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REVIEW

The contribution of astrocytes and microglia to traumatic brain injury

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Traumatic brain injury (TBI) represents a major cause of death and disability in developed countries. Brain injuries are highly heterogeneous and can also trigger other neurological complications, including epilepsy, depression and dementia. The initial injury often leads to the development of secondary sequelae; cellular hyperexcitability, vasogenic and cytotoxic oedema, hypoxia-ischaemia, oxidative stress and inflammation, all of which influence expansion of the primary lesion. It is widely known that inflammatory events in the brain following TBI contribute to the widespread cell death and chronic tissue degeneration. Neuroinflammation is a multifaceted response involving a number of cell types, both within the CNS and in the peripheral circulation. Astrocytes and microglia, cells of the CNS, are considered key players in initiating an inflammatory response after injury. These cells are capable of secreting various cytokines, chemokines and growth factors, and following injury to the CNS, undergo changes in morphology. Ultimately, these changes can influence the local microenvironment and thus determine the extent of damage and subsequent repair. This review will focus on the roles of microglia and astrocytes following TBI, highlighting some of the key processes, pathways and mediators involved in this response. Additionally, both the beneficial and the detrimental aspects of these cellular responses will be examined using evidence from animal models and human post-mortem TBI studies.

LINKED ARTICLES

This article is part of a themed section on Inflammation: maladies, models, mechanisms and molecules. To view the other articles in this section visit http://dx.doi.org/10.1111/bph.2016.173.issue-4

Abbreviations

BBB, blood–brain barrier; CCI, controlled cortical impact; DAMP, danger-associated molecular pattern; GFAP, glial fibrillary acidic protein; Iba-1, ionized calcium binding adapter molecule 1; MHC, major histocompatibility complex; PRR, pathogen recognition receptor; TBI, traumatic brain injury; TLR, Toll-like receptor

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TARGETS
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TNF-α

These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Pawson *et al.*, 2014) and are permanently archived in the Concise Guide to PHARMACOLOGY 2013/14 ([a,b,c,d]Alexander *et al.*, 2013a,b,c,d).

Introduction

The pathology of traumatic brain injury (TBI) is complex and multifactorial, with TBIs commonly categorized into primary and secondary injuries. Primary injury results from mechanical disruption of brain tissue, often resulting in axonal shearing and can lead to the formation of contusions and haemorrhage (Werner and Engelhard, 2007). As a result of these events, a secondary cascade of molecular and biochemical changes is initiated within minutes of the initial impact, termed secondary injury. Secondary injury processes, such as excitotoxicity, ischaemia, apoptosis, necrosis and inflammation, are critical in determining the extent of injury expansion and damage to brain tissue following the primary insult (Greve and Zink, 2009). Much of the research and studies performed in the field of TBI are focused on targeting various aspects of the secondary injury cascade in order to control cell death and tissue degeneration post-injury; however, most have demonstrated limited translational success (Menon, 2009).

Neuroinflammation is as a major pathological process in the secondary response after TBI (Cederberg and Siesjo, 2010). This inflammatory response is designated the term 'sterile inflammation' or inflammation in the absence of a pathogenic stimulus and involves multiple cell types within the CNS (Rock *et al.*, 2010). Furthermore, brain injury compromises the integrity of the blood–brain barrier (BBB), a physical barrier separating the brain parenchyma from the body's circulation (Rodriguez-Baeza *et al.*, 2003; Shlosberg *et al.*, 2010). Injury-induced BBB damage allows infiltration of blood-borne cells, adding a layer of complexity to the neuroinflammatory response (Beschorner *et al.*, 2002; Jin *et al.*, 2012). Moreover, increased permeability of the BBB after injury allows passage of both small and large molecules into the brain (Habgood *et al.*, 2007).

In addition to the peripheral response, dying or damaged cells within the lesional and peri-lesional areas in the brain release cellular debris into the microenvironment, priming local microglia and astrocytes. These resident cells express a variety of pattern recognition receptors (PRRs) at their cell surface and intracellularly, allowing them to mount an immune response (Gorina *et al.*, 2011; Holm *et al.*, 2012; de Rivero Vaccari *et al.*, 2012; Fellner *et al.*, 2013). Toll-like receptors (TLRs) are a class of membrane-bound PRRs, which are activated by a variety of endogenous pathogen-associ ated molecular patterns or danger-associated molecular patterns (DAMPs), including dsDNA and RNA, CpG motifs and chaperone proteins. These molecules are released by pathogens, or in the case of brain injury, dying cells (Anderson, 2000). Signalling through TLRs can also induce the production of inflammatory cytokines and chemokines, allowing them to signal to cells within damaged tissue, and potentially exacerbate the neuroinflammatory cascade (Lafon *et al.*, 2006; Goodall *et al.*, 2014; Li *et al.*, 2014). Importantly, neurons also express TLRs, allowing them to respond to, and mount, a TLR-driven inflammatory response (Lafon *et al.*, 2006).

The brain's innate response to injury is crucial; resident astrocytes and microglia are often the primary cell types to initiate an inflammatory cascade upon sensing danger, and proteins associated with the activation of these cells are often used as biomarkers in TBI (Hernandez-Ontiveros *et al.*, 2013; Diaz-Arrastia *et al.*, 2014). Their responses include, but are not limited to, secretion of pro- and anti-inflammatory cytokines, chemokines and growth factors, barrier formation around lesional areas, phagocytosis of dying cells and cellular debris, and modulation of cellular responses.

Reactive astrocytes, for example, can influence the responses of other cell types after TBI, both within the brain and in the periphery. Ablation of reactive astrocytes has resulted in an increase in leukocyte infiltration in a stab

wound injury model, leading to neurodegeneration (Bush *et al.*, 1999). Contrasting evidence suggest that the glial cell-mediated invasion of peripheral cells can also be considered a mechanism of protection in a hippocampal entorhinodentate lesion model (Babcock *et al.*, 2003).

Pleiotropic responses of glial cells have been shown to facilitate both inflammation resolution and exacerbation (Johnson *et al.*, 2013; Roth *et al.*, 2014). Because of this, a better understanding of the nature of the inflammatory response generated by astrocytes and microglia will aid in developing therapies to combat cell death and degeneration, and protect viable brain tissue after TBI. The inflammatory responses of both astrocytes and microglia will be discussed in this review, with an examination of the dual nature of these responses, and how these cells modulate the surrounding environment after brain injury.

Astrocytes

Astrocytes form part of the macroglia, cell types comprising oligodendrocytes, radial glia and ependymal cells (Kimelberg and Nedergaard, 2010). They are critical in maintaining physiological homeostasis within the CNS, with important roles in supporting neuronal function, glial transmission and signalling via Ca^{2+} release and uptake (Chen and Swanson, 2003). Studies have reported that human cortical astrocytes displayed a greater degree of heterogeneity and complexity than their rodent counterparts (Oberheim *et al.*, 2009; Sosunov *et al.*, 2014). Furthermore, astrocytes isolated from human temporal neocortex were larger than rodent astrocytes and capable of transmitting Ca^{2+} waves much faster than rodent astrocytes (Oberheim *et al.*, 2009). These studies indicate that human astrocytes appear to be much more complex than those in the rodent brain. However, the study of astrocytes after injuries such as TBI is most commonly performed in rodents due to the ease of manipulating pathways and mediators in these models.

Astrocytes also play a role in maintaining BBB integrity, by forming astrocytic end feet around endothelial cells (Risau and Wolburg, 1990; Abbott *et al.*, 2006). Astrocytic interactions with endothelial cells are a critical component of the induction and maintenance of the BBB (see Abbott *et al.*, 2006) and involve processes such as inter- and intra-cellular communication. Disruption of the BBB is associated with high levels of secreted factors from damaged tissue, such as S100B protein (used as a marker of BBB leakage) and MMP (Vajtr *et al.*, 2009). Additionally, other mediators can influence the integrity of the BBB. For example, bradykinin-induced astrocytic secretion of IL-6 leads to opening of the BBB (Schwaninger *et al.*, 1999). It is suggested that BBB breakdown follows a biphasic pattern after injury, with an initial increase in permeability hours after TBI, and declining thereafter, and a secondary delayed phase 3–7 days following injury, as found in controlled cortical impact (CCI) and closed head injury models (Shapira *et al.*, 1993; Baskaya *et al.*, 1997). Damage to the BBB after injury can also cause infiltration of peripheral immune cells (Jin *et al.*, 2012). These cells are thought to play roles in repair but can also exacerbate neuroinflammation in the secondary phase of injury. The role of peripheral cells in injury is extensive and is outside the scope of this review. Therefore, this review will focus specifically on astrocytes and microglia in TBI.

Astrocytes and brain injury

An increase in astrocyte reactivity in response to injury is termed astrogliosis (Sofroniew and Vinters, 2010). This response involves changes in morphology, increased expression of the intermediate filament proteins, glial fibrillary acidic protein (GFAP) and vimentin, heightened proliferation and secretion of inflammatory mediators and growth factors (Pekny *et al.*, 1999; Gorina *et al.*, 2011; Zamanian *et al.*, 2012; Paintlia *et al.*, 2013). Many of these factors act in an autocrine and paracrine fashion to facilitate astrocytic reactivity of the cells surrounding them. Interestingly, it has been demonstrated that FGF serves as an inhibitory molecule in rendering astrocytes reactive, both in resting states and after stab wound injury (Kang *et al.*, 2014). This body of work indicates the delicate balance between reactivity and suppression of function in astrocytes after injury.

Reactive astrocytes can acquire a hypertrophic morphology after injury, involving extension of processes and swelling of cell bodies. A recent study conducted in a mouse CCI model reported hypertrophic astrocytes in the lesional and peri-lesional area 3 days after TBI (Villapol *et al.*, 2014). Further changes in morphology were evident 7 days after injury, with glial scar formation. Reactive gliosis was maintained up to 60 days after injury in this model, demonstrating an ongoing response of astrocytes to brain injury.

Excitotoxicity is another common mechanism of secondary brain injury after TBI (Palmer *et al.*, 1993). It is appreciated that while neurons are highly vulnerable to excitotoxicity, astrocytes have key roles in the re-uptake of glutamate from synapses, preventing excessive extracellular glutamate accumulation (Chen and Swanson, 2003). Glutamate transporters, such as EAAT1 and EAAT2, are essential for glutamate re-uptake. It has been demonstrated that blocking these transporters on astrocytes by administering antisense oligonucleotides in rats resulted in increased extracellular glutamate concentration, leading to excitotoxicity and neurodegeneration (Rothstein *et al.*, 1996). Human TBI studies have observed decreases in glial expression of EAAT1 and EAAT2 after TBI in lesional and peri-lesional areas, suggesting down-regulation of these transporters after TBI (van Landeghem *et al.*, 2006; Beschorner *et al.*, 2007). This suggests that astrocytic down-regulation in humans after TBI promotes accumulation of glutamate in extracellular areas and may contribute to excessive excitotoxicity leading to neurodegeneration.

Glial scar formation is commonly seen post-injury and consists largely of astrocytes, along with microglia, endothelial cells and fibroblasts and extracellular matrix (Silver and Miller, 2004). Several mediators have been implicated in inducing glial scar formation, including TGF-β1 and TGF-2, IFN-γ, FGF and fibrinogen (DiProspero *et al.*, 1997; Moon and Fawcett, 2001; Schachtrup *et al.*, 2010). Knock-out mice studies have also demonstrated a role for GFAP and vimentin in proper glial scar formation (Pekny *et al.*, 1999; Wilhelmsson *et al.*, 2004). Scarring is thought to act as a physical barrier to encapsulate damaged tissue in order to prevent toxic molecules and DAMPs from leaking out into healthy tissue and to prevent access to invading cell types

after injury. It has however also been shown to have an inhibitory effect on axonal regrowth and regeneration (Ribotta *et al.*, 2004; Silver and Miller, 2004).

Another prominent aspect of astrogliosis is the ability of astrocytes to migrate or proliferate towards lesional or damaged tissue. The proliferative capacity of astrocytes as part of the injury-induced response has been extensively studied in a stab wound model of injury, where the lesion is induced in the somatosensory cortex (Bardehle *et al.*, 2013). Using two-photon laser scanning microscopy, GFP-labelled astrocytes were found to up-regulate GFAP expression and display elongated processes and enlarged cell bodies, indicative of hypertrophy at 7 days post-injury. Interestingly, proliferation rather than migration of astrocytes was detected at 5–7 days post-injury. In a mouse CCI model, GFAP-positive astrocytes were found to proliferate at 1, 3 and 7 days post-injury, with numbers of proliferating astrocytes peaking 3 days post-injury (Susarla *et al.*, 2014). These astrocytes were located in close proximity to the lesion and were hypertrophic with extended processes. These studies demonstrate a predominant proliferative response in astrocytes, which peaks in the acute phase after experimental TBI. Collectively, these studies point to a critical role of astrocytes in modulating barrier formation, secretion of inflammatory factors and glial scar formation after injury. Additionally, astrocytes can elicit both protective and deleterious actions, which can influence the extent of brain damage or repair after injury.

Damage versus repair: dual roles of astrocytes following brain injury

Astrogliosis has been defined in the context of both neuroprotection and neurodegeneration (Myer *et al.*, 2006; Zamanian *et al.*, 2012). Reactive astrocytes are capable of producing pro-inflammatory cytokines, chemokines and MMP that degrade the extracellular matrix and cause further BBB disruption (Carpentier *et al.*, 2005; Kim *et al.*, 2005). However, astrocytes are also capable of producing factors to support repair and regeneration after CNS damage (Kim *et al.*, 2010; Madathil *et al.*, 2013).

Specific roles of reactive astrocytes have been studied in models of moderate and severe CCI TBI (Myer *et al.*, 2006). Selective ablation of proliferating reactive astrocytes allows researchers to elucidate their roles (Bush *et al.*, 1999; Myer *et al.*, 2006). In mice, the removal of proliferating reactive astrocytes resulted in neuronal degeneration and inflammation, and thus, has confirmed their essential role in preserving neuronal tissue after moderate, but not severe TBI (Myer *et al.*, 2006). Furthermore, reactive astrocytes were shown to have a critical role in preventing the infiltration of inflammatory cells in regions containing intact cortical neurons. However, in direct contrast, blocking astrocytic proliferation using agents that disrupt various stages of the cell cycle leads to reduced neuronal cell death after fluid percussion injury in rats (Di Giovanni *et al.*, 2005). Reduced astrocytic proliferation was also accompanied by reduced glial scar formation, microglial activation and improved histological and cognitive outcome after TBI, suggesting that the presence of reactive astrocytes can create an environment permissive to degeneration.

Factors secreted from reactive astrocytes can influence their actions and thus could explain the discrepancy between studies observing favourable roles versus those finding damaging roles for reactive astrocytes. For instance, astrocyte-specific overexpression of the growth factor IGF-1 resulted in increased gliosis accompanied by reduced hippocampal neurodegeneration after CCI injury (Madathil *et al.*, 2013). Interestingly, astrocyte-specific overexpression of the pro-inflammatory cytokine IL-6 also resulted in increased wound healing concurrently with increased reactive gliosis (Penkowa *et al.*, 2003; Quintana *et al.*, 2008). In contrast, astrocyte-specific deletion of a Ca^{2+}-dependent N cadherin demonstrated a crucial role for this protein in mediating astrogliosis and consequent neurodegeneration (Kanemaru *et al.*, 2013). These studies highlight the diversity of reactive gliosis and how the loss or gain of a single factor in reactive astrocytes can influence inflammatory responses and neuronal outcome.

Additionally, responses of astrocytes can be dependent upon their ability to assume different morphologies and phenotypes. Astrocytic heterogeneity was observed in a study examining the role of astrocytes after LPS injury and mid-cerebral artery occlusion (Zamanian *et al.*, 2012). LPS-injured astrocytes assumed deleterious phenotypes, while astrocytes in the brains of mice subjected to mid-cerebral artery occlusion assume reparative phenotypes. It is possible that after TBI, heterogeneous groups of astrocytes also emerge. Accumulating evidence from morphological and genetic studies reveals that astrocytes can acquire different morphologies and up-regulate activation markers in varying levels after brain injury (Hill *et al.*, 1996; Bardehle *et al.*, 2013; Martin-Lopez *et al.*, 2013). Astrocytic responses are influenced by their location with respect to the injury, the signals they receive from their environment and also factors during their development (Martin-Lopez *et al.*, 2013).

Astrocytes have also been found to affect microglial responses. In a stab wound TBI model, impaired astrocyte recruitment after depletion of the RhoGTPase Cdc42 resulted in increased microglial activation (Robel *et al.*, 2011). Additionally, increased astrocyte activation in an *in vitro* oxygen-glucose deprivation model influenced the production of anti-inflammatory mediators and suppressed microglial activation (Kim *et al.*, 2010). These responses imply that microglia can heighten their activity as a compensatory mechanism for depleted astrocytic numbers, and conversely, excessive activation of astrocytes can dampen microglial responses. It is evident from these studies that astrocytes can affect the local environment after TBI, either in concert with, or by affecting neighbouring cells. Indeed, like astrocytes, microglial cells play instrumental roles in shaping the microenvironment after TBI.

Microglia

Microglia are specialized immune cells of the brain with phagocytic and antigen-presenting capabilities (Hickey and Kimura, 1988). Originating from mesodermal cells of the yolk sac, microglia are derived from erythromyeloid precursor cells, and recently, their development has been shown to be dependent upon the transcription factors Pu.1 and interferon regulatory factor-8 (Ginhoux *et al.*, 2010; Kierdorf *et al.*, 2013). There is a large degree of heterogeneity in microglial

structure and shape, depending upon their activation state. Broadly, microglia are described as 'ramified' when in a resting or quiescent state (Glenn *et al.*, 1992). Upon activation, microglia can transform to a hypertrophic or bushy morphology (Tambuyzer *et al.*, 2009). Additionally, microglia are described as acquiring an amoeboid morphology during early stages of development or when actively phagocytosing cellular debris. In the healthy brain, microglia are often described as 'resting', although they are constantly surveying their environment in preparation for insult or injury (Nimmerjahn *et al.*, 2005). Additionally, microglia are also capable of pruning synapses on neighbouring neurons during development by direct engulfment, thus serving a homeostatic function (Paolicelli *et al.*, 2011).

Microglia express a variety of molecules at their cell surface, thus allowing their identification, and in certain cases, distinction from peripheral macrophages. Microglia are Mac 1/CD11b^{+} CD45^{low}-expressing cells, whereas macrophages are Mac-1/CD11b^{+} CD45^{high}-expressing cells (D'Mello *et al.*, 2009). The recent development of CCR2-RFP knock-in mice crossed with CX_3CR1-GFP mice has allowed the differentiation of monocytes from microglia (Saederup *et al.*, 2010). Additionally, microglia express high levels of the chemokine receptor, CX_3CR1, and the intracellular Ca^{2+}-binding protein, ionized calcium binding adapter molecule 1 (Iba-1) (Fukuda *et al.*, 1996; Nishiyori *et al.*, 1998). Microglia in activated states up-regulate the expression of Iba-1, which can occur in cases of infection or injury to the CNS. Additionally, CD68 (ED1) is also used as a marker to confirm microglial activation (Graeber *et al.*, 1990), and CD68 immunoreactivity is observed after both mouse and human TBI (Frugier *et al.*, 2010; Loane *et al.*, 2014). Following CNS trauma, microglia undergo morphological changes and can secrete a variety of factors, which either exacerbate or limit tissue damage.

Microglia in TBI

Microglia are instrumental in mounting an immune response after TBI. A study investigating pathological changes in injured post-mortem human brains showed a prolonged and persistent activation of microglia, which was present even years after injury (Johnson *et al.*, 2013). With chronic trauma, patients displayed increased levels of the major histocompatibility complex (MHC) Class II molecule CR3/43 and CD68 on activated microglia compared with acutely injured and non-injured controls. In addition, increased white matter degeneration and disruption of myelinated fibres were associated with the chronic cohort of patients, clearly demonstrating an association between chronic microglial activation after injury and neurodegeneration.

Similarly, in a mouse CCI model, neuropathological and inflammatory changes were monitored up to 1 year after experimental TBI (Loane *et al.*, 2014). Microglia were chronically activated, with increased levels of Iba-1, CD68 and the MHC Class II molecule CR3/43 compared with controls. Morphology was assessed as being hypertrophic or bushy. This was accompanied with lesion expansion, loss of hippocampal neurons, white matter damage and loss of myelin. This study is corroborated by earlier work that reported the presence of mononuclear phagocytes in injured rat brains 3 months after weight-drop injury, along with an up-regulation in MHC Class II and the release of IL-1β and TNF-α (Holmin and Mathiesen, 1999). These smaller scale studies in rodents confirm the results found in the longitudinal study in humans, demonstrating the presence of chronically activated microglia and tissue degeneration after injury.

Evidence from alternative TBI models suggests that microglia have various roles after injury, adding to the complexity of microglial responses in the context of brain injury. In a novel closed head injury model, it was revealed that skull thinning and compression in mice resulted in cell death of the meningeal layers (Roth *et al.*, 2014). This was associated with an extension of microglial processes surrounding the lesion and microglia surrounding dying astrocytes in the glial limitans. The microglial response was found to be dependent upon purinergic signalling, and blocking this signalling resulted in increased permeability of the glial limitans and subsequent cell death around the meninges. Similarly, in a laser-induced focal injury model, the microglial response was determined to be dependent upon ATP release and activation of P2Y receptors (Davalos *et al.*, 2005). In response to injury, it was established that ATP mediated extension of microglial processes to surround the injury site, allowing a barrier to be formed between damaged and intact tissues. Together, these significant studies emphasize that the microglial response in injury can be dependent upon context and injury severity (highlighted through the use of different injury models), and therefore, a better understanding of the pleiotropic nature of these responses is needed to advance our understanding of microglial contribution in injury.

The dual nature of the microglial response; M1 and M2 phenotypes

The microglial response in TBI is dualistic; highly dependent upon timing and the nature of the injury itself. Macrophages/microglia can acquire heterogeneous phenotypes following CNS insult in response to varying environmental cues. These cells are ascribed the nomenclature of 'M1' or 'M2', with varying classes of M2 cells (Mantovani and Locati, 2009; Sica and Mantovani, 2012). Additionally, the terms 'classical activation' for M1, 'alternative activation (M2a)' and 'acquired deactivation (M2c)' are commonly used to group microglia (Cao *et al.*, 2012; Kumar *et al.*, 2013). Cells polarize from a 'resting' or 'M0' phenotype into an M1 phenotype after LPS or IFN-γ exposure and are considered neurotoxic following CNS injury (Kigerl *et al.*, 2009). It has been suggested that M1 microglia exhibit reduced phagocytosing capability compared with M0 microglia in response to oxygen-glucose deprivation *in vitro*, and M2 exhibit increased activity (Hu *et al.*, 2012; Wang *et al.*, 2013). In contrast, an M2 phenotype is acquired following exposure to IL-4 or IL-13, and these cells are considered neuroprotective (Kigerl *et al.*, 2009). M1 microglia are known to produce mediators such as pro-inflammatory cytokines and inducible NOS to elicit their deleterious effects, and M2 microglia produce scavenger receptors, growth factors such as TGF-β and the anti-inflammatory cytokine IL-10 (Figure 1). As such, these mediators or cell surface receptors for corresponding M1 and M2 cells are used as markers to broadly differentiate the two cell types in injury states. Table 1 lists the different markers used to study M1/M2 polarization following TBI.

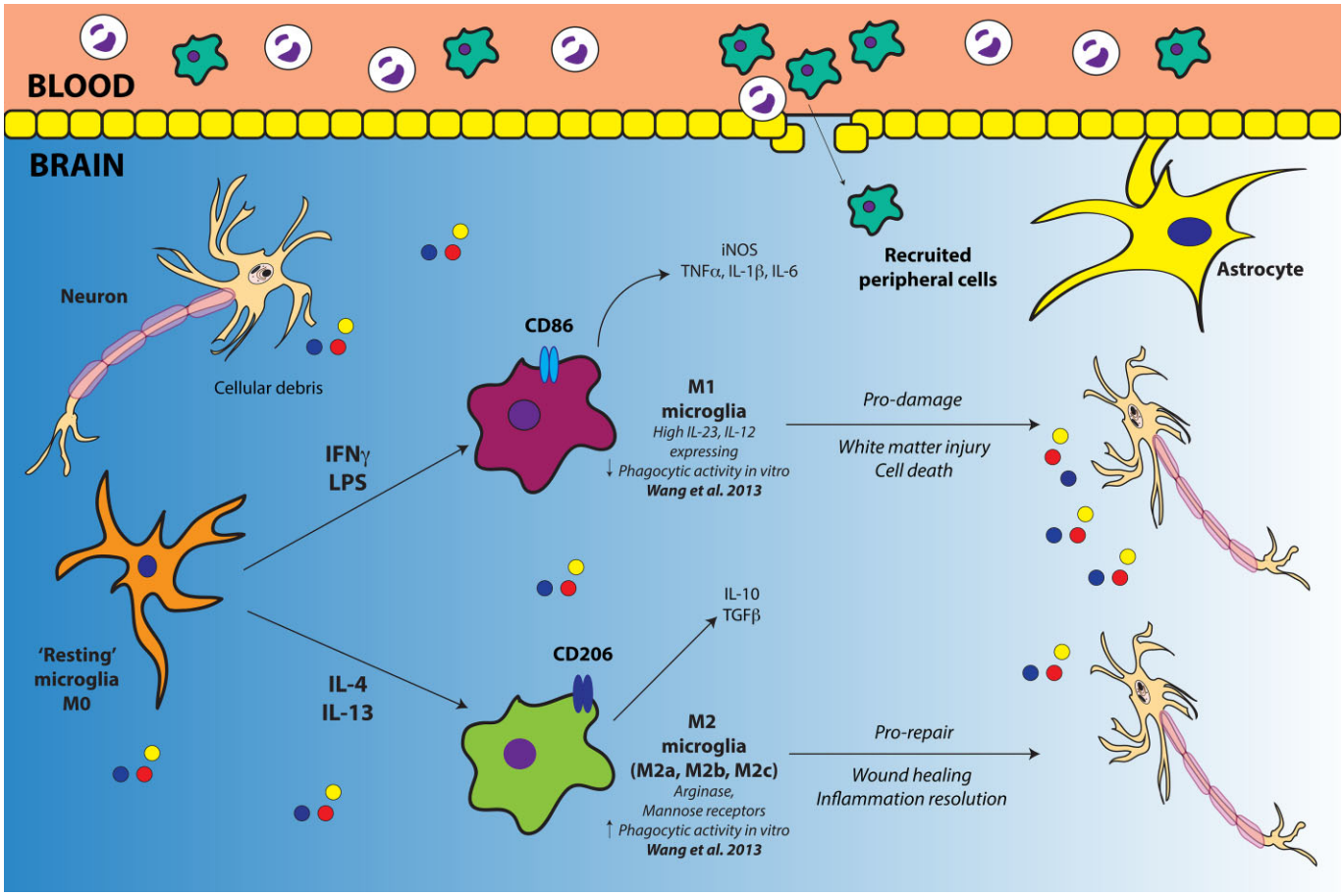

Figure 1

Diagrammatic representation of M1/M2 polarization in microglia. For simplicity, M2 microglia have been represented as just the one phenotype, as opposed to illustrating the various classes. Cellular debris and other mediators released by dying neurons after injury prime microglia. An environment rich with the classical pro-inflammatory stimuli, such as IFN-γ and LPS, promotes the polarization of resting microglia into an M1 phenotype. M1 microglia release pro-inflammatory cytokines, chemokines and iNOS. An M1 environment is neurotoxic, facilitating white matter injury and cell death. In contrast, a neuroinflammatory environment rich in anti-inflammatory IL-4 or IL-13 drives the development of an M2 phenotype. M2 microglia release IL-10 and TGF-β, while promoting repair and inflammation resolution. In addition to microglia, peripherally infiltrating macrophages can also undergo polarization into M1 and M2 phenotypes, and recently, this has shown to be true of neutrophils in a murine ischaemic stroke model (Cuartero *et al.*, 2013).

There is still some debate over the use of specific markers to distinguish M1 and M2 cells. Furthermore, the grouping of cells into 'M1' and 'M2' categories is not a unanimous view; there are reports suggesting that macrophage/microglial polarization fits onto a spectrum rather than into two distinct groups (reviewed in Mosser and Edwards, 2008). These conflicting reports may be a reflection of the studies, with changes observed in whole tissue specimens rather than in specific cell types (Kumar *et al.*, 2013; Wang *et al.*, 2013). Many studies therefore describe microglial polarization as being skewed towards either an 'M1-like' or 'M2-like' state. Additionally, it is possible that that M1/M2 polarization may be reversible, as previously polarized cells may be able to transform to different phenotypes depending upon the presence of additional environmental stimuli (Butovsky *et al.*, 2005; Schwartz *et al.*, 2006). Furthermore, cells may be undergoing phenotypic switches when isolated, influencing levels of receptors/marker proteins. A recent review has addressed complexities regarding macrophage nomenclature (Murray *et al.*, 2014). It is suggested that researchers use more standardized methods of isolation and use a wider range of markers to designate categories for polarized macrophages. Recently, using a direct RNA sequencing technique, a unique set of genes, termed the 'microglial sensome', was identified in microglia isolated from aged mice (Hickman *et al.*, 2013). Furthermore, microglial-specific genes that were up-regulated during classical and alternative microglial priming were identified in both young and aged mice, allowing a greater understanding of microglial polarization states. Evidently, similar such studies in microglia after TBI are warranted and will increase our knowledge of microglial-specific polarization following injury. As such, the M1/M2 paradigm is no doubt an evolving one; and as new evidence comes to light, these categories and definitions will be subject to re-evaluation.

Studies performed in CCI models indicate that the onset of activation varies significantly between M1 and M2 micro-

Table 1

Markers to classify M1 and M2 microglia commonly used in studies of TBI

Study	Microglial polarization		Other microglial activation markers used
	M1	M2	
Jin *et al.* (2012)	CD86	CD206	Iba-1, $CD11b^{+}$ $CD45^{low}$-expressing cells characterized as microglia
Wang *et al.* (2013)	CD32, CD16, iNOS, CD11b, CD86	CD206, IL-10, Ym1/2, TGF-β, Arg-1, CCL22	Iba-1
Kumar *et al.* (2013)	IL-1β, TNF-α, CD86, iNOS, CCL2, CCL3 (classical activation)	Arg-1, Ym1, Mrc, Fizz-1 (alternative activation; M2a) and IL-4rα, SOCS3, TGF-β (acquired deactivation; M2c)	Iba-1, CD11b, ED1, MHC II
Cao *et al.* (2012)	TNF-α, CD45 (classical activation)	Arg-1 (alternative activation), TGF-βI, TGF-βRII (acquired deactivation)	Iba-1, MHC I, MHC II, TSPO
Bachstetter *et al.* (2013)	IL-1β, IL-6, TNF-α, CCL2, CCL3 (classical activation)	Arg-1, Ym1 (alternative activation)	Iba-1, CD68, CD45, MHC II
Dohi *et al.* (2010)	iNOS, NO, TNF-α (classical activation)	Arg-1, Ym1 (alternative activation)	CD11B

Some studies use the nomenclature M2a and M2c ('alternative activation' and 'acquired deactivation') to describe M2 microglia and 'classical activation' for M1 microglia.
Arg-1, arginase-1; iNOS, inducible NOS.

glia (Jin *et al.*, 2012; Wang *et al.*, 2013). By qPCR and immunohistochemistry, Wang *et al.* demonstrated an immediately increased and transient M2-like environment after TBI, in contrast to the delayed but slightly more prolonged M1-like environment (Wang *et al.*, 2013). However, in the absence of cell-specific markers, it was suggested that this environment could be attributed to both microglial and macrophage populations. However, in distinguishing resident microglia from peripheral macrophages by assessing CD45/CD11B and Iba-1 expression, Jin *et al.* confirmed a similar pattern of M1/M2 activation with an initial and transient M2 peak, followed by a predominant M1 response at 21 days post-injury (Jin *et al.*, 2012). Specifically, microglial numbers followed a multiphasic pattern, with increased levels 7 days post-injury, a decrease in microglial numbers 14 days post-injury and, finally, a gradual increase after 21 days. The exact period of activation differed slightly between these studies, perhaps reflecting the different methods of analysis of M1/M2 populations.

Evidence from stroke models also suggests that there is a propensity for an M2-favoured environment early after injury, followed by a delayed onset of M1 microglia/macrophages *in vivo* (Hu *et al.*, 2012). These results are also observed with astrocyte populations after ischaemic stroke, suggesting the presence of beneficial and detrimental subsets of astrocyte, with a protective astrocytic phenotype observed in a murine stroke model (Zamanian *et al.*, 2012). Collectively, this body of evidence suggests the need to further characterize the polarization properties of microglia specifically rather than in whole brain specimens. The studies reviewed here provide evidence for M1/M2 environments in the brain following injury, and when combined with information about the specific phenotypic changes of microglia exclusively, a greater understanding of M1/M2 dynamics following brain injury will be achieved. Additionally, studies that selectively investigate microglial properties following injury will aid our understanding of the exact nature and timing of both beneficial and detrimental microglial responses following injury.

Discussion

It is clear that the diverse roles of astrocytes and microglia in TBI arise as a result of divergent stimuli they receive from the surrounding cells and the local microenvironment. Substrates produced in injury, such as inflammatory mediators, proteases, complement factors and DAMPs, trigger complex cascades, promoting a variety of cellular responses. Importantly, responses of astrocytes and microglia in experimental TBI studies can be largely dependent upon the nature and severity of injury; focal models, such as stab wound models, can induce different responses in these cells compared with diffuse models, such as weight-drop injury. This is highlighted nicely in the suite of microglial studies, which demonstrated both reparative and deleterious roles for these cells in various models of experimental TBI (Davalos *et al.*, 2005; Loane *et al.*, 2014; Roth *et al.*, 2014). Studies investigating responses of reactive glial cells must therefore take this into account and ideally incorporate results from more than just one TBI model. Additionally, the outcomes of glial responses depend upon both environmental cues and other cell types, emphasizing the complex nature of reactive gliosis in injury.

Studies of reactive astro- and microgliosis have identified somewhat opposing roles in injury: those promoting neurotoxicity and degeneration and those promoting repair and regeneration. Microglia are not exclusive in their ability to polarize into different phenotypes; recently, it has been shown that astrocytes can assume both pro-repair and pro-damage phenotypes depending upon their stimulus, and accordingly, these astrocytes up-regulate phenotype-specific

markers (Zamanian *et al*., 2012). The identification of glial polarization in the context of brain injuries is crucial in advancing our understanding of glial heterogeneity, thereby allowing us to manipulate these properties to limit damage and support regeneration following TBI.

It is important to note that these cellular responses may not occur in isolation. Although not extensively discussed within this review, cells within the brain can act in concert with recruited haematopoietic cells after injury to exert their biological function. Signals released by microglia, for example, have been shown to recruit monocytes into the brain in response to peripheral organ inflammation (D'Mello *et al*., 2009). Neuroimmune crosstalk is emerging as a critical concept in studies of brain injury and stroke, and the use of chimeric mice and the selective depletion of brain-derived or peripheral cells is allowing investigators to tease apart the roles of these different cell types (Gliem *et al*., 2012; Downes *et al*., 2013; Low *et al*., 2014).

Therapies targeting CNS injuries must take into account the multifaceted nature of cellular responses if they are going to be effective in limiting neuronal damage after TBI. Inflammatory responses of astrocytes and microglia in TBI represent an interesting therapeutic opportunity. By harnessing the protective or reparative effects of these responses while simultaneously dampening their deleterious effects, we may be able to control the progression and exacerbation of inflammation and protect viable brain tissue. Therefore, a greater understanding of the mechanisms governing reactive gliosis and how gliosis affects other cell types will aid in the development of better therapeutics for TBI.

Conflict of interest

The authors declare no conflicts of interest.

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