The emerging roles of microglia are currently being investigated in the healthy and diseased brain with a growing interest in their diverse functions. In recent years, it has been demonstrated that microglia are not only immunocentric, but also neurobiological and can impact neural development and the maintenance of neuronal cell function in both healthy and pathological contexts. In the disease context, there is widespread consensus that microglia are dynamic cells with a potential to contribute to both central nervous system damage and repair. Indeed, a number of studies have found that microenvironmental conditions can selectively modify unique microglia phenotypes and functions. One novel mechanism that has garnered interest involves the regulation of microglial function by microRNAs, which has therapeutic implications such as enhancing microglia-mediated suppression of brain injury and promoting repair following inflammatory injury. Furthermore, recently published articles have identified molecular signatures of myeloid cells, suggesting that microglia are a distinct cell population compared to other cells of myeloid lineage that access the central nervous system under pathological conditions. Thus, new opportunities exist to help distinguish microglia in the brain and permit the study of their unique functions in health and disease.

Introduction

Microglia are brain-resident myeloid cells that belong to the mononuclear phagocyte system, which includes bone-marrow precursors, circulating monocytes and tissue-resident macrophages. In both the healthy and diseased CNS, microglia are perpetually exposed to diverse environmental stimuli that can influence their function and often correspond to a unique molecular and morphological profile. Microglia are highly dynamic innate immune cells that are capable of robust chemotaxis, phagocytosis, antigen presentation, and cytokine production, in addition to a diverse set of newly emerging neurobiological functions. In this review, we explore...
how microglia can impact overall brain function at the molecular and cellular level in both the healthy and pathological context. We discuss the putative roles of microglia throughout mammalian brain development, ageing, and in response to infection and/or injury. Furthermore, discussion is included that provides translational context whereby we can now exploit the phenotypic and functional properties of microglia to help future neuroscience research and provide novel targets of future therapies.

**Microglia in the developing CNS**

**Unique aspects of microglia ontogeny**

Microglia are mesodermal-derived mononuclear cells of the CNS that arise during primitive haematopoiesis in yolk sac blood islands (Cuadros et al., 1993; Ginhoux et al., 2010; Golub and Cumano, 2013). In humans, microglia can be identified in the extracerebral mesenchyme as early as 4.5 gestational weeks and invade the parenchyma at ~5 gestational weeks (Monier et al., 2006, 2007; Verney et al., 2010). In rodents, microglia are first detected as primitive tyrosine protein kinase c-kit (now known as KIT) positive erythromyeloid precursors at embryonic Day 8 (Kierdorf et al., 2013), which is consistent with observations that microglia progenitors arise before embryonic Day 8 in rodents (Alliot et al., 1999; Ginhoux et al., 2010). Primitive haematopoiesis may also contribute precursors to the adult haematopoietic stem cell population. Haematopoietic stem cell progeny are responsible for the circulating or ‘peripheral’ compartment of monocytes, which infiltrate tissues during injury/immune challenge and can replenish populations of certain tissue macrophages throughout life (further discussed in subsequent sections and more detailed in reviews by Sieweke and Allen, 2013; Haldar and Murphy, 2014; Jenkins and Hume, 2014).

In humans, the functional significance of microglia’s unique ontogeny remains to be clarified; however, initial insights have revealed that some differences exist in developmental genetic programming when compared to peripheral blood-derived myeloid cell types. Studies using proto-oncogene c-myb−/− mice, a transcription factor that regulates haematopoiesis, demonstrate that unlike haematopoietic stem cells, erythromyeloid progenitors resulting from primitive haematopoiesis do not require c-myb for self-renewal and proliferation (Hulshof et al., 2003; Lieu and Reddy, 2009). However, interpreting these data may be complicated given c-myb regulates the colony stimulating factor 1 receptor (CSF1R), which is involved in maintaining and differentiating both monocytes and microglia (Jenkins and Hume, 2014). Microglia differentiation is further subjected to molecular regulation by the transcription factors SPI1 (previously known as PU.1) and interferon regulatory factor 8 (IRF8), which are both implicated in normal myeloid cell development. The fractalkine receptor (CX3CR1) is also commonly used for lineage tracing of microglia in rodent models and is expressed in human microglia (Hulshof et al., 2003; Ginhoux et al., 2010), yet Kierdorf et al. (2013) have described genetic and cell-surface markers of rodent microglia before CX3CR1 expression. An early protein tyrosine phosphatase receptor type C positive (CD45a) and CX3CR1 negative (CX3CR1−) myeloid precursor population is dependent on the transcription factor SPI1, while loss of IRF8 causes a maturational defect and apoptosis of microglia precursors before acquiring CX3CR1 expression. IRF8 is linked downstream of SPI1, thus implicating this factor in early microglia differentiation and development (Kierdorf et al., 2013). Interestingly, the C57Bl/6 genetic background requires SPI1 for yolk sac macrophage development, whereas outbred Spi1−/− mice do not (Lichanska et al., 1999; Jenkins and Hume, 2014). Additionally, Minten et al. (2012) reported an increase in microglia number in the same IRF8 knockouts crossed with CSF-1R-eGFP C57Bl/6 mice (Holtscshe et al., 1996; Minten et al., 2012). These discrepancies highlight the complexity and importance of genetic background in animal studies focusing on microglia; it is unclear how these findings translate to humans.

In humans, the spatial distribution of embryo-colonizing microglia in histological sections suggests they enter the brain primordium via the developing meninges, ventricular zone, and choroid plexus (Monier et al., 2006, 2007; Verney et al., 2010). Initially, microglia have an amoeboid morphology and eventually acquire a mature, ramified morphology. This developmental amoeboid shape suggests they may be activated during development (Verney et al., 2012; Supramaniam et al., 2013). Immature rodent microglia express high levels of specific chemokines and their cognate receptors (e.g. CX3CR1, CCR2, CCR1, CXCR3) during maturation in the brain parenchyma (Goings et al., 2006; Verney et al., 2010; Kierdorf et al., 2013; Shigemoto-Mogami et al., 2014). In the respective gene knockout animals, the morphology and numbers of microglia remain constant and suggests these molecules are not necessary for distribution in the developing parenchyma (Kierdorf et al., 2013). Members of the matrix metalloproteinase (MMP) family, including MMP8 and MMP9, also regulate distribution and microglia number in the embryonic mouse brain (Kierdorf et al., 2013). With an increasing interest in anatomically spatial differences amongst microglia in various brain regions, the individual factors regulating the migration and positioning of microglia in regions of the developing brain are currently being investigated (de Haas et al., 2008; Doorn et al., 2014; Filho et al., 2014; Kim et al., 2014a; Llorens et al., 2014; Silverman et al., 2014).
Bidirectional interaction of microglia and the developing neural architecture

The human cerebral cortex is composed of 20–25 billion neurons that arise from the ventricular and subventricular zones during embryonic development. It has been estimated that microglia make up 6–18% of neocortical cells in the human brain (Peligvig et al., 2008; Lyck et al., 2009). The early arrival of microglia in the developing brain, together with their activated phenotype and evidence of microgliosis during neurodevelopmental disorders, suggests the possibility of developmental microglia-neuron crosstalk (Monier et al., 2006, 2007; Pelvig et al., 2008; Lyck et al., 2009; Ginhoux et al., 2010; Verney et al., 2010, 2012; Supramaniam et al., 2013; Baburamani et al., 2014; Shigemoto-Mogami et al., 2014). Indeed, a number of studies have implicated microglia in neurodevelopmental contexts including autism spectrum disorders (Gupta et al., 2014), obsessive compulsive disorder (Chen et al., 2010; Nayak et al., 2014), schizophrenia (Kenk et al., 2014; de Baumont et al., 2015), Tourette’s syndrome (Lennington et al., 2014), cerebral palsy (Mallar et al., 2014), foetal alcohol spectrum disorders (Guizetti et al., 2014), fragile X syndrome (Alokam et al., 2014; Gholizadeh et al., 2015), and others.

It has been suggested that soluble factors may be involved during developmental microglia-neuron crosstalk. Pharmacological inhibition of microglia activation using minocycline reduces overall neurogenesis and oligodendrogenesis, whereas neural precursors are sensitive to combinations of the cytokines interleukin 1 beta (IL1β) and interferon gamma (IFNγ) released by microglia (Shigemoto-Mogami et al., 2014). Microglia also enhance oligodendrogenesis via a combination of IL1β and interleukin 6 (IL6); however, the observed effects are targeted towards later stages of oligodendroglial development (Shigemoto-Mogami et al., 2014). In vitro, subventricular zone neural stem cell cultures from postnatal Day 8 mice maintain the ability to form neurospheres, but progressively lose the ability to generate neuroblasts; co-culture of subventricular zone neural stem cells with microglia or microglia-conditioned media rescues their ability to generate neuroblasts (Walton et al., 2006). Some studies have suggested that microglia in neurogenic regions behave differently than those in non-neurogenic areas (Goings et al., 2006; Marshall et al., 2014). Indeed, Mosher et al. (2012) found that secretory products derived from neural progenitor cells can modulate the cytokine profiles of microglia, while augmenting phagocytosis and migration functions in vitro. These effects were apparently due to neural progenitor cell-derived vascular endothelial growth factor (Mosher et al., 2012) and help highlight the bidirectional nature of the microglia-neuron relationship during brain development.

In addition to secretory products, microglia also physically regulate the number of neural progenitor cells in the developing cerebral cortex. In rodents and non-human primates, this phenomenon occurs via preferential phagocytosis of viable neurons (independent of apoptotic markers) within proliferative zones in latter stages of cortical neurogenesis (Cunningham et al., 2013). Large numbers of activated microglia are found within neural proliferative zones, while few microglia are present within the cortical plate and is consistent with studies performed in humans (Monier et al., 2006, 2007; Verney et al., 2010). Interestingly, maternal lipopolysaccharide (LPS) administration negatively impacts the neural progenitor cell population in rodents, presumably by promoting a pro-inflammatory microglia phenotype. Conversely, doxycycline skews the phenotypic ratio towards an anti-inflammatory phenotype and significantly increases the neural progenitor cell population (Cunningham et al., 2013). The mechanisms responsible for the phagocytosis of viable neurons in the developing human CNS are currently unknown; however, relevant phagocytosis mediators in the adult CNS may provide a starting point (discussed below).

In the developing brain, microglia interact specifically with neuronal synapses. One study addressed the developmental interactions of microglia and synapses by examining the visual cortex during a critical developmental period using the CX3CR1+EGFP/Thy-1 YFP transgenic mouse (Tremblay et al., 2010). A number of features of visual perception are established during this period in mice, and are associated with well-described changes in dendritic spines (Majewska and Sur, 2003; Bence and Levelt, 2005; Hooks and Chen, 2007; Schafer et al., 2013). This study demonstrated physical contact between microglia and dendritic spines, which were demonstrated to change size and often eliminated. During this developmental period, in response to re-exposure to light following dark adaptation, microglia phagocytosed more synaptic elements compared with dark-adapted controls. These data suggest that in response to visual experience, spine elimination/remodelling is mediated at least partially by microglial phagocytosis (Tremblay et al., 2010; Schafer et al., 2013). Microglia have been shown to directly phagocytose developmental synapses that express C1q via complement receptors on microglia; the loss of C1q expression in the mouse brain results in synapse elimination defects, including a failure to refine synaptic connections (Steens et al., 2007). In a more recent study, Stevens’ group demonstrated that developing retinal ganglion cell presynaptic terminals are pruned in a complement-dependent manner (Schafer et al., 2012; Tyler and Boulander, 2012). Paolicelli and colleagues used stimulated emission depletion (STED) microscopy to demonstrate that post-synaptic density protein-95 (PSD95, now known as DLG4) immunoreactivity was present within microglial processes in the mouse hippocampus during normal development, suggesting that microglia can uptake pre- or postsynaptic material using clathrin and non-clathrin...
coated vesicles (Westphal et al., 2008; Paolicelli et al., 2011).

During development, microglia-synapse interactions are critical. CX3CR1 is expressed by microglia (Jung et al., 2000; Gundra et al., 2014), while its ligand, fractalkine (CX3CL1), can be either secreted by neurons or found at the cell surface and helps to establish an interaction between microglia and neurons in both the healthy and pathological CNS (Harrison et al., 1998; Hughes et al., 2002). CX3CR1 knockout mice display transient deficits in synaptic pruning, consistent with observations wherein single-cell recordings of CA1 pyramidal neurons revealed a decreased spontaneous excitatory postsynaptic current/miniature excitatory postsynaptic current amplitude ratio, indicating immature connectivity in knockout animals (Paolicelli et al., 2011). In a follow-up study, the authors quantify the effect of this pruning deficit on synaptic transmission, functional connectivity, and behavioural outcomes in mice, suggesting that a repetitive behavioural phenotype is reminiscent of neurodevelopmental disorder in humans (Zhan et al., 2014). CX3CR1 deficit has specifically been shown to impair learning and is consistent with a substantial role for microglia in normal synaptic regulation and network development (Boulanger, 2009; Pernot et al., 2011; Rogers et al., 2011; Voineagu et al., 2011; Fillman et al., 2013). The possibility that defects in microglia function could lead to diverse developmental neuropathologies is an exciting future avenue for a multitude of therapeutic targets.

**Pathology and homeostasis in the adult CNS**

**Maintenance of microglia populations, microgliosis and parenchymal precursors**

We are now beginning to understand how microglia are maintained in the adult brain and the factors responsible for regulating their population. It seems that microglia are the dominant tissue-resident macrophages, which maintain their population without contribution from the periphery throughout life (Fig. 1) (Ajami et al., 2007; Mildner et al., 2007; Sieweke and Allen, 2013; Jenkins and Hume, 2014). Colony stimulating factor 1 (CSF1), also known as macrophage colony stimulating factor (MCSF), maintains macrophage populations through its protein tyrosine kinase receptor, CSF1R, which is expressed on committed macrophage precursors, monocytes, and microglia (Pixley and Stanley, 2004; Nandi et al., 2012; Jenkins and Hume, 2014). The osteopetrosic mouse is effectively Csf1−/−, causing a myriad of skeletal and haematopoietic defects, including a reduction in microglia that ranges from 30% to complete depletion depending on the region (Wegiel et al., 1998; Kondo et al., 2007; Wei et al., 2010; Nandi et al., 2012). Mutations in the CSF1R gene can cause more severe defects, including a >99% microglia reduction brain-wide (Erblich et al., 2011). Elmore et al. (2014) found that blocking CSF1R with a multi-targeted tyrosine kinase inhibitor, depleted adult mice of 99% of microglia, reinforcing the importance of CSF1R signalling in microglia maintenance.

In a large functional screen of the human extracellular proteome, Lin et al. identified interleukin 34 (IL34), a previously unknown ligand for CSF1R (Lin et al., 2008). IL34 is more abundantly (~10-fold) and broadly expressed compared to CSF1, while their differential regional expression accounts for differences between CSF1 and CSF1R knockout phenotypes (Wei et al., 2010). Using IL34 deficient mice, it was demonstrated that this cytokine is a critical factor for microglia differentiation; neurons represented the primary source of IL34 (Wang et al., 2012). In the adult mouse brain, CSF1 and IL34 are maintained at high levels, whereas CSF1R levels decrease during postnatal development (Nandi et al., 2012). Recently, an alternate receptor for IL34 has been identified, suggesting the possibility of distinct functions for IL34 and CSF1 (Hamilton and Achuthan, 2013; Nandi et al., 2013). Whether IL34 and CSF1 have differential influences on human microglial function is unknown.

Following brain injury, microglia numbers are increased (termed microgliosis) in tissues proximal to injury, exhibit an activated morphology and phenotype, and increase their expression of MHCII/HLA-DR (Monier et al., 2007) and inducible nitric oxide synthase (NOS2) (Verney et al., 2010). In the inflamed brain, the lack of specific markers capable of distinguishing circulating and invading peripheral blood-derived macrophages from microglia in histological sections precludes making accurate distinctions between these cell types. Initially, irradiation and bone marrow reconstitution experiments using congenic mice were used to examine the peripheral and central contributions to the microglia pool under various conditions; however, this model system generated various confounds (Ransohoff, 2007). In parabiosis, syngeneic mice have their circulatory systems joined such that host or donor cells can be tracked using specific markers. Thus, the host microglia population can be distinguished from peripheral cell types under both steady-state conditions and following experimental manipulation. Using this method, it was observed that blood-derived cells did not contribute to microglia turnover under steady-state conditions, nor microgliosis in models using facial nerve axotomy, or over-expression of a transgenic mutant superoxide dismutase 1 (SOD1G93A) (Ajami et al., 2007). Using a similar design, yet relying on a more controlled, modified irradiation-reconstitution method, Mildner et al. (2007) reported no participation of blood-derived cells in homeostatic microglial turnover, or in experimentally induced microgliosis (facial axotomy, and cuprizone-induced demyelination models).
The literature describing the numerous molecular factors regulating microglia population dynamics at a mechanistic level is vast and are not discussed in detail as they are reviewed sufficiently elsewhere (Kettenmann et al., 2011). Proliferation regulation signals include positive regulation via intracellular and volume-sensitive chloride channels, adenosine receptors, and cannabinoid receptors (Fiebich et al., 1996; Gebicke-Haerter et al., 1996; Schlichter et al., 1996, 2011; Carrier et al., 2004; Hartzell et al., 2005; Ducharme et al., 2007; Milton et al., 2008). Negative regulation may occur via voltage-dependent K+ channels, somatostatin receptors, glucocorticoid receptors, and beta-2 adrenergic receptors (Gehrman and Banati, 1995; Tanaka et al., 1997; Fujita et al., 1998; Brough et al., 2002). Albumin may also cause microglia proliferation through intracellular Ca²⁺ signalling, providing an interesting candidate signal in the context of injuries that compromise blood–brain barrier integrity.
Immunocentric microglia: activation, polarization and resting states

Microglia possess both immunocentric and neurobiological functions during development and throughout adulthood. In light of the recent expansion of research in the field of macrophage activation, polarization, and plasticity, we support the recent endeavour of Murray et al. (2014) to generate standardized myeloid activation/polarization nomenclature and experimental practices (Table 1). This new nomenclature attempts to define polarization by the molecular constituents used to activate the cells. Importantly, in contrast to working with rodent macrophages and microglia in culture, human microglia do not require growth factors for maintenance and/or differentiation in vitro (Duraafourt et al., 2012). When making direct comparisons with peripheral-derived macrophages, growth factors may or may not be used in attempts to make direct in vitro comparisons between peripheral-derived macrophages and brain-resident microglia. Therefore, the null condition M(−) should be clearly defined in each microglia study with these facts in mind (Table 1). ‘Polarization’ refers to perturbation of microglia with exogenous agents to achieve a particular phenotype, and is evident by the presence of one or more molecular markers and a shift in molecular profile. In vivo, the term ‘polarization’ attempts to simulate environmental conditions that are indicative of microglia function via receptors and mechanisms collectively referred to as the sensome (Hickman et al., 2013). The term ‘activation’ can be difficult to interpret in the neuroimmunological context because it is derived from a purely immunological context. Although correct in many myeloid cell immunological contexts, activation implies that microglia are ‘inactive’ under certain conditions. This in fact may not be the case even in vitro where human microglia display a high degree of motility and activity, similar to what has been observed in vivo (Supplementary Video 1). Use of the term ‘basal’ instead of ‘inactive’ may partially resolve this quandary, for now. Alternate considerations for myeloid cell nomenclature may be found in Svanh et al. (2014). Given the nature of our review, we use previous widely-cited nomenclature (e.g. M1/M2), which directly relates to the present literature. A table is provided here to aid in relating previous published materials with the novel proposed nomenclature (Table 1).

Polarized myeloid cells, including blood-derived macrophages and CNS-resident microglia, have been historically classified along a spectrum similar to the T helper (T<sub>H</sub>1/ T<sub>H</sub>2) classification of T lymphocytes (Fig. 2). Myeloid cell polarization was initially characterized in peripheral cells whereby classical activation of macrophages was observed following exposure to T<sub>H</sub>1-type cytokines such as IFNγ (Mantovani et al., 2004). Building on the observation that in murine macrophages the T<sub>H</sub>2-associated cytokine IL4 could induce expression of the mannose receptor CD206 (Stein et al., 1992), a T<sub>H</sub>2-driven alternative activation phenotype was described (Gordon, 2003). In the literature, ‘classically activated’ myeloid cells (LPS ± IFNγ) have been referred to as ‘M1’, whereas ‘alternatively activated’ cells (IL4 treatment in the presence or absence of IL13) as ‘M2’. At the extremes of this spectrum, the properties of myeloid cells have been often studied by generating monocyte-derived macrophages, followed by exposure to either T<sub>H</sub>1 or T<sub>H</sub>2 type cytokines to polarize cells into the M1 or M2 lineage, respectively (Sierra-Filardi et al., 2010). Standardized protocols for human myeloid cells, including microglia, have been described previously (Duraafourt et al., 2013). The M2 phenotype has been further subclassified according to specific activation stimuli, with M2a generated by exposure to IL4 and IL13, M2b using immune complexes and LPS, and M2c using IL10 and transforming growth factor beta (TGFβ1) (Leidi et al., 2009). The generation of M2a cells with IL4 and IL13 relies on signalling through IL4RA, leading to inhibition of nuclear factor kappa-light-chain-enhancer of

(Hooper et al., 2005; Kettenmann et al., 2011). A recent study found that ganciclovir, an anti-retroviral drug, was a potent inhibitor of rodent microglia/macrophages within the inflamed CNS, without noticeably affecting the peripheral myeloid proliferative response, or subsets of other immune cells. This finding raises the intriguing possibility of therapeutics selectively targeting myeloid proliferation within the CNS (Ding et al., 2014). Furthermore, with the recent molecular profiles for human and rodent microglia, which can be used to help distinguish them from peripheral-derived cells, this may become a therapeutic possibility (Gautier et al., 2012; Chiu et al., 2013; Butovsky et al., 2014).

Despite the suggestion of local maintenance, little is known about microglia precursors in the adult brain. Following experimental injury, nestin expression is upregulated in microglia, but can also be found in many other cell types near stroke infarcts, focal spinal cord injury, and in other injury models (Namiki and Tator, 1999; Sahin et al., 1999; Shibuya et al., 2002; Mothe and Tator, 2005; Takamori et al., 2009; Wohl et al., 2011). It remains unknown whether erythromyeloid precursors express nestin, or if this phenotype is indicative of a more mature precursor cell type found in the brain alone. It is also worth noting that no studies have yet demonstrated a CNS-derived cell with stem characteristics that is capable of generating microglia. Elmore et al. (2014) have shown that nestin-positive microglia precursors expressed Iba1, the lectin IB4, and CD45 (PTPRC), with a fraction of cells also expressing Ki67, CD34 and c-Kit (KIT), which decreased in expression upon stabilization of their population (Elmore et al., 2014). In injury models, nestin expression following brain injury often occurs in cells of the perivascular region, suggesting at least one putative niche for microglial precursors (Shin et al., 2013; Hughes and Bergles, 2014).
activated B cells (NF-κB) signalling and an anti-inflammatory phenotype (Taylor et al., 2005). M2b cells are the least well studied; they continue to express M1-associated co-stimulatory molecules and produce nitric oxide (NO), but also secrete anti-inflammatory cytokines, such as IL10 (Edwards et al., 2006). M2c macrophages, originally termed deactivated, are associated with immune suppression and tissue remodelling (Mantovani et al., 2004).

Classically-activated peripheral myeloid cells have been characterized by their rounded amoeboid morphology, which is consistent with a state of hyperactivity. These cells express surface markers such as co-stimulatory molecules CD80 and CD86 and the chemokine receptor CCR7, produced reactive nitrogen species and pro-inflammatory cytokines such as IL12 (Van Ginderachter et al., 2006; Fairweather and Cihakova, 2009). In contrast, M2 cells possess a typical elongated morphology, express cell surface markers including the scavenger receptor CD163, C-type lectins CD206 and CD209, and produce anti-inflammatory cytokines such as TGFβ1 and IL10 (Mantovani et al., 2004; Martinez et al., 2006; Van Ginderachter et al., 2006). In line with this anti-inflammatory profile, the M2 phenotype has been associated with the repair process in animal models of stroke (Desestret et al., 2013), spinal cord injury (Shechter et al., 2013), and demyelination (Miron et al., 2013).

The concept of microglia polarization was first explored in the murine system by Ponomarev et al. (2007) and demonstrated that microglia expressed the M2-associated protein Ym1 (now known as CHIL3) in an IL4 dependent manner (Ponomarev et al., 2007). Ym1 is a chitinase-like lectin and has been identified as a marker of M2 macrophages in the murine system, but not in humans (Raes et al., 2002, 2005). Expression of the enzyme arginase 1 has also been reported as a marker of M2 macrophages; however, it remains controversial whether selective expression also translates to humans. Studies characterizing human adult and foetal microglia demonstrated that human microglia can also adopt M1 and M2-like characteristics, while possessing distinct phenotypic and functional properties compared to monocyte-derived macrophages (Durafourt et al., 2012). M1-polarized human adult microglia express similar surface markers as their macrophage counterparts, including CD80 and CCR7. Following IL4 and IL13 activation, human microglia can also adopt M1 and M2-like characteristics, while possessing distinct phenotypic and functional properties compared to monocyte-derived macrophages (Durafourt et al., 2012). In the injured CNS, microglia express fewer M2-associated surface markers than macrophages, with CD209 being the most reliable marker. While alternatively-activated macrophages more efficiently phagocytose opsonized targets than their M1 counterparts (Leidi et al., 2009), M2 microglia were also more efficient at phagocytosing myelin compared to either M1 microglia or any polarized macrophage phenotype (Durafourt et al., 2012). In the injured CNS, microglia may therefore be more likely than monocyte-derived macrophages to contribute to the clearance of debris, thus allowing for an efficient repair process to occur (Kotter et al., 2006).

Targeting myeloid cell polarization remains a clinically relevant objective. Murine studies have shown that lack of IL4 in the CNS, but not the periphery, impaired the M2 polarization of microglia and exacerbated symptoms of experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis (Ponomarev et al., 2007). Examination of post-mortem tissue from patients with multiple sclerosis revealed that myeloid cells in active multiple sclerosis lesions abundantly expressed M1 markers, whereas the majority of these cells also co-expressed M2 markers (Vogel et al., 2013). In a demyelination model, following

Table 1 Nomenclature of activated macrophages/microglia

<table>
<thead>
<tr>
<th>Classic nomenclature</th>
<th>Novel nomenclature</th>
<th>Activating molecules</th>
<th>Notes</th>
<th>Citations</th>
</tr>
</thead>
<tbody>
<tr>
<td>M0/Unpolarized</td>
<td>M(-)</td>
<td>None, M-CSF, or GM-CSF</td>
<td>M-CSF or GM-CSF may be used to compare with macrophages</td>
<td>Durafourt et al., 2012</td>
</tr>
<tr>
<td>M1</td>
<td>M(LPS)</td>
<td>LPS, IFNγ or both</td>
<td>a.k.a. Classical activation</td>
<td>Mantovani et al., 2004</td>
</tr>
<tr>
<td>M2</td>
<td>M(IL4)</td>
<td>IL4</td>
<td>a.k.a. Alternative activation</td>
<td>Stein et al., 1992; Gordon, 2003; Sierra-Filardi et al., 2010</td>
</tr>
<tr>
<td>M2a</td>
<td>M(IL4 + IL13)</td>
<td>IL4 and IL13</td>
<td>Profile associated with anti-inflammatory phenotype in macrophages</td>
<td>Taylor et al., 2005; Leidi et al., 2009</td>
</tr>
<tr>
<td>M2b</td>
<td>M(IG + LPS)</td>
<td>Immune complexes and LPS</td>
<td>Profile associated with pro-inflammatory cell-surface protein expression but IL10 secretion in macrophages</td>
<td>Edwards et al., 2006; Leidi et al., 2009</td>
</tr>
<tr>
<td>M2c</td>
<td>M(IL10 + TGFβ1)</td>
<td>IL10 and TGFβ1</td>
<td>Profile associated with tissue remodelling and immune suppression in macrophages</td>
<td>Mantovani et al., 2004; Leidi et al., 2009</td>
</tr>
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</table>

GM-CSF = granulocyte-macrophage colony stimulating factor, now known as CSF2; M-CSF = macrophage colony stimulating factor, now known as CSF1.
stereotactic injection of lysolecithin, murine microglia undergo a switch from the M1 to M2 phenotype. This process was shown to drive oligodendrocyte differentiation and promote remyelination (Miron et al., 2013). In the injured CNS, TNF prevents the phagocytosis-mediated conversion from M1 to M2 type cells, while iron promotes a rapid switch from the M2 to M1 phenotype (Kroner et al., 2014). Dysregulation of myeloid cell polarization has also been implicated in acute neuroinflammatory conditions, including spinal cord injury, traumatic brain injury, and stroke, where M1 cells predominate (Cherry et al., 2014). In a rodent model of spinal cord injury, mesenchymal stem cells were shown to promote M2 macrophage differentiation and improve functional recovery (Cherry et al., 2014). In another rodent model, M2 macrophages were found to be recruited though the choroid plexus and to contribute to the repair process (Shechter et al., 2013). These studies demonstrate that M2 myeloid cells contribute not only to neuroprotection, but may also promote repair process in the injured CNS and may represent novel therapeutic strategies for treating neurodegenerative conditions.

**MicroRNA regulation of microglia status in pathology**

Over the past decade, only a small number of molecules have emerged as clinically-used biomarkers of pathogenic processes or treatment responses. Unfortunately, many putative biomarkers do not meet all the criteria for an ‘ideal’ biomarker (Rajasekaran and Bar-Or, 2012). In several clinical fields of medicine, microRNAs have emerged as important post-transcriptional regulators of gene expression and putative biomarkers that may either indicate or predict disease activity, clinical prognosis, and/or treatment to
individual therapies. MicroRNAs are short non-coding RNAs (18–22 nucleotides) that are stable and abundant molecules present in all biological fluids. Not only have they been suggested as ideal biomarkers, but can also possess disease-relevant biological activity. Most recently, the expression and biological function of several distinct microRNA molecules have been described in myeloid cells, including microglia (Moore et al., 2013; Ponomarev et al., 2013). Compared to other cells of the innate and adaptive immune systems, the relatively high plasticity of microglia, in terms of their contributions to both disease-promoting and tissue-regenerative activities, may be at least in part due to their microRNA expression profiles.

It has been suggested that distinct microRNA expression patterns in microglia may play a significant role in their phenotype and function. The development and differentiation of microglia is controlled by the transcription factors CEBPA and NFIA, which are directly controlled by mir-223 (Fazi et al., 2005). In additional studies by Ponomarev et al. (2011) mir-124 was abundantly expressed in the brain and responsible for maintaining microglia quiescence by restricting SPI1 (PU.1) and CSF1 expression. In vitro studies measuring microRNA expression in primary rodent microglia have also demonstrated that under standard culture conditions, mir-124 and mir-155 levels decreased over time, whereas mir-146a was increased, suggesting these microRNAs may be responsible for the altered cell phenotype and function associated with ageing (Caldeira et al., 2014). Many additional microRNAs have also been suggested to regulate monocyte differentiation (Forrest et al., 2010), however, the role(s) of these microRNAs in human microglia remains uncertain (Pospisil et al., 2011).

Several microRNAs have been reported to both promote and inhibit innate inflammatory responses (Table 2). In the literature, mir-155 is among one of the most highly referenced pro-inflammatory microRNAs. In primary cells (rodent and human) and several microglia cell lines, mir-155 is upregulated upon activation with several pro-inflammatory factors, including LPS, IFNγ and TNF. In human myeloid cells, including both human fetal and human adult microglia, mir-155 expression is significantly elevated under M1-polarizing conditions (Moore et al., 2013). In human microglia, transfection with a mir-155 mimic alone significantly increases pro-inflammatory cytokine expression, co-stimulatory molecule expression (e.g. CD80, CD86, CCR7), and enhances allogeneic T cell responses in co-culture (Moore et al., 2013); inhibition of mir-155 decreased these phenotypes. This pro-inflammatory phenotype is driven in part by the ability of mir-155 to block SOCS1 expression, however, additional studies have also demonstrated that mir-155 can also decrease the expression of molecules/receptors associated with promoting the M2 phenotype (Martinez-Nunez et al., 2011). In addition to mir-155, several other microRNAs can also promote M1-like phenotypes in microglia, including mir-9 (Yao et al., 2014) and mir-125b (Parisi et al., 2013). Other microRNAs, including mir-146a and mir-200b, are also thought to contribute to microglia activation, however induction of these particular microRNAs is not thought to promote an inflammatory phenotype, but rather help resolve chronic inflammation (Rom et al., 2010; JadHAV et al., 2014a). Studies performed in vitro using human microglia and monocyte-derived macrophages suggest these cell types are phenotypically and functionally distinct upon activation (Durafourt et al., 2012). Differences in cell migration and phagocytosis have also been reported between monocyte-derived macrophages and microglia. The reasons for these differences may be in part due to their unique mRNA and/or microRNA profiles. Recent studies have investigated the molecular signature of microglia compared with other myeloid and immune cell types demonstrating unique microRNAs expression in microglia, including mir-99a, mir-342-3p, and mir-125b-5p (Butovsky et al., 2014). In vitro, mir-155 levels are significantly elevated in human blood-derived myeloid cells compared to microglia (Moore et al., 2013). Together with these unique myeloid cell signatures, it may be possible to selectively target biological activities of individual myeloid cell populations, which may have pharmacological and therapeutic value in the context of brain injury and repair.

In several different neurological conditions, including Alzheimer’s disease, amyotrophic lateral sclerosis, stroke, multiple sclerosis, and Parkinson’s disease, the expression levels of microRNAs are altered in microglia. In multiple sclerosis lesions, activated macrophages/microglia have significantly elevated mir-155 levels compared with microglia in normal autopsied brain tissue. Indeed, in vivo silencing of mir-155 ameliorates clinical and pathological outcomes in EAE. In a genetic model of Alzheimer’s disease, mir-155 levels are significantly elevated and induced simultaneously with microglia activation (Guedes et al., 2014). Mir-146a, a negative regulator of inflammatory responses, is downregulated in microglia lacking presenilin 2 (PSEN2), a gene that is mutated in autosomal dominant Alzheimer’s disease, thus increasing the pro-inflammatory phenotype of microglia (Jayadev et al., 2013). In microglia cultured from mice lacking PARK7 (previously known as DJ-1), a Parkinson’s disease-associated gene linked to disease onset and progression, mir-155 is increased following activation with IFNγ, but not in wild-type cells (Kim et al., 2014b). In an animal model of cerebral ischaemia, decreased mir-424 expression is associated with increased microglial activation and neuronal apoptosis (Zhao et al., 2013), whereas increased mir-181c expression is associated with decreasing TNF and protected neurons from damaging effects of activated microglia (Zhang et al., 2012a). A comparison between microRNA expression levels in activated microglia derived from the Sod1G93A mouse versus wild-type microglia identified several dysregulated microRNAs, including mir-155, mir-146b, mir-365, and mir-125b, which were linked to dysregulated IL6 and TNF expression (Parisi et al., 2013). In an unbiased analysis of microRNA expression using microglia and...
monocytes derived from SOD1 mice, differential expression of several microRNAs were also observed between cell types with changes also being observed over disease course (Butovsky et al., 2012).

In neurodegenerative diseases, the participation of microglia and their respective polarized phenotypes in contributing to both tissue injury and repair is becoming increasingly recognized. With the altered microRNA expression profiles in several brain-related conditions, we are now beginning to assign distinct molecular profiles with subtypes of myeloid cells and determine their unique functional roles as they relate to inflammatory and tissue regenerative processes. In the future, not only will microRNAs help elucidate the molecular mechanisms responsible for driving disease pathogenesis, but may also serve as putative pharmacological targets and biomarkers of disease and therapeutic efficacy.

### Microglia and the ageing CNS

Microglia are activated in response to numerous signals in the CNS, including viruses, bacteria, cytokines, growth factors, and other molecules involved in immune responses. This activation can lead to the transformation of microglia into a variety of phenotypes, each with distinct functions. For example, M1 microglia are pro-inflammatory, whereas M2 microglia are anti-inflammatory and involved in wound healing and tissue repair.

Table 2: MicroRNAs implicated in microglia function

<table>
<thead>
<tr>
<th>MicroRNA</th>
<th>Reference</th>
<th>Reported involvement/function</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inflammation-related</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mir-9</td>
<td>Akerblom et al., 2013; Kong et al., 2014; Yao et al., 2014</td>
<td>Ischaemia, MCPIP1 and NF-κB signalling</td>
</tr>
<tr>
<td>Mir-17</td>
<td>Jadhav et al., 2014b</td>
<td>NADPH oxidases and reactive oxygen species production</td>
</tr>
<tr>
<td>Mir-21</td>
<td>Kong et al., 2014</td>
<td>Ischaemia</td>
</tr>
<tr>
<td>Mir-22</td>
<td>Parisi et al., 2013</td>
<td>Purinergic signalling</td>
</tr>
<tr>
<td>Mir-29b</td>
<td>Fenn et al., 2013; Thounaojam et al., 2014</td>
<td>Aging, TNFAIP3 and NF-κB signalling</td>
</tr>
<tr>
<td>Mir-32</td>
<td>Mishra et al., 2012</td>
<td>TRAF3 signalling</td>
</tr>
<tr>
<td>Mir-34a</td>
<td>Alexandrov et al., 2013; Zhao et al., 2013; Su et al., 2014</td>
<td>NF-κB signalling and TREM2-regulated phagocytosis activity, p53 signalling</td>
</tr>
<tr>
<td>Mir-124a</td>
<td>Ponomarev et al., 2011; Willemen et al., 2012; Freilich et al., 2013; Kong et al., 2014</td>
<td>CEBPA–SPI1 (PU.1) signalling, cell activation, cell quiescence</td>
</tr>
<tr>
<td>Mir-125b</td>
<td>Parisi et al., 2013</td>
<td>Purinergic signalling, STAT3 signalling</td>
</tr>
<tr>
<td>Mir-132</td>
<td>Kong et al., 2014</td>
<td>Ischaemia</td>
</tr>
<tr>
<td>Mir-134</td>
<td>Kong et al., 2014</td>
<td>Ischaemia</td>
</tr>
<tr>
<td>Mir-138</td>
<td>Kong et al., 2014</td>
<td>Ischaemia</td>
</tr>
<tr>
<td>Mir-142</td>
<td>Chaudhuri et al., 2013</td>
<td>Viral infections, SIRT1 regulation</td>
</tr>
<tr>
<td>Mir-145</td>
<td>Freilich et al., 2013; Su et al., 2014</td>
<td>Contribute to alternative activation, IL4/STAT6 signalling, p53 signalling</td>
</tr>
<tr>
<td>Mir-146a</td>
<td>Faraco et al., 2009; Rom et al., 2010; Li et al., 2011; Saba et al., 2012; Pareek et al., 2014</td>
<td>Promoting and resolving inflammation, tumour suppression, NF-κB and JAK-STAT signalling</td>
</tr>
<tr>
<td>Mir-146b</td>
<td>Parisi et al., 2013</td>
<td>Purinergic signalling</td>
</tr>
<tr>
<td>Mir-155</td>
<td>Tarassishin et al., 2011; Cardoso et al., 2012; Freilich et al., 2013; Lippai et al., 2013; Moore et al., 2013; Parisi et al., 2013; Pareek et al., 2014</td>
<td>Contribute to classical activation state, SOCS1 activity</td>
</tr>
<tr>
<td>Mir-181a</td>
<td>Dave and Khalili, 2010; Kong et al., 2014</td>
<td>Ischaemia</td>
</tr>
<tr>
<td>Mir-195</td>
<td>Shi et al., 2013</td>
<td>Autophagy</td>
</tr>
<tr>
<td>Mir-200b</td>
<td>Jadhav et al., 2014a</td>
<td>cJun/MAPK signalling</td>
</tr>
<tr>
<td>Mir-221</td>
<td>Kong et al., 2014</td>
<td>Ischaemia</td>
</tr>
<tr>
<td>Mir-222</td>
<td>Kong et al., 2014</td>
<td>Ischaemia</td>
</tr>
<tr>
<td>Mir-365</td>
<td>Parisi et al., 2013</td>
<td>NFKB signalling, IL6/STAT3 signalling</td>
</tr>
<tr>
<td>Mir-424</td>
<td>Zhao et al., 2013</td>
<td>Cell cycle regulation and pro-inflammatory pathways</td>
</tr>
<tr>
<td>Mir-689</td>
<td>Freilich et al., 2013</td>
<td>Canonical pro-inflammatory pathway</td>
</tr>
<tr>
<td>Mir-711</td>
<td>Freilich et al., 2013</td>
<td>Canonical pro-inflammatory pathway</td>
</tr>
<tr>
<td><strong>Growth factor-related</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Let7f</td>
<td>Selvamani et al., 2012</td>
<td>Ischaemia and IGF1 signalling</td>
</tr>
<tr>
<td>Mir-29b</td>
<td>Fenn et al., 2013</td>
<td>IGF1 and CX3CL1 signalling</td>
</tr>
<tr>
<td><strong>Apoptosis-related</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mir-21</td>
<td>Zhang et al., 2012b</td>
<td>Hypoxia and FasL signalling</td>
</tr>
</tbody>
</table>
chemokines, or aberrant aggregation of endogenous disease-promoting proteins. Alternatively, microglial activation can also occur following the loss of neurally-derived homeostatic signals. For example, the CD200R present on microglia interacts with CD200 and induces a resting phenotype. CD200 is expressed on the surface of various brain cells, including neurons, oligodendrocytes and astrocytes (Wright et al., 2003; Biber et al., 2007). In the hippocampus of aged rats, a CD200 fusion protein decreased microglial activation (Cox et al., 2012). ‘Normal’ ageing is often accompanied by a decline in neural function, which can lead to cognitive impairment and thought to be caused by changes in the brain independent of those associated with neurodegenerative disease states. In neurons, such changes include morphological changes (e.g. decreased dendritic length, reduced spine and synapse density), deficits in synaptic plasticity and altered electrophysiological properties (de Brabander et al., 1998; Dickstein et al., 2013). These changes are thought to be due to synaptic remodelling, a decrease in synaptic structures and associated deficits in synaptic plasticity (Jacobs et al., 1997; Duan et al., 2003; Hof and Morrison, 2004). However, neuronal alterations are most likely in response to wider, age-related shifts in glial cell phenotypes, thus resulting in increased release of neurotoxic molecules and decreased glial cell-mediated neuroprotective abilities.

Multiple phenotypic changes have been described for microglia over the lifespan of the cell (reviewed in Harry, 2013) (Fig. 3). Ageing affects microglia in a number of ways by altering the cells’ endogenous characteristics and inducing a switch to a less responsive, ‘dystrophic’ phenotype. Dystrophic changes are morphological changes distinct from the previously described responses to acute challenge, including blebbing of the plasma membrane, reduction/loss of ramified processes and an increase in cytoplasmic inclusions. In aged mice, changes in gene expression reflect the morphologic and functional plasticity of dystrophic cells whereby changes in cytokine production have also been observed; aged microglia produce higher levels of NOS2, TNF, IL1β, and IL6 (Williams et al., 1992). Aged microglia also show elevated expression of markers generally associated with an activated pro-inflammatory phenotype, including, MHC Class II-associated molecules, pathogen-associated molecular pattern (PAMP) receptors and CD11b (Perry et al., 1993). An increase in the number of dystrophic microglia in the aged human brain has been reported, which have a decreased capacity to mount conventional cellular responses to injury (Streit et al., 2004, 2009). A recent in vivo study using young, adult and aged mice demonstrated an age-related increase in microglia soma volume, a truncation of cell processes and a more heterogeneous CNS distribution. In addition, the previously described dynamic microglial response to tissue injury was shown to be markedly reduced in the aged animals (Hefendehl et al., 2014). The concept of dystrophic microglia has been challenged with the suggestion that aged microglia...
are less altered in terms of morphology and function as previously described. For instance, it has been suggested that aged microglia are merely primed to produce higher levels of both pro- and anti-inflammatory cytokines, relative to an already increased basal expression (Sierra et al., 2007).

In the aged mouse brain, microglia can accumulate phagocytic inclusions, in addition to being less evenly distributed in the cerebral cortex and increasing in overall number (Tremblay et al., 2012). At 12 months of age, murine microglia adopt an M1-like phenotype as shown by increased expression of several pro-inflammatory molecules and cytokines, including NOS2, TNF, IL1β, and IL6 (Crain et al., 2013), while changes in anti-inflammatory cytokines have also been observed (Ye and Johnson, 2001; Sierra et al., 2007).

Research in the Alzheimer’s field has yielded much of the work pertaining to the function of aged microglia. In aged mice (14–16 months), microglia produce higher basal levels of pro-inflammatory cytokines compared to microglia derived from young animals (1–2 months) and have reduced glutathione (antioxidant molecule) levels. Aged microglia also display reduced proficiency in the internalization of amyloid-β42 (Njie et al., 2012). A clear correlation between microglia and plaque maintenance/clearance in Alzheimer’s disease has been established (El Khoury et al., 2007; Bolmont et al., 2008; Floden and Combs, 2011). A study in the APP/PSEN1 mouse model of Alzheimer’s disease showed that microglia adopt an M1-like phenotype that progresses with age and correlates with decreased CD11b expression. Over time, the reduced capacity to internalize amyloid-β42 suggests that aged microglia have a reduced innate ability to clear disease-relevant protein aggregations (Njie et al., 2012).

**Microglial senescence**

In response to injury, microglia are thought to undergo a burst of proliferative activity and eventually become apoptotic in attempt to normalize cell numbers to pre-injury levels. Like all somatic cells, it is believed that microglia undergo ‘replicative senescence’, a phenomenon linked to telomere shortening (Flanary et al., 2007). Cellular senescence is causally linked to the development of age-related pathologies, whereas proof-of-concept experiments suggest that selective removal of senescent cells can delay or prevent age-related tissue deterioration and dysfunction (Naylor et al., 2013). Outside the CNS, senescent cells are rapidly removed from circulation via phagocytosis; however, as microglia are the innate phagocytes of the brain, there lacks a compensatory mechanism for their removal. This observation is supported by studies that show an age-dependent accumulation of microglia within the brain parenchyma. It is suggested that microglial senescence can contribute to the development of dystrophic microglia, which in turn contributes to the pathogenesis of neurodegenerative diseases such as Alzheimer’s disease (Flanary, 2005). In a disease context, one might expect that any loss or decrease of phagocytic ability would result in the accumulation of disease-related proteins, such as amyloid-β, apolipoprotein E (APOE), and α-synuclein. It has been hypothesized that microglia become compromised because of an onset of senescence, which hinders their homeostatic capability. Consequently, the ability of microglia to fulfill their role in the CNS has a marked effect in the brain parenchyma and may increase the susceptibility to neurodegenerative disorders, such as Alzheimer’s disease, Parkinson’s disease and multiple sclerosis.

**Microglia, neurobiology, endogenous repair in the adult CNS and clinical applications**

**Microglia-synapse interaction in the adult brain**

Since Kreutzberg popularized the facial axotomy model, it has been observed that microglia associate with injured nerve perikarya during synaptic stripping (Kreutzberg, 1966; Blinzinger and Kreutzberg, 1968). Although some have described a potential role for glia-derived ATP or astrocytes in synaptic stripping of motor neurons (Svensson and Aldskogius, 1993; Linda et al., 2000; Yamada et al., 2008), others favour a model involving the direct apposition of microglia with neuronal perikarya and apical dendrites (Blinzinger and Kreutzberg, 1968; Graeb et al., 1993; Cullheim and Thams, 2007; Trapp et al., 2007). Interestingly, the fate of particular synapse terminals seems to depend on the type of input, since Linda et al. (2000) found that GABA and glycine terminals were preferentially preserved after motor neuron axotomy, although both excitatory and inhibitory inputs were drastically reduced overall. This may support a transition from information processing towards a mode of survival and repair, protecting injured neurons from over-activity and the secondary effects of excitotoxic molecules, thus helping recovery from injury. Candidate molecular mechanistic targets for the stripping response include the CX3CR1 signalling axis and MHC class I molecules as reviewed in Cullheim and Thams (2007). Using two-photon imaging strategies in which microglia and neurons are fluorescently labeled, it has been shown that under basal conditions microglia survey their microenvironment for signals and clearance of debris using highly dynamic processes (Davalos et al., 2005; Nimmerjahn et al., 2005). Wake et al. (2009) demonstrated that basal microglia processes make brief and direct contact with synaptic terminals at a rate of approximately once per hour. The frequency of these homeostatic contacts is
activity-dependent and is reduced concomitantly with neuronal activity. In that study, ischaemic insult increased the duration of contact drastically, and was associated with a disappearance of the presynaptic bouton due to engulfment by microglia. Whether this activity is absolutely required for elements of synaptic homeostasis is still unknown, but the mechanisms of this activity are now becoming clearer.

It is conceivable that microglia could engage in crosstalk with neurons and release neurotrophins (Elkabes et al., 1996; Kohsaka et al., 1996; Morgan et al., 2004) or other signalling factors that can regulate synaptic structure and transmission, thereby engaging in modulating synaptic homeostasis. Microglia have a myriad of receptors that can respond to secreted neuronal products and possess the ability to modulate neuronal functions, supporting a bidirectional model of communication (Kettenmann et al., 2011). Brain-derived neurotrophic factor (BDNF) derived from microglia was shown to modulate the neuronal anion gradient underlying neuropathic pain, and directly regulate synaptic function (Coull et al., 2005). Parkhurst et al. (2013) recently and definitively showed that microglia depletion in the otherwise healthy rodent brain induced deficiencies in multiple learning tasks and a specific reduction in motor-learning dependent synapse formation. This was largely regulated by microglial BDNF and reproduced in microglia-depleted rodents. In the context of Alzheimer’s disease, it was shown that amyloid-β stimulation of NOS2 and reactive oxygen species production in microglia inhibited NMDA-receptor dependent long-term potentiation and provides further evidence for a diverse array of neuronal and synaptic activities that can be modulated by microglia (Wang et al., 2004). Furthermore, the neuroimmune interface with classical neurobiological activities is not reserved for microglia alone, as they have been shown to regulate hippocampal neurogenesis and maintain spatial learning abilities in adulthood through interactions with T cells (Ziv et al., 2006).

Neuronal repair in the adult CNS: the central role of microglia

In the adult CNS, microglia are attractive candidate therapeutic cellular targets for augmenting brain repair. In theory, this involves investigating a means to reduce microglia-induced damage while also boosting pro-neurogenic and/or oligodendrogenic mechanisms that are known to exist in adult microglia. Given the several known mechanisms by which microglia are directly involved in promoting neuroinflammatory damage, we will not detail these mechanisms in this review; for reviews, see (Barres, 2008; Perry et al., 2010; Saijo and Glass, 2011; Rawji and Yong, 2012; Xanthos and Sandkuhler, 2014).

In vitro, LPS-activated microglia blocks neurogenesis in organotypic hippocampal slice cultures; microglia activated by IL4 and IFNγ stimulate neurogenesis. Butovsky et al. attributed the blocking activity of LPS-activated microglia to TNF, whereas the augmenting effect of IL4 and IFNγ was attributed to insulin-like growth factor 1 (IGF1) (Butovsky et al., 2005). In a rat model, microglia were found to be pro-neurogenic to striatal neuroblasts derived from the ipsilateral subventricular zone following ischaemia (Thored et al., 2009). Using allogeneic co-cultures of human microglia and neural progenitor cells, Liu et al. (2013) showed that co-cultures had higher survival and proliferation rates of both neural progenitor cells and microglia compared to monocultures. Despite evidence that microglia have pro-neurogenic activity (Aarum et al., 2003; Battista et al., 2006), some groups propose that microglia also possess anti-neurogenic activities (Ekdahl et al., 2003; Monje et al., 2003; Goings et al., 2006), highlighting the dual role of microglia during adult neurogenesis, reviewed in Ekdahl et al. (2009).

Oligodendrocyte precursor cell differentiation can also be influenced by microglia, making microglia attractive targets for augmenting repair in the context of demyelinating diseases. In vitro, Butovsky et al. (2005) demonstrated the differential effects of IL4 and IFNγ treated microglia towards augmenting oligodendrogenesis. In a follow-up study, elevated IFNγ conferred a phenotype that blocked oligodendrogenesis with an associated increase in TNF and decrease in IGF1. This phenotype could be reversed by IL4, whereby IL4 activated microglia injected into the CSF of EAE mice improved clinical symptoms and increased oligodendrogenesis in the spinal cord (Butovsky et al., 2006). In a human oligodendrocyte precursor cell model, supernatants derived from LPS and IFNγ-stimulated human microglia (M1) significantly decreased oligodendrocyte precursor cell differentiation; IL4 and IL13 simulated microglia (i.e. M2a) did not exhibit an effect (Moore et al., 2015). Another line of evidence that activated microglia influence neural progenitor cell and oligodendrocyte precursor cell differentiation includes ischaemia studies where activated microglia secretory products influenced neurosphere differentiation with a bias towards oligodendrocytes and neurons (Deierborg et al., 2010). Miron et al. (2013) note that M2 cells drive oligodendrocyte differentiation during CNS remyelination in a focal demyelination model. In human gliomas, Sarkar et al. (2014) found that brain-tumour initiating cells sphere-forming capacity was markedly reduced by microglia and macrophages derived from non-glioma human subjects and involved the induction of cell cycle arrest and differentiation genes. The authors demonstrated that low concentrations of amphotericin B increased glioma-derived microglial production of IL8 and CCL2 (previously known as MCP-1), which curbed autologous brain-tumour initiating cell growth (Sarkar et al., 2014).

Another interesting prospect is the modulation of phagocytotic properties in microglia, which are often beneficial in models of disease and injury. The surface receptor triggering receptor expressed on myeloid cells-2 (TREM2) is expressed by microglia and often associated with an anti-inflammatory phenotype (Colonna, 2003;
Klesney-Tait et al., 2006). Upon treatment with LPS and IFNγ, TREM2 expression is decreased (Schmid et al., 2002). When overexpressed in microglia, TREM2 increased the capacity to phagocytose apoptotic cell membranes; decreased TREM2 impaired clearance of apoptotic neurons, and increased TNF and NOS2 (Takahashi et al., 2005). In EAE, TREM2 transduced myeloid cells ameliorated disease course by increasing phagocytic activity and inducing an anti-inflammatory cytokine milieu (Takahashi et al., 2007). In humans, mutations in TREM2 and its adaptor DAP-12 (now known as TYROBP) lead to abnormalities in TREM2 function, and are associated with Nasu-Hakola disease, which is characterized by frontal lobe dementia and bone cysts (Paloneva et al., 2002). A rare variant of TREM2 has also been demonstrated to increase susceptibility to late onset Alzheimer’s disease (Jones, 2013). By contrast, in Alzheimer’s disease brains, the expression pattern of TREM2 is partially associated with amyloid deposition (Jiang et al., 2013); the mRNA for TYROBP is augmented in tissue plaques (Frank et al., 2008). In the CSF of patients with multiple sclerosis, increased soluble forms of TREM2 have been also observed (Piccio et al., 2008).

Similar to TREM2, SIRP-1α (previously known as CD172a) is expressed by microglia and involved in phagocytosis. The interaction between SIRP-1α and its ligand CD47, which is expressed by neurons and astrocytes, inhibits phagocytosis and delivers a ‘do not eat me signal’ to microglia (van Beek et al., 2005; Biber et al., 2007). This interaction has clinical relevance and has been implicated in multiple sclerosis pathophysiology. Indeed, the upregulation of several microRNAs, including miR-34a, miR-155 and miR-326 within multiple sclerosis lesions, has been suggested to decrease CD47 expression on astrocytes, hence promoting phagocytosis by microglia (Junker et al., 2009). Together, these studies highlight that in the adult brain, microglia can regulate oligodendrocyte precursor cell and neural progenitor cell proliferation and differentiation, a characteristic that can be harnessed for therapeutic application.

**Quantifying microglia in vivo using PET imaging**

PET is a molecular imaging technique able to quantify a molecular target in vivo. PET imaging requires the availability of a radiolabelled ligand (radioligand) to bind the target in question with only minimal non-specific binding. The vast majority of PET radioligands used in neuroscience research target neurotransmitter receptors, particularly dopamine and 5-HT receptors. Currently, the 18 kDa translocator protein (TSPO) is the only microglial target for which radioligands are available for clinical use. TSPO is richly expressed in microglia and localised mainly to the outer mitochondrial membrane. Various in vitro studies using post mortem human brain tissue have shown increased TSPO density (measured by specific binding of the TSPO ligand; Banati et al., 2000) in diseases characterized by microglial infiltration. These include studies in multiple sclerosis (Banati et al., 2000), Alzheimer’s disease (Diorio et al., 1991; Venneti et al., 2008; Gulyas et al., 2009), Huntington’s disease (Messmer and Reynolds, 1998), stroke (Venneti et al., 2008), frontotemporal dementia (Venneti et al., 2008), and ALS (Sitte et al., 2001; Venneti et al., 2008).

**The limitations of using PET imaging to quantify microglia density**

Although the predominant source of TSPO expression in the brain is thought to represent microglia, TSPO is expressed in other myeloid cells, including macrophages. For diseases involving blood–brain barrier disruption and/or monocyte recruitment, PET imaging is unable to extract the microglial component of the myeloid signal. Second, astrocytes can also express TSPO under certain conditions in culture (Itzhak et al., 1993) and in animal models (Maeda et al., 2007; Ji et al., 2008). Nevertheless, a substantial autoradiographical literature demonstrates that TSPO expression follows the spatiotemporal pattern of microglial activation (Dubois et al., 1988; Myers et al., 1991; Shah et al., 1994; Stephenson et al., 1995; Conway et al., 1998; Banati et al., 2000; Raghavendra Rao et al., 2000). Hence, it is generally accepted that microglial cells are responsible for the majority of TSPO expression in CNS diseases.

Currently, the mechanisms responsible for increased TSPO signal are not well understood. It is unclear whether the increase in TSPO signal results from increased cell and/or mitochondrial number, or an actual increase in protein expression within mitochondria. Although in vitro experiments in Leydig cells (Rey et al., 2000) and pancreatic islet cells (Trincavelli et al., 2002) show cellular increases in TSPO mRNA in response to activation by cytokines, no data is available for microglia. Furthermore, the relationship between microglial phenotype and TSPO expression is poorly understood. It is unknown whether PET signal reflects an increase in cell number or a change in cell phenotype.

**Clinical TSPO ligands**

$^{11}$C-PK11195 was first used in humans in 1986 (Charbonneau et al., 1986) and until recently, was the only available TSPO radioligand. Unfortunately, quantification of the $^{11}$C-PK11195 signal is complicated by various technical factors, including its high non-specific binding and its adherence to plastic and glass, preventing accurate plasma measurements that are required for signal modelling. In recent years there has been considerable interest in developing new generation TSPO radioligands to overcome these problems.
Hundreds of potential new generation tracers have been synthesized (Chauveau et al., 2008), however, only a few have passed the physiochemical hurdles required to reach clinical evaluation. Early clinical studies with second generation ligands demonstrated the technical improvements over 11C-PK11195, but were compromised by the existence of a polymorphism in the coding region of the gene encoding TSPO, which dramatically alters binding affinity (Owen et al., 2010, 2012). This polymorphism, rs6971, causes an amino acid substitution (Ala147Thr) that reduced the binding affinity of second generation ligands to TSPO in subjects expressing 147Thr (either as a homozygote or a heterozygote) relative to Ala-Ala wild-types. The specific PET signal is determined by both target expression and binding affinity of the radioligand for the target. Therefore, for a given level of TSPO expression, Thr-Thr and Ala-Thr subjects will have a misleadingly low PET signal relative to wild-types. Alanine 147 is highly conserved across species; however, the mutation is only found in humans and was not detected in preclinical development. Current studies now involve genotyping at rs6971 and either enrol Ala-Ala subjects only, or enrol all subjects but correct the data for genotype. Third generation radioligands are now being developed, which have low sensitivity to the polymorphism (Brouwer et al., 2014).

The future directions of PET ligands and CNS research

With the variability caused by the rs6971 polymorphism now understood, PET studies with second generation ligands are in a position to start delivering valuable insights into mechanisms of CNS disease in vivo. Ongoing in vitro studies into the relationship between microglial phenotype and binding of TSPO ligands will facilitate our understanding of the molecular mechanisms underlying the change in PET signal. In the future, more specific ligands targeting TSPO in the myeloid cell population are likely to become available. New cell surface targets identified in recent studies that distinguish microglia from macrophages (Butovsky et al., 2014) should be amenable to PET and offer the potential to dissect the microglial and macrophage component of the overall myeloid cell signal. Finally, it is likely that ligands will be available to identify specific myeloid cell phenotypes, and will be invaluable in investigating the immunomodulatory effect of novel pharmaceuticals. New generation PET scanners are currently being constructed with much larger gantries. This will markedly improve the sensitivity of the technique, allowing for administering lower doses of radioligand, and more scans in the same patient before reaching radiation safety limits.

Conclusions

This comprehensive review summarizes and emphasizes the importance of microglia in overall brain function. Microglia are dynamic surveyors of their milieu, informing and responding to neurobiological functions intrinsic to CNS development, homeostasis, ageing, and injury. Exciting new pharmacological agents targeting not only the deleterious functions of microglia, but also mechanisms which promote endogenous repair will likely soon be a reality. Furthermore, the development of novel PET ligands for microglia will provide insight into in vivo biology of human microglia, providing further correlates to animal data. Overall, future studies will facilitate clinical advancement and interrogation of the microglial role in many aspects of nervous system function.

Supplementary material

Supplementary material is available at Brain online.

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