Cellular senescence at the crossroads of inflammation and Alzheimer's disease

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Abstract

Ageing is a key risk factor for Alzheimer's disease (AD), but the reasons for this association are not well understood. Senescent cells accumulate in aged tissues and have been shown to play causal roles in age-related pathologies through their pro-inflammatory secretome. The question arises whether senescence-induced inflammation might contribute to AD and bridge the gap between ageing and AD. Here, we highlight the role of cellular senescence as a driver of the ageing phenotype and discuss the current evidence that connects senescence with AD and neurodegeneration.

Cellular senescence: a promising hotspot in age-related neuropathies

By 2030, **dementia** (see Glossary) is expected to affect more than 70 million people worldwide [1]. The most common cause of dementia is **Alzheimer´s Disease (AD)**, which contributes to 60-80% of dementia cases, and is characterized by both the accumulation of misfolded proteins and **neuroinflammation** [2]. The greatest risk factor for AD is **ageing**, with most AD cases diagnosed in people over 65 years old. Nevertheless, despite the tremendous social and financial implications of an increasing elderly population, current understanding of the biology of ageing is still limited. This, alongside the lack of effective treatments to prevent or slow down the progression of AD, emphasises the need to explore concepts and hypotheses beyond the currently dominant ones in the field.

Among the hallmarks of ageing is **cellular senescence** [3], which has been gaining broadening recognition, presumably for two main reasons. First, the selective elimination of senescent cells in naturally aged mice lengthens **healthspan** and delays age-related disorders, suggesting a causal role of senescent cells in ageing [4]. This is likely to be mediated by the **senescence-associated secretory phenotype (SASP)** [5] that is linked to the chronic inflammation seen in advanced age (**inflammaging**) [6]. Second, although cellular senescence was initially discussed mostly in the context of cancer, the number of senescence-associated diseases being identified is now growing and, as result, the therapeutic potential of targeting senescent cells clearly goes beyond tumour suppression [7]. Indeed, recent evidence of cellular senescence in tau-dependent pathology [8, 9] and AD [10, 11], opens up the possibility that senescence might be one of the mechanisms underlying the complex cellular response during AD progression [12].

In this Review, we summarize current understanding of cellular senescence and its associated inflammatory response, with a focus on its potential contribution to AD pathology (Figure 1). We then discuss new research strategies needed to fill the knowledge gap at the crossroads of inflammation and AD.

Cellular senescence as a source of chronic inflammation

In early studies, senescence was considered a cellular response to halt proliferation after the maximum number of replication cycles has been reached [13]. However, in the decades that followed, the concept of senescence was expanded to include not just replicative but also premature senescence such as the one triggered by oncogenic activation [14], or by exposure to genotoxic agents or mitochondrial dysfunction [15]. As opposed to quiescence, the senescence growth arrest is irreversible even in the presence of mitogenic stimuli, and it relies on activation of the p53/p21 and $p16^{INK4a}$/Rb pathways [16]. Senescent cells display enlarged morphology [17] and an expanded lysosomal compartment, which facilitates beta-galactosidase activity even at a suboptimal pH 6, the so called senescence-associated beta-galactosidase (SA-β-Gal) activity [18]. SA-β-Gal activity is indeed one of the most widely used markers to identify senescent cells, although it must be combined with additional markers [19].

Senescent cells have a number of typical characteristics and they affect their respective tissues in various ways. Senescent cells are resistant to apoptosis, due to upregulation of certain BCL-2 family proteins [20, 21]. Other salient features of senescent cells include chromatin remodelling and metabolic alterations (Box 1). Cellular senescence causes tissue ageing via both intrinsic and extrinsic mechanisms. Intrinsically, senescence might compromise the tissue's regenerative capacity if the irreversible cell growth arrest of

senescence reaches the stem cell compartment [22]. The extrinsic effects on tissue ageing are mainly mediated by the secretion of a complex mixture of interleukins, chemokines, growth factors, and proteases constituting the SASP [23]. The SASP is one of the most intriguing features of senescent cells. Its heterogeneous and dynamic composition and its specific array of functions vary with the inducer of senescence and the cell type being affected [24, 25]. However, generally speaking, the SASP has three main functions. First, the SASP plays an important cell-autonomous role by reinforcing the growth arrest and the secretory phenotype itself through a positive-feedback loop which, in part, accounts for the tumour suppressor properties of senescence [26, 27]. Second, multiple components of the SASP can propagate senescence to neighbouring cells in a process called **paracrine senescence** [28]. Third, the SASP eventually recruits the immune system to clear the senescent cells and halt the **inflammation**. Interestingly, whereas immunosurveillance of senescent cells is beneficial in the context of cancer [29] and embryonic development [30, 31], surveillance of senescent neuroblasts in the aged brain might come at the price of cognitive decline [32].

Although senescent cells in aged tissues typically represent no more than 15% of the total number of cells [33], they contribute to chronic inflammation in two ways. First, additive or synergistic effects of paracrine senescence amplify the SASP, and thereby exacerbate inflammation. Mediators of paracrine senescence include ROS signalling through gap junctions [34], growth factors and chemokines [28], and small extracellular vesicles [35]. Second, **immunosenescence** (Box 2) results in a declined immune function and a subsequent lower rate of senescent cells clearance [36, 37]. The resulting accumulation of senescent cells further reinforces the inflammation [6]. In support of this idea, an age-dependent increase of certain circulating SASP factors was observed in human plasma, and this increase is positively associated with frailty and adverse postsurgical outcomes [38].

Moreover, elimination of senescent cells in ageing mouse models reduces the levels of systemic inflammatory mediators, pointing to senescent cells as a key source of inflammation [4]. Conversely, transplantation of senescent cells into young mice increases systemic inflammation and causes physical dysfunction [39]. Therefore, senescence-induced inflammation is a contributing factor to age-related pathologies, including neurodegenerative diseases [40].

Inflammation in AD

The neuropathological hallmarks of AD include extracellular plaques of abnormally folded amyloid-β (Aβ), intracellular aggregation of the microtubule protein tau in neurofibrillary tangles (NFT), and neuroinflammation [41]. The immune response may be beneficial in the early stages of the disease but is generally thought to be deleterious when it becomes chronic [41]. Genome-wide association studies (GWAS) have identified several polymorphic variants in genes expressed by **microglia** that modulate the risk of AD. These genetic associations suggest a prominent role of immunity and inflammation in AD that goes beyond phagocytosis and clearance of Aβ plaques. Coupled with other lines of evidence, these findings indicate that neuroinflammation represents much more than a by-product of AD pathology [42].

Two of the major AD risk genes are *APOE* and *TREM2* [43]. Mechanistic studies in animal models coupled with post-mortem analyses of microglia from human tissue indicate that coordinated activation of the TREM2-APOE pathway in response to neuritic plaques leads to a phenotypic switch from homeostatic to neurodegenerative microglia, characterized by augmented local inflammatory responses [44]. Interestingly, this microglial transformation might be mediated, at least in part, by the induction of cellular senescence [11]. Despite a major role of microglia in neuroinflammation, **astrocytes** also play their part through release

of cytokines enabling bidirectional communication with glial cells, neurons, and endothelial cells [45].

Evidence for the centrality of the crosstalk among the different brain cell types in the context of AD pathogenesis contributed to the changing perspectives from a neuron-centric view, as exemplified in the amyloid-cascade hypothesis, to more recent frameworks that capture non-linear and more complex aspects of the disease's progression including, for instance, the description of a prolonged cellular phase of AD involving feedback and feedforward responses of astrocytes, microglia and vasculature [12]. Although the causal chain of events underlying AD's progression is not yet clear, insights from transcriptomic studies are helping get a better understanding of the specific inflammatory reaction during AD, and have placed new early players like **oligodendrocytes** under the spotlight [46, 47].

Importantly, the fact that clinical diagnosis of AD typically comes after decades of a long preclinical stage opens up the possibility that targeting early events and identifying early markers may well be an essential step to halt or at least delay the progression of the disease [12]. There is clinical evidence that neuroinflammation is already present in the mild cognitive impairment (MCI) stage, before the onset of dementia [48]. This early neuroinflammation may be driven or exacerbated by the accumulation of senescent cells in the ageing brain. In line with these ideas, a transgenic mouse model characterized by low-grade chronic inflammation shows early onset of memory loss, enhanced neuroinflammation and increased accumulation of senescent cells in the brain [49]. The augmented neuroinflammation can also drive amyloid deposition through enhanced expression of IFITM3 [50]. Of note, IFITM3 has been found as cargo of small extracellular vesicles released by senescent cells during ageing [35], further reinforcing the potential crosstalk between senescence and chronic inflammation during brain

ageing. This raises the possibility that by specifically modulating the secretome of senescent cells one could restrain neuroinflammation and perhaps slow down the progression from MCI to AD. A putative role of senescence and the SASP during MCI and AD is strengthened by the fact that **senolytic** therapy in mouse models of ageing [51], tau-dependent pathology [8], and AD [10] decreases neuroinflammation. In addition to ageing, there are other sources of neuroinflammation that have been associated with an increased risk of AD. Traumatic brain injury (TBI) increases neuroinflammation by inducing both acute and chronic changes in the immune system and an augmented susceptibility to infection [52]. Interestingly, TBI induces the expression of certain senescence markers in microglia, potentially linking TBI-induced inflammation with cellular senescence [53]. However, more work is needed to unravel the mechanisms of TBI-induced inflammation in AD and whether cellular senescence may be a common link facilitating the pathological response.

Systemic factors reaching the brain due to age-related vascular damage constitute another important source of neuroinflammation [54]. Cerebrovascular diseases, such as cerebral small vessel disease, increase the risk of cognitive impairment and dementia and constitute a potential preventive and therapeutic target for AD [55]. **Blood-brain barrier (BBB)** breakdown often precedes dementia [56] and amyloid deposits or tau pathology have been shown to exacerbate vascular damage [57, 58]. Pericytes participate in the regulation of cerebral blood flow, support the BBB function and are essential to preserve brain homeostasis [59]. It has been argued that the deposition of oligomeric Aβ is causally linked to an increase in capillary constriction via dysfunctional pericytes, thereby providing an explanation for the lower cerebral blood flow seen early in AD [60]. Moreover, due to their frontline position, certain groups of pericytes are the first cell type activated in the CNS in response to systemic inflammation [61]. Interestingly, those pericytes signal to neurons

mainly through secretion of the chemokine Ccl2, one of the key components of the SASP mediating paracrine senescence [28].

Two recent papers have presented evidence that obesity [62] and hyperinsulinemia [63] may trigger senescence in the brain. These studies underscore the idea that cells in the brain exposed to systemic factors can undergo senescence and warrants further investigation. One of the questions that merits further examination is whether the pro-inflammatory cytokines released after systemic insults come, at least in part, from senescent cells.

Potential role of cellular senescence in AD

Exciting new evidence regarding a role of senescent cells in neurodegeneration is starting to emerge (Figure 2) [64]. Certain cellular changes seen during AD coalesce with cellular changes seen during senescence. Intrinsic sources of DNA damage typical for senescent cells, such as telomeric alterations and hyperproliferation, are seen in AD [11, 65]. DNA double-strand breaks (DSBs), a common underlying cause of senescence [22], accumulate in the human hippocampus during AD [66]. Additional examples are impaired proteostasis and mitochondrial dysfunction, both hallmarks of ageing and AD [3]. Defective **autophagy** has been suggested as a possible cause for the accumulation of misfolded proteins seen in AD [67]. Interestingly, a decline in autophagy has been shown to lead to the accumulation of transcriptional factor GATA4, a known driver of senescence and inflammation [68]. Preliminary evidence from a human cell co-culture system suggests that senescent microglial cells show decreased autophagy which exacerbates the accumulation of Aβ [69]. This observation is in line with another report pointing to a role of autophagy in preventing senescence in neurons [70]. Finally, mitochondrial research provides additional evidence of the similarities between the cellular alterations seen in senescence and AD. Mitochondrial

dysfunction triggers senescence with a distinct secretory phenotype [71]. Similarly, signs of defective **mitophagy**, accumulation of dysfunctional mitochondria and increased oxidative stress, are seen in the AD brain [72]. Importantly, mitophagy mitigates inflammation [73] and its restoration prevents cognitive decline [74].

It is likely that AD pathology triggers senescence [8-11, 75] further increasing the burden of senescent cells and inflammation in the aged brain, and possibly starting a positive feedback loop that might exacerbate disease. This potential scenario highlights the need to choose appropriate AD models that recapitulate the aged brain microenvironment in order not to underestimate the influence that senescence and chronic inflammation might have during neurodegeneration. Additionally, cellular dysfunction and impaired intercellular communication due to senescence combined with a high-risk genetic background could lead to AD (Figure 1). Therefore, understanding the key events that enable the shift from healthy brain ageing to pathological ageing and neurodegeneration is essential. In this regard, comparing the spatial distribution and identity of brain senescent cells during healthy ageing and pathological ageing at different stages of AD could shed some light on the potential roles of cellular senescence during disease progression.

Based on the current evidence, we hypothesize two primary sources of senescent cells in the AD brain: age-related senescence and pathology-induced senescence (Table 1).

Age-related senescence as a driver of neuroinflammation. Alongside other risk factors, age-related build-up of senescent cells in the brain could set an ideal pro-inflammatory environment for the onset of AD. Following this line of thought, a recent transcriptomic comparison of the hippocampus of young and aged mice shows age-related accumulation of

senescence markers in microglia and oligodendrocyte progenitor cells (OPCs) and how the removal of senescent microglia prevents signs of cognitive decline while decreasing neuroinflammation [51]. The evidence regarding which cell types could undergo age-related senescence in the brain is still sparse, but the literature is growing, and we next briefly discuss some of the cell types that have been examined in this context.

Microglia. Several studies have identified age-related changes in microglia. For instance, the presence of dystrophic microglia likely precedes the spread of tau pathology [76] and other neurodegenerative processes [77]. Ageing, apart from causing morphological changes in microglia, lowers the microglial threshold to trigger an inflammatory response, as the cells enter a “priming” state [48]. Evidence from human tissue suggests that ageing alters microglial gene expression in virtually every brain area [78]. Ageing also correlates with **myelin** degradation, and clearance of the resulting myelin pieces by microglia leads to **lipofuscin** accumulation in lysosomes [79], a well-known senescence marker [80]. Accumulation of myelin debris over a certain threshold could overwhelm the phagocytic capacity of microglia and trigger senescence [81]. Under stress or pathological states, certain microglial cells show signs which correlate with typical features seen in senescent cells such as oxidative stress and nuclear chromatin remodelling that gives them a “dark” appearance in electron micrographs [82]. Overall, these results suggest that at least some of these subsets of age-related dystrophic, primed, lipofuscin-positive, or dark microglia are senescent.

Another important feature of aged microglia is the intracellular accumulation of lipid droplets alongside an increase in the number of lysosomes [83]. In other cell types, lipid accumulation is in fact a marker of senescence, as for instance in the case of foamy **macrophages** [84]

and certain glial cells [62]. However, whether there is a connection between age-related lipid accumulation in microglia and cellular senescence remains to be elucidated.

Based on these observations, it is intriguing to speculate that both cell-autonomous and non-autonomous factors might be inducers of senescence in microglia, or at least serve as senescence markers in this cell type. Cell-autonomously, for instance, senescent microglia might result from cell-intrinsic ageing processes or from prolonged excessive phagocytosis (for instance of Aβ). Cell non-autonomous mechanisms could involve, for instance, signals from senescent neurons (e.g. as a consequence of tau pathology). Further research is needed to better understand whether microglial senescence is a primary event in AD pathology or more of a by-product of the disease, and more generally, to clarify microglia's role in neurodegeneration.

Astrocytes. Upon injury, disease, or ageing, astrocytes enter a reactive state that involves transcriptional changes, and can result in a neurotoxic phenotype that challenges the viability of neurons and oligodendrocytes. Interestingly, the transition to the reactive state is indirectly mediated by paracrine communication with activated microglia [85]. Preliminary findings suggest that defects in nuclear morphology and loss of lamin B1 are hallmarks of astrocyte senescence [86]. There is evidence that senescent astrocytes accumulate in the human brain of aged donors, and the senescent cell burden is exacerbated in AD patients [87, 88]. Relatedly, RNA sequencing analyses indicate a disease-associated astrocyte population whose abundance increases with age and during AD progression [89]. Therefore, it seems reasonable to speculate that a at least a certain subset of reactive and disease-associated astrocytes are senescent. Notably, the accumulation of the senescent astrocytes might be directly linked to neurodegeneration by promoting excitotoxicity [90].

Due to the paracrine effects of microglia on astrocytes, it is possible that microglial cells undergo senescence first, and transmit senescence to astrocytes at a later stage. Given the tight crosstalk between brain cell types, a priority goal for future research is to clarify the potential hierarchies or different waves of senescence in the brain. This information will be critical in attempts to design effective therapeutic strategies that target senescence.

Neurons. Neurons are terminally differentiated cells and whether, as such, they can undergo senescence remains a matter of controversy. However, there is growing evidence to support an affirmative answer [9, 63, 70, 91-93]. Most neurons die when exposed to amyloid-β, however, certain neurons abnormally re-enter the cell cycle and are protected from cell death [94]. This raises the possibility that the cell cycle re-entry events seen in post-mitotic neurons trigger a senescence-like phenotype. In line with this hypothesis, the prevalence of senescence-like neurons increases with age [92]. Interestingly, senescent cells are resistant to apoptosis [20, 21] and, coincidentally or not, an alternative mechanism of cell death, necroptosis, has been associated with the neuronal loss seen in the AD brain [95]. The choice of senescence over cell death could be beneficial as a way of preserving cell numbers in tissues with restricted regenerative capacity, but at the price of promoting inflammation [96].

Endothelial cells. Endothelial dysfunction in the ageing brain leads to neurovascular uncoupling, impaired cerebral blood flow and a leaky BBB. Interestingly, primary endothelial cells from an accelerated ageing mouse model, which displays higher BBB permeability than WT littermates, are found to be prematurely senescent, adding to the possibility that endothelial dysfunction could be senescence driven [97]. In this regard, a single-cell RNAseq study has revealed accumulation of senescent brain endothelial cells in aged mice [98], and

human microvessels isolated from the prefrontal cortex at advanced Braak stages show upregulation of genes associated with endothelial cell senescence [99]. Relatedly, impaired senescence response in endothelial cells has been linked to cerebral vascular malformations [100] and new evidence suggests that certain senescent endothelial cells in the liver show an enhanced detoxifying function and removal of these is detrimental for the animal [101]. Of note, a recent preprint challenges the prevailing assumption that microglial cells express the majority of risk genes for AD, and reports that particularly in humans, the vasculature plays a more prominent role than previously appreciated, with striking differences between mouse models and humans [102]. As such, the role of endothelial senescence in the brain during ageing and AD warrants further investigation.

Pathology-induced senescence exacerbates AD. Recent evidence shows a correlation between senescent cells in the brain and Aβ plaques and tau pathology [8-11]. This raises the possibility that senescent cells might start a positive feedback loop that accelerates the progression of AD (Figure 2).

Aβ-driven senescence. One of the early responses to amyloid lesions is the reactivation of microglia proliferation and the appearance of the disease-associated microglia (DAM) phenotype [103]. A recent publication demonstrates that the augmented microglia proliferative rate triggers replicative senescence and that senescent microglia result in the DAM phenotype [11]. Interestingly, this mechanism of Aβ-induced senescence draws parallels with the hyperproliferative state triggered by oncogenic activation during oncogene-induced senescence and opens up the possibility of additional shared pathways between cancer and neurodegeneration with cellular senescence as a central phenotype.

Aβ plaques also induce senescence in OPCs based on observations in the brains of patients with AD and in an Aβ AD mouse model [10]. Eliminating the senescent OPCs using a brief course of senolytics decreases inflammation and microglial activation. Senolytic treatment for longer periods also decreases Aβ plaque size and improves cognition, suggesting that senescent cells exacerbate Aβ pathology. This study proposes paracrine effects of senescent cells that merit additional investigation, particularly the influence of the senescent microenvironment as a trigger of microglia activation.

Tau-driven senescence. In a mouse model of tau-dependent neurodegeneration, the accumulation of tau in neurons triggered senescence in astrocytes and microglia [8]. Importantly, elimination of the senescent glia ameliorated neurodegeneration, which suggests a causal role of senescence in tau-dependent pathology. It is important to note that in the aforementioned study, senescent cells were eliminated using pharmacological manipulations applied systemically and via genetic approaches. This raises the intriguing question of whether the beneficial effects of the elimination of senescent cells in the brain might be enhanced by the elimination of senescent cells in the periphery, and the consequent lower levels of systemic inflammation.

The possibility that senescence spreads from neurons to glia in a paracrine manner should be further investigated, as well as the question of the identity of the SASP components being involved. In this regard, a recent preprint suggests that astrocyte senescence is triggered by uptake of neuronal tau, which results in HMGB1 release [104]. HMGB1 is a danger-associated molecular pattern (DAMP) molecule secreted by senescent cells shortly after induction [105]. Interestingly, increased HMGB1 levels in the AD brain have been previously

associated with the microglia response to amyloid plaques [106]. Therefore, HMGB1 could be an early marker of neuroinflammation driven by senescent glial cells.

Transcriptomic analyses of NFT-containing neurons from post-mortem AD brains unveiled an expression profile compatible with cellular senescence [9]. Validation with different AD transgenic mouse models further suggests that tau accumulation triggers neuronal senescence, and that the specific elimination of these senescent neurons reduces neurodegeneration [9]. Conversely, crossing a tauopathy model with senescence-accelerated mice shows an exacerbation of tau pathology [107], which reinforces the idea that ageing and senescence-induced inflammation contribute to neurodegeneration.

New therapeutic strategies

Despite the prevalence of Alzheimer's disease, there is still no cure. Therapies aiming to reduce amyloid beta load have all failed at clinical trials and so there is an urgent need to develop new strategies. Cellular senescence is emerging as an interesting player in the pathophysiology of AD making senotherapies an attractive therapeutic alternative. Senolytic therapies (Box 3) have already shown promising results in mouse models of tau-dependent neurodegeneration [8, 9] and AD [10] and are now entering the clinical stage: two upcoming clinical trials (NCT04785300[I] (ALSENLITE) and NCT04685590[II] (SToMP-AD)) will assess the therapeutic potential of the senolytic cocktail dasatinib and quercetin (D+Q) in older individuals with MCI. ALSENLITE is an open-label pilot study of intermittent administration of dasatinib (phase 1) and quercetin (phase 2) whereas SToMP-AD is a phase 2 multi-site, randomized, double-blind placebo-controlled trial that will determine safety and efficacy in older adults with MCI or early-stage AD. However, in order to avoid potential negative effects of eliminating senescent cells [101, 108] alternative senotherapeutic approaches should be

tested to modulate neuroinflammation, for instance, with strategies aimed at suppressing the SASP. Moreover, senescent cells are heterogeneous and dynamic and so is their associated secretory phenotype [24, 25, 109]. There is therefore an urgent need to elucidate which cell types undergo senescence in AD as well as the timeline and nature of the SASP. A joint effort towards the establishment of a tissue-specific senescence cell atlas is essential and will provide a key resource for testing the therapeutic potential of future approaches [110]. Whereas many therapies have shown promise in rodent models, it is important to note that most have failed in human trials thereby stressing the need to use models which rely on human cells [111-115] when investigating possible therapeutics for AD.

Concluding remarks

Thinking outside the amyloid box and acknowledging the relevance of the complex cellular changes and crosstalk between cell types seen during the preclinical phase of AD will be crucial in the search for new therapies. As an example, senescence and senescence-induced inflammation could have profound cell autonomous and non-autonomous effects, impairing the cellular homeostatic responses in the brain. However, the involvement of cellular senescence in the healthy brain or during pathological ageing remains underexplored (see Outstanding Questions). Age-related build-up of senescent cells in the brain might be responsible for creating the perfect pro-inflammatory conditions to favour the onset of AD in the presence of other risk factors. Later, it is conceivable that positive feedback loops between senescent cells and neuropathological hallmarks of AD, particularly Aβ plaques and NFT-accumulating neurons, could accelerate neurodegeneration worsening cognitive impairment, although these possibilities require further investigation.

399 It may well be the case that current evidence of cellular senescence in AD represents just
400 the tip of the iceberg. Of note, studies on age-related diseases outside the CNS increasingly
401 suggest causal links between cellular senescence and disease progression. These research
402 domains may offer an important foundation for further investigation into the roles of
403 senescence in healthy and pathological brain ageing.
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Brain cell type	Markers of age-related senescence	Markers of pathology-induced senescence
Neurons	Elevated p16, p21, γH2A.X, p-p38, SASP, mH2A, SA-βGal, lipofuscin, GATA4; loss of lamin B1 and HMGB1 [51, 68, 70, 92].	Elevated p16, p21, γH2A.X, SASP [9].
Microglia	Dystrophic morphology; elevated p16, p21, SASP, lipofuscin [51, 76, 79].	Dystrophic morphology; elevated p16, p21, SASP, SA-βGal; telomere shortening [8, 11, 76].
Astrocytes	Elevated p16, p21, GATA4, SASP; loss of lamin B1 [51, 68, 86, 87].	Elevated p16, p21, SASP, SA-βGal [8, 87, 104].
OPCs	Elevated p16, p21 [51].	Elevated p16, p21, SASP, SA-βGal [10].
Oligodendrocytes	Elevated p21 [51].	
Endothelial cells	Elevated p16, SA-βGal [97].	Elevated p21, SASP [99].

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407 **Table 1.** Senescence markers in the brain during age- or pathology-induced senescence.

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Figure 1. Senescence-induced inflammation during brain ageing and Alzheimer´s Disease (AD). Recent evidence suggests increased inflammation observed in AD may be partly driven by an increase in cellular senescence triggered by amyloid beta and/or tau pathology. Similarly, ageing, the biggest risk factor for AD, also leads to an accumulation of senescent cells which contribute to chronic inflammation through the senescence-associated secretory phenotype (SASP). Senescence-induced inflammation may increase AD risk alongside additional genetic and/or environmental factors. NFT: neurofibrillary tangles.

Figure 2. Putative pathways of senescence-induced inflammation in AD. Age-related senescence of vascular cells contributes to blood-brain barrier (BBB) breakdown and facilitates entry into the brain of systemic factors that may elicit an inflammatory response. Hallmarks of AD - Aβ plaques, neurofibrillary tangles (NFT), and neuroinflammation – induce senescence of neurons and glial cells (OPCs, astrocytes and microglia). Pro-inflammatory cytokines released by brain senescent cells (including IL-6, IL-1β, PAI-1, TNF-alpha, HMGB1) exacerbate AD pathology, leading to neurodegeneration and cognitive decline.

Box 1 – Mechanisms of cellular senescence

Cellular senescence is characterized by a stable cell growth arrest in response to different types of stress, including replicative exhaustion, oncogenic activation, or mitochondrial dysfunction [19]. Senescent cells accumulate cyclin-dependent kinase inhibitors to preserve the proliferative arrest, such as p16, that is routinely used as a marker of senescence [22]. Senescence is normally triggered in response to persistent DNA damage response (DDR) activation [116]. For example, critically short telomeres elicit a DDR that leads to replicative senescence [117]. Alternatively, telomeric DNA damage independent of telomere length triggers senescence in non-proliferating cells [118]. Other sources of DNA damage include the hyperproliferation stress seen during oncogene-induced senescence and around amyloid plaques [11, 119], and the accumulation of dysfunctional mitochondria that increases oxidative stress and promotes the SASP [120]. The persistent DDR activation is key for the initiation and maintenance of the SASP [121]. SASP is also regulated by activation of the p38MAPK pathway [122], transcriptional factors like NF-κB, C/EBP-β, and GATA4 [26, 27, 68], and epigenetic mechanisms [123]. Another hallmark of cellular senescence is the loss of nuclear lamina protein lamin B1 [124], leading to large-scale changes in the chromatin landscape [125] and compromised integrity of the nuclear envelope. As a result, leakage of fragments of nuclear chromatin into the cytoplasm of senescent cells trigger activation of the innate immunity cytosolic DNA-sensing cGAS/STING pathway, leading to both short-term and chronic inflammation [126, 127]. SASP is subject to temporal regulation, including the late activation of LINE-1 retrotransposable elements leading to a type-1 interferon response (IFN-1) that contributes to chronic inflammation [109]. The roles of autophagy during senescence are not yet fully clear. DNA damage and ageing prevent autophagic degradation of transcription factor GATA4 leading to its subsequent accumulation that reinforces senescence and the SASP [68]. Therefore, autophagy might act as a negative regulator of

senescence. Supporting this hypothesis, there is a decline in general autophagy and mitophagy in aged muscle stem cells that, if reverted, prevents senescence [128]. Conversely, autophagy degrades nuclear SIRT1 promoting senescence [129] and is required to support the establishment and implementation of the senescence phenotype including the SASP [130].

Box 2 – The impact of ageing on the immune system

Immunosenescence affects both the adaptive and the innate immune system. Lymphocytes showing impaired function, low proliferative activity, and high secretory profile accumulate during ageing and are possibly senescent [131]. Examples include exhausted memory B cells [132] and effector memory CD8+ T cells re-expressing CD45RA (T_{EMRA}) [133]. In a recent report, this specific type of CD8+ T cells was found in both peripheral blood and cerebrospinal fluid (CSF) of AD patients and negatively correlated with cognitive decline [134]. Macrophages are recruited by the SASP to clear senescent cells and, as a consequence, they upregulate *p16Ink4a* and increase SA-β-galactosidase activity [135]. These senescence-associated macrophages (SAM) accumulate with age and can therefore contribute to chronic inflammation. Macrophages and senescent cells share indeed many phenotypes, including lysosomal expansion, metabolic reprogramming, a secretory phenotype, phagocytic capacity and growth arrest [136]. Consequently, some senolytic drugs target macrophages resulting in therapeutic side-effects [84, 137]. However, this senescence-like state in macrophages is reversible at least under certain circumstances [138]. Importantly, reversing age-related metabolic changes in peripheral macrophages is sufficient to lower chronic inflammation and improve cognition [139], which highlights the influence of systemic inflammation on the aged brain. Other markers of senescence seen in the aged immune system include telomere attrition [140] and accumulation of lipofuscin [141].

Box 3 – A snapshot on senolytics.

Naturally aged mice genetically engineered to eliminate $p16^{Ink4a}$-positive senescent cells show less age-related pathologies than same-aged counterparts [4]. This discovery started a race to develop senolytics [142] with the hope to translate the results from mouse models into the clinic. The most widely used senolytic in proof-of-concept experiments, ABT263, causes thrombocytopenia which restrains its clinical potential [21]. Newer senolytic approaches to limit side-effects include the repurposing of FDA-approved drug digoxin [143, 144], the use of galactose-modified prodrugs to increase selectivity towards senescent cells [145-147], immunotherapy using CAR T cells designed to target a cell-surface protein specifically expressed by senescent cells [148], or inhibition of glutamine metabolism [149]. However, the knowledge about the role of cellular senescence in neurodegenerative diseases like AD is still sparse in comparison to other diseases. Therefore, the potential use of senolytics to treat dementias like AD should be approached with caution until we have a clearer picture of the functions of senescence during brain ageing and AD.

Glossary

Ageing: accumulation of molecular and cellular damage over time that affects most living organisms. It leads to functional decline, growing risk of disease and, ultimately, death.

Alzheimer's disease: progressive and irreversible brain disorder that constitutes the most common cause of dementia. The neuropathological hallmarks include amyloid plaques and neurofibrillary tangles.

Astrocytes: abundant glial cells that maintain homeostasis of the brain by providing trophic and metabolic support to neurons.

Autophagy: intracellular degradation system that facilitates lysosomal degradation of misfolded or unfolded proteins and of damaged organelles.

Blood-brain barrier (BBB): a semi-permeable barrier separating the blood from the cerebrospinal fluid, and constituting a barrier to the passage of cells, particles and large molecules. It mainly consists of a tightly sealed endothelium sheathed by mural vascular cells and perivascular astrocyte end-feet.

Cellular senescence: state of irreversible cell cycle arrest elicited in response to different type of stress to restrain the expansion of old and damaged cells. Senescent cells undergo many other phenotypic alterations besides the growth arrest, including a secretory phenotype.

Dementia: general term for a particular group of symptoms, including difficulties with memory, language, problem-solving and other thinking skills that affect a person's ability to perform everyday activities.

Healthspan: the average length of an organism's life during which they are in good health.

Immunosenescence: gradual deterioration of the immune system with advanced age, mainly affecting the adaptive immunity.

Inflammation: defence mechanism to resolve tissue damage and preserve tissue homeostasis after injury or infection. It normally involves innate and adaptive immune responses, and the release of pro-inflammatory cytokine mediators. When acute, is protective. However, chronic inflammation promotes an array of diseases.

Inflammaging: low-grade and "sterile" (not induced by pathogens) chronic systemic inflammation that develops with advanced age.

Lifespan: the average length of an organism's life. Period of time between birth and death.

Lipofuscin: age-related intracellular aggregates composed of oxidized proteins and lipid-containing residues of lysosomal digestion.

Macrophages: effector cells of the innate immune system. They recognize, phagocytose and destroy microorganisms, diseased or damaged cells. Macrophages in the brain are called microglia.

Microglia: resident immune cells of the brain that constantly monitor the cerebral microenvironment to respond to pathogens and damage.

Mitophagy: specialized type of autophagy aimed at eliminating dysfunctional mitochondria and with a prominent role in preventing age-related pathology.

Myelin: insulating layer around nerves made up of protein and fatty substances. The myelin sheath allows electrical impulses to transmit quickly and efficiently along the nerve cells.

Neuroinflammation: inflammation of the nervous system seen in most neurological disorders.

Oligodendrocytes: type of glial cells whose main function is to form and maintain the myelin that surrounds and insulates neuronal axons.

Paracrine senescence: bystander or secondary senescence as a result of non-cell autonomous exposure to specific components of the senescence-associated secretory phenotype.

Senescence-associated secretory phenotype (SASP): heterogeneous, complex and dynamic mixture of cytokines, pro-inflammatory mediators and growth factors secreted by senescent cells and responsible for most of the physiological and pathophysiological roles of senescence.

Senolytic: a drug that selectively induces death of senescent cells.

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Declaration of Interests

A.G. is a named inventor in an MRC patent related to senolytic therapies. B.D.S. is a founder of the company K5 Tx which develops Alzheimer's Disease drug targets.

Resources

[I] https://clinicaltrials.gov/ct2/show/NCT04785300

[II] https://clinicaltrials.gov/ct2/show/NCT04685590

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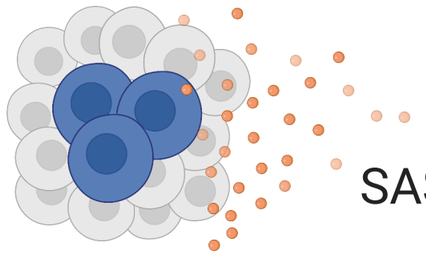
Highlights

- Senescent cells accumulate in aged tissues and often play a causal role in age-related pathologies, partly due to their pro-inflammatory secretome.
- In mouse models of neurodegeneration, clearance of senescent glial cells ameliorates tau-dependent neurodegeneration and decreases inflammation as well as beta-amyloid plaque size.
- Senescence emerges as a pivotal player in the complex cellular landscape of AD.
- The senescence secretome constitutes a promising therapeutic target to balance neuroinflammation during AD progression.

Outstanding Questions

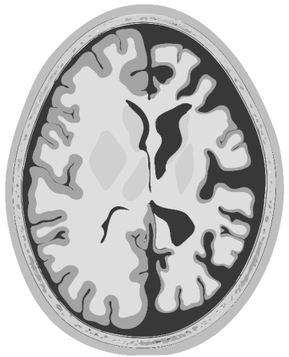
- Senescent or senescent-like cells have been identified in AD rodent models and human tissue. Is cellular senescence a by-product or a driving force of Alzheimer's Disease?
- Targeting senescent cells for therapeutic benefit shows efficacy in pre-clinical studies and is at the verge of clinical trials. However, senescent cells are heterogenous, and recent data challenges the assumption that all senescent cells are detrimental. Which brain cell types undergo senescence? Do brain senescent cells play any beneficial roles? And what is the spatio-temporal pattern of senescent cell accumulation during disease progression?
- An alternative path to the use of senolytics as a therapeutic strategy involves the modulation of the senescence-associated secretory phenotype (SASP). The SASP holds the ability to spread the senescence phenotype to the neighbouring cells. Does the SASP mediate paracrine senescence in the brain? Which is the nature of the senescence secretome in the brain? Could we balance neuroinflammation by modulating the SASP?
- Disruption of the BBB is an age-related event that is accelerated by AD. Could systemic inflammatory mediators reaching the brain trigger senescence and contribute to neuroinflammation?
- Various sources of inflammation (e.g. traumatic brain injury and systemic inflammation) have been identified as a risk factor for Alzheimer's disease. Do the mechanistic pathways of these acute sources of inflammation differ from those of senescence-induced inflammation? Are there instances where these pathways converge, leading to exacerbated pathology?

Senescent cells



senescence
induced
inflammation

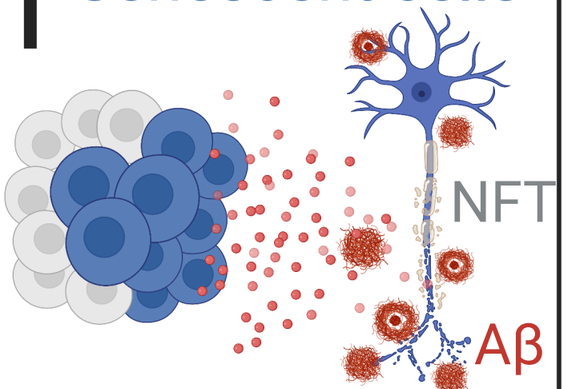
Healthy,
aged



Alzheimer´s
Disease

genetic risk, environment

Senescent cells



senescence
induced
inflammation

Systemic inflammation

Endothelial cells (senescent)

BBB

Pericytes (senescent?)

Astrocyte

inflammation

Aβ induced OPC senescence

IL6, IL1B, PAI1, TNFA, HMGB1,...

microglia & astrocyte senescence

Neurodegeneration

NFT neurons (senescent)

senescence