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UPDATE

Immunosenescence of microglia and macrophages: impact on the ageing central nervous system

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Ageing of the central nervous system results in a loss of both grey and white matter, leading to cognitive decline. Additional injury to both the grey and white matter is documented in many neurological disorders with ageing, including Alzheimer's disease, traumatic brain and spinal cord injury, stroke, and multiple sclerosis. Accompanying neuronal and glial damage is an inflammatory response consisting of activated macrophages and microglia, innate immune cells demonstrated to be both beneficial and detrimental in neurological repair. This article will propose the following: (i) infiltrating macrophages age differently from central nervous system-intrinsic microglia; (ii) several mechanisms underlie the differential ageing process of these two distinct cell types; and (iii) therapeutic strategies that selectively target these diverse mechanisms may rejuvenate macrophages and microglia for repair in the ageing central nervous system. Most responses of macrophages are diminished with senescence, but activated microglia increase their expression of pro-inflammatory cytokines while diminishing chemotactic and phagocytic activities. The senescence of macrophages and microglia has a negative impact on several neurological diseases, and the mechanisms underlying their age-dependent phenotypic changes vary from extrinsic microenvironmental changes to intrinsic changes in genomic integrity. We discuss the negative effects of age on neurological diseases, examine the response of senescent macrophages and microglia in these conditions, and propose a theoretical framework of therapeutic strategies that target the different mechanisms contributing to the ageing phenotype in these two distinct cell types. Rejuvenation of ageing macrophage/microglia may preserve neurological integrity and promote regeneration in the ageing central nervous system.

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Abbreviations: HSC = hematopoietic stem cell; IL = interleukin; TLR = Toll-like receptor

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Introduction

Grey and white matter injury is common in many neurological diseases and numerous studies have demonstrated a decreased capacity for neurological repair with ageing (Sacco, 1997; Dai, 2001; Blasko *et al.*, 2004; Marquez de la Plata *et al.*, 2008; Goldschmidt *et al.*, 2009). Major components of the inflammatory response accompanying neurological disorders are microglia and macrophages, innate immune cells important for CNS regeneration. These cells undergo senescence in distinct ways, negatively impacting the degenerative and repair response in the ageing CNS (Shaw *et al.*, 2013). Studies using transcriptome profiling and genetic strategies have demonstrated important differences in gene expression and function between CNS-resident microglia and peripheral macrophage populations during health as well as injury (Butovsky *et al.*, 2014; Gosselin *et al.*, 2014; Greenhalgh and David, 2014; Lavin *et al.*, 2014; Yamasaki *et al.*, 2014; Shemer *et al.*, 2015). Understanding how the ageing process affects these different cell types should reveal important insights into potential mechanistic targets that can be harnessed for therapeutic attenuation of neurodegenerative processes as well as enhancement of reparative activities in the ageing CNS. Furthermore, as many potential pharmacological agents may not be able to penetrate the CNS, and monocytes in different inflammatory states may have divergent routes of entry into the CNS, understanding how ageing affects peripherally-derived monocytes differently from CNS-resident microglia should help direct systemic therapeutics for rejuvenation of ageing monocytes to repair the ageing CNS (Shechter *et al.*, 2013). This article will discuss the evidence that CNS-intrinsic microglia age differently from peripherally-derived macrophages. Mechanisms potentially explaining the divergent effects of ageing on these cell types will be presented. Finally, a theoretical framework is proposed on how best to rejuvenate microglia and macrophages for repair of the ageing CNS.

Microglia and macrophages

Genesis of cells

Microglia and macrophages are two distinct myeloid populations with separate developmental origins (Ginhoux *et al.*, 2010; Schulz *et al.*, 2012; Kierdorf *et al.*, 2013). In mice, microglia derive from erythromyeloid progenitors in the foetal yolk sac prior to embryonic Day 8 and then migrate to the developing CNS by embryonic Day 9.5. Macrophages derive from extravasated monocytes that are produced from erythromyeloid progenitors initially in the aorta-gonad-mesonephros at embryonic Day 10.5 and then in the foetal liver at embryonic Day 12.5 (Perdiguero *et al.*, 2015; Prinz and Priller, 2014). Postnatally, monocytes are produced from hematopoietic stem cells (HSCs) in the bone marrow, which then circulate in the blood and differentiate into macrophages following extravasation into tissues (Prinz and Priller, 2014).

Activation of microglia and macrophages

Microglia are the resident immune cells of the CNS and are thought to be self-sustaining throughout adulthood (Ajami *et al.*, 2007; Bruttger *et al.*, 2015). In the healthy, uninjured CNS, microglia have a ramified morphology and are maintained in a quiescent state through transforming growth factor-β and inhibitory ligand-receptor interactions with neurons, astrocytes, and oligodendrocytes (Perry and Holmes, 2014). Despite this relatively calm state, microglia are constantly extending and retracting their processes, surveying the surrounding parenchyma for any abnormalities (Nimmerjahn *et al.*, 2005). Microglia and infiltrating monocytes express pattern recognition receptors which detect molecules that are released by injured CNS cells or that are inherent on the surfaces of invading pathogens. Detection of these molecules induces pro-inflammatory signalling cascades, stimulating microglia and infiltrating monocytes to adopt an activated phenotype that displays an amoeboid morphology with an enlarged cytoplasm. The activated microglia and differentiated monocyte-derived macrophages upregulate many pro-inflammatory markers, secrete a variety of pro-inflammatory cytokines, and express many molecules important for antigen presentation (Prinz and Priller, 2014).

Upon injury to the CNS, activated microglia and infiltrating monocyte-derived macrophages appear phenotypically similar, possessing many of the same cell surface receptors and appearing amoeboid in morphology. Due to this phenotypic similarity after injury, many researchers have grouped these distinct cell types as macrophages/microglia (Rawji and Yong, 2013). However, these two cell types have different genetic and transcriptomic signatures, suggesting different functions of these cells within the CNS injury site (Butovsky *et al.*, 2014; Gosselin *et al.*, 2014; Lavin *et al.*, 2014). Furthermore, attempts using bone marrow and parabiosis chimeras, as well as novel transgenic mice, have uncovered different roles that these two cell types perform following CNS injury (Ajami *et al.*, 2011; Greenhalgh and David, 2014; Yamasaki *et al.*, 2014). Despite these significant advances, this review will refer to these two cell types as macrophages/microglia as methodological limitations of previous studies have not permitted their separate identification.

Microglia and macrophage subsets

The activation status of macrophages/microglia have been classified under two broad phenotypic states, termed M1 and M2, where M1 cells are generally associated with the secretion of pro-inflammatory cytokines, and M2 cells with the production of regulatory or anti-inflammatory cytokines (Martinez *et al.*, 2008). It is now appreciated, however, that the M1/M2 classification is insufficient to address the spectrum of macrophage programmes that arise upon activation

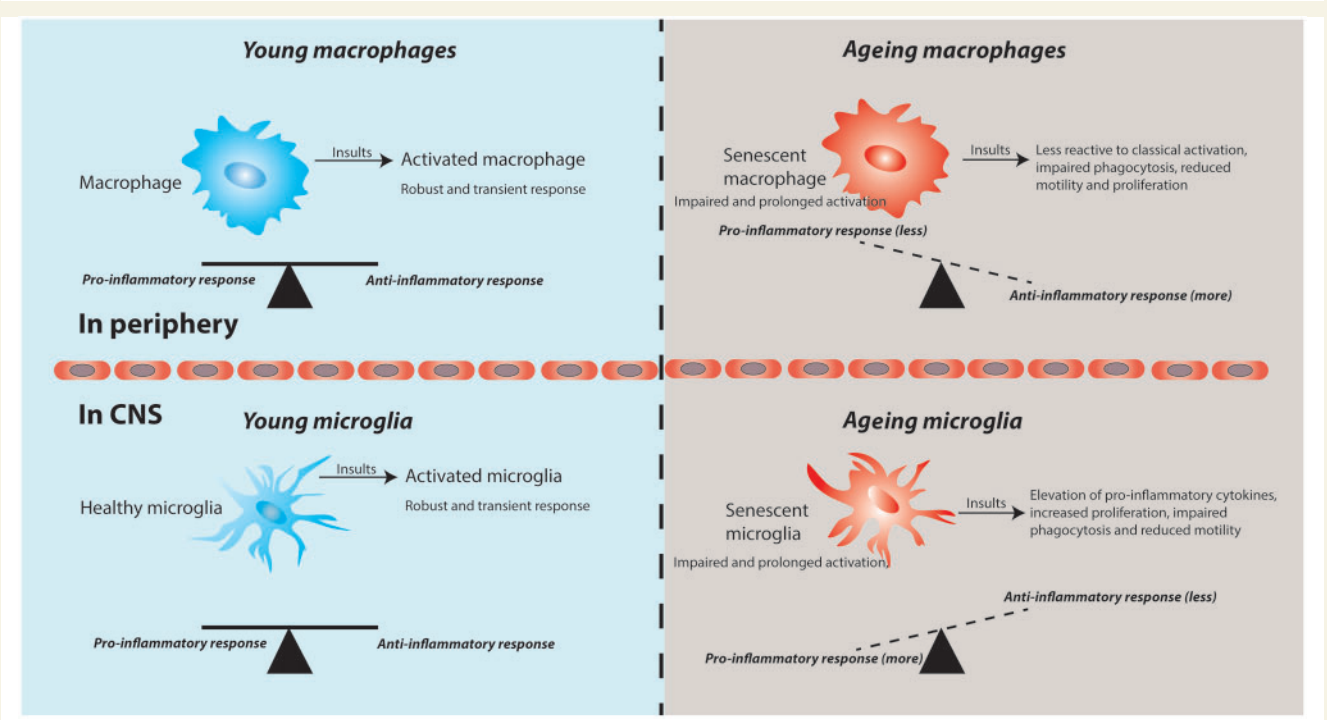

Figure 1 Phenotypic changes associated with macrophages and microglia during normal ageing. During ageing, senescent macrophages have decreased pro-inflammatory cytokine secretion, impaired phagocytosis and chemotaxis, and decreased proliferation. In contrast, senescent microglia display an elevation in pro-inflammatory cytokines and proliferative capacity, while showing a deficit in phagocytosis and motility.

by different stimuli (Martinez and Gordon, 2014); remarkably, at least nine distinct subsets of activated macrophages have been proposed based on transcriptomic signatures (Xue *et al.*, 2014). Thus, while some of the reports below address senescent macrophages/microglia in terms of so-called M1 or M2 polarization, we will refer to these cells as pro-inflammatory and regulatory, respectively.

Epigenetic regulation of global gene expression, in addition to a complex array of transcription factors, signalling pathways, and post-transcriptional regulators, are thought to underlie the diversity of macrophage plasticity not only after infection or tissue injury, but also within different tissue environments (Lawrence and Natoli, 2011; Butovsky *et al.*, 2014; Gosselin *et al.*, 2014; Lavin *et al.*, 2014). It was recently shown that in response to different tissue environments, the macrophage lineage-determining factor PU.1 interacts with diverse secondary transcription factors to induce a tissue-specific enhancer landscape (Gosselin *et al.*, 2014). In addition, microglial function is regulated by the host microbiota (Erny *et al.*, 2015), introducing further complexity to the diversity of macrophages/microglia.

Phenotype accompanying macrophage and microglia senescence

While both macrophages and microglia display diminished phagocytosis and chemotaxis with ageing, the effects of age manifest differently with regards to their pro-inflammatory status. With ageing, macrophages are less able to produce a functional pro-inflammatory response (Shaw *et al.*, 2013). Microglia, on the other hand, exhibit an exaggerated pro-inflammatory response, a phenomenon referred to as microglia priming (Perry and Holmes, 2014; Michell-Robinson *et al.*, 2015). This section will discuss the phenotypic changes that occur in macrophages and microglia with ageing (Fig. 1).

Both ageing microglia and macrophages exhibit deficits in phagocytic and chemotactic functions. In studies examining the capability for microglia to engulf amyloid-β fibrils, microglia isolated from ageing but not young mice lost the ability for phagocytosis (Floden and Combs, 2011; Njie *et al.*, 2012). In a model of focal white matter demyelination, the age-associated delay in remyelination is associated with impaired clearance of inhibitory myelin debris, accompanied by alteration of the retinoid X receptor pathway in macrophages (Kotter *et al.*, 2006; Ruckh *et al.*, 2012; Natrajan *et al.*, 2015). When young macrophages are introduced to the ageing demyelinated CNS, clearance of myelin debris and remyelination is enhanced. In addition to reduced phagocytic activity, both ageing microglia and macrophages have deficits in chemotaxis. Ageing microglia display decreased process motility and cellular migration in response to laser-induced injury and extracellular ATP, when compared to young microglia (Damani *et al.*, 2011). In a demyelinating model, the age-related decrease in remyelination is associated with a reduction in macrophage/microglia recruitment (Zhao *et al.*, 2006).

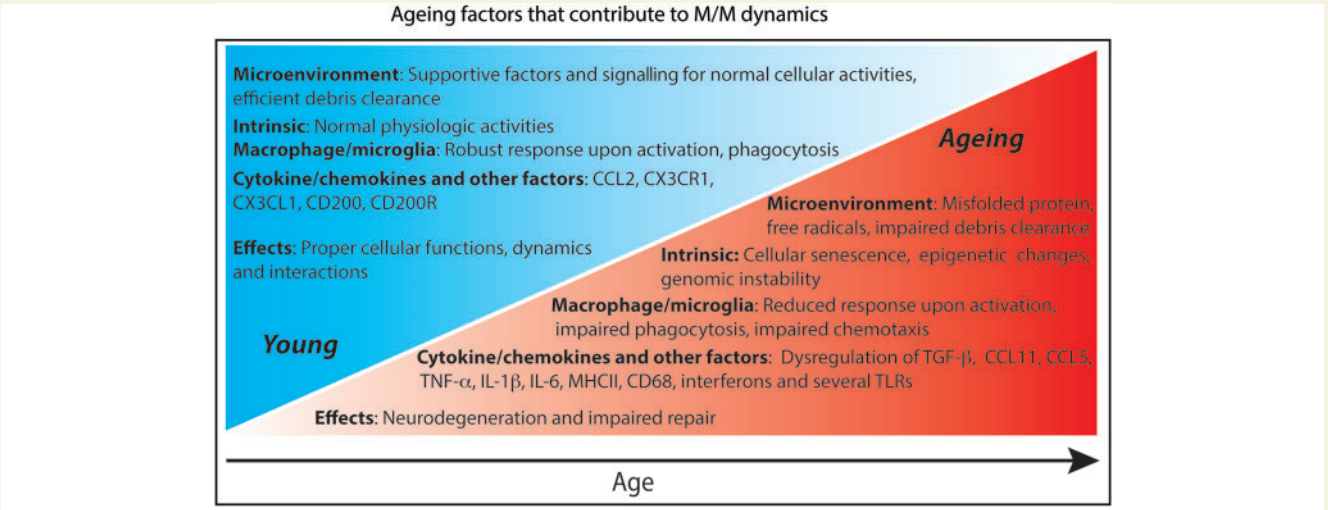

Figure 2 Factors associated with ageing that negatively impact CNS regeneration. During normal ageing, changes in HSCs, macrophages, microglia, systemic peripheral and central factors, as well as shifts in the microenvironment composition may all contribute to the increased neurodegeneration and decreased repair observed in CNS injury in ageing subjects. CX3CR1 = chemokine (C-X3-C motif) receptor 1; CX3CL1 = chemokine (C-X3-C motif) ligand 1; CD200 = cluster of differentiation 200; CD200R = cluster of differentiation 200 receptor; TGF-β = transforming growth factor β; TNF-α = tumour necrosis factor α; MHCII = major histocompatibility complex II; CD68 = cluster of differentiation 68.

Though ageing microglia and macrophages both have deficits in phagocytosis and chemotaxis, ageing macrophages seem to be less able to produce a functional pro-inflammatory response, whereas ageing microglia exhibit an exaggerated pro-inflammatory response. When stimulated with strong activating agents such as the TLR4 agonist, lipopolysaccharide, or the TLR2 agonist, zymosan, ageing macrophages significantly decreased their production of the pro-inflammatory cytokines TNF-α and IL-6 when compared to young macrophages (Boehmer *et al.*, 2004, 2005). This group, in addition to other groups, associated these observations with decreased TLR4 expression and reduced activation of NF-κB (Boehmer *et al.*, 2005; Chelvarajan *et al.*, 2005). In human monocytes isolated from elderly patients, diminished pro-inflammatory cytokine production was observed when cells were stimulated with Toll-like receptor (TLR) ligands, correlating with decreased TLR1 surface expression (Gon *et al.*, 1996; van Duin *et al.*, 2007). In contrast, several studies found an increased propensity for ageing microglia to initiate a stronger response to an inflammatory stimulus. Upon peripheral injection of lipopolysaccharide, ageing mice had a pronounced increase in pro-inflammatory cytokines and microglial activity in the CNS compared to young mice (Godbout *et al.*, 2005; Sierra *et al.*, 2007). In addition, *ex vivo* cultures of microglia from ageing mice increased their basal and stimulated secretion of TNF-α and IL-6 relative to microglia from young mice (Njie *et al.*, 2012). Morphologically, ageing microglia have enlarged processes, cytoplasmic hypertrophy, and a less ramified appearance (Sheng *et al.*, 1998; Damani *et al.*, 2011; Tremblay *et al.*, 2012). Moreover, there is elevated immunoreactivity for markers of activation, including IL-1 and major histocompatibility complex II (Sheng *et al.*, 1998; Conde and Streit, 2006).

Though difficult to distinguish between peripherally-derived macrophages and CNS-resident microglia, *in vivo* studies show that the capacity for ageing macrophages and microglia to adopt a regulatory phenotype is compromised with ageing. In a model of spinal cord injury, ageing macrophages and microglia are impaired in the induction of IL-4 receptor α, an important receptor in stimulating a regulatory phenotype (Fenn *et al.*, 2014). This observation was associated with impaired recovery. In a model of cortical traumatic brain injury, ageing mice display an exaggerated pro-inflammatory macrophage/microglia response along with increased lesion size (Kumar *et al.*, 2013). Another study found that the impaired remyelination in ageing mice was associated with a decrease in regulatory macrophages and microglia (Miron *et al.*, 2013). Rejuvenation of remyelination in ageing mice correlated with an increase in regulatory macrophages and microglia.

Mechanisms underlying microglial and macrophage senescence

As discussed, ageing microglia show a propensity to increase pro-inflammatory cytokines, but are deficient in phagocytosis and chemotaxis. There are several possible mechanisms that may underlie this ageing phenotype (Fig. 2). First, as neurons become damaged with age, there is a loss of inhibitory ligand-receptor interactions with microglia (Perry and Holmes, 2014). Second, misfolded proteins such as amyloid-β accumulate during normal ageing; amyloid-β has been found to elevate pro-inflammatory cytokines in microglia (Flanary *et al.*, 2007; Perry and Holmes, 2014). Third, the increase of

transforming growth factor-β expression with age, and chronic exposure of microglia to this cytokine, may impair the capacity of microglia to secrete anti-inflammatory cytokines (Doyle *et al.*, 2010; Cohen *et al.*, 2014). This transforming growth factor-β-mediated impairment is attributed to a downregulation of interferon regulatory factor-7, a transcription factor important in switching microglia from a pro-inflammatory to an anti-inflammatory phenotype (Cohen *et al.*, 2014). In addition, it was found that the anti-inflammatory cytokine IL-4 was elevated at the choroid plexus with ageing (Baruch *et al.*, 2013); IL-4 is thought to induce the choroid plexus epithelial cells to produce high levels of CCL11, which may skew microglia to a more pro-inflammatory state (Villeda *et al.*, 2011; Baruch and Schwartz, 2013; Schwartz *et al.*, 2013). The same group further demonstrated that the choroid plexus of ageing mice have an increased type I interferon response, and that neutralization of this response not only restored CCL11 levels, but also decreased age-related chronic neuroinflammation (Baruch *et al.*, 2014). In addition to these changes in the ageing CNS microenvironment, differences in the localization of injury may also influence the ageing microglia response. Microglia responding to injuries in the white matter may differ from injuries predominantly present in the grey matter. In studies in which controlled cortical impact injuries were inflicted to the hippocampus and thalamus, ageing rodents have an increased macrophage/microglia response compared to young rodents (Sandhir *et al.*, 2008; Kumar *et al.*, 2013). In contrast, in a study in which focal demyelination was induced in a white matter tract, ageing rodents have a decreased macrophage/microglia response (Zhao *et al.*, 2006).

In leukocortical multiple sclerosis lesions, there is an enhanced density of macrophages/microglia in the white matter compared to the adjacent grey matter (Peterson *et al.*, 2001). Reasons for these differences are unknown, but may be a function of the microenvironment, such as the presence of the extracellular matrix molecules versican and hyaluronan, that are predominantly present in white matter lesions (Chang *et al.*, 2012) and which may affect the functions of myeloid antigen-presenting cells. This is an interesting area requiring further study.

In contrast to microglia, ageing monocyte-derived macrophages appear deficient in elevating pro-inflammatory cytokines, and are impaired in phagocytic and chemotactic responses. As circulating monocytes have a half-life of ~71 h in humans, it is conceivable that the mechanisms underlying the senescent phenotype arise at early stages in monocyte development (Whitelaw, 1972). Indeed, HSCs undergo senescence and have decreased regenerative potential (Florian *et al.*, 2012; Geiger *et al.*, 2013). Studies suggest that senescence of HSCs, and thus by extension, monocytes, is influenced mainly by intrinsic factors and partially by extrinsic factors (Geiger *et al.*, 2013). Examples of intrinsic features include telomere shortening, epigenetic changes, and accumulation of DNA damage (Rube *et al.*, 2011; Beerman *et al.*, 2013). When ageing HSCs are transplanted into young recipient mice, there is retention of the senescent phenotype, indicating the influence intrinsic changes have on the HSCs (Geiger *et al.*, 2013). Genes important in maintaining the integrity of the genome are among 1600 genes that are downregulated in ageing HSCs, suggesting that loss of genomic preservation underlies HSC ageing (Chambers *et al.*, 2007). Furthermore, numerous cell cycle regulatory proteins become dysregulated in ageing HSCs, providing a possible explanation underlying the decreased self-renewal and the loss of cell polarity witnessed in ageing HSCs (Janzen *et al.*, 2006; Florian *et al.*, 2012; Geiger *et al.*, 2013).

Though HSC ageing is thought to be mainly influenced by intrinsic factors, there is evidence emerging to suggest that extrinsic factors in the microenvironment also play a role. For example, the ageing microenvironment contains high levels of CCL5, which stimulate an increase in myeloid progenitors and a decrease in lymphoid progenitors, classical hallmarks of HSC senescence (Ergen *et al.*, 2012). Another study using heterochronic parabiosis demonstrated the capability of a youthful circulation to reverse characteristics of ageing HSCs, emphasizing the influence of systemic factors on HSC ageing (Mayack *et al.*, 2010). Altogether, it appears that the many phenotypic changes of monocytes with ageing are likely initiated at earlier stages of hematopoiesis (Fig. 2).

Strategies to rejuvenate macrophages/microglia for CNS regeneration

Promoting ageing microglial phagocytosis and chemotaxis whilst reducing pro-inflammatory cytokine levels

As ageing microglia produce more pro-inflammatory cytokines, agents that penetrate into the CNS to inhibit microglia, such as minocycline or laquinimod, may be beneficial in tempering an excessive pro-inflammatory response (Fan *et al.*, 2007; Kobayashi *et al.*, 2013; Samanani *et al.*, 2013; Mishra *et al.*, 2014). This approach may only be partially effective, however, as beneficial effects of microglia such as amyloid-β phagocytosis may also be hindered. Thus, a more desired therapy is one that preferentially enhances phagocytosis and chemotaxis, but not excessive pro-inflammatory cytokine secretion. Such an agent could be monophosphoryl lipid A, a modified form of lipopolysaccharide that stimulates mainly the TRIF (TIR-domain-containing adapter-inducing interferon-β) and not the more pro-inflammatory MyD88 pathway downstream of TLR4 (Mata-Haro *et al.*, 2007). Our group administered monophosphoryl lipid A in an Alzheimer's disease mouse model and found increased microglial phagocytosis, reduced amyloid-β load and improved clinical outcome (Michaud *et al.*, 2013). Another group pharmacologically upregulated class A1 scavenger receptors on mononuclear phagocytes, resulting in increased amyloid-β clearance in an Alzheimer's disease mouse model (Frenkel *et al.*, 2013). This approach of stimulating microglial

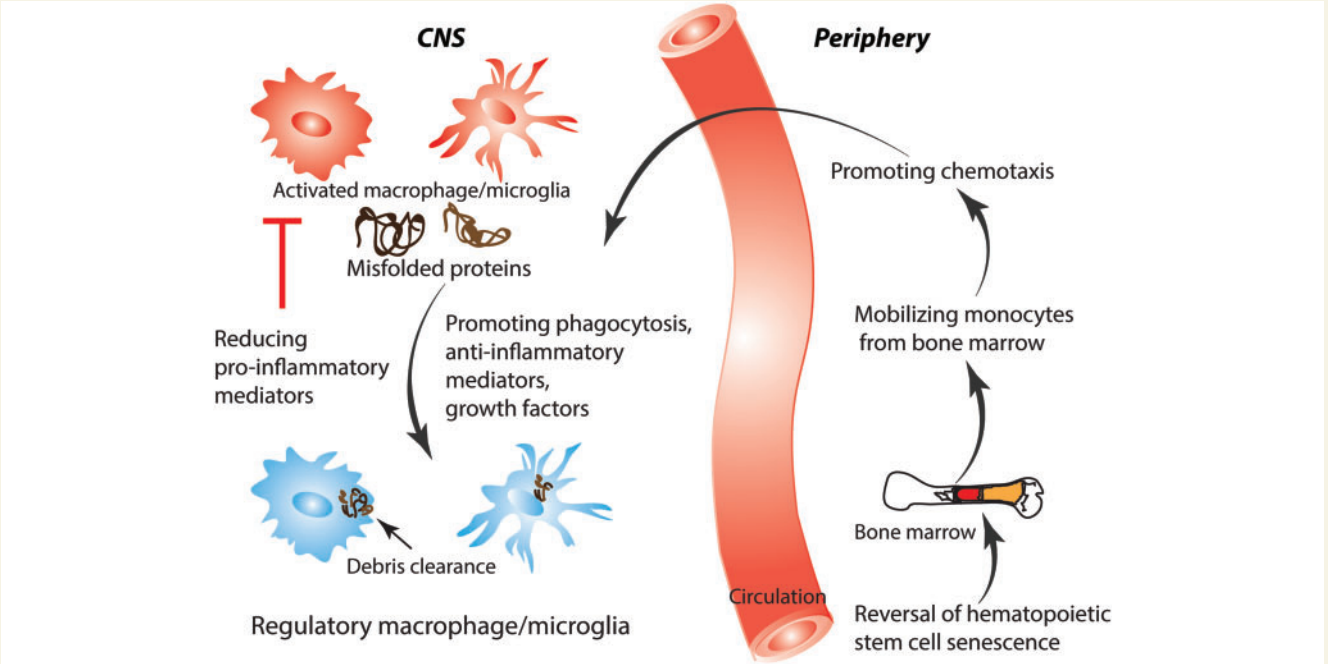

Figure 3 Therapeutic manipulation of ageing macrophages and microglia. As monocyte-derived macrophages age differently from CNS-intrinsic microglia, different therapeutic strategies are required to harness the benefits of these cells for CNS repair. Therapies that reverse HSC senescence, increase monocyte chemotaxis, enhance microglia and macrophage phagocytosis, generate a regulatory macrophage/microglia phenotype, and reduce toxic pro-inflammatory mediators are promising for repair of the injured and ageing CNS.

phagocytosis but not pro-inflammatory cytokine secretion would conceivably be cytoprotective in many injuries in the ageing CNS. Moreover, stimulating microglia to phagocytose inhibitory myelin debris without an excessive pro-inflammatory cytokine response could enhance axon regeneration and remyelination. Several groups have also examined strategies to enhance a more regulatory microglial phenotype (Yamanaka *et al.*, 2012; Cohen *et al.*, 2014; Wang *et al.*, 2015). Such treatments target transcriptional regulators important in promoting a regulatory microglia phenotype, such as upregulation of interferon regulatory factor-7 or activation of peroxisome proliferator-activated receptor γ (Yamanaka *et al.*, 2012; Cohen *et al.*, 2014). Strategies in which a regulatory phenotype is promoted is promising as beneficial functions such as phagocytosis would be enhanced, while detrimental functions such as production of toxic pro-inflammatory molecules would be reduced (Fig. 3 and Table 1).

Several heterochronic parabiosis studies have now demonstrated the negative impact that the ageing systemic circulation has on muscle regeneration, remyelination, and neurogenesis (Conboy *et al.*, 2005; Villeda *et al.*, 2011; Ruckh *et al.*, 2012). Therefore, supplementing the ageing CNS microenvironment with rejuvenating factors present in the young circulation may correct the dysregulated microglial phenotype.

Stimulating ageing monocytes and macrophages

As monocytes age differently from microglia, agents that mobilize monocytes from the bone marrow and enhance functional aspects of ageing circulating monocytes, in addition to strategies that reverse HSC senescence, are promising avenues to rejuvenate the monocyte-derived macrophage population for neurological repair. To mobilize monocytes from the bone marrow, macrophage colony stimulating factor may be considered. In models of Alzheimer's disease or stroke, macrophage colony stimulating factor promoted the mobilization and migration of bone marrow-derived macrophages into the CNS and reduced injury (Boissonneault *et al.*, 2009; Lampron *et al.*, 2013). In a model of demyelination in mice, macrophage colony stimulating factor facilitated the increased representation of macrophages in the CNS (Doring *et al.*, 2015).

Pharmacological agents may also be considered for direct stimulation of monocytes and macrophages, so as to aid in the secretion of beneficial factors and in the promotion of phagocytosis and the clearance of inhibitory debris in CNS lesions to allow repair to occur. One such medication is amphotericin B, an anti-fungal agent that stimulates monocytes and macrophages to produce molecules such as TNF-α, IL-10, and CCL2, in addition to increasing their phagocytic activity (Doring *et al.*, 2015). Indeed, we found that amphotericin B stimulation of monocytes, macrophages and microglia reprogrammed the compromised cells around brain tumours to curb cancer growth (Sarkar *et al.*, 2014). Amphotericin B and macrophage colony stimulating factor combinational treatment of mice with demyelination of the spinal cord improved subsequent remyelination (Doring *et al.*, 2015). The severe hepato- and

Table 1 Pharmacological approaches to rejuvenate macrophages and microglia in CNS disorders with ageing

Pharmacological approach	Proposed mode of action	Reference
Enhancement of regulatory phenotype		
Interferon-β1	Upregulation of interferon regulatory factor-7	Cohen *et al.* (2014)
Scriptaid	Promotion of GSK3β/PTEN/Akt through inhibition of class I/II histone deacetylases	Wang *et al.* (2015)
DSP-8658	Activation of PPARγ, promoting a regulatory phenotype, and enhancing amyloid-β phagocytosis	Yamanaka *et al.* (2012)
Promotion of chemotaxis and phagocytosis		
Monophosphoryl lipid A	TRIF-mediated TLR4 agonist	Michaud *et al.* (2013)
Protollin	Upregulation of class A1 scavenger receptors and CCL2	Frenkel *et al.* (2013)
Macrophage colony stimulating factor	Increased mobilization of bone marrow-derived monocytes	Boissonneault *et al.* (2009); Lampron *et al.* (2013); Doring *et al.* (2015)
Amphotericin B	MyD88/TRIF-dependent upregulation of pro-inflammatory cytokines	Sarkar *et al.* (2014); Doring *et al.* (2015)
Reversal of HSC senescence		
Rapamycin	Inhibition of mTOR-dependent HSC senescence	Chen *et al.* (2009)
CASIN	Inhibition of cell division control protein 42-mediated HSC senescence	Florian *et al.* (2012)
Inhibition of excess microglial pro-inflammatory cytokine secretion		
Minocycline	Inhibition of NF-κB	Fan *et al.* (2007); Kobayashi *et al.* (2013)
Laquinimod	Reduction of several pro-inflammatory signalling pathways	Mishra *et al.* (2014)

GSK3β =glycogen synthase kinase 3 beta; PTEN = phosphatase and tensin homologue; Akt = protein kinase B; PPARγ = peroxisome proliferator-activated receptor γ; TRIF = TIR-domain-containing adapter-inducing interferon-β; TLR4 = Toll-like receptor 4; CCL2 = chemokine (C-C motif) ligand 2; MyD88 = myeloid differentiation primary response protein 88; mTOR = mechanistic target of rapamycin; NF-κB = nuclear factor kappa-light-chain-enhancer of activated B cells.

nephrotoxicity of amphotericin B in humans would preclude its widespread use, however.

Many studies have derived proof-of-principle evidence for the capability to reverse HSC senescence. As ageing HSCs have increased mTOR levels, pharmacological inhibition of this kinase using rapamycin has reversed many characteristics associated with HSC ageing (Chen *et al.*, 2009). As the young systemic circulation has been shown to rejuvenate different stem and progenitor cells through heterochronic parabiosis, identifying specific rejuvenating factors are potential therapeutic strategies to rejuvenate HSCs (Oh *et al.*, 2014). Another therapeutic strategy is HSC transplantation, in which an ageing, dysregulated HSC population is replaced by a more functional population (Prinz and Priller, 2014).

Conclusion

The effects of ageing on CNS regeneration culminate in an exacerbated lesion accompanied by impaired repair. This article proposes that, during ageing, monocyte-derived macrophages manifest differently from CNS-intrinsic microglia. Ageing macrophages show decreased pro-inflammatory cytokine secretion, phagocytosis, and chemotaxis. In contrast, senescent microglia display a primed, more pro-inflammatory phenotype, but also deficient phagocytosis and chemotaxis. Differential mechanisms, such as extrinsic changes in the microenvironment as well as intrinsic changes including genomic instability, underlie the divergent phenotypes manifested in these two cell types with age. We propose that therapeutic strategies that selectively target these distinct cell types through the underlying mechanisms are promising approaches for rejuvenation (Fig. 3 and Table 1). One has to be mindful, however, not to worsen detrimental aspects, when attempting to stimulate beneficial features such as chemotaxis. Restoring the dysregulated macrophage/microglia phenotype that occurs with ageing is a promising strategy for regeneration in the ageing CNS.

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