372.

Herbomel P, Thisse B, Thisse C (2001) Zebrafish early macrophages colonize cephalic mesenchyme and developing brain, retina, and epidermis through a M-CSF receptor-dependent invasive process. Developmental biology 238:274-288.

Hernangomez M, Mestre L, Correa FG, Loria F, Mecha M, Inigo PM, Docagne F, Williams RO, Borrell J, Guaza C (2012) CD200-CD200R1 interaction contributes to neuroprotective effects of anandamide on experimentally induced inflammation. Glia 60:1437-1450.

Hinks GL, Franklin RJ (1999) Distinctive patterns of PDGF-A, FGF-2, IGF-I, and TGF-beta1 gene expression during remyelination of experimentally-induced spinal cord demyelination. Molecular and cellular neurosciences 14:153-168.

Hoek RM, Ruuls SR, Murphy CA, Wright GJ, Goddard R, Zurawski SM, Blom B, Homola ME, Streit WJ, Brown MH, Barclay AN, Sedgwick JD (2000) Down-regulation of the macrophage lineage through interaction with OX2 (CD200). Science 290:1768-1771.

Horky LL, Galimi F, Gage FH, Horner PJ (2006) Fate of endogenous stem/progenitor cells following spinal cord injury. The Journal of comparative neurology 498:525-538.

Hoshiko M, Arnoux I, Avignone E, Yamamoto N, Audinat E (2012) Deficiency of the microglial receptor CX3CR1 impairs postnatal functional development of thalamocortical synapses in the barrel cortex. The Journal of neuroscience : the official journal of the Society for Neuroscience 32:15106-15111.

Howard M, O'Garra A (1992) Biological properties of interleukin 10. Immunology today 13:198-200.

Hu J CF, Guevara J L, Van Eldik L J. (1996) S100 beta Stimulates Inducible Nitric Oxide Synthase Activity and mRNA Levels in Rat Cortical Astrocytes. J Biol Chem 271:2543–2547.

Hu X, Hicks CW, He W, Wong P, Macklin WB, Trapp BD, Yan R (2006) Bace1 modulates myelination in the central and peripheral nervous system. Nature neuroscience 9:1520-1525.

Hu X, Li P, Guo Y, Wang H, Leak RK, Chen S, Gao Y, Chen J (2012) Microglia/macrophage polarization dynamics reveal novel mechanism of injury expansion after focal cerebral ischemia. Stroke 43:3063-3070.

Hu X, Liou AK, Leak RK, Xu M, An C, Suenaga J, Shi Y, Gao Y, Zheng P, Chen J (2014) Neurobiology of microglial action in CNS injuries: receptor-mediated signaling mechanisms and functional roles. Progress in neurobiology 119-120:60-84.

Hur J, Lee P, Kim MJ, Kim Y, Cho YW (2010) Ischemia-activated microglia induces neuronal injury via activation of gp91phox NADPH oxidase. Biochemical and biophysical research communications 391:1526-1530.

Ihrie RA, Alvarez-Buylla A (2011) Lake-front property: a unique germinal niche by the lateral ventricles of the adult brain. Neuron 70:674-686.

Ilieva H, Polymenidou M, Cleveland DW (2009) Non-cell autonomous toxicity in neurodegenerative disorders: ALS and beyond. The Journal of cell biology 187:761-772.

Imitola J, Raddassi K, Park KI, Mueller FJ, Nieto M, Teng YD, Frenkel D, Li J, Sidman RL, Walsh CA, Snyder EY, Khoury SJ (2004) Directed migration of neural stem cells to sites of CNS injury by the stromal cell-derived factor 1alpha/CXC chemokine receptor 4 pathway. Proceedings of the National Academy of Sciences of the United States of America 101:18117-18122.

Iosif RE, Ekdahl CT, Ahlenius H, Pronk CJ, Bonde S, Kokaia Z, Jacobsen SE, Lindvall O (2006) Tumor necrosis factor receptor 1 is a negative regulator of progenitor proliferation in adult

hippocampal neurogenesis. The Journal of neuroscience : the official journal of the Society for Neuroscience 26:9703-9712.

Issazadeh S, Navikas V, Schaub M, Sayegh M, Khoury S (1998) Kinetics of expression of costimulatory molecules and their ligands in murine relapsing experimental autoimmune encephalomyelitis in vivo. Journal of immunology 161:1104-1112.

Ito U, Nagasao J, Kawakami E, Oyanagi K (2007) Fate of disseminated dead neurons in the cortical ischemic penumbra: ultrastructure indicating a novel scavenger mechanism of microglia and astrocytes. Stroke 38:2577-2583.

Jandial G, Chen MY (2012) Regenerative Biology of the Spine and Spinal Cord. New York, USA.

Janzer RC, Raff MC (1987) Astrocytes induce blood-brain barrier properties in endothelial cells. Nature 325:253-257.

Jessell TM, Siegel RE, Fischbach GD (1979) Induction of acetylcholine receptors on cultured skeletal muscle by a factor extracted from brain and spinal cord. Proceedings of the National Academy of Sciences of the United States of America 76:5397-5401.

Kambayashi T, Assarsson E, Lukacher AE, Ljunggren HG, Jensen PE (2003) Memory CD8+ T cells provide an early source of IFN-gamma. Journal of immunology 170:2399-2408.

Karimi-Abdolrezaee S, Billakanti R (2012) Reactive astrogliosis after spinal cord injury-beneficial and detrimental effects. Molecular neurobiology 46:251-264.

Karimi-Abdolrezaee S, Schut D, Wang J, Fehlings MG (2012) Chondroitinase and growth factors enhance activation and oligodendrocyte differentiation of endogenous neural precursor cells after spinal cord injury. PloS one 7:e37589.

Karimi-Abdolrezaee S, Eftekharpour E, Wang J, Morshead CM, Fehlings MG (2006) Delayed transplantation of adult neural precursor cells promotes remyelination and functional

neurological recovery after spinal cord injury. The Journal of neuroscience : the official journal of the Society for Neuroscience 26:3377-3389.

Karimi-Abdolrezaee S, Eftekharpour E, Wang J, Schut D, Fehlings MG (2010) Synergistic effects of transplanted adult neural stem/progenitor cells, chondroitinase, and growth factors promote functional repair and plasticity of the chronically injured spinal cord. The Journal of neuroscience : the official journal of the Society for Neuroscience 30:1657-1676.

Kastin AJ, Akerstrom V, Pan W (2004) Neuregulin-1-beta1 enters brain and spinal cord by receptor-mediated transport. Journal of neurochemistry 88:965-970.

Kataria H, Alizadeh A, Shahriary GM, Saboktakin Rizi S, Henrie R, Santhosh KT, Thliveris JA, Karimi-Abdolrezaee S (2018) Neuregulin-1 promotes remyelination and fosters a pro-regenerative inflammatory response in focal demyelinating lesions of the spinal cord. Glia 66:538-561.

Kaur C, Rathnasamy G, Ling EA (2013) Roles of activated microglia in hypoxia induced neuroinflammation in the developing brain and the retina. Journal of neuroimmune pharmacology : the official journal of the Society on NeuroImmune Pharmacology 8:66-78.

Kawabori M, Yenari MA (2015) The role of the microglia in acute CNS injury. Metabolic brain disease 30:381-392.

Kawanokuchi J, Mizuno T, Takeuchi H, Kato H, Wang J, Mitsuma N, Suzumura A (2006) Production of interferon-gamma by microglia. Multiple sclerosis 12:558-564.

Kelker HC, Le J, Rubin BY, Yip YK, Nagler C, Vilcek J (1984) Three molecular weight forms of natural human interferon-gamma revealed by immunoprecipitation with monoclonal antibody. The Journal of biological chemistry 259:4301-4304.

Kerschensteiner M, Meinl E, Hohlfeld R (2009) Neuro-immune crosstalk in CNS diseases. Neuroscience 158:1122-1132.

Kettenmann H, Kirchhoff F, Verkhratsky A (2013) Microglia: new roles for the synaptic stripper. Neuron 77:10-18.

Kettenmann H, Hanisch UK, Noda M, Verkhratsky A (2011) Physiology of microglia. Physiological reviews 91:461-553.

Kigerl KA, Gensel JC, Ankeny DP, Alexander JK, Donnelly DJ, Popovich PG (2009) Identification of two distinct macrophage subsets with divergent effects causing either neurotoxicity or regeneration in the injured mouse spinal cord. The Journal of neuroscience : the official journal of the Society for Neuroscience 29:13435-13444.

Kim JY, Sun Q, Oglesbee M, Yoon SO (2003) The role of ErbB2 signaling in the onset of terminal differentiation of oligodendrocytes in vivo. The Journal of neuroscience : the official journal of the Society for Neuroscience 23:5561-5571.

Kitayama M, Ueno M, Itakura T, Yamashita T (2011) Activated microglia inhibit axonal growth through RGMa. PloS one 6:e25234.

Klein RS, Hunter CA (2017) Protective and Pathological Immunity during Central Nervous System Infections. Immunity 46:891-909.

Koenigsknecht-Talboo J, Landreth GE (2005) Microglial phagocytosis induced by fibrillar beta-amyloid and IgGs are differentially regulated by proinflammatory cytokines. The Journal of neuroscience : the official journal of the Society for Neuroscience 25:8240-8249.

Kokaia Z, Martino G, Schwartz M, Lindvall O (2012) Cross-talk between neural stem cells and immune cells: the key to better brain repair? Nature neuroscience 15:1078-1087.

Koning N, Swaab DF, Hoek RM, Huitinga I (2009) Distribution of the immune inhibitory molecules CD200 and CD200R in the normal central nervous system and multiple sclerosis lesions suggests neuron-glia and glia-glia interactions. Journal of neuropathology and experimental neurology 68:159-167.

Kramer R, Bucay N, Kane DJ, Martin LE, Tarpley JE, Theill LE (1996) Neuregulins with an Ig-like domain are essential for mouse myocardial and neuronal development. Proceedings of the National Academy of Sciences of the United States of America 93:4833-4838.

Kriegstein A, Alvarez-Buylla A (2009) The glial nature of embryonic and adult neural stem cells. Annual review of neuroscience 32:149-184.

Kroner A, Greenhalgh AD, Zarruk JG, Passos Dos Santos R, Gaestel M, David S (2014) TNF and increased intracellular iron alter macrophage polarization to a detrimental M1 phenotype in the injured spinal cord. Neuron 83:1098-1116.

Kurant E (2011) Keeping the CNS clear: glial phagocytic functions in Drosophila. Glia 59:1304-1311.

Lacmann A, Hess D, Gohla G, Roussa E, Krieglstein K (2007) Activity-dependent release of transforming growth factor-beta in a neuronal network in vitro. Neuroscience 150:647-657.

Lai C, Feng L (2004) Neuregulin induces proliferation of neural progenitor cells via PLC/PKC pathway. Biochemical and biophysical research communications 319:603-611.

Lampron A, Larochelle A, Laflamme N, Prefontaine P, Plante MM, Sanchez MG, Yong VW, Stys PK, Tremblay ME, Rivest S (2015) Inefficient clearance of myelin debris by microglia impairs remyelinating processes. The Journal of experimental medicine 212:481-495.

Lau LW, Keough MB, Haylock-Jacobs S, Cua R, Doring A, Sloka S, Stirling DP, Rivest S, Yong VW (2012) Chondroitin sulfate proteoglycans in demyelinated lesions impair remyelination. Annals of neurology 72:419-432.

Lawson LJ, Perry VH, Dri P, Gordon S (1990) Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain. Neuroscience 39:151-170.

Lee KF, Simon H, Chen H, Bates B, Hung MC, Hauser C (1995) Requirement for neuregulin receptor erbB2 in neural and cardiac development. Nature 378:394-398.

Lemke G (1996) Neuregulins in development. Molecular and cellular neurosciences 7:247-262.

Lewis DA, Moghaddam B (2006) Cognitive dysfunction in schizophrenia: convergence of gamma-aminobutyric acid and glutamate alterations. Archives of neurology 63:1372-1376.

Li T, Zhang S (2016) Microgliosis in the Injured Brain: Infiltrating Cells and Reactive Microglia Both Play a Role. The Neuroscientist : a review journal bringing neurobiology, neurology and psychiatry 22:165-170.

Li Y, Du XF, Liu CS, Wen ZL, Du JL (2012) Reciprocal regulation between resting microglial dynamics and neuronal activity in vivo. Developmental cell 23:1189-1202.

Li Y, Xu Z, Ford GD, Croslan DR, Cairobe T, Li Z, Ford BD (2007) Neuroprotection by neuregulin-1 in a rat model of permanent focal cerebral ischemia. Brain research 1184:277-283.

Li Y, Lein PJ, Ford GD, Liu C, Stovall KC, White TE, Bruun DA, Tewolde T, Gates AS, Distel TJ, Surles-Zeigler MC, Ford BD (2015) Neuregulin-1 inhibits neuroinflammatory responses in a rat model of organophosphate-nerve agent-induced delayed neuronal injury. Journal of neuroinflammation 12:64.

Lian H, Litvinchuk A, Chiang AC, Aithmitti N, Jankowsky JL, Zheng H (2016) Astrocyte-Microglia Cross Talk through Complement Activation Modulates Amyloid Pathology in Mouse Models of Alzheimer's Disease. The Journal of neuroscience : the official journal of the Society for Neuroscience 36:577-589.

Liao B, Zhao W, Beers DR, Henkel JS, Appel SH (2012) Transformation from a neuroprotective to a neurotoxic microglial phenotype in a mouse model of ALS. Experimental neurology 237:147-152.

Lichanska AM, Browne CM, Henkel GW, Murphy KM, Ostrowski MC, McKercher SR, Maki RA, Hume DA (1999) Differentiation of the mononuclear phagocyte system during mouse embryogenesis: the role of transcription factor PU.1. Blood 94:127-138.

Lin W, Lin Y (2010) Interferon-gamma inhibits central nervous system myelination through both STAT1-dependent and STAT1-independent pathways. Journal of neuroscience research 88:2569-2577.

Lin W, Kemper A, Dupree JL, Harding HP, Ron D, Popko B (2006) Interferon-gamma inhibits central nervous system remyelination through a process modulated by endoplasmic reticulum stress. Brain : a journal of neurology 129:1306-1318.

Liu J, Hjorth E, Zhu M, Calzarossa C, Samuelsson EB, Schultzberg M, Akesson E (2013a) Interplay between human microglia and neural stem/progenitor cells in an allogeneic co-culture model. Journal of cellular and molecular medicine 17:1434-1443.

Liu M, Bing G (2011) Lipopolysaccharide animal models for Parkinson's disease. Parkinson's disease 2011:327089.

Liu X, Su H, Chu TH, Guo A, Wu W (2013b) Minocycline inhibited the pro-apoptotic effect of microglia on neural progenitor cells and protected their neuronal differentiation in vitro. Neuroscience letters 542:30-36.

Liu Y, Ford B, Mann MA, Fischbach GD (2001) Neuregulins increase alpha7 nicotinic acetylcholine receptors and enhance excitatory synaptic transmission in GABAergic interneurons of the hippocampus. The Journal of neuroscience : the official journal of the Society for Neuroscience 21:5660-5669.

Liu ZG, Hsu H, Goeddel DV, Karin M (1996) Dissection of TNF receptor 1 effector functions: JNK activation is not linked to apoptosis while NF-kappaB activation prevents cell death. Cell 87:565-576.

Loane DJ, Byrnes KR (2010) Role of microglia in neurotrauma. Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics 7:366-377.

Loeb JA, Susanto ET, Fischbach GD (1998) The neuregulin precursor proARIA is processed to ARIA after expression on the cell surface by a protein kinase C-enhanced mechanism. Molecular and cellular neurosciences 11:77-91.

Logan MA, Freeman MR (2007) The scoop on the fly brain: glial engulfment functions in Drosophila. Neuron glia biology 3:63-74.

Long-Smith CM, Sullivan AM, Nolan YM (2009) The influence of microglia on the pathogenesis of Parkinson's disease. Progress in neurobiology 89:277-287.

Lopez-Bendito G, Cautinat A, Sanchez JA, Bielle F, Flames N, Garratt AN, Talmage DA, Role LW, Charnay P, Marin O, Garel S (2006) Tangential neuronal migration controls axon guidance: a role for neuregulin-1 in thalamocortical axon navigation. Cell 125:127-142.

Lu H, Hu J, Li J, Pang W, Hu Y, Yang H, Li W, Huang C, Zhang M, Jiang Y (2013) Optimal dose of zinc supplementation for preventing aluminum-induced neurotoxicity in rats. Neural regeneration research 8:2754-2762.

Lull ME, Block ML (2010) Microglial activation and chronic neurodegeneration. Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics 7:354-365.

Luo XQ, Li A, Yang X, Xiao X, Hu R, Wang TW, Dou XY, Yang DJ, Dong Z (2018) Paeoniflorin exerts neuroprotective effects by modulating the M1/M2 subset polarization of microglia/macrophages in the hippocampal CA1 region of vascular dementia rats via cannabinoid receptor 2. Chinese medicine 13:14.

Luskin MB (1993) Restricted proliferation and migration of postnatally generated neurons derived from the forebrain subventricular zone. Neuron 11:173-189.

Lyman M, Lloyd DG, Ji X, Vizcaychipi MP, Ma D (2014) Neuroinflammation: the role and consequences. Neuroscience research 79:1-12.

Lytle JM, Chittajallu R, Wrathall JR, Gallo V (2009) NG2 cell response in the CNP-EGFP mouse after contusive spinal cord injury. Glia 57:270-285.

Ma M, Ma Y, Yi X, Guo R, Zhu W, Fan X, Xu G, Frey WH, 2nd, Liu X (2008) Intranasal delivery of transforming growth factor-beta1 in mice after stroke reduces infarct volume and increases neurogenesis in the subventricular zone. BMC neuroscience 9:117.

Mack CL, Vanderlugt-Castaneda CL, Neville KL, Miller SD (2003) Microglia are activated to become competent antigen presenting and effector cells in the inflammatory environment of the Theiler's virus model of multiple sclerosis. Journal of neuroimmunology 144:68-79.

Maimone D, Gregory S, Arnason BG, Reder AT (1991) Cytokine levels in the cerebrospinal fluid and serum of patients with multiple sclerosis. Journal of neuroimmunology 32:67-74.

Makinodan M, Rosen KM, Ito S, Corfas G (2012) A critical period for social experience-dependent oligodendrocyte maturation and myelination. Science 337:1357-1360.

Mallat M, Chamak B (1994) Brain macrophages: neurotoxic or neurotrophic effector cells? Journal of leukocyte biology 56:416-422.

Mantovani A, Biswas SK, Galdiero MR, Sica A, Locati M (2013) Macrophage plasticity and polarization in tissue repair and remodelling. The Journal of pathology 229:176-185.

Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M (2004) The chemokine system in diverse forms of macrophage activation and polarization. Trends in immunology 25:677-686.

Mao Y, Nguyen T, Sutherland T, Gorrie CA (2016) Endogenous neural progenitor cells in the repair of the injured spinal cord. Neural regeneration research 11:1075-1076.

Marin-Teva JL, Cuadros MA, Martin-Oliva D, Navascues J (2011) Microglia and neuronal cell death. Neuron glia biology 7:25-40.

Marin-Teva JL, Dusart I, Colin C, Gervais A, van Rooijen N, Mallat M (2004) Microglia promote the death of developing Purkinje cells. Neuron 41:535-547.

Martino G, Pluchino S (2006) The therapeutic potential of neural stem cells. Nature reviews Neuroscience 7:395-406.

Mason JL, Toews A, Hostettler JD, Morell P, Suzuki K, Goldman JE, Matsushima GK (2004) Oligodendrocytes and progenitors become progressively depleted within chronically demyelinated lesions. The American journal of pathology 164:1673-1682.

Mattson MP (2005) NF-kappaB in the survival and plasticity of neurons. Neurochemical research 30:883-893.

Mei L, Xiong WC (2008) Neuregulin 1 in neural development, synaptic plasticity and schizophrenia. Nature reviews Neuroscience 9:437-452.

Meletis K, Barnabe-Heider F, Carlen M, Evergren E, Tomilin N, Shupliakov O, Frisen J (2008) Spinal cord injury reveals multilineage differentiation of ependymal cells. PLoS biology 6:e182.

Memarian A, Nourizadeh M, Masoumi F, Tabrizi M, Emami AH, Alimoghaddam K, Hadjati J, Mirahmadian M, Jeddi-Tehrani M (2013) Upregulation of CD200 is associated with Foxp3+ regulatory T cell expansion and disease progression in acute myeloid leukemia. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine 34:531-542.

Mencel M, Nash M, Jacobson C (2013) Neuregulin upregulates microglial alpha7 nicotinic acetylcholine receptor expression in immortalized cell lines: implications for regulating neuroinflammation. PloS one 8:e70338.

Menn B, Garcia-Verdugo JM, Yaschine C, Gonzalez-Perez O, Rowitch D, Alvarez-Buylla A (2006) Origin of oligodendrocytes in the subventricular zone of the adult brain. The Journal of neuroscience : the official journal of the Society for Neuroscience 26:7907-7918.

Merrill JE, Ignarro LJ, Sherman MP, Melinek J, Lane TE (1993) Microglial cell cytotoxicity of oligodendrocytes is mediated through nitric oxide. Journal of immunology 151:2132-2141.

Mettang M, Reichel SN, Lattke M, Palmer A, Abaei A, Rasche V, Huber-Lang M, Baumann B, Wirth T (2018) IKK2/NF-kappaB signaling protects neurons after traumatic brain injury.

FASEB journal : official publication of the Federation of American Societies for Experimental Biology 32:1916-1932.

Meuth SG, Simon OJ, Grimm A, Melzer N, Herrmann AM, Spitzer P, Landgraf P, Wiendl H (2008) CNS inflammation and neuronal degeneration is aggravated by impaired CD200-CD200R-mediated macrophage silencing. Journal of neuroimmunology 194:62-69.

Michel PP, Ruberg M, Agid Y (1997) Rescue of mesencephalic dopamine neurons by anticancer drug cytosine arabinoside. Journal of neurochemistry 69:1499-1507.

Mildner A, Schmidt H, Nitsche M, Merkler D, Hanisch UK, Mack M, Heikenwalder M, Bruck W, Priller J, Prinz M (2007) Microglia in the adult brain arise from Ly-6ChiCCR2+ monocytes only under defined host conditions. Nature neuroscience 10:1544-1553.

Miller AM, Stella N (2009) Microglial cell migration stimulated by ATP and C5a involve distinct molecular mechanisms: quantification of migration by a novel near-infrared method. Glia 57:875-883.

Miller RH (2002) Regulation of oligodendrocyte development in the vertebrate CNS. Progress in neurobiology 67:451-467.

Mills CD, Kincaid K, Alt JM, Heilman MJ, Hill AM (2000) M-1/M-2 macrophages and the Th1/Th2 paradigm. J Immunol 164:6166-6173.

Mills E, Dong XP, Wang F, Xu H (2010) Mechanisms of brain iron transport: insight into neurodegeneration and CNS disorders. Future medicinal chemistry 2:51-64.

Miron VE, Boyd A, Zhao JW, Yuen TJ, Ruckh JM, Shadrach JL, van Wijngaarden P, Wagers AJ, Williams A, Franklin RJM, Ffrench-Constant C (2013) M2 microglia and macrophages drive oligodendrocyte differentiation during CNS remyelination. Nature neuroscience 16:1211-1218.

Moghaddam B (2004) Targeting metabotropic glutamate receptors for treatment of the cognitive symptoms of schizophrenia. Psychopharmacology 174:39-44.

Monje ML, Toda H, Palmer TD (2003) Inflammatory blockade restores adult hippocampal neurogenesis. Science 302:1760-1765.

Montero JC, Rodriguez-Barrueco R, Yuste L, Juanes PP, Borges J, Esparis-Ogando A, Pandiella A (2007) The extracellular linker of pro-neuregulin-alpha2c is required for efficient sorting and juxtacrine function. Molecular biology of the cell 18:380-393.

Moondra V, Sarma S, Buxton T, Safa R, Cote G, Storer T, Lebrasseur NK, Sawyer DB (2009) Serum Neuregulin-1beta as a Biomarker of Cardiovascular Fitness. The open biomarkers journal 2:1-5.

Morgan SC, Taylor DL, Pocock JM (2004) Microglia release activators of neuronal proliferation mediated by activation of mitogen-activated protein kinase, phosphatidylinositol-3-kinase/Akt and delta-Notch signalling cascades. Journal of neurochemistry 90:89-101.

Morrens J, Van Den Broeck W, Kempermann G (2012) Glial cells in adult neurogenesis. Glia 60:159-174.

Mosher KI, Andres RH, Fukuhara T, Bieri G, Hasegawa-Moriyama M, He Y, Guzman R, Wyss-Coray T (2012) Neural progenitor cells regulate microglia functions and activity. Nature neuroscience 15:1485-1487.

Mosser CA, Baptista S, Arnoux I, Audinat E (2017) Microglia in CNS development: Shaping the brain for the future. Progress in neurobiology 149-150:1-20.

Mosser DM, Edwards JP (2008) Exploring the full spectrum of macrophage activation. Nature reviews Immunology 8:958-969.

Mount MP, Lira A, Grimes D, Smith PD, Faucher S, Slack R, Anisman H, Hayley S, Park DS (2007) Involvement of interferon-gamma in microglial-mediated loss of dopaminergic neurons. The Journal of neuroscience : the official journal of the Society for Neuroscience 27:3328-3337.

Nagata K, Nakajima K, Takemoto N, Saito H, Kohsaka S (1993) Microglia-derived plasminogen enhances neurite outgrowth from explant cultures of rat brain. International journal of developmental neuroscience : the official journal of the International Society for Developmental Neuroscience 11:227-237.

Nakamura M, Okada S, Toyama Y, Okano H (2005) Role of IL-6 in spinal cord injury in a mouse model. Clinical reviews in allergy & immunology 28:197-204.

Nakanishi M, Niidome T, Matsuda S, Akaike A, Kihara T, Sugimoto H (2007) Microglia-derived interleukin-6 and leukaemia inhibitory factor promote astrocytic differentiation of neural stem/progenitor cells. The European journal of neuroscience 25:649-658.

Nakazawa T, Hisatomi T, Nakazawa C, Noda K, Maruyama K, She H, Matsubara A, Miyahara S, Nakao S, Yin Y, Benowitz L, Hafezi-Moghadam A, Miller JW (2007) Monocyte chemoattractant protein 1 mediates retinal detachment-induced photoreceptor apoptosis. Proceedings of the National Academy of Sciences of the United States of America 104:2425-2430.

Napoli I, Neumann H (2010) Protective effects of microglia in multiple sclerosis. Experimental neurology 225:24-28.

Nave KA (2010) Myelination and support of axonal integrity by glia. Nature 468:244-252.

Nave KA, Werner HB (2014) Myelination of the nervous system: mechanisms and functions. Annual review of cell and developmental biology 30:503-533.

Neddens J, Vullhorst D, Paredes D, Buonanno A (2009) Neuregulin links dopaminergic and glutamatergic neurotransmission to control hippocampal synaptic plasticity. Communicative & integrative biology 2:261-264.

Neher JJ, Neniskyte U, Zhao JW, Bal-Price A, Tolkovsky AM, Brown GC (2011a) Inhibition of Microglial Phagocytosis Is Sufficient To Prevent Inflammatory Neuronal Death. J Immunol 186:4973-4983.

Neher JJ, Neniskyte U, Zhao JW, Bal-Price A, Tolkovsky AM, Brown GC (2011b) Inhibition of microglial phagocytosis is sufficient to prevent inflammatory neuronal death. Journal of immunology 186:4973-4983.

Neniskyte U, Gross CT (2017) Errant gardeners: glial-cell-dependent synaptic pruning and neurodevelopmental disorders. Nature reviews Neuroscience 18:658-670.

Neniskyte U, Neher JJ, Brown GC (2011) Neuronal death induced by nanomolar amyloid beta is mediated by primary phagocytosis of neurons by microglia. The Journal of biological chemistry 286:39904-39913.

Neumann H, Kotter MR, Franklin RJ (2009) Debris clearance by microglia: an essential link between degeneration and regeneration. Brain : a journal of neurology 132:288-295.

Neumann H, Misgeld T, Matsumuro K, Wekerle H (1998) Neurotrophins inhibit major histocompatibility class II inducibility of microglia: involvement of the p75 neurotrophin receptor. Proceedings of the National Academy of Sciences of the United States of America 95:5779-5784.

Nicholas RS, Wing MG, Compston A (2001) Nonactivated microglia promote oligodendrocyte precursor survival and maturation through the transcription factor NF-kappa B. The European journal of neuroscience 13:959-967.

Nimmerjahn A, Kirchhoff F, Helmchen F (2005) Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science 308:1314-1318.

Nissl F (1899) Ueber einige Beziehungen zwishcen Nerven zellerkrankungen und gliosen Erscheinnungen bei verschiedenen Psychosen. Archives of psychiatry 32:1-21.

Niswender CM, Conn PJ (2010) Metabotropic glutamate receptors: physiology, pharmacology, and disease. Annual review of pharmacology and toxicology 50:295-322.

O'Kusky J, Ye P (2012) Neurodevelopmental effects of insulin-like growth factor signaling. Frontiers in neuroendocrinology 33:230-251.

O'Kusky JR, Ye P, D'Ercole AJ (2000) Insulin-like growth factor-I promotes neurogenesis and synaptogenesis in the hippocampal dentate gyrus during postnatal development. The Journal of neuroscience : the official journal of the Society for Neuroscience 20:8435-8442.

Obermeier B, Daneman R, Ransohoff RM (2013) Development, maintenance and disruption of the blood-brain barrier. Nature medicine 19:1584-1596.

Okada M, Corfas G (2004) Neuregulin1 downregulates postsynaptic GABAA receptors at the hippocampal inhibitory synapse. Hippocampus 14:337-344.

Olson JK, Girvin AM, Miller SD (2001) Direct activation of innate and antigen-presenting functions of microglia following infection with Theiler's virus. Journal of virology 75:9780-9789.

Ortega MC, Bribian A, Peregrin S, Gil MT, Marin O, de Castro F (2012) Neuregulin-1/ErbB4 signaling controls the migration of oligodendrocyte precursor cells during development. Experimental neurology 235:610-620.

Oswald IP, Wynn TA, Sher A, James SL (1992) Interleukin 10 inhibits macrophage microbicidal activity by blocking the endogenous production of tumor necrosis factor alpha required as

a costimulatory factor for interferon gamma-induced activation. Proceedings of the National Academy of Sciences of the United States of America 89:8676-8680.

Ousman SS, David S (2000) Lysophosphatidylcholine induces rapid recruitment and activation of macrophages in the adult mouse spinal cord. Glia 30:92-104.

Ozaki M, Sasner M, Yano R, Lu HS, Buonanno A (1997) Neuregulin-beta induces expression of an NMDA-receptor subunit. Nature 390:691-694.

Ozenci V, Kouwenhoven M, Huang YM, Xiao B, Kivisakk P, Fredrikson S, Link H (1999) Multiple sclerosis: levels of interleukin-10-secreting blood mononuclear cells are low in untreated patients but augmented during interferon-beta-1b treatment. Scandinavian journal of immunology 49:554-561.

Palmer TD, Markakis EA, Willhoite AR, Safar F, Gage FH (1999) Fibroblast growth factor-2 activates a latent neurogenic program in neural stem cells from diverse regions of the adult CNS. The Journal of neuroscience : the official journal of the Society for Neuroscience 19:8487-8497.

Pang Y, Cai Z, Rhodes PG (2003) Disturbance of oligodendrocyte development, hypomyelination and white matter injury in the neonatal rat brain after intracerebral injection of lipopolysaccharide. Brain research Developmental brain research 140:205-214.

Pang Y, Campbell L, Zheng B, Fan L, Cai Z, Rhodes P (2010) Lipopolysaccharide-activated microglia induce death of oligodendrocyte progenitor cells and impede their development. Neuroscience 166:464-475.

Pang Y, Fan LW, Tien LT, Dai X, Zheng B, Cai Z, Lin RC, Bhatt A (2013) Differential roles of astrocyte and microglia in supporting oligodendrocyte development and myelination in vitro. Brain and behavior 3:503-514.

Paolicelli RC, Bolasco G, Pagani F, Maggi L, Scianni M, Panzanelli P, Giustetto M, Ferreira TA, Guiducci E, Dumas L, Ragozzino D, Gross CT (2011) Synaptic pruning by microglia is necessary for normal brain development. Science 333:1456-1458.

Park KW, Lee DY, Joe EH, Kim SU, Jin BK (2005) Neuroprotective role of microglia expressing interleukin-4. Journal of neuroscience research 81:397-402.

Park SK, Solomon D, Vartanian T (2001) Growth factor control of CNS myelination. Developmental neuroscience 23:327-337.

Peferoen L, Kipp M, van der Valk P, van Noort JM, Amor S (2014) Oligodendrocyte-microglia cross-talk in the central nervous system. Immunology 141:302-313.

Peress NS, Fleit HB, Perillo E, Kuljis R, Pezzullo C (1993) Identification of Fc gamma RI, II and III on normal human brain ramified microglia and on microglia in senile plaques in Alzheimer's disease. Journal of neuroimmunology 48:71-79.

Peri F, Nusslein-Volhard C (2008) Live imaging of neuronal degradation by microglia reveals a role for v0-ATPase a1 in phagosomal fusion in vivo. Cell 133:916-927.

Perry VH, Gordon S (1991) Macrophages and the nervous system. International review of cytology 125:203-244.

Peruzzotti-Jametti L, Bernstock JD, Vicario N, Costa ASH, Kwok CK, Leonardi T, Booty LM, Bicci I, Balzarotti B, Volpe G, Mallucci G, Manferrari G, Donega M, Iraci N, Braga A, Hallenbeck JM, Murphy MP, Edenhofer F, Frezza C, Pluchino S (2018) Macrophage-Derived Extracellular Succinate Licenses Neural Stem Cells to Suppress Chronic Neuroinflammation. Cell stem cell 22:355-368 e313.

Petrovic-Djergovic D, Goonewardena SN, Pinsky DJ (2016) Inflammatory Disequilibrium in Stroke. Circulation research 119:142-158.

Pfeiffer SE, Warrington AE, Bansal R (1993) The oligodendrocyte and its many cellular processes. Trends in cell biology 3:191-197.

Pluchino S, Martino G (2005) The therapeutic use of stem cells for myelin repair in autoimmune demyelinating disorders. Journal of the neurological sciences 233:117-119.

Pocock JM, Kettenmann H (2007) Neurotransmitter receptors on microglia. Trends in neurosciences 30:527-535.

Prinz M, Tay TL, Wolf Y, Jung S (2014) Microglia: unique and common features with other tissue macrophages. Acta neuropathologica 128:319-331.

Prokop S, Miller KR, Heppner FL (2013) Microglia actions in Alzheimer's disease. Acta neuropathologica 126:461-477.

Puntambekar SS, Hinton DR, Yin X, Savarin C, Bergmann CC, Trapp BD, Stohlman SA (2015) Interleukin-10 is a critical regulator of white matter lesion containment following viral induced demyelination. Glia.

Qin L, Liu Y, Wang T, Wei SJ, Block ML, Wilson B, Liu B, Hong JS (2004) NADPH oxidase mediates lipopolysaccharide-induced neurotoxicity and proinflammatory gene expression in activated microglia. The Journal of biological chemistry 279:1415-1421.

Ramesh G, MacLean AG, Philipp MT (2013) Cytokines and chemokines at the crossroads of neuroinflammation, neurodegeneration, and neuropathic pain. Mediators of inflammation 2013:480739.

Ramesh G, Benge S, Pahar B, Philipp MT (2012) A possible role for inflammation in mediating apoptosis of oligodendrocytes as induced by the Lyme disease spirochete Borrelia burgdorferi. Journal of neuroinflammation 9:72.

Ransohoff RM, Perry VH (2009) Microglial physiology: unique stimuli, specialized responses. Annual review of immunology 27:119-145.

Rath M, Muller I, Kropf P, Closs EI, Munder M (2014) Metabolism via Arginase or Nitric Oxide Synthase: Two Competing Arginine Pathways in Macrophages. Frontiers in immunology 5:532.

Redondo-Castro E, Hernandez J, Mahy N, Navarro X (2013) Phagocytic microglial phenotype induced by glibenclamide improves functional recovery but worsens hyperalgesia after spinal cord injury in adult rats. The European journal of neuroscience 38:3786-3798.

Reinke E, Fabry Z (2006) Breaking or making immunological privilege in the central nervous system: the regulation of immunity by neuropeptides. Immunology letters 104:102-109.

Rezaie P, Patel K, Male DK (1999) Microglia in the human fetal spinal cord--patterns of distribution, morphology and phenotype. Brain research Developmental brain research 115:71-81.

Ribeiro Xavier AL, Kress BT, Goldman SA, Lacerda de Menezes JR, Nedergaard M (2015) A Distinct Population of Microglia Supports Adult Neurogenesis in the Subventricular Zone. The Journal of neuroscience : the official journal of the Society for Neuroscience 35:11848-11861.

Riccomagno MM, Kolodkin AL (2015) Sculpting neural circuits by axon and dendrite pruning. Annual review of cell and developmental biology 31:779-805.

Rieckmann P, Albrecht M, Kitze B, Weber T, Tumani H, Broocks A, Luer W, Helwig A, Poser S (1995) Tumor necrosis factor-alpha messenger RNA expression in patients with relapsing-remitting multiple sclerosis is associated with disease activity. Annals of neurology 37:82-88.

Rieff HI, Raetzman LT, Sapp DW, Yeh HH, Siegel RE, Corfas G (1999) Neuregulin induces GABA(A) receptor subunit expression and neurite outgrowth in cerebellar granule cells. The Journal of neuroscience : the official journal of the Society for Neuroscience 19:10757-10766.

Rigato C, Buckinx R, Le-Corronc H, Rigo JM, Legendre P (2011) Pattern of invasion of the embryonic mouse spinal cord by microglial cells at the time of the onset of functional neuronal networks. Glia 59:675-695.

Rio C, Rieff HI, Qi P, Khurana TS, Corfas G (1997) Neuregulin and erbB receptors play a critical role in neuronal migration. Neuron 19:39-50.

Robertson W (1900) A microscopic demonstration of the normal and pathological histology of mesoglia cells. Journal of mental science 46:733–752.

Rohl C, Lucius R, Sievers J (2007) The effect of activated microglia on astrogliosis parameters in astrocyte cultures. Brain research 1129:43-52.

Rott O, Fleischer B, Cash E (1994) Interleukin-10 prevents experimental allergic encephalomyelitis in rats. European journal of immunology 24:1434-1440.

Rousseau E, Michel PP, Hirsch EC (2013) The iron-binding protein lactoferrin protects vulnerable dopamine neurons from degeneration by preserving mitochondrial calcium homeostasis. Molecular pharmacology 84:888-898.

Roy K, Murtie JC, El-Khodor BF, Edgar N, Sardi SP, Hooks BM, Benoit-Marand M, Chen C, Moore H, O'Donnell P, Brunner D, Corfas G (2007) Loss of erbB signaling in oligodendrocytes alters myelin and dopaminergic function, a potential mechanism for neuropsychiatric disorders. Proceedings of the National Academy of Sciences of the United States of America 104:8131-8136.

Roy NS, Wang S, Jiang L, Kang J, Benraiss A, Harrison-Restelli C, Fraser RA, Couldwell WT, Kawaguchi A, Okano H, Nedergaard M, Goldman SA (2000) In vitro neurogenesis by progenitor cells isolated from the adult human hippocampus. Nature medicine 6:271-277.

Ryu KY, Cho GS, Piao HZ, Kim WK (2012) Role of TGF-beta in Survival of Phagocytizing Microglia: Autocrine Suppression of TNF-alpha Production and Oxidative Stress. Experimental neurobiology 21:151-157.

Saijo K, Glass CK (2011) Microglial cell origin and phenotypes in health and disease. Nature reviews Immunology 11:775-787.

Saijo K, Winner B, Carson CT, Collier JG, Boyer L, Rosenfeld MG, Gage FH, Glass CK (2009) A Nurr1/CoREST pathway in microglia and astrocytes protects dopaminergic neurons from inflammation-induced death. Cell 137:47-59.

Salmaggi A, Dufour A, Eoli M, Corsini E, La Mantia L, Massa G, Nespolo A, Milanese C (1996) Low serum interleukin-10 levels in multiple sclerosis: further evidence for decreased systemic immunosuppression? Journal of neurology 243:13-17.

Salter MW, Beggs S (2014) Sublime microglia: expanding roles for the guardians of the CNS. Cell 158:15-24.

Saraiva M, O'Garra A (2010) The regulation of IL-10 production by immune cells. Nature reviews Immunology 10:170-181.

Schafer DP, Stevens B (2015) Microglia Function in Central Nervous System Development and Plasticity. Cold Spring Harbor perspectives in biology 7:a020545.

Schafer DP, Lehrman EK, Stevens B (2013) The "quad-partite" synapse: microglia-synapse interactions in the developing and mature CNS. Glia 61:24-36.

Schafer DP, Lehrman EK, Kautzman AG, Koyama R, Mardinly AR, Yamasaki R, Ransohoff RM, Greenberg ME, Barres BA, Stevens B (2012) Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. Neuron 74:691-705.

Schmid CD, Melchior B, Masek K, Puntambekar SS, Danielson PE, Lo DD, Sutcliffe JG, Carson MJ (2009) Differential gene expression in LPS/IFNgamma activated microglia and macrophages: in vitro versus in vivo. Journal of neurochemistry 109 Suppl 1:117-125.

Schuldiner O, Yaron A (2015) Mechanisms of developmental neurite pruning. Cellular and molecular life sciences : CMLS 72:101-119.

Sedel F, Bechade C, Vyas S, Triller A (2004) Macrophage-derived tumor necrosis factor alpha, an early developmental signal for motoneuron death. The Journal of neuroscience : the official journal of the Society for Neuroscience 24:2236-2246.

Shigemoto-Mogami Y, Hoshikawa K, Goldman JE, Sekino Y, Sato K (2014) Microglia enhance neurogenesis and oligodendrogenesis in the early postnatal subventricular zone. The Journal of neuroscience : the official journal of the Society for Neuroscience 34:2231-2243.

Shrivastava K, Gonzalez P, Acarin L (2012) The immune inhibitory complex CD200/CD200R is developmentally regulated in the mouse brain. The Journal of comparative neurology 520:2657-2675.

Sica A, Mantovani A (2012) Macrophage plasticity and polarization: in vivo veritas. The Journal of clinical investigation 122:787-795.

Sierra A, Abiega O, Shahraz A, Neumann H (2013) Janus-faced microglia: beneficial and detrimental consequences of microglial phagocytosis. Frontiers in cellular neuroscience 7:6.

Sierra A, Encinas JM, Deudero JJ, Chancey JH, Enikolopov G, Overstreet-Wadiche LS, Tsirka SE, Maletic-Savatic M (2010) Microglia shape adult hippocampal neurogenesis through apoptosis-coupled phagocytosis. Cell stem cell 7:483-495.

Siglienti I, Chan A, Kleinschnitz C, Jander S, Toyka KV, Gold R, Stoll G (2007) Downregulation of transforming growth factor-beta2 facilitates inflammation in the central nervous system by reciprocal astrocyte/microglia interactions. Journal of neuropathology and experimental neurology 66:47-56.

Simard AR, Rivest S (2004) Bone marrow stem cells have the ability to populate the entire central nervous system into fully differentiated parenchymal microglia. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 18:998-1000.

Simmons LJ, Surles-Zeigler MC, Li Y, Ford GD, Newman GD, Ford BD (2016) Regulation of inflammatory responses by neuregulin-1 in brain ischemia and microglial cells in vitro involves the NF-kappa B pathway. Journal of neuroinflammation 13:237.

Skeen MJ, Miller MA, Shinnick TM, Ziegler HK (1996) Regulation of murine macrophage IL-12 production. Activation of macrophages in vivo, restimulation in vitro, and modulation by other cytokines. Journal of immunology 156:1196-1206.

Smith JA, Das A, Ray SK, Banik NL (2012) Role of pro-inflammatory cytokines released from microglia in neurodegenerative diseases. Brain research bulletin 87:10-20.

Smith ME (1999) Phagocytosis of myelin in demyelinative disease: a review. Neurochemical research 24:261-268.

Solomon W, Wilson NO, Anderson L, Pitts S, Patrickson J, Liu M, Ford BD, Stiles JK (2014) Neuregulin-1 attenuates mortality associated with experimental cerebral malaria. Journal of neuroinflammation 11:9.

Song GJ, Suk K (2017) Pharmacological Modulation of Functional Phenotypes of Microglia in Neurodegenerative Diseases. Frontiers in aging neuroscience 9:139.

Squarzoni P, Oller G, Hoeffel G, Pont-Lezica L, Rostaing P, Low D, Bessis A, Ginhoux F, Garel S (2014) Microglia modulate wiring of the embryonic forebrain. Cell reports 8:1271-1279.

Sriram S (2011) Role of glial cells in innate immunity and their role in CNS demyelination. Journal of neuroimmunology 239:13-20.

Stefansson H et al. (2002) Neuregulin 1 and susceptibility to schizophrenia. American journal of human genetics 71:877-892.

Stein M, Keshav S, Harris N, Gordon S (1992) Interleukin 4 potently enhances murine macrophage mannose receptor activity: a marker of alternative immunologic macrophage activation. The Journal of experimental medicine 176:287-292.

Steiner H, Blum M, Kitai ST, Fedi P (1999) Differential expression of ErbB3 and ErbB4 neuregulin receptors in dopamine neurons and forebrain areas of the adult rat. Experimental neurology 159:494-503.

Stence N, Waite M, Dailey ME (2001) Dynamics of microglial activation: a confocal time-lapse analysis in hippocampal slices. Glia 33:256-266.

Stephenson EL, Mishra MK, Moussienko D, Laflamme N, Rivest S, Ling CC, Yong VW (2018) Chondroitin sulfate proteoglycans as novel drivers of leucocyte infiltration in multiple sclerosis. Brain : a journal of neurology 141:1094-1110.

Streit WJ (2002) Microglia as neuroprotective, immunocompetent cells of the CNS. Glia 40:133-139.

Streit WJ, Graeber MB (1993) Heterogeneity of microglial and perivascular cell populations: insights gained from the facial nucleus paradigm. Glia 7:68-74.

Sun D (2014) The potential of endogenous neurogenesis for brain repair and regeneration following traumatic brain injury. Neural regeneration research 9:688-692.

Suzuki SO, Goldman JE (2003) Multiple cell populations in the early postnatal subventricular zone take distinct migratory pathways: a dynamic study of glial and neuronal progenitor migration. The Journal of neuroscience : the official journal of the Society for Neuroscience 23:4240-4250.

Swinnen N, Smolders S, Avila A, Notelaers K, Paesen R, Ameloot M, Brone B, Legendre P, Rigo JM (2013) Complex invasion pattern of the cerebral cortex bymicroglial cells during development of the mouse embryo. Glia 61:150-163.

Szelenyi J, Vizi ES (2007) The catecholamine cytokine balance: interaction between the brain and the immune system. Annals of the New York Academy of Sciences 1113:311-324.

Talaveron R, Matarredona ER, de la Cruz RR, Macias D, Galvez V, Pastor AM (2014) Implanted neural progenitor cells regulate glial reaction to brain injury and establish gap junctions with host glial cells. Glia 62:623-638.

Talmage DA (2008) Mechanisms of neuregulin action. Novartis Foundation symposium 289:74-84; discussion 84-93.

Taves S, Berta T, Chen G, Ji RR (2013) Microglia and spinal cord synaptic plasticity in persistent pain. Neural plasticity 2013:753656.

Taylor PR, Martinez-Pomares L, Stacey M, Lin HH, Brown GD, Gordon S (2005) Macrophage receptors and immune recognition. Annual review of immunology 23:901-944.

Taylor RA, Chang CF, Goods BA, Hammond MD, Mac Grory B, Ai Y, Steinschneider AF, Renfroe SC, Askenase MH, McCullough LD, Kasner SE, Mullen MT, Hafler DA, Love JC, Sansing LH (2017) TGF-beta1 modulates microglial phenotype and promotes recovery after intracerebral hemorrhage. The Journal of clinical investigation 127:280-292.

Tian L, Rauvala H, Gahmberg CG (2009) Neuronal regulation of immune responses in the central nervous system. Trends in immunology 30:91-99.

Tian L, Ma L, Kaarela T, Li Z (2012) Neuroimmune crosstalk in the central nervous system and its significance for neurological diseases. Journal of neuroinflammation 9:155.

Tremblay ME (2011) The role of microglia at synapses in the healthy CNS: novel insights from recent imaging studies. Neuron glia biology 7:67-76.

Tremblay ME, Lowery RL, Majewska AK (2010) Microglial interactions with synapses are modulated by visual experience. PLoS biology 8:e1000527.

Tremblay ME, Stevens B, Sierra A, Wake H, Bessis A, Nimmerjahn A (2011) The role of microglia in the healthy brain. The Journal of neuroscience : the official journal of the Society for Neuroscience 31:16064-16069.

Tronnes AA, Koschnitzky J, Daza R, Hitti J, Ramirez JM, Hevner R (2016) Effects of Lipopolysaccharide and Progesterone Exposures on Embryonic Cerebral Cortex Development in Mice. Reproductive sciences 23:771-778.

Tzahar E, Waterman H, Chen X, Levkowitz G, Karunagaran D, Lavi S, Ratzkin BJ, Yarden Y (1996) A hierarchical network of interreceptor interactions determines signal transduction

by Neu differentiation factor/neuregulin and epidermal growth factor. Molecular and cellular biology 16:5276-5287.

Vartanian T, Fischbach G, Miller R (1999) Failure of spinal cord oligodendrocyte development in mice lacking neuregulin. Proceedings of the National Academy of Sciences of the United States of America 96:731-735.

Vartanian T, Corfas G, Li Y, Fischbach GD, Stefansson K (1994) A role for the acetylcholine receptor-inducing protein ARIA in oligodendrocyte development. Proceedings of the National Academy of Sciences of the United States of America 91:11626-11630.

von Bernhardi R, Tichauer JE, Eugenin J (2010) Aging-dependent changes of microglial cells and their relevance for neurodegenerative disorders. Journal of neurochemistry 112:1099-1114.

Voss EV, Skuljec J, Gudi V, Skripuletz T, Pul R, Trebst C, Stangel M (2012) Characterisation of microglia during de- and remyelination: can they create a repair promoting environment? Neurobiology of disease 45:519-528.

Wahl SM, Wen J, Moutsopoulos N (2006) TGF-beta: a mobile purveyor of immune privilege. Immunological reviews 213:213-227.

Wake H, Moorhouse AJ, Miyamoto A, Nabekura J (2013) Microglia: actively surveying and shaping neuronal circuit structure and function. Trends in neurosciences 36:209-217.

Wake H, Moorhouse AJ, Jinno S, Kohsaka S, Nabekura J (2009) Resting microglia directly monitor the functional state of synapses in vivo and determine the fate of ischemic terminals. The Journal of neuroscience : the official journal of the Society for Neuroscience 29:3974-3980.

Walsh JT, Zheng J, Smirnov I, Lorenz U, Tung K, Kipnis J (2014) Regulatory T cells in central nervous system injury: a double-edged sword. Journal of immunology 193:5013-5022.

Walter L, Neumann H (2009) Role of microglia in neuronal degeneration and regeneration. Seminars in immunopathology 31:513-525.

Walton NM, Sutter BM, Laywell ED, Levkoff LH, Kearns SM, Marshall GP, 2nd, Scheffler B, Steindler DA (2006) Microglia instruct subventricular zone neurogenesis. Glia 54:815-825.

Wang F, Baba N, Shen Y, Yamashita T, Tsuru E, Tsuda M, Maeda N, Sagara Y (2017) CCL11 promotes migration and proliferation of mouse neural progenitor cells. Stem cell research & therapy 8:26.

Wang N, Liang H, Zen K (2014) Molecular mechanisms that influence the macrophage m1-m2 polarization balance. Frontiers in immunology 5:614.

Wang X, Suzuki Y (2007) Microglia produce IFN-gamma independently from T cells during acute toxoplasmosis in the brain. Journal of interferon & cytokine research : the official journal of the International Society for Interferon and Cytokine Research 27:599-605.

Wang X, Sam-Wah Tay S, Ng YK (2000) Nitric oxide, microglial activities and neuronal cell death in the lateral geniculate nucleus of glaucomatous rats. Brain research 878:136-147.

Wang X, Fu S, Wang Y, Yu P, Hu J, Gu W, Xu XM, Lu P (2007) Interleukin-1beta mediates proliferation and differentiation of multipotent neural precursor cells through the activation of SAPK/JNK pathway. Molecular and cellular neurosciences 36:343-354.

Watkins S, Robel S, Kimbrough IF, Robert SM, Ellis-Davies G, Sontheimer H (2014) Disruption of astrocyte-vascular coupling and the blood-brain barrier by invading glioma cells. Nature communications 5:4196.

Wei R, Jonakait GM (1999) Neurotrophins and the anti-inflammatory agents interleukin-4 (IL-4), IL-10, IL-11 and transforming growth factor-beta1 (TGF-beta1) down-regulate T cell

costimulatory molecules B7 and CD40 on cultured rat microglia. Journal of neuroimmunology 95:8-18.

Weldon DT, Rogers SD, Ghilardi JR, Finke MP, Cleary JP, O'Hare E, Esler WP, Maggio JE, Mantyh PW (1998) Fibrillar beta-amyloid induces microglial phagocytosis, expression of inducible nitric oxide synthase, and loss of a select population of neurons in the rat CNS in vivo. The Journal of neuroscience : the official journal of the Society for Neuroscience 18:2161-2173.

Welser-Alves JV, Milner R (2013) Microglia are the major source of TNF-alpha and TGF-beta1 in postnatal glial cultures; regulation by cytokines, lipopolysaccharide, and vitronectin. Neurochemistry international 63:47-53.

Whittaker MT, Zai LJ, Lee HJ, Pajoohesh-Ganji A, Wu J, Sharp A, Wyse R, Wrathall JR (2012) GGF2 (Nrg1-beta3) treatment enhances NG2+ cell response and improves functional recovery after spinal cord injury. Glia 60:281-294.

Willem M, Garratt AN, Novak B, Citron M, Kaufmann S, Rittger A, DeStrooper B, Saftig P, Birchmeier C, Haass C (2006) Control of peripheral nerve myelination by the beta-secretase BACE1. Science 314:664-666.

Williamson LL, Sholar PW, Mistry RS, Smith SH, Bilbo SD (2011) Microglia and memory: modulation by early-life infection. The Journal of neuroscience : the official journal of the Society for Neuroscience 31:15511-15521.

Wolf SA, Boddeke HW, Kettenmann H (2017) Microglia in Physiology and Disease. Annual review of physiology 79:619-643.

Wu Y, Dissing-Olesen L, MacVicar BA, Stevens B (2015) Microglia: Dynamic Mediators of Synapse Development and Plasticity. Trends in immunology 36:605-613.

Wu Z, Hayashi Y, Zhang J, Nakanishi H (2007) Involvement of prostaglandin E2 released from leptomeningeal cells in increased expression of transforming growth factor-beta in glial cells and cortical neurons during systemic inflammation. Journal of neuroscience research 85:184-192.

Xiong X, Barreto GE, Xu L, Ouyang YB, Xie X, Giffard RG (2011) Increased brain injury and worsened neurological outcome in interleukin-4 knockout mice after transient focal cerebral ischemia. Stroke 42:2026-2032.

Xu Z, Croslan DR, Harris AE, Ford GD, Ford BD (2006) Extended therapeutic window and functional recovery after intraarterial administration of neuregulin-1 after focal ischemic stroke. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism 26:527-535.

Xu Z, Ford GD, Croslan DR, Jiang J, Gates A, Allen R, Ford BD (2005) Neuroprotection by neuregulin-1 following focal stroke is associated with the attenuation of ischemia-induced pro-inflammatory and stress gene expression. Neurobiology of disease 19:461-470.

Yan K, Zhang R, Sun C, Chen L, Li P, Liu Y, Peng L, Sun H, Qin K, Chen F, Huang W, Chen Y, Lv B, Du M, Zou Y, Cai Y, Qin L, Tang Y, Jiang X (2013) Bone marrow-derived mesenchymal stem cells maintain the resting phenotype of microglia and inhibit microglial activation. PloS one 8:e84116.

Yang J, Jiang Z, Fitzgerald DC, Ma C, Yu S, Li H, Zhao Z, Li Y, Ciric B, Curtis M, Rostami A, Zhang GX (2009) Adult neural stem cells expressing IL-10 confer potent immunomodulation and remyelination in experimental autoimmune encephalitis. The Journal of clinical investigation 119:3678-3691.

Yarden Y, Sliwkowski MX (2001) Untangling the ErbB signalling network. Nature reviews Molecular cell biology 2:127-137.

Ye L, Huang Y, Zhao L, Li Y, Sun L, Zhou Y, Qian G, Zheng JC (2013) IL-1beta and TNF-alpha induce neurotoxicity through glutamate production: a potential role for neuronal glutaminase. Journal of neurochemistry 125:897-908.

Yenari MA, Kauppinen TM, Swanson RA (2010) Microglial activation in stroke: therapeutic targets. Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics 7:378-391.

Yi MH, Zhang E, Kang JW, Shin YN, Byun JY, Oh SH, Seo JH, Lee YH, Kim DW (2012) Expression of CD200 in alternative activation of microglia following an excitotoxic lesion in the mouse hippocampus. Brain research 1481:90-96.

Yokozeki T, Wakatsuki S, Hatsuzawa K, Black RA, Wada I, Sehara-Fujisawa A (2007) Meltrin beta (ADAM19) mediates ectodomain shedding of Neuregulin beta1 in the Golgi apparatus: fluorescence correlation spectroscopic observation of the dynamics of ectodomain shedding in living cells. Genes to cells : devoted to molecular & cellular mechanisms 12:329-343.

Yu Z, Fukushima H, Ono C, Sakai M, Kasahara Y, Kikuchi Y, Gunawansa N, Takahashi Y, Matsuoka H, Kida S, Tomita H (2017) Microglial production of TNF-alpha is a key element of sustained fear memory. Brain, behavior, and immunity 59:313-321.

Yuan J, Ge H, Liu W, Zhu H, Chen Y, Zhang X, Yang Y, Yin Y, Chen W, Wu W, Yang Y, Lin J (2017) M2 microglia promotes neurogenesis and oligodendrogenesis from neural stem/progenitor cells via the PPARgamma signaling pathway. Oncotarget 8:19855-19865.

Zai LJ, Wrathall JR (2005) Cell proliferation and replacement following contusive spinal cord injury. Glia 50:247-257.

Zhang F, Zhong R, Li S, Fu Z, Cheng C, Cai H, Le W (2017) Acute Hypoxia Induced an Imbalanced M1/M2 Activation of Microglia through NF-kappaB Signaling in Alzheimer's Disease Mice and Wild-Type Littermates. Frontiers in aging neuroscience 9:282.

Zhang X, Surguladze N, Slagle-Webb B, Cozzi A, Connor JR (2006) Cellular iron status influences the functional relationship between microglia and oligodendrocytes. Glia 54:795-804.

Zhang Y, Feng S, Nie K, Li Y, Gao Y, Gan R, Wang L, Li B, Sun X, Wang L, Zhang Y (2018) TREM2 modulates microglia phenotypes in the neuroinflammation of Parkinson's disease. Biochemical and biophysical research communications 499:797-802.

Zhao WJ (2013) The expression and localization of neuregulin-1 (Nrg1) in the gastrointestinal system of the rhesus monkey. Folia histochemica et cytobiologica 51:38-44.

Zhu P, Hata R, Cao F, Gu F, Hanakawa Y, Hashimoto K, Sakanaka M (2008) Ramified microglial cells promote astrogliogenesis and maintenance of neural stem cells through activation of Stat3 function. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 22:3866-3877.

Zimmerman LE (1956) Pathology of the demyelinating diseases. Transactions - American Academy of Ophthalmology and Otolaryngology American Academy of Ophthalmology and Otolaryngology 60:46-58.

Ziv Y, Ron N, Butovsky O, Landa G, Sudai E, Greenberg N, Cohen H, Kipnis J, Schwartz M (2006) Immune cells contribute to the maintenance of neurogenesis and spatial learning abilities in adulthood. Nature neuroscience 9:268-275.