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Untangling senescent and damage-associated microglia in the aging and diseased brain

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Abstract

Microglial homeostasis has emerged as a critical mediator of health and disease in the central nervous system. In their neuroprotective role as the predominant immune cells of the brain, microglia surveil the microenvironment for debris and pathogens, while also promoting neurogenesis and performing maintenance on synapses. Chronological aging, disease onset, or traumatic injury promotes irreparable damage or deregulated signaling to reinforce neurotoxic phenotypes in microglia. These insults may include cellular senescence, a stable growth arrest often accompanied by the production of a distinctive pro-inflammatory secretory phenotype, which may contribute to age- or disease-driven decline in neuronal health and cognition and is a potential novel therapeutic target. Despite this increased scrutiny, unanswered questions remain about what distinguishes senescent microglia and non-senescent microglia reacting to insults occurring in aging, disease, and injury, and how central the development of senescence is in their pivot from guardian to assailant. To intelligently design future studies to untangle senescent microglia from other primed and reactionary states, specific criteria must be developed that define this population and allow for comparisons between different model systems. Comparing microglial activity seen in homeostasis, aging, disease, and injury allows for a more coherent understanding of when and how senescent and other harmful microglial subpopulations should be targeted.

Keywords

senescence; microglia; aging; TBI; neurodegenerative disease

Introduction

Globally, the number of people aged 65+ years will likely be greater than 1.5 billion by 2050; comprising ~16% of the world's population [1]. With this aging population comes

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a predicted increase in various age-related diseases, including many neurodegenerative diseases (ND) like Alzheimer's disease (AD) and Parkinson's disease. Exploring the link between increased age and increased risk of ND has been an attractive area of research, especially given the high costs involved with the treatment and care of the afflicted. For example, the cost of AD and other dementias on the United States healthcare system alone is estimated to reach $355 billion in 2021, without even considering informal costs to caregivers [2]. Unfortunately, there is currently no cure for most age-related NDs, and treatment strategies usually center around simply managing the symptoms of a disease. This deficiency highlights the need for the development of new targets and therapeutic strategies to combat these ailments.

Another area of recent concern in neurological health has been the implication of traumatic brain injury in contact sport athletes and soldiers. Kinetic insults to the central nervous system (CNS) may not only be acutely disabling, but can promote persistent changes in the cellular populations and microenvironment of the CNS for years post-injury [3]. Compelling similarities exist between the post-injury phenotype seen in traumatic brain injury (TBI) and the disease phenotype observed in the brains of patients with AD and other dementias, including dysregulation of amyloid and tau proteins and a sustained alteration of glial phenotype [4–8]. Recent meta-analyses have even found potential connections between TBI occurring early in life and the risk of developing ND with age in humans [9,10], although issues with self-reporting and grading of TBI severity complicate the correlation [11,12]. Similar to geriatric NDs, no therapies exist to relieve the long-term effects of TBI beyond preventative measures, which is problematic as approximately 69 million individuals worldwide are estimated to sustain a TBI every year [13].

An attractive emerging therapeutic target in many maladies of aging is cellular senescence, which could provide a possible link between a dysfunctional CNS microenvironment and increased risk of ND. Senescence describes a cellular state where an irreversible cell-cycle arrest is induced by the expression of cyclin-dependent kinase inhibitors Cdkn1a ($p21^{CIP1}$, hereafter p21) and/or Cdkn2a ($p16^{INK4a}$, hereafter p16) [14–16]. Senescent cells produce a distinct inflammatory secretome and have been found to accumulate with age and in many diseases; potentially contributing to pathology [17–21]. A challenge in targeting senescence is the apparent heterogeneity of this cellular state, wherein a variety of insults may incite senescence, the inflammatory secretory phenotype is modified depending on cell type, and the senescent phenotype may even change based on the stage of senescence [22–24]. Even defining senescence is difficult, with different groups using different criteria to define a senescent cell. To address this, the International Cell Senescence Association recently put forth a recommendation for determining the presence of senescence, describing a combination of cell-cycle arrest, macromolecular damage, deregulated metabolism, and the production of a senescent-associated secretory phenotype (SASP) [25]. Another group has also proposed a two-step algorithm to assess senescence [24]. These features of senescence in the context of microglia will be explored later in this review.

If senescent cells are active drivers of neurodegeneration, then identifying the cellular population responsible becomes critical. Putative senescent states have been suggested in a variety of neural subpopulations with aging and in various disease contexts, including

microglia [26–30], neurons [31–33], astrocytes [26,34,35], and oligodendrocyte progenitor cells [36]. Vascular and endothelial cells of the blood-brain barrier have also demonstrated senescence properties [37,38]. It is also likely that multiple cell types become senescent in complex diseases, which further complicates the cause-effect relationship between specific populations of senescent cells and disease processes.

Microglia, the resident mononuclear phagocytes of the CNS, play key roles in immune surveillance and defense [39], synaptic remodeling [40], and homeostasis [41]. They have also been identified as a putative senescent population in natural aging in both humans [42] and mice [27], mouse models of AD pathology [26], and in TBI [28,29]. However, in a reactionary or dystrophic state, microglia also contribute to the pathogenesis of various NDs and post-TBI syndromes [43–45]. The overlapping phenotypes of reactive and senescent microglia together with the heterogeneity of microglial phenotypes described in aging and disease [45,46] present a nuanced challenge to defining senescent microglia and parsing out their contribution to dysfunction.

In this review, we discuss the features of microglia phenotypes associated with aging, disease, and injury, and propose how senescent microglia may contribute to these conditions. With the known phenotypical overlaps between microglia across these contexts, we will also suggest ways to distinguish senescent microglia from other microglial populations.

Phenotypic complexity of microglia in aging, neurodegenerative disease, and injury

Microglial states are usually defined in relation to the conditions present at homeostasis (Figure 1). Although the term ‘homeostasis’ can be over-simplified – indeed the exact nature of these homeostatic microglia may differ based on many factors including brain region and temporal heterogeneity [47] – we will refer to the state of microglia in non-pathological contexts as ‘homeostatic’ for the purposes of this review. In this state, microglia are associated with immune protection, the support of neuron health, and synaptic remodeling [40,47].

Dysfunctional microglia are distinguished by a loss of homeostatic function, including impaired phagocytic capacity and the acquisition of a pro-inflammatory profile [48]. For the purposes of this review, these pro-inflammatory (yet non-senescent) microglia associated with pathological conditions will be referred to as ‘activated’ microglia (Figure 1). While we acknowledge that the use of the label ‘activated’ can be vague, it is outside the scope of this review to dissect the various states of microglia that have been described in aging, neurodegenerative disease and injury. Other reviews [47,49,50] have more closely examined the nuanced heterogeneity of microglia in those states.

Senescent and dysfunctional microglia, under various names, are often referenced interchangeably in studies of aging, ND, and TBI. We postulate that senescence is a distinct cellular state which can be distinguished from otherwise non-senescent inflammatory microglia (Figure 2). Given the putative differentiating features between microglial

phenotypes outlined below, unique roles played by senescent microglia may be delineated and not attributed to non-senescent dysfunctional microglia.

Aging

Several changes are associated with the microglial populations in the aged brain. An accumulation of microglia with a 'dystrophic' appearance or de-ramified microglia with cytoplasmic fragmentations have been noted in aged human brains [42,51]. Aged microglia also tend to accumulate lipofuscin [52–54], aggregations of highly oxidized proteins cross-linked with sugars and lipids [55], which have been associated with dysfunction and inflammation [56,57]. Additionally, microglia in naturally aged mice appear to have lower phagocytic activity compared to young mice [48]. This, combined with an apparent decrease in motility [58,59], has been suggested to contribute to dysfunctional synaptic pruning and potentially to the cognitive deficits associated with age [60,61].

In both humans [62] and mice [52,63,64], aged microglia adopt a pro-inflammatory profile including higher expression of genes including TNFα, IL-6, and IL-1β, as well as markers of 'priming' such as CD11b and MHC-II (Figure 1). This state of 'priming' is thought to make aged microglia disproportionately reactive to immunological challenges, leading to prolonged neuroinflammation and the cognitive deficits associated with age [65,66]. The exact cause for the shifting of microglia to this pro-inflammatory state with age is unknown, although several theories such as the increased permeability of the blood-brain barrier introducing more 'priming' signals [67,68] and/or monocyte-derived cells [69], or age-related myelin fragment accumulation [70] have been proposed. It is also interesting to note that while the existing body of literature strongly indicates systemic organismal aging influences microglia function, the reverse is also true – that a change in microglial function could also result in age-related cognitive changes. For example, microglial repopulation in naturally aged mice led to a rescue in age-related cognitive, synaptic and neuronal deficits [71].

The concept of increased microglial senescence in aged brains has gained traction in recent years. An accumulation of senescent microglia with age has been shown in rodents both *in vivo* [72] and *ex vivo* [51,72], although the evidence of senescent microglia accumulation in aged human brains is limited [42]. Further developing the concept of microglial senescence remains challenging though, given the lack of consensus on the hallmarks needed to define its presence. In particular, better resolution of the expression patterns and composition of the SASP is needed, especially in comparison to a normal responsive state.

Neurodegenerative disease

The loss of homeostatic function and an 'activated' phenotype in microglia has also been associated with ND. Previously, disease-relevant microglia were often compared to M1 pro-inflammatory 'activated' macrophages [73], but increasingly other labels like disease-associated microglia (DAMs) [44], 'dark' microglia [74], 'primed' microglia [65,66], or more nuanced references to the exact cell markers expressed by specific microglial populations, have grown in popularity to better reflect the heterogeneous nature of

microglial biology [75]. Morphologically speaking, microglia with a dystrophic appearance are also increased in several NDs [42,76].

It remains to be seen if there is a common microglial phenotype that is associated with ND. The downregulation of genes associated with microglial homeostasis, Tmem119, P2ry12, and Cx3cr1, have been identified in many murine models representing AD [44,77–80], amyotrophic lateral sclerosis (ALS) [44,77,79], and multiple sclerosis [79]; suggesting similar signaling pathways are present. It has also been suggested that microglia could mechanistically contribute to ND through aberrant synaptic pruning via the complement system [81]. These losses of homeostatic function are also not unique to microglia in ND and are seen in the context of aging [44,60,61,77,82]. This concurrence could explain the strong link between increased age and ND [83].

There is of yet no consensus on the identifying markers or genes for the ‘activated’ inflammatory microglia associated with disease [47]. It’s likely that different populations of microglia can play variable roles depending on the disease, stage of the disease, or brain region affected, making the comparison between studies especially challenging. Some markers commonly identified as upregulated in microglia associated with ND are Cd11c [44,84–86], Iba1 [74,87,88], Trem2 variants [89,90], and MHC-II [91,92]. Pro-inflammatory molecules like IL-1β, IL-6, TNFα, and MCP-1 are also associated with ‘activated’ microglia in disease [93].

Senescent microglia have also been identified as contributing to the pathogenesis of NDs. Whole-body clearance of p16-expressing cells in a mouse model of tauopathy led to an amelioration of disease, with microglia identified as a senescent cell population in this disease model [26]. Another study in a mouse model of AD found that replicative senescence in microglia was associated with early pathology [30]. A mouse model of ALS also found gliosis and motor neuron loss was associated with increased senescence indicators [94]. However, it is likely microglia are but one neural subpopulation that becomes senescent in age and disease. Recent studies have produced evidence for senescence in neurons and oligodendrocyte progenitors in other mouse models of tauopathy and AD [33,36]. Regardless of the hypothesized identity of the senescent population, each of these studies demonstrated pathology mitigation following pharmacological removal of senescent cells [26,33,36].

The relationship between senescent and ‘activated’ microglia in the context of disease remains unclear – one state could promote the other, or they may be overlapping populations. This is especially challenging since the SASP, a key feature of senescent cells, commonly encompasses pro-inflammatory molecules also associated with ‘activated’ microglia like TNFα, IL-1β, and IL-6 [95]. It’s also unclear whether senescent microglia upregulate markers associated with ‘activated’ microglia or downregulate microglial homeostatic genes.

Injury

Traumatic brain injury (TBI) differs from aging and disease in that the inciting factor for its associated pathology is external and more acute. A variety of mechanical insults

including contusion, closed-head impact, and blast injury can produce mild, moderate, or severe TBI depending on individual circumstances [96–99]. TBI is a biphasic injury: after the mechanical primary injury, a secondary dystrophic state produces long-term dysfunction in the brain that includes elevated neuroinflammation as a result of blood-brain barrier disruption, increased oxidative stress, and persistent changes in the microglial population [100–106].

In the minutes following TBI, microglia are recruited via purinergic signaling to clear necrotic debris and adopt a phagocytic state [100,107,108]. The mode of activation following TBI is somewhat age-dependent and may be less neuroprotective and more toxic in older animals [109]. In the initial week following injury, the microglia population adopts a balanced distribution between pro- and anti-inflammatory states to promote neurogenesis and enhance the immune response to injury [89,110,111]. However, as time passes following the primary injury the neuroprotective functions are lost, and microglia become the prime motivators of neuroinflammatory gene expression in the post-TBI brain [112]. *In vitro,* conditioned media derived from microglia in an induced pro-inflammatory ‘M1-like’ state reduces survival of oligodendrocytes *in vitro*, whereas conditioned media from microglia in an induced anti-inflammatory ‘M2-like’ state promotes remyelination [111,113]. In human patients, increased microglial activation is even detectable by PET scan 17 years after the primary injury [114].

Microglia associated with the long-term inflammatory phenotype following TBI are commonly defined as MHC-II/CD86/Cd11b positive, with upregulated expression of NOX24, Tlr4, Trem2, CD68, Clec7a, and Stat1, and higher expression of interferon- and immune-associated signaling genes [100,112,115,116]. This resembles the ‘primed’ state microglia appear to enter with aging and is also determined both by cell-intrinsic changes and external influences from the altered microenvironment [105]. Of recent interest is how post-injury microglia promote increased oxidative damage leading to neurodegeneration through a NOX2-dependent mechanism [100,117,118]. The activity of NOX2 in glia may in fact track with the severity of TBI in patients [119]. While microglial NOX2 is chronically active in neurodegenerative contexts, its rapid increase in activity in microglia following TBI is relevant to their role in driving pathology and an important distinguishing feature [120,121]. Importantly, a recent study in mice demonstrated pharmacological clearance of microglia post-injury is neuroprotective and reduces cognitive impairment [112].

Inquiry into pro-inflammatory microglia as a therapeutic target in TBI secondary injury has coincided with increasing interest in microglial senescence. Elderly individuals are at greater risk to suffer significant cognitive impairment and early morbidity following TBI, potentially due to an already present dysfunction in the microglial compartment [109,122,123]. Due to the difficulty of distinguishing senescent microglia from other primed or dystrophic states, the evidence for these cells becoming senescent following TBI remains preliminary. There is evidence of reductions in telomere length and deficiencies in DNA repair following TBI which could predispose neural cells to senescence [124–126]. Two recent publications identified a possible senescence signature following TBI in adult mice [28,29]. Although their ultimate role in the secondary injury cascade is still under

investigation, this emerging evidence points to senescent microglia as contributors to the post-TBI phenotype.

Distinguishing microglia in aging, neurodegenerative disease, and injury

In the past decade, the study of microglial biology has evolved beyond the M1 vs. M2 paradigm, acknowledging the broad spectrum of intermediate states these cells exist in depending on context. There is a large overlap between microglial phenotypes in aging, neurodegenerative disease, and injury – mainly a loss of homeostasis and a pro-inflammatory phenotype. For now, there is no cellular marker or component of the secretome that is specific for microglia found in aging, disease, or injury.

Attempting to distinguish microglia found in the aging and neurodegenerative contexts implies that it's possible to have an aged 'healthy' brain without ND. Some may argue that aging itself is a pathology – neuropathological features like neurofibrillary tangles and amyloid plaques have been identified post-mortem in individuals who otherwise did not demonstrate overt cognitive impairment [127–130]. It is unknown if these pathological features indicate that these individuals were in the pre-clinical stages of a disease or if they would continue to be nondemented despite their neuropathology [131].

Regardless, there are some studies that have offered putative ways to distinguish these closely related microglial populations. An interesting example is iron accumulation and metabolism. One study by Shahidehpour *et al.* suggests that there is increased ferritin accumulation in human microglia in ND but not old age, and proposed altered iron homeostasis as a distinguishing factor between aged 'healthy' microglia and those associated with neurodegeneration [132]. Indeed, elevated iron in the CNS is associated with ND (reviewed in [133] and [134]), possibly at levels higher than with normal aging [133]. Given that increased iron levels are also associated with advanced age [133–135] more studies are needed to ascertain if altered iron homeostasis is in fact specific to ND-associated microglia. Additionally, increased cellular iron accumulation has been posited as a feature of senescence, although it's not critical to maintaining this fate [136,137]. Whether changes in iron homeostasis reflect senescent cell accumulation with CNS aging or is in fact relevant to the emergence of disease remains to be seen.

Some investigators have turned to the resolution provided by single-cell sequencing technology to clarify any differing roles played by microglia. Several single-cell studies in murine microglia have been carried out to delineate the distinct roles microglia could play in these closely related contexts. For example, a study by Hammond *et al.* compared microglia from developing, aged, and mice injured from a focal white matter injury and identified two populations highly concentrated in aged mice – one defined by Ccl4, and another enriched in several interferon-response genes [45]. In addition, a cluster predominantly in injured mice characterized by Ifi27l2a was also identified [45]. Other studies have delineated specific populations of microglia associated with disease [44,138–140], however, the specific characteristics of the microglia of interest in each study differed. This potentially highlights a general difficulty in comparing single-cell studies – that the high resolution provided is extremely sensitive to the exact model used and even the isolation and preparation method of the microglia. Another difficulty in interpreting the results from single-cell studies is

the lack of validating studies showing functional implications of the unique microglial populations identified. Regardless, single-cell profiling of microglia remains an important tool in parsing apart the differences between the different microglial states.

Several of the aforementioned studies incompletely investigate the role of senescent microglia in the various microglia phenotypes described. This could be because the common features seen in senescent cells and 'activated' inflammatory microglia make it difficult to distinguish how these populations contribute to pathology.

Distinguishing between senescent microglia and 'activated' microglia

The potential overlap between senescent microglia and 'activated' microglia remains an underexplored area in microglial research (Figure 2). Further investigation into this relationship could help explain neurodegeneration in age, disease, and injury and provide novel treatment options in the removal of senescent cells with pharmaceutical interventions known as senolytic drugs.

Perhaps one reason why studies on senescent microglia have been inconsistent is due to a lack of stringent criteria in labeling a cellular state as 'senescent'. In immunological terms, senescence is often used loosely or interchangeably with immunosenescence, to describe a general decline in immune function with age [141]. However, cellular senescence as described here refers to a specific cellular state, often defined by a combination of cell-cycle arrest, macromolecular damage, deregulated metabolism, and a SASP [25]. Even with the latter definition in mind, many papers have not investigated all of these aspects of the cellular state when describing senescence [22,25], and the 'senescent' microglia previously reported may in fact be referring to different groups of 'activated' microglia.

Another roadblock in trying to define senescence in microglia is the overlap with 'activated' microglia. For example, the presence of a **SASP** is often used to show that a cellular population is senescent [25]. However, a SASP factor specific to senescent microglia has not been identified, and several components of the SASP can be attributed to the secreted inflammatory milieu of non-senescent 'activated' microglia [93]. To add extra confusion, the SASP can also differ based on cell type, cause of senescence, or even stage of senescence [37,95]. This makes defining microglial senescence by this one aspect difficult.

A common marker used to define senescent cells, **senescence-associated β-galactosidase** (SA-β-gal), may not be specific to senescence in microglia. SA-β-gal is a colorimetric assay to detect senescent cells through histochemical means and is thought to rely on the increased lysosomal mass [142,143]. However, it is becoming increasingly evident that SA-β-gal may not be a marker of senescence in all contexts [144]. In particular, non-senescent macrophages may exhibit SA-β-gal positivity [145,146], and microglia can also stain positive in non-senescent contexts due to their phagocytic function and the possible associated change in lysosomal numbers [147]. Still, the SA-β-gal assay could potentially be used with the right controls and assessed in terms of an increase in SA-β-gal activity rather than a presence/absence assay [30,148]. To validate this method, the specificity of SA-β-gal in microglia needs to be formally assessed.

Another possible alternative to the SA-β-gal assay particularly in the context of microglia is the use of Sudan Black B, a stain for **lipofuscin** [149]. Lipofuscin has been shown to accumulate in aged microglia [52–54], and is generally considered a hallmark of aging [150]. It has been suggested that Sudan Black B has a high overlap with SA-β-gal staining, which is especially relevant in contexts where the latter is not suitable such as in formalin-fixed, paraffin-embedded tissues [151,152], or in microglia where SA-β-gal is likely to stain false positive. However, whether the Sudan Black B assay is specific to senescence, its rate of false positivity, and its overlap with SA-β-gal in microglia remains to be thoroughly investigated.

A possible differentiating factor between 'activated' and senescent microglia is **cell cycle arrest**. A senescent cell, by definition, is in a state of cell cycle arrest [25]. In contrast, increased proliferation has been associated with 'activated' pro-inflammatory microglia in animal models [84,153–155]. This makes the assessment of cell cycle arrest, whether it be through the upregulation of certain senescence-associated cell cycle inhibitors like p16 and p21 [25] and/or through directly assessing proliferation by incorporation of EdU or cell tracking, a possible avenue to differentiate senescent cells from 'activated' cells. However, using the expression of cell cycle inhibitors as a surrogate for cell cycle arrest comes with its own limitations. For example, p21 may have roles outside of cell cycle inhibition [156] and its upregulation is not specific to senescence [157]. p21 levels may also decrease in 'late' senescence or when senescence has been achieved [115,158], whereas high levels of p16 are thought to be maintained throughout senescence [158,159]. Although p16 is often considered a more reliable marker of senescence, it can also be upregulated in non-senescent contexts [18,48,72]. The uncertain specificity of p16 and p21 in microglia should be taken into consideration, re-enforcing the need for a combinatorial approach to senescence markers.

The increasing adoption of **single-cell RNA sequencing** has also led some to use the technology to define senescent populations. However, the considerable variability of SASP expression [22,37] and non-specificity to senescence in microglia [93] still applies. A gene specific to cellular senescence also has not been identified, and different papers will use different marker genes to describe their senescent cell population [37,160]. Regardless, single-cell RNA sequencing could be used to support the presence of a senescent cell population if multiple aspects of senescence are addressed – including that of cell cycle arrest (especially with an upregulation of the senescence-associated Cdkn2a and Cdkn1a), a pro-inflammatory secretome, and possible other context-specific senescent markers. Pathway analyses may also be useful, with cellular senescence available as a gene ontology term that has been used to define senescence in the brain [161]. This should be done with caution, as several genes in that pathway are not specific to senescence, and using pathway analyses should be a supportive tool and not used alone to define senescence.

Another possible way to support the presence of senescence in a microglia population is to probe their response to **senolytics**. Senolytics are pharmacological interventions that selectively clear senescent cells, and mostly work by exploiting senescent cell reliance on anti-apoptotic and pro-survival pathways [162], which have been termed the Senescent Cell Anti-Apoptotic Pathways (SCAPs) [163]. The targets of commonly used senolytic agents

can vary. Navitoclax inhibits the B-cell lymphoma 2 (BCL-2) family of anti-apoptotic factors [164,165], and while fisetin inhibits some of these and other SCAP network components it may also exert its senolytic effect through other activities like the inhibition of the PI3K/AKT/mTOR pathway [166–168]. The combination of dasatinib and quercetin also inhibits SCAP network components and multiple tyrosine kinases [169]. When SCAPs are targeted, there is theoretically preferential death of senescent cells by senolytics at doses that will not affect non-senescent cells [170]. Perhaps an increased reliance on pro-survival pathways and susceptibility to senolytics could help differentiate senescent microglia from non-senescent inflammatory microglia. The first-generation senolytics currently employed have the potential for off-target effects and are effective in different cell types, and the correct dose and class of senolytics necessary for the selective clearance of senescent microglia are still undetermined. Nevertheless, some senolytics have been effectively used in mouse models of neurological disease to alleviate pathology including dasatinib and quercetin [36] and navitoclax [26], which has led to senolytic therapies entering clinical trials for the modulation of AD [171]. Given the global effects of all current senolytics, a major consideration for future studies should be differentiating CNS-specific vs. systemic results of senescent cell clearance.

It is critical that studies aimed at rigorously defining microglial senescence in aging, disease, and injury must demonstrate a clear senescent signature. This is particularly important due to the absence of a single, unambiguous senescence biomarker, which complicates definitive claims. Techniques such as bulk or single-cell RNA sequencing on sorted microglia can be expensive and time-consuming, but such methods provide a solid foundation from which to form hypotheses about senescence involvement in an experimental system. Due to the highly situational nature of the senescence program, gene-expression profiling is essential to determine a particular microglia subpopulation to assay for further senescence markers (cell-cycle arrest, p16/p21 upregulation, macromolecular damage, etc.). Transgenic mouse lines where senescent cells can be cleared by a genetic construct and commercially available senolytic drugs should also be considered as sensitivity to senolytic methods is one of the strongest lines of evidence currently used in the literature [167,172–174]. Finally, although published evidence for microglial senescence is highly variable it can still be valuable as a reference point when designing a study and should be expanded upon to provide a comprehensive picture of how senescent microglia drive neurodegeneration. Distinguishing senescent microglia and defining how they drive neurodegeneration will have major implications in developing new treatment strategies, given the unique and exploitable features of senescent cells.

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Conflicts of Interest

D.J.B. is a shareholder and co-inventor on patent applications licensed to or filed by Unity Biotechnology, a company developing senolytic medicines, including small molecules that selectively eliminate senescent cells.

Research in his lab has been reviewed by the Mayo Clinic Conflict of Interest Review Board and is being conducted in compliance with Mayo Clinic Conflict of Interest policies. The funders had no role in the writing of the manuscript or in the decision to publish.

Abbreviations:

AD	Alzheimer's disease
BCL-2	B-cell lymphoma 2
CNS	central nervous system
ND	neurodegenerative disease
SA-β-gal	senescent-associated β-galactosidase
SASP	senescence-associated secretory phenotype
SCAP	Senescent Cell Anti-Apoptotic Pathway
TBI	traumatic brain injury

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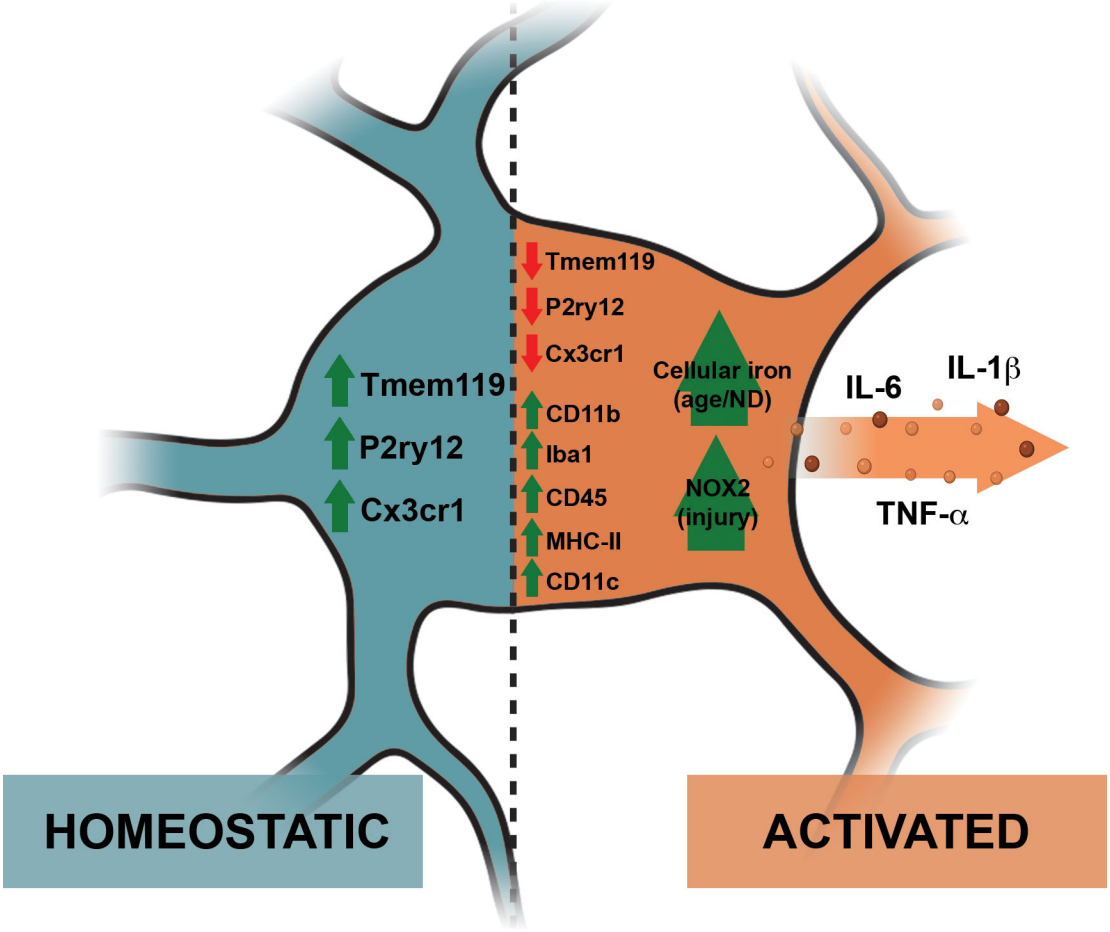

Figure 1. Contrasting features of microglia in homeostatic and inflammatory 'activated' states.

The 'activated' inflammatory microglial state differs from its resting homeostatic state in many ways, such as the downregulation of homeostatic markers (Tmem119, P2ry12, Cx3cr1) and the upregulation of other markers like CD11b, Iba1, CD45, MHC-II, and Cd11c. 'Activated' microglia also have a pro-inflammatory secretome, which includes IL-6, IL-1β, and TNF-α. They also differ in morphology, with homeostatic microglia having highly ramified processes and 'activated' inflammatory microglia having shorter processes with a larger cell body. Some putative markers proposed for 'activated' inflammatory microglia in specific contexts include an increase in cellular iron with age and neurodegenerative disease (ND) and an upregulation of NOX2 in injury. Figure created in BioRender.

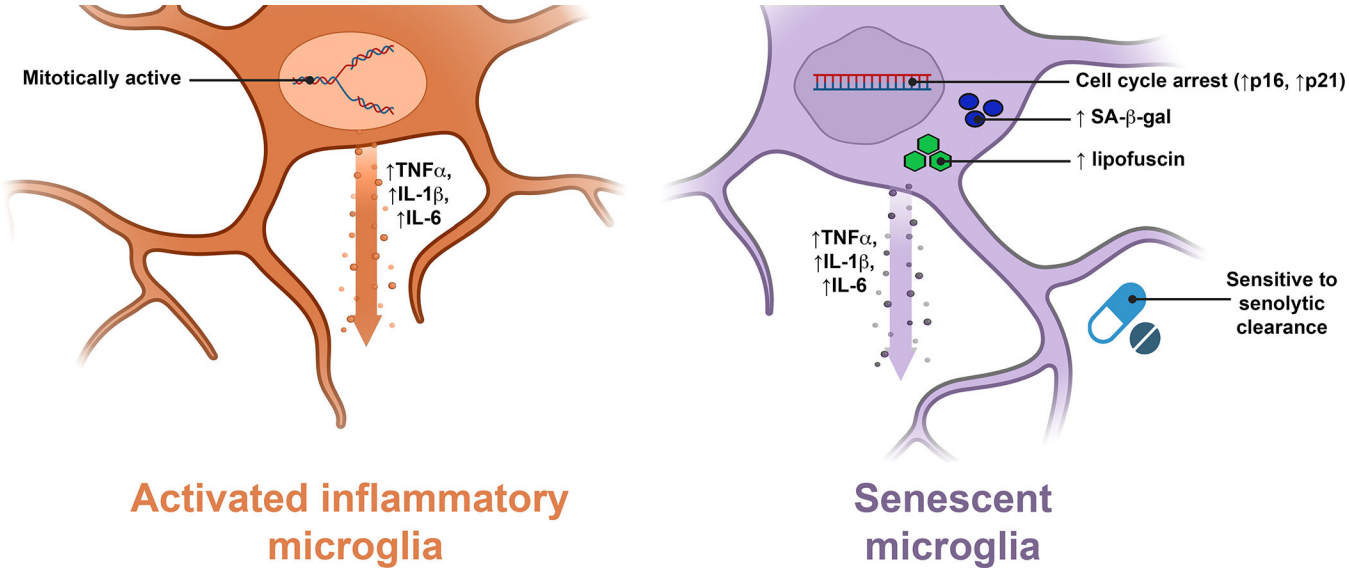

Figure 2. Similarities and differences between 'activated' inflammatory and senescent microglia.

Senescent microglia can be difficult to differentiate from an 'activated' inflammatory state as they share many characteristics, such as an inflammatory secretome which may include TNF-α, IL-1β, and IL-6. Some features we propose that can differentiate senescent microglia include cell cycle arrest (especially with an upregulation in p16 and p21), an increase in SA-β-gal staining, an increase in lipofuscin staining, as well as sensitivity to senolytic clearance. Figure created in BioRender.