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Microglia in the TBI Brain: The Good, The Bad, And The Dysregulated

David J. Loane* and Alok Kumar

Department of Anesthesiology, and Shock, Trauma, and Anesthesiology Research (STAR) Center, University of Maryland School of Medicine, Baltimore, MD

Abstract

As the major cellular component of the innate immune system in the central nervous system (CNS) and the first line of defense whenever injury or disease occurs, microglia play a critical role in neuroinflammation following a traumatic brain injury (TBI). In the injured brain microglia can produce neuroprotective factors, clear cellular debris and orchestrate neurorestorative processes that are beneficial for neurological recovery after TBI. However, microglia can also become dysregulated and can produce high levels of pro-inflammatory and cytotoxic mediators that hinder CNS repair and contribute to neuronal dysfunction and cell death. The dual role of microglial activation in promoting beneficial and detrimental effects on neurons may be accounted for by their polarization state and functional responses after injury. In this review article we discuss emerging research on microglial activation phenotypes in the context of acute brain injury, and the potential role of microglia in phenotype-specific neurorestorative processes such as neurogenesis, angiogenesis, oligodendrogenesis and regeneration. We also describe some of the known molecular mechanisms that regulate phenotype switching, and highlight new therapeutic approaches that alter microglial activation state balance to enhance long-term functional recovery after TBI. An improved understanding of the regulatory mechanisms that control microglial phenotypic shifts may advance our knowledge of post-injury recovery and repair, and provide opportunities for the development of novel therapeutic strategies for TBI.

Keywords

Traumatic brain injury; neuroinflammation; microglia; macrophage; phenotype; M1-like; M2-like; polarization; neurodegeneration; repair

Introduction

Neuroinflammation is a prominent feature of many neurodegenerative diseases (Eikelenboom et al., 2010; Perry et al., 2010), and is increasingly being recognized as an

*Address correspondence to: David J. Loane PhD, Assistant Professor, Department of Anesthesiology, University of Maryland School of Medicine, Baltimore, MD.

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important pathophysiological mechanism underlying chronic neurodegeneration following traumatic brain injury (TBI) (Faden and Loane, 2015; Johnson et al., 2013; Smith et al., 2013; Smith et al., 2012). As the primary mediators of the innate immune response in the central nervous system (CNS), microglia play a critical role in neuroinflammation and secondary injury after TBI. The understanding of the functional role of microglia in the injured brain and spinal cord has developed significantly in recent years, with strong support for dual beneficial and detrimental roles for microglia, resulting in tissue repair or neurodegeneration respectively (David and Kroner, 2011; Kumar and Loane, 2012). By removing cellular debris by phagocytosis and releasing neurotrophic factors and anti-inflammatory cytokines microglia can prevent neuronal injury and restore tissue integrity in the injured brain. However, it is clear that the development of a disinhibited and highly reactive microglial activation state results in the release of high levels of pro-inflammatory and cytotoxic mediators that contribute to neuronal dysfunction and cell death (David and Kroner, 2011; Kumar and Loane, 2012). Immune cells within the CNS milieu such as microglia and infiltrating macrophages appear to be heterogeneous with diverse functional phenotypes that range from pro-inflammatory (M1-like) phenotypes to immunosuppressive (M2-like) phenotypes. The “M1/M2” paradigm has been increasingly studied in neurodegenerative diseases in an attempt to uncover mechanisms of immunopathogenesis, and advances in understanding of molecular and functional states of microglia and macrophages may provide a framework to dissect out and interrogate the dual beneficial and destructive roles of these cells after TBI.

In this review, we focus on microglia and macrophage phenotypes in the context of acute CNS injury and post-traumatic repair. We discuss their role in phenotype-specific neurorestorative processes such as neurogenesis, angiogenesis, oligodendrogenesis and regeneration, and the extracellular and molecular signals that regulate phenotype switching. We also highlight new therapeutic approaches that alter M1-/M2-like balance following TBI and enhance long-term functional recovery via promotion of tissue repair processes and suppression of neurodegeneration.

Microglia: origin and function in the uninjured brain

Microglia represent the major cellular component of the innate immune system of the brain, and constitute up to 10% of the total cells in the adult CNS (Kettenmann et al., 2011). Up until recently microglia were considered to be macrophages of the CNS, however a series of recent findings has established that microglia have a distinctive lineage and molecular signature as compared with circulating monocytes (Butovsky et al., 2014; Ginhoux et al., 2010; Hickman et al., 2013). Fate mapping analysis revealed that adult microglia derive from primitive erythromyeloid progenitors that leave the yolk sac on E8.5–E9.0, enter the neural tube via the primitive blood stream, and journey to the CNS where they gain lineage specific gene expression and differentiate into mature microglia (Ginhoux et al., 2010). Microglia development is dependent on Pu.1 and Irf8 transcription factors (Kierdorf et al., 2013), whereas essential bone-marrow derived macrophage transcription factors Myb and colony-stimulating factor 1 (CSF1) are not required (Schulz et al., 2012). In addition, microglial development is primarily regulated by CSF-1 receptor (CSF-1R) (Erblich et al., 2011) and its alternate ligand interleukin (IL)-34 (IL-34) (Greter et al., 2012), and

transforming growth factor- β (TGF β) has been recently identified as a critical differentiation factor beginning at E14.5 (Butovsky et al., 2014). Yolk-sac-derived microglia are maintained independently of circulating monocytes throughout life (Ajami et al., 2007), with the population being maintained by self-renewal in the healthy CNS, perhaps by a progenitor source within the CNS (Elmore et al., 2014). Microglia and brain macrophages express a number of common protein markers such as CD11b and CX₃CR1, however recent transcriptomic analyses have revealed that microglia have a unique molecular signature as compared to circulating monocytes and other tissue-resident macrophages derived from primitive yolk-sac (Butovsky et al., 2014; Hickman et al., 2013). This unique cluster of microglial-specific genes, which includes p2yr12, Fcrls, Tmem119, Offml3, Tgfb1 (Butovsky et al., 2014; Hickman et al., 2013), can now be used to accurately distinguish microglia from macrophages and monocytes.

Up until recently highly ramified microglia were presumed to be resting and inactive in the healthy brain, but elegant *in vivo* imaging studies revealed that microglia are in fact highly active, and their long cellular processes undergo continuous cycles of extension, withdrawal and de novo formation to continuously scan their environment for disruptions in homeostasis (Davalos et al., 2005; Nimmerjahn et al., 2005). This physiological activity enables microglia to patrol the brain microenvironment and clear accumulated metabolic products or tissue debris by phagocytosis. More than a case of solely maintaining CNS homeostasis, it is now clear that microglia play a critical role in brain development, activity-dependent synaptic plasticity, and learning in the adult CNS, via activities in regulating cell death, synapse elimination, neurogenesis and neuronal surveillance (for review see (Katsumoto et al., 2014; Salter and Beggs, 2014; Tremblay et al., 2011)). Thus, the latest research indicates that microglia refine neuronal circuits and connectivity, and contribute to plasticity. Therefore, in addition to responding to CNS injury and disease, microglia also play an active role in shaping activity in the healthy brain (Salter and Beggs, 2014).

In the healthy CNS, microglia remove cellular debris without changing their ramified phenotype. In contrast, in response to injury or infection microglia become activated which results in dramatic morphological transformation and gene expression changes. Similar to peripheral cells microglia express pathogen recognition receptors such as toll-like receptors (TLRs) and NOD-like receptors (NLRs), and therefore respond to pathogen-associated molecular patterns (PAMPs) and endogenously produced danger-associated molecular patterns (DAMPs), which are secreted by damaged neurons and others cells of the CNS (Hanisch and Kettenmann, 2007). They also express receptors for a number of other factors that are released by damaged neurons, including ATP, glutamate, growth factors and cytokines. Microglia are antigen presenting cells and communicate with T lymphocytes, and upon activation upregulate cell surface markers such as MHC II and CD86 among others, as well as adhesion molecules and complement receptors (Hanisch and Kettenmann, 2007). A key to maintaining microglia in a normal physiological state is the immunosuppressive potential of the CNS. In the healthy brain neurons express a number of immunosuppressive proteins that act as “off” signals, that when paired with specific microglial receptors maintain microglia in a non-activated state (Harrison et al., 1998; Hoek et al., 2000). Such neuronal-microglial signaling mechanisms include fractalkine-CX3CR1, CD200-CD200R1,

CD47-CD172a/SIRP α , among others (Hanisch and Kettenmann, 2007). In addition, soluble factors such as neurotrophins, anti-inflammatory cytokines and prostaglandins released locally by neurons, astrocytes and microglia downregulate the immune response thereby maintaining microglia in a surveillant state (Hanisch and Kettenmann, 2007).

Heterogeneity of microglial activation

It is now recognized that there is considerable heterogeneity of microglial activation in the brain. Macrophages in non-neuronal tissues have remarkable plasticity that allows them to efficiently respond to environmental signals and change their phenotype and function following pathogen exposure or tissue damage (Gordon, 2003; Sica and Mantovani, 2012). This process is called macrophage polarization, and enables the adaptive responses of innate immunity to take place. Two distinct macrophage polarization states have been described, termed M1 and M2, which represent both ends of a spectrum of functional macrophage activation (Gordon, 2003; Sica and Mantovani, 2012). Within this activation spectrum there is one M1 and three M2 polarization subtypes, M2a, M2b and M2c, each with a specific function and pattern of phenotypic marker expression (Gordon, 2003; Sica and Mantovani, 2012). The presence of multiple activation phenotypes for microglia is a relatively new concept (Colton, 2009), and it should not be assumed that phenotypes and functions of peripheral macrophages will translate accurately to microglia in the brain. In fact, the latest research indicates that the stimulus-induced transcriptional plasticity of microglia does not directly correspond to the M1 or M2 plasticity observed with macrophages (Butovsky et al., 2014). However, despite their different ontogeny, microglia also have the capacity to become polarized into 'M1-like' and 'M2-like' activation phenotypes, although their roles in brain injury progression and repair have yet to be fully determined.

Microglia and macrophages respond to pro-inflammatory molecules, such as bacterial lipopolysaccharide (LPS) or the T_H1 cytokine, interferon- γ (IFN γ), to adopt a 'classical' M1-like phenotype, which produces high levels of pro-inflammatory cytokines (IL-1 β , IL-12, tumor necrosis factor- α (TNF α)), chemokines (CCL2, CXCL9, CXCL10), and reactive oxygen species (ROS) that are essential for host defense (Colton, 2009; Gordon, 2003; Sica and Mantovani, 2012). M1-like phenotype markers include CD16, CD32, CD86, MHC II, and iNOS. M1-like activation is associated with phagocytosis, ability to kill pathogens by iron restriction, phagosome acidification, and ROS release (Colton, 2009; Gordon, 2003; Sica and Mantovani, 2012). In many cases, the M1-like response is protective and is downregulated once the damage or pathogen has been removed; however, a dysregulated or excessive M1-like activation in the brain can induce neurotoxicity due to release of pro-inflammatory factors and neurotoxic mediators that set off vicious cycles of microglial-mediated neurodegeneration (Gao et al., 2003; Qin et al., 2004). Microglia and macrophages also respond to T_H2 cytokines such as IL-4 and IL-13 to induce an 'alternative' M2a-like state that is associated with immunity against parasites, Th2 cell recruitment, tissue repair, and growth stimulation (Colton, 2009; Gordon, 2003; Sica and Mantovani, 2012). In addition to producing anti-inflammatory cytokines (IL-10), M2a-polarized cells upregulate several phenotypic markers such as arginase 1, CD206, Ym1, Fizz1, inhibit NF κ B isoforms, and increase production of scavenger receptors for phagocytosis (Colton, 2009; Gordon, 2003; Sica and Mantovani, 2012). Microglia/

macrophages adopt a ‘deactivated’ M2c-like phenotype in response to IL-10, glucocorticoids, or uptake of apoptotic cells, and this phenotype appears to be involved in tissue remodeling and matrix deposition after inflammation has been downregulated (Colton, 2009; Gordon, 2003; Sica and Mantovani, 2012). M2c-polarized cells upregulate phenotypic markers such as CD163, CD206, TGF β , and Sphk-1. Finally, microglia/macrophages can adopt an intermediate M2b-like phenotype in response to immune complex exposure and toll like receptors (TLRs) ligands (Colton, 2009; Gordon, 2003; Sica and Mantovani, 2012). M2b has either pro- or anti-inflammatory function and is associated with memory immune responses (B-cell class switching and recruitment of Treg cells). The M2b phenotype has characteristics of both M1 (MHCII, CD86) and M2 (IL-10^{high}, IL-12^{low}) cells, and when M2b macrophages stimulate T cells they are biased towards a Th2 response (Filardy et al., 2010), which suggests that they may be a potential regulator of the M2 response in general.

Phenotypic markers for each polarization state were established using *in vitro* model systems, and defining specific phenotypes *in vivo* is much more challenging due to various environmental and tissue specific stimuli that drive polarization (Novak and Koh, 2013). After CNS injury or in neurodegenerative disorders microglia or infiltrating macrophages often present mixed phenotypes indicating their plastic nature and their ability to acquire multiple activation phenotypes in response to local environmental signals and dynamic changes in the inflammatory milieu (Sica and Mantovani, 2012). Clearly, the M1-M2 paradigm is an oversimplified model that only represents two extreme states within an activation continuum. However, given the limited data on the roles of microglia and infiltrating macrophage subsets after TBI, their classification into M1-like and M2-like provides a framework to dissect out and interrogate the dual beneficial and destructive roles of resident microglia and infiltrating macrophages after TBI (Figure 1), and for exploration of new therapeutic strategies.

The immune system response to TBI

Within minutes of TBI there is a robust neuroinflammatory response that is mediated by complex molecular and cellular inflammatory events. The temporal profile of these events have been studied using animal models of TBI, as well as human surgical and post-mortem tissue samples and analysis of cerebrospinal fluid (CSF) and plasma from TBI patients (Ziebell and Morganti-Kossmann, 2010). Depending on the intensity of the injury and whether it is penetrating or not, TBI induces instantaneous cell death at the site of impact (primary injury). Damaged cells release DAMPs that signal to other resident and infiltrating immune cells via pattern recognition receptors, and astrocytes, microglia and damaged neurons at the site of injury secrete cytokines and chemokines. These potent immune mediators activate microglia and astrocytes at the site of injury and recruit peripheral immune cells that traffic through the damaged blood-brain barrier during the acute post-traumatic period.

Neutrophils are the first peripheral cells to accumulate in the brain after TBI (Clark et al., 1994), and they attempt to clear cell debris by phagocytosis. However, neutrophils also contribute to ongoing tissue damage by releasing toxic mediators such as ROS (Rhodes,

2011). Experimental studies indicate that neutrophil infiltration in the TBI brain is maximal at 1 day post-injury, and is followed by accumulation of leukocyte subsets that peak at about 3 days post-injury (Soares et al., 1995). Monocytes are recruited to the damage brain in response to local chemokine signals (e.g. CCL2, CXCL10, CCL5 etc.), and once in the brain they differentiate into macrophages. Two subpopulations of monocytes have recently been defined based on their relative cell-surface expression of the chemokine receptors CCR2 and CX3CR1, with CCR2⁺ cells representing 'inflammatory' (CD11b⁺CD45^{hi}CCR2⁺Ly6C^{hi}) monocytes and the CX3CR1⁺ cells representing 'patrolling' (CD11b⁺CD45^{hi}CX3CR1⁺) monocytes (Auffray et al., 2007). It is the inflammatory monocytes that are preferentially recruited to the TBI brain (Hsieh et al., 2013), and they predominate the lesion site at 3 days post-injury. Dendritic cells (DCs), T lymphocytes and natural killer (NK) cells are also recruited to the TBI brain during this period (Jin et al., 2012), but at much lower numbers than infiltrating monocytes. The exact functional role of DCs and T cells in TBI pathology has yet to be established, but distinct T cell subsets may modulate local inflammatory responses to be either harmful or protective (Walsh et al., 2014). Within this time frame brain resident glial cells become highly activated. Astrocytes surrounding the lesion are reactive and up-regulate GFAP and produce cytokines and chemokines that contribute to additional recruitment and activation of resident microglia and peripheral immune cells. Microglia transform from a ramified to an amoeboid morphology and when activated they are morphologically indistinguishable from recruited blood-derived macrophages. They secrete pro-inflammatory cytokines and free radicals that are cytotoxic to neurons and can contribute to neurodegeneration after TBI. Thus, the inflammatory response to TBI is highly complex with several interrelated molecular and cellular events initiated after injury to activate resident microglia and astrocytes, recruit additional peripheral immune cells, and damage neurons. Determining the relative contribution and functional role of M1- and M2-like polarized microglia and infiltrating macrophages in this complex tissue injury environment is challenging, but experimental studies are beginning to shed light on these phenotypic responses.

M1- and M2-like polarization after TBI

It would be expected that M1- and M2-like microglia and macrophages would work in concert to fine-tune inflammatory responses, scavenge debris, and promote remodeling and repair after TBI; thereby contributing to successful wound healing and promoting a return towards homeostasis after injury. However, experimental and clinical studies demonstrate a chronic and persistent M1-like phenotype for months to years after a single moderate-level TBI or repeated mild TBI (Acosta et al., 2013a; Aungst et al., 2014; Johnson et al., 2013; Loane et al., 2014a; Mouzon et al., 2014; Nagamoto-Combs et al., 2007; Nonaka et al., 1999; Ramlackhansingh et al., 2011; Smith et al., 2013), with limited capacity for tissue repair, particularly after moderate-to-severe TBI.

Experimental studies in models of spinal cord and ischemic brain injury have shown that the majority of microglia and recruited macrophages at the site of injury have mixed M1- and M2-like activation profiles, but that the M2-like response is short-lived and there is phenotypic shift towards an M1-like dominant response within one week of injury (Hu et al., 2012; Kigerl et al., 2009). M1-like cells that dominate the lesion have reduced phagocytic

activity, and increased secretion of pro-inflammatory and neurotoxic mediators that can exacerbate injury and contribute to pathology (David and Kroner, 2011; Hu et al., 2012; Kigerl et al., 2009). Furthermore, M1-like macrophages have also been shown to impair axon regeneration via upregulation of chondroitin sulphate proteoglycans that are a potent inhibitor of axon regrowth (Galtrey and Fawcett, 2007; Martinez et al., 2006).

Similar M1- and M2-like activation dynamics have been reported in the TBI brain. Focal contusion TBI induced by controlled cortical impact (CCI) results in extensive local tissue damage, activation of microglia (CD11b+/CD45^{low}), as well as a rapid and robust cellular infiltration of neutrophils (CD11b+/CD45+/Ly6G+) and inflammatory monocytes (CD11b+/CD45^{hi}/CCR2+/Ly6C^{hi}) in the injured cortex within 24 hours of CCI (Hsieh et al., 2013; Jin et al., 2012). Using flow cytometry in combination with markers for M1- and M2-like activation Jin et al., 2012 described a bimodal microglial activation response after CCI, with an initial peak at 7 days post-injury characterized by CD206+/CD45^{low}/CD11b+ M2-like activation and the secondary peak at 28 days post-injury characterized by CD86+/CD45^{low}/CD11b+ M1-like activation (Jin et al., 2012). Such temporal dynamics are supported by histological studies that showed transient CD206+/Iba1+ M2-like activation that peaked at 5 days after CCI followed by a transition to a dominant CD16/32+/Iba1+ M1-like activation at later time points that was associated with increased white matter injury (Wang et al., 2013b). These data indicate that different subsets of microglia and/or macrophages are being activated in the acute and chronic phases after CCI.

Recently, pharmacological studies combined with transgenic mouse models have provided important information on the relative contribution of resident microglia versus infiltrating monocytes to M1/M2-like polarization after TBI. Morganti and colleagues took advantage of CX3CR1^{GFP/+}CCR2^{RFP/+} reporter mice (Saederup et al., 2010) to study the temporal kinetics of TBI-induced CCR2+ monocyte accumulation in the brain (Morganti et al., 2015). Using this model it is possible to distinguish between peripheral inflammatory monocytes (CCR2^{RFP/+}) and resident microglia (CX3CR1^{GFP/+}) based on their relative expression of CCR2 because microglia do not express CCR2 natively or following CNS injury (Saederup et al., 2010; Schilling et al., 2009). CCL2 is the cognate ligand for CCR2, and its expression is significantly upregulated within hours of head trauma as detected in surgically resected samples and CSF from TBI patients (Semple et al., 2010; Stefani et al., 2008), or in rodent TBI models (Dalgard et al., 2012; Israelsson et al., 2008; Rhodes et al., 2009; Semple et al., 2010). The functional consequences of CCL2/CCR2 signaling after TBI has been evaluated using knockout mice, and deficiency of either CCL2 or CCR2 results in improved neurological and histological outcomes. When compared to wildtype mice, CCL2^{-/-} mice subjected to a closed head TBI model had increased neurological function, reduced lesion volume, astrogliosis and accumulation of F4/80+ macrophages at delayed time points post-injury (Semple et al., 2010). Similarly, CCR2^{-/-} mice subjected to CCI had reduced lesion volumes, decreased pro-inflammatory gene expression, and decreased macrophage infiltration (CD45^{hi}CCR2+ and F4/80+ cells) in the injured cortex when compared to wildtype mice (Hsieh et al., 2014; Israelsson et al., 2014), and CCR2^{-/-} CCI mice also had improved cognitive performance in the Morris water maze test and reduced hippocampal neurodegeneration at 8 weeks post-injury (Hsieh et al., 2014). Studies using CCR2 antagonists have also improved outcomes in experimental TBI models. When the CCR2

antagonist RS504393 was administered after weight-drop TBI in rats a high dose treatment reduced early apoptotic cell death and improved cognitive function in the Morris water maze test (Liu et al., 2013). More recently Morganti and colleagues evaluated a new CCR2 antagonist (CCX872) in CX3CR1^{GFP/+}CCR2^{RFP/+} reporter mice using the CCI model, and demonstrated that CCX872 pre-treatment resulted in reduced macrophage accumulation (CD45^{hi}CCR2+F4/80+ cells) acutely after CCI (Morganti et al., 2015). CCR2 antagonism also improved cognitive function recovery after TBI as demonstrated by increased performance of CCX872-treated mice in a radial arm water maze test at 28 days post-injury (Morganti et al., 2015). They also showed that CCR2+ infiltrating macrophages in the brain expressed both M1- and M2-like markers acutely after CCI, with sequential activation of M1-, followed by M2a-, and finally M2c- marker expression (Morganti et al., 2015). The M1-/M2-like activation profile of CCR2+ brain macrophages after CCI was similar to the damage response of wound-healing macrophages outside the CNS, such that proinflammatory and proteolytic macrophages accumulate rapidly after injury, followed by macrophages that promote wound-healing and anti-inflammatory responses (Deonarine et al., 2007; Gensel and Zhang, 2015). However, the accumulation of CCR2+ macrophages into the TBI brain ultimately resulted in upregulation of pro-inflammatory M1-like cells with neurotoxic potential at chronic time points, and CCR2 antagonism robustly reduced this pro-inflammatory profile and ameliorated the long-term cognitive dysfunction induced by TBI (Morganti et al., 2015). In another interesting study Hsieh and colleagues used a reporter mouse model for M2-polarized macrophages (eYFP arginase 1; YARG) to investigate the role of infiltrating brain macrophage subsets after CCI, and demonstrated robust infiltration of arginase 1 (Arg1+)-positive macrophages that differentiated into at least two distinct subpopulations with unique chemokine expression patterns (Hsieh et al., 2013). Expression profiling of Arg1+ and Arg1- macrophage subpopulations that infiltrated the TBI brain revealed that they do not separate into true M1- or M2-like transcriptional profiles, but rather adopt mixed molecular phenotypes, and the ratio of macrophage subsets change over time after TBI (Hsieh et al., 2013). These later two studies highlight the plasticity of the macrophages response to TBI, and how phenotypes and/or functions may switch over time in response to changing stimuli within the local lesion microenvironment.

It is important to note that the majority of existing data on M1-/M2-like phenotypes after TBI have been developed using focal TBI models that have significant tissue damage/loss and robust cellular infiltration. Diffuse brain injury models such as the midline fluid percussion injury (mFPI) model also cause chronic microglial activation that have M1-like characteristics but without significant contributions from infiltrating macrophage populations (Fenn et al., 2014a). Following diffuse brain injury IL-1 β and TNF α are transiently increased in the cortex and hippocampus as early as 4 hours post-injury, before returning to baseline levels by 72 hours (Fenn et al., 2014a), and enriched microglia show increased expression of M1-like markers (IL-1 β , CD14, iNOS) and M2-like markers (arginase 1) at 24 hours post-injury when compared to sham-injured controls (Fenn et al., 2015). At chronic time points up to 30 days post-mFPI there is increased MHC II expression on isolated microglia, and this 'primed' phenotype results in a hyperinflammatory response to secondary immune challenges such as LPS that persist for weeks to months, and is associated with development depressive-like behavior (Fenn et al., 2014a). There are subtle

changes in microglial morphology in the cortex and hippocampus following diffuse brain injury; one study described microglia with a unique rod-like morphology and train arrangement along axon tracks that were predominantly detected in the primary sensory barrel fields of mFPI rats (Ziebell et al., 2012). These cells may participate in circuit reorganization following diffuse brain injury, but whether they contribute to detrimental (M1-like) or beneficial (M2-like) responses has yet to be determined. Interestingly, rod-like microglia have neuroprotective properties in *in vitro* model systems (Tam and Ma, 2014).

Several other factors influence M1-/M2-like polarization in the injured brain, such as injury severity, location within grey or white matter, or aging. Models of ischemic injury demonstrate that microglia/macrophages in the ischemic core are phenotypically different from those in the penumbra. For example, M2-like markers Ym1 and CD206 are solely expressed in amoeboid-like cells in the lesion core, while CD16/32 (M1-like marker) is highly expressed in amoeboid-like cells in the ischemic core, but also in ramified cells in the penumbral regions (Hu et al., 2012; Perego et al., 2011). Interestingly, Ym1 is differentially expressed after focal TBI; it is highly expressed in amoeboid cells that lack CD68 expression in the cortical contusion zone, but it is also expressed in ramified cells in subcortical regions (Kumar et al., 2013). In addition, microglial phenotypes can vary depending on tissue type, with a more rapid transition from M2- to M1-dominant phenotypes in the white matter after TBI when compared to phenotypic switches in grey matter tissue (Wang et al., 2013b). Therefore, injury severity and location greatly influence morphological and phenotypic changes in microglia/macrophages after TBI, and future research needs to investigate the functional role of microglia in different CNS regions following a diverse range of injury severities and in different TBI models (e.g. focal versus diffuse TBI models).

With aging, microglia develop an altered profile consistent with a heightened inflammatory or primed state (Norden et al., 2014). Primed microglia have higher baseline expression of inflammatory markers and mediators, a lower threshold to be activated and switched to a pro-inflammatory (M1-like) state, and an exaggerated inflammatory response following immune activation. Microglial priming in the aged brain can result in development of cognitive deficits, impaired synaptic plasticity and accelerated neurodegeneration (Norden et al., 2014). Several studies investigating phenotypic changes in microglia in the aged brain identified an overall increase in pro-inflammatory (M1-like) gene expression, with diminished alternative (M2-like) gene expression (Cribbs et al., 2012; Lee et al., 2013). However, a recent study using direct RNA sequencing demonstrated that primed microglia in the aged brain are M2 polarized and change the microglial phenotype towards a more neuroprotective state (Hickman et al., 2013). Interestingly, phenotypic analysis of post-mortem frontal cortex and cerebellar tissue from early and late Alzheimer's disease (AD) revealed that early AD samples were biased towards the M1 phenotype, while patients with later stage AD had an M2a bias (Wilcock, 2014). Given the lack of consensus, and the complexities involved in analyzing phenotypic and functional roles of microglia in the brain, further studies are needed to determine the effects of aging on microglia phenotypes in CNS injury and neurodegenerative disease. Nonetheless, when aged mice (24 month old) are subjected focal TBI they have an exaggerated neuroinflammatory response in the

hippocampus and cortex when compared with young TBI mice (3 month old), that is associated with increased neurodegeneration and worse outcomes (Kumar et al., 2013; Sandhir et al., 2008). Microglia from aged mice migrate more slowly to sites of laser-induced focal injury, and remain at the lesion site for more extended periods than that of microglia in young mice (Damani et al., 2011), and aged microglia also have less phagocytic capacity and clear less myelin after toxin-induced demyelination injury than young microglia (Zhao et al., 2006). Phenotypically, aged TBI mice have a significantly increase M1-like gene expression profile (IL-1 β , TNF, iNOS) when compared to young TBI mice, and the M2-like gene expression profile in aged TBI mice appears to be dysregulated, with several M2-like genes significantly upregulated (arginase 1, Ym1), and others downregulated (Fizz-1, TGF β , IL-4R α), when compared to young TBI mice (Kumar et al., 2013). The blunted IL-4R α and TGF β expression in aged TBI mice suggests that aged microglia have an impaired M2-like regulatory phenotype, and are less sensitive to feedback from anti-inflammatory pathways. This data is supported by other studies in which aged microglia fail to upregulate IL-4R α after LPS injection or following isolated SCI (Fenn et al., 2014b; Fenn et al., 2012), resulting in reduced sensitivity to IL-4 dependent programming towards an M2a-like or pro-repair microglial phenotype in aged mice.

Phenotype-specific roles of microglia and macrophages in CNS repair and regeneration

A significant body of *in vitro* and *in vivo* research now supports a role for M2-polarized microglia and macrophages in promoting phenotype-specific CNS repair and regeneration. Therefore, a greater understanding of the molecular mechanisms that regulate the functional responses of microglia/macrophages after TBI may guide the development of future therapeutic interventions that target phenotypic switching to improve functional recovery after TBI. In the following sections we review how phenotypic changes in microglial/macrophage activation can impact multiple steps in CNS repair and regeneration.

Neurogenesis

In the adult mammalian brain neurogenesis occurs predominantly in the subgranular zone (SGZ) of the dentate gyrus in the hippocampal formation and in the forebrain subventricular zone (SVZ) of the lateral ventricle (Gage, 2000). Ramified microglia are an essential component of the neurogenic niche in the SGZ of the adult hippocampus, and direct the migration and differentiation of neural stem/progenitor cells (NSC) by secreting soluble factors that promote neurogenesis (Walton et al., 2006). Brain injury induces endogenous adult neurogenesis in the SVZ and SGZ, as well as in non-neurogenic regions such as the striatum and cerebral cortex adjacent to the injury (Arvidsson et al., 2002; Bengzon et al., 1997). However, only a fraction of new neurons will survive long-term, and this is largely due to the pathological environment encountering the newborn neurons.

Neuroinflammation is a key component of the pathological environment, and depending on cell types involved or activation status, post-traumatic neuroinflammation can be detrimental or supportive for adult neurogenesis (Ek Dahl et al., 2009). For example, LPS-induced inflammation, which drives a pronounced M1-like activation profile in microglia, strongly

impairs basal hippocampal neurogenesis levels in adult rats (Ekdahl et al., 2003; Monje et al., 2003); an effect that can be restored when anti-inflammatory drugs, such as indomethacin or minocycline, are administered to LPS-treated rats. Further, LPS-stimulated microglia or their conditioned medium, reduce NSC survival and prevent differentiation *in vitro* (Cacci et al., 2008). Numerous additional studies have demonstrated that several pro-inflammatory cytokines produced by M1-like microglia and macrophages, such as IFN γ , IL-1 β , TNF α , and IL-6, can also suppress neurogenesis (Ben-Hur et al., 2003; Cacci et al., 2005; Iosif et al., 2008; Koo and Duman, 2008; Monje et al., 2003; Vallieres et al., 2002). In contrast, IL-4 stimulation of microglia, which polarizes microglia towards an M2-like phenotype, promotes neurogenesis of NSCs in co-culture systems (Butovsky et al., 2006b), by increasing levels of insulin-like growth factor-1 (IGF-1), a cytokine that has been correlated with increased hippocampal proliferation and neurogenesis (Aberg et al., 2003; Choi et al., 2008; O'Kusky et al., 2000; Trejo et al., 2001). Further support for a beneficial role of microglial in adult neurogenesis is provided by *in vitro* studies of NSCs co-cultured with microglia, or grown in conditioned media from microglia containing neurotrophic factors such as basic fibroblast growth factor (bFGF), brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) (Aarum et al., 2003; Morgan et al., 2004; Walton et al., 2006). For example, microglia and microglia-conditioned medium rescued the *in vitro* formation of neuroblasts from SVZ NSCs, which otherwise would have been lost with continued culture (Aarum et al., 2003; Walton et al., 2006). Therefore these, and other protective factors released by microglia (e.g. protease serine 2; (Nikolakopoulou et al., 2013)), can increase neural precursor cell proliferation, neuroblast migration and functional integration of new born neurons into the SGZ of the adult hippocampus (Ekdahl et al., 2009).

TBI increases the rates of NSC proliferation in focal and diffuse injury models without increasing neurogenesis in the adult hippocampus (Bye et al., 2011; Gao and Chen, 2013). In addition, TBI promotes cell proliferation of glial cells (astrocytes and microglia) that are robustly activated at chronic time points (Acosta et al., 2013b; Bye et al., 2011; Gao and Chen, 2013). These studies suggest that TBI activates through promotion of NSC proliferation an innate repair response and/or plasticity mechanism in the brain. However, long-term upregulation of chronic pro-inflammatory pathways may suppress functional integration of newborn neurons into niche zones, such that additional support/intervention is required to increase endogenous neurogenesis to successful repair the damage brain after TBI. Indeed, various treatments that increase endogenous neurogenesis lead to improved post-TBI recovery in adult rodents (Han et al., 2011; Zhang et al., 2012). Further, it would appear that IGF-1 plays an important role in neurogenesis after TBI as conditional IGF-1 overexpression in regions of neuronal damage results in a marked increase in immature neuron densities in the SGZ at 10 days after CCI (Carlson et al., 2014), and this results in increased cognitive function recovery after TBI (Madathil et al., 2013). Interestingly, when CCI mice undergo a delayed voluntary exercise program using in-cage running wheels, chronic M1-like microglial activation is robustly reduced after TBI, and IL-10, IGF-1, CREB and BDNF levels are significantly increased in the hippocampus, which results in enhanced neurogenesis in the SGZ and improved cognitive function recovery at chronic time points (Piao et al., 2013).

Angiogenesis

The adult CNS vasculature is extremely stable under physiological conditions, but is activated upon injury (Greener and Jin, 2005). Adult vascular remodeling includes angiogenesis by mature endothelial cells and vasculogenesis by endothelial progenitor cells (EPCs). EPCs are present in the bone marrow and peripheral blood, and mobilize in the blood and accumulate in the brain after TBI (Guo et al., 2009). Angiogenesis may play a significant role in mediating functional recovery following TBI (Xiong et al., 2010), and several pharmacological agents that increase angiogenesis have been shown to improve functional outcome following experimental TBI (Wu et al., 2008; Zhang et al., 2009).

In the injured brain angiogenic remodeling occurs in the ischemic penumbra within days of ischemic insult (Chen et al., 1994; Wei et al., 2001), and angiogenic vessels are often surrounded by microglia and macrophages (Jander et al., 1998; Manoonkitiwongsa et al., 2001), suggesting that activated microglia/macrophages may be instrumental in promoting the angiogenic response to cerebral ischemia. Although the phenotype-specific role of microglia in post-traumatic angiogenesis and vascular repair is currently unknown, M2-polarized macrophages play a critical role in vascular repair and wound healing (Jetten et al., 2014; Medina et al., 2011; Willenborg et al., 2012; Zajac et al., 2013). M2 macrophages promote angiogenesis by producing proangiogenic cytokines and growth factors such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (FGF-2) (Jetten et al., 2014). Both factors have dose dependent proangiogenic effects and have been shown to act synergistically *in vitro* (Xue and Greisler, 2002), and they induce proliferation and migration of endothelial cells and formation of vascular sprouts (Pakala et al., 2002; Polverini et al., 1977; Sunderkotter et al., 1991). In addition, a recent study unveiled a molecular mechanism for high angiogenic capacity whereby M2-like macrophages release matrix metalloproteinase 9 propeptide (proMMP9) via downregulation of tissue inhibitor of metalloproteinases 1 (TIMP-1) to induce angiogenesis and vasculature repair in complex pathological microenvironments (Zajac et al., 2013). Importantly, overexpression of proangiogenic factors (VEGF and FGF) after experimental TBI is neuroprotective, and increases post-traumatic angiogenesis and neurogenesis resulting in improved functional recovery (Siddiq et al., 2012; Thau-Zuchman et al., 2010, 2012a, b).

The activation state of microglia has been shown to regulate brain endothelial cell proliferation, an early event in cerebral angiogenesis, by altering the balance of the M1-like cytokine TNF α and the M2-like cytokine TGF β (Welser et al., 2010). In addition, treatment of brain endothelial cells with conditioned medium collected from metformin-polarized M2 microglia, promotes angiogenesis *in vitro* (Jin et al., 2014). Furthermore, chronic metformin treatment of mice subjected to middle cerebral artery occlusion (MCAO) enhances M2-polarization of microglia/macrophages, increases angiogenesis and neurogenesis, and results in improved functional recovery after ischemic injury (Jin et al., 2014). Taken together, the *in vitro* and *in vivo* data support a potential role of M2-polarized microglia and macrophages in post-traumatic angiogenesis and vascular repair following TBI.

Oligodendrogenesis and remyelination

Recent studies indicate that M2-polarized microglia and macrophages are essential for efficient remyelination after CNS injury. In the experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis, there is a switch from an M1- to an M2-dominant phenotype during the remyelination phase of disease, whereas the relapsing phase is characterized by a dominant M1 response and suppression of immunomodulatory M2-polarized cells at the lesion site (Miron et al., 2013). Oligodendrocyte differentiation is also modulated by phenotype, because conditioned media from M2-polarized microglia promotes differentiation of precursors into mature oligodendrocytes *in vitro*, and oligodendrocyte differentiation is significantly impaired when M2-polarized cells are depleted in EAE mice (Miron et al., 2013). These data suggested that M2-polarized microglia/macrophages support CNS remyelination by driving oligodendrocyte differentiation. Additional studies support these findings and show that microglia/macrophage phenotypes influence oligodendrocyte survival and fate. Using an *in vitro* model of hypoxia and ischemia, Wang and colleagues demonstrated that conditioned media from M1-polarized microglia exacerbated oxygen glucose deprivation (OGD)-induced oligodendrocyte death (Wang et al., 2015; Wang et al., 2013b), whereas M2-conditioned media protected oligodendrocyte viability after OGD (Wang et al., 2015). Inflammation also impacts oligodendrogenesis in the adult CNS, such that M1-polarized microglia block oligodendrogenesis via a TNF α -dependent mechanism (Butovsky et al., 2006b), whereas M2-polarized microglia overcome this blockage, and promote oligodendrocyte formation via mechanisms that include the release of IGF-I by microglia (Butovsky et al., 2006a; Butovsky et al., 2006b). Therefore, the latest research suggests that microglia and macrophage polarization plays an important role in oligodendrocyte fate and remyelination after CNS injury.

Therapeutic strategies to alter M1-/M2-like balance and improve outcomes after TBI

As mentioned earlier the lesion microenvironment plays a critical role in determining the polarization state of microglia and macrophages after CNS injury, with extracellular signals from within the lesion biochemical milieu driving phenotypic shifts and functional cellular responses. For example, when conditioned medium from ischemic neurons is added to cultured microglia, the microglia adopt an M1-like phenotype with increased production of pro-inflammatory mediators (TNF α , nitric oxide) that inhibit microglial phagocytic activity (Hu et al., 2012), indicating that injured neurons release soluble factors that drive an M2 to M1 phenotypic shift. Several soluble factors found in the lesion microenvironment, such as TNF α , IFN γ and lipocalin-2, can drive this M2 to M1 transition (Jang et al., 2013; Kroner et al., 2014). T cell subsets can also infiltrate the lesion and release T-cell-derived factors that can drive microglial/macrophage phenotypic changes after CNS injury (Hooten et al., 2015; Walsh et al., 2014). Furthermore, a complex network of signaling pathways, transcription factors, epigenetic mechanisms, and post-transcriptional regulators control microglial/macrophage polarization. These include IRF/STAT/SOCS signaling, NF κ B activation, nuclear receptors (PPAR γ , PPAR δ , RXR), redox signaling (Nrf2, NOX2, HIF-1 α), histone demethylases (Jmdj3), and microRNAs (miR; miR-155/miR-124), among others (for review see: (Sica and Mantovani, 2012)). Therefore, both extracellular signals from the lesion

microenvironment and intracellular molecular mechanisms control phenotypic switching of microglia and macrophages. From a therapeutic perspective targeting factors within the lesion microenvironment and/or intracellular signaling pathways may dynamically alter phenotypic and functional responses of microglia/macrophages after TBI, and offer novel therapeutic approaches.

Cell-based therapies are being developed for TBI, and given that M2-like microglia and macrophages are anti-inflammatory and can promote regeneration and repair, transplant of M2-polarized cells to counteract the M2 to M1 transition after TBI might be an appealing strategy to reduced neurodegeneration and promote long-term functional recovery. This approach has had mixed results in CNS injury models. In one study adoptive transfer of M2 macrophages into adult rats after SCI resulted in a shift in inflammatory profile from M1-dominated to M2-like through the production of anti-inflammatory cytokines (IL-10, TGF β), which in turn created a local microenvironment that was conducive to the rescue of residual myelin and neurons, and preservation of locomotor function (Ma et al., 2015). However, despite showing promise in *in vitro* models, adoptive transfer of M2 macrophages into ischemic rat brain at 3 days post-MCAO failed to improve functional or histological outcomes at 2 weeks post-injury (Desestret et al., 2013). To date, adoptive transfer of M2 macrophages in TBI models has not been evaluated, however, when human bone marrow mesenchymal stem cells (MSC) are infused into TBI mice they promote robust M2-like polarization of microglia and alter the lesion microenvironment, resulting in M2-mediated repair responses and long-term neurological recovery (Zanier et al., 2014). These effects may be mediated in part by STAT3/NF κ B signaling because MSC induce M2 polarization of macrophage via activation of STAT3 and inhibition of NF κ B pathways (Gao et al., 2014). Despite encouraging findings in some experimental models it is unclear how plastic M2-polarized cells will respond when transplanted into a reactive injury environment, which may contain a vast array of extracellular signals that could reverse or reduce the M2-properties required for immunosuppression, resolution and repair. Further investigation is needed to determine the true potential of adoptive transfer strategies to promote M2-mediated neuroprotection and repair for TBI.

Several classes of drugs have been demonstrated to alter M1-/M2-like balance of microglia and macrophages, and may be useful as therapeutic agents for TBI. The nuclear hormone receptor peroxisome proliferator-activated receptors (PPAR) act as master regulators of the M2-like phenotype in macrophages (Chawla, 2010), and PPAR γ activation primes macrophages into an M2-like state with broad anti-inflammatory and tissue repair properties (Bouhlef et al., 2007). PPAR γ activation produces similar effects in microglia (Storer et al., 2005), and PPAR activation of microglia promotes increased phagocytic uptake of amyloid- β plaques and is neuroprotective in mouse models of AD (Mandrekar-Colucci et al., 2012; Yamanaka et al., 2012). Considering that TBI produces large amounts of myelin and cell debris, PPAR-induced M2-like polarization of microglia/macrophages may be beneficial by promoting removal of debris. PPAR agonists are neuroprotective in TBI models, and the PPAR α receptor agonist, fenofibrate, reduced post-traumatic neuroinflammation, oxidative stress and cerebral edema, and improved neurological function when administered after TBI (Besson et al., 2005; Chen et al., 2007). Similarly, the PPAR γ receptor agonists,

pioglitazone and rosiglitazone, reduced post-traumatic microglial activation and promoted anti-oxidant (Mn-SOD) and neuroprotective chaperone protein (HSP27/70) expression that resulted in reduced neurodegeneration and improved functional recovery after TBI (Sauerbeck et al., 2011; Thal et al., 2011; Yi et al., 2008). Rosiglitazone also induces IL-4 expression in the brain, and reduces age-dependent M1-like microglial activation in an IL-4 dependent manner (Loane et al., 2009a). As such, the anti-inflammatory and neuroprotective properties of PPAR γ agonists in TBI models may be mediated in part via M2-like polarization of microglia/macrophages.

Selective activation of metabotropic glutamate receptor 5 (mGluR5) on microglia is a novel mechanism to attenuate M1-like microglial activation and associated microglial-mediated neurotoxicity (Byrnes et al., 2009a; Loane et al., 2009b). Activation of mGluR5 has powerful neuroprotective effects in experimental models of CNS injury (Bao et al., 2001; Byrnes et al., 2012; Byrnes et al., 2009b; Chen et al., 2012; Loane et al., 2013; Wang et al., 2013c), and the mGluR5 orthosteric agonist, CHPG, attenuates chronic M1-like microglial activation following TBI via inhibition of microglial NOX2 (Byrnes et al., 2012; Loane et al., 2013; Loane et al., 2009b). Interestingly, NOX2 has been proposed to act as a molecular switch between M1-like and M2-like microglial activation states (Choi et al., 2012), as it serves to promote the former and suppress the latter. Recently it has been demonstrated that a positive allosteric modulator for mGluR5, VU0360172, repolarizes cultured microglia towards an M2-like phenotype following LPS or IFN γ stimulation (Loane et al., 2014b). Furthermore, in addition to attenuating chronic NOX2 expression in activated microglia, VU0360172 alters the M1-/M2-like balance following TBI. VU0360172 treatment results in decreased iNOS expression (M1-like marker) and increased expression of arginase 1 (M2-like marker) after TBI, and this predominant M2-like microglial activation profile is associated with reduced neurodegeneration and improved long-term motor function recovery in VU0360172-treated TBI mice (Loane et al., 2014b). VU0360172 is also neuroprotective in an experimental subarachnoid hemorrhage model in rats via attenuation of microglial activation and the upregulation of the Bcl-2 anti-apoptotic pathway (Zhang et al., 2015)

Macrophage phenotypic transitions can be regulated by epigenetic mechanisms (Ivashkiv, 2013). For example, IL-4 induces the upregulation of the histone demethylase Jmjd3 in macrophages, which alters chromatin modifications to promote M2 gene expression and inhibition of M1 genes (Ishii et al., 2009; Satoh et al., 2010). Similar mechanisms control microglia polarization; Jmjd3 enhances the polarization of M2-like microglia by modifying histone H3K27me3, and suppression of Jmjd3 in the MPTP model of Parkinson's disease results in microglial overactivation and exacerbated dopaminergic neuron cell death in the substantia nigra (Tang et al., 2014). Histone deacetylases (HDAC) remove acetyl groups from ϵ -N-acetyl lysine amino acids on a histone to allow DNA to be wrapped more tightly around histones. This blocks gene transcription and opposes histone acetyltransferase activity that promotes transcription. Several HDAC inhibitors provide neuroprotection against TBI (Dash et al., 2010; Lu et al., 2013; Shein et al., 2009; Wang et al., 2013a; Zhang et al., 2008). Scriptaid, a novel inhibitor of class I/II HDACs, offers dose-dependent neuroprotection that results in long-lasting functional recovery following focal TBI, even when administered up to 12 hours post-injury (Wang et al., 2013a). Moreover, it has been shown that Scriptaid prevents white matter injury by polarizing microglial/macrophage towards the M2-like

phenotype (Wang et al., 2015). Specifically, HDAC inhibition promotes an M1- to M2-like phenotypic shift by upregulating glycogen synthase kinase 3 beta (GSK3 β), which inactivates phosphatase and tensin homologue (PTEN) through phosphorylation to initiate PI3K/Akt signaling. (Wang et al., 2015). The GSK3 β -dependent M2-like phenotype exerts potent anti-inflammatory effects that protect myelin-forming oligodendrocytes resulting in diminished white matter injury. Thus, inhibition of HDACs in microglia alters M1-/M2-like balance via the GSK3 β /PTEN/Akt axis, and offer a novel therapeutic target to promote tissue repair and functional recovery after TBI.

Several other drugs have been used effectively to alter M1-/M2-like polarization of microglia and macrophages following acute brain injury. These include drugs that modulate endogenous endocannabinoid system (Tchantchou et al., 2014; Tchantchou and Zhang, 2013), CB2 inverse agonists (Reiner et al., 2015), α -7 nicotinic acetylcholine receptor agonists (Han et al., 2014a; Han et al., 2014b), endogenous antagonists of bone morphogenetic proteins (Shin et al., 2014), and CCR2 antagonists (Morganti et al., 2015), among others. Therefore, given the role for M2-like polarized microglia/macrophages in promoting phenotype-specific brain injury repair, drug treatment strategies that alter phenotypic and functional responses of microglia and/or macrophages after TBI offer considerable promise as new treatment options for TBI.

Considerations for future development of therapeutic strategies that alter microglial/macrophage phenotypes

Historically, broad-spectrum anti-inflammatory therapies for TBI have translated poorly to the clinic for human head injury (Kumar and Loane, 2012; Maas et al., 2010), which may in part reflect loss of beneficial M2-like effects in microglia. It is likely that efficient tissue repair after TBI requires both M1-like and M2-like functional responses, including coordinated transitions between phenotypes that fine-tune the sequential inflammatory, proliferative and remodeling phases of active repair (Gurtner et al., 2008; Novak and Koh, 2013). Therefore caution is advised against initiating a poorly timed M1- to M2-like phenotypic shift because the M1-like response plays a critical role in early repair processes, and additional beneficial roles for M1-like microglia following TBI may still be discovered. Now that microglia are known to play an active role in sculpting neuronal circuits and synapse remodeling (Salter and Beggs, 2014), it is not inconceivable that M1-like microglia may also drive the clearance of cellular debris from damaged synapses early after brain injury. Therefore, when developing therapeutic strategies to modulate M1-/M2-like phenotypes, timing the transitions between polarization states to facilitate efficient tissue repair needs to be carefully considered, as may tapering M2-like augmenting strategies to avoid chronic immunosuppressive effects and maladaptive repair processes. A long-term repair phase after a rapid pro-inflammatory response that is driven by M2-like macrophages has been demonstrated to result in fibrosis and other aberrant repair (Hesse et al., 2001), because elevated arginase activity shifts the metabolism of L-arginine significantly to produce increased ornithine and proline that simulate cell division leading to hyperplasia and fibrosis. In addition, a long-term increase in arginase activity leads to an uncoupled decrease in NO production, which has been shown to precipitate endothelial dysfunction

(Horowitz et al., 2007). Thus, it is critical to boost, and diminish, the correct microglial and/or macrophage phenotypes at the right time in order to drive endogenous repair pathways following acute brain injury and to avoid maladaptive responses. Finally, a note of caution is needed with regard to the translation of M1-/M2-like polarization states developed in rodents to humans because several commonly used markers of the M2-like phenotype, such as arginase 1 and Ym1, are not expressed in human myeloid cells (Raes et al., 2005). However, others such as CD206 and CD163, appear to be consistent (Vogel et al., 2013). Therefore, if markers for M2-like polarization are carefully selected then it may be possible to identify M2-like microglia and macrophages in human brain injury and translate promising therapeutic strategies to the clinic.

Conclusion

The latest research implicates M1/M2-like polarization of microglia and/or macrophages in acute brain injury, and activated microglia/macrophages with distinct molecular phenotypes can either promote neurorestorative processes, or hinder CNS repair and expand tissue damage leading to chronic neurodegeneration after TBI. In the context of CNS remodeling, the M2-like phenotype can support enhanced functional recovery after TBI via the induction of key neurorestorative processes, such as neurogenesis, angiogenesis, oligodendrogenesis and remyelination. Future therapeutic approaches that target post-traumatic neuroinflammation should avoid broad suppression of microglial activation, and instead should alter the balance between different phenotypes to promote maximal CNS remodeling and repair. This concept is supported by recent experimental studies that modulated M1/M2-like polarization to protect against post-traumatic neurodegeneration and enhance CNS repair. Therefore, future research is needed to determine key regulatory mechanisms that control phenotype switching in microglia in order to boost their “good” and suppress their “bad” activation states, and promote maximal functional recovery following TBI.

Supplementary Material

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Highlights

- Microglia play a critical role in neuroinflammation following TBI
- Microglial activation has beneficial and detrimental effects on neurons after TBI
- Microglial phenotypic and functional responses may account for these dual effects
- Treatments that alter M1/M2-like balance may enhance CNS repair after TBI

Figure 1. Microglial phenotypic and functional responses after TBI

In response to DAMPs and other extracellular signals released by injured neurons microglia can become polarized towards M1-like and M2-like activation states that can have distinct roles in neurodegeneration and tissue repair. M1-like microglia are characterized by upregulated expression of phenotypic protein markers such as IL-1β, TNFα, IL-6, iNOS, and IL-12p40. They release pro-inflammatory cytokines, chemokines and free radicals that impair brain repair and contribute to chronic neuroinflammation, oxidative stress and long-term neurological impairments. In contrast, M2-like microglia upregulate protein markers such as CD206, CD163, FCγR, arginase 1, Ym1, and TGFβ. M2-like microglia release anti-inflammatory cytokines, neurotrophic factors and proteases, and they have increased phagocytic activity. M2-like microglia promote immunosuppression and resolution of M1-mediated neuroinflammation, and participate in CNS remodeling and repair by modulating neurorestorative processes such as neurogenesis, angiogenesis, oligodendrogenesis and remyelination. Abbreviations: DAMPs, danger-associated molecular patterns; PRR, pathogen recognition receptors; TLR, toll-like receptors.