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The Glial Aging & Senescence Hypothesis of Late-onset Alzheimer's

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Abstract

Alzheimer's disease (AD) predominantly occurs as a late-onset form (LOAD), involving neurodegeneration and cognitive decline with progressive memory loss. Over time, risk factors and aging promote accumulation of well-known AD pathologies in oxidative stress, amyloid-beta and tau protein pathology, as well as inflammation. Homeostatic glial functions regulate and suppress these AD pathologies; however, other glial states involve increased pro-inflammatory cytokine release and further pathology accumulation. Different stresses can additionally induce cellular senescence, or an irreversible differentiation process resulting in decreased supportive functions and increased, pro-inflammatory cytokine release. While these pathophysiological underpinnings all contribute to LOAD, they require temporal and mechanistic integration. This perspective hypothesizes that when individuals have threshold senescent glia accumulation, they transition from healthy cognition into mild cognitive impairment and LOAD diagnosis. Particularly, senescent microglia are predicted to represent a final threshold required for the tau pathology burden and spreading that corresponds to sustained neurodegeneration and dementia severity. We first explore age-related decline in glia that promote increases in AD pathologies, and then discuss emerging evidence linking oxidative stress, neurons containing tau pathology, and amyloid-beta to microglia, oligodendrocyte progenitor cell, and astrocyte senescence. Our hypothesis proposes that senescent astrocytes and oligodendrocyte progenitors pressure microglia to phagocytose neurons containing tau pathology. The resulting senescent microglia would form neuritic plaques and induce paracrine senescence transitioning into a progressive clinical dementia presentation. This predictive hypothesis can potentially account for why medications used to treat LOAD fail, as previous treatments have not reduced senescent glial burden. It is also coherent with the predominant hypotheses surrounding LOAD, generates testable hypotheses about LOAD, and increases rationale in testing senolytics as targeted treatments for LOAD arrest and reversal.

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List of Abbreviations

APP/Aβ: Amyloid precursor protein; Amyloid-beta
AD: Alzheimer's disease
ATM-NEMO-IKK: Ataxia-telangiectasia-mutated; NF-κβ essential modulator; Inhibitor of κβ kinase
APOE: Apolipoprotein E
CDK2NA / $p16^{INK4A}$: Cyclin-dependent kinase inhibitor 2A
cGAS-cGAMP-STING: Cyclic GMP-AMP synthase; cyclic GMP-AMP; Stimulator of interferon genes
CNS: Central nervous system
CR1/3/4: Complement receptor 1/3/4
CSF1R: Colony stimulating factor 1 receptor
C/EBP: CCAAT-enhancer-binding protein
C1q: Complement component 1q
C3b/iC3b: Complement component 3b; inactivated C3b
CX3CR1: Fractalkine receptor
DAM1/2/MGnD: Disease-associated microglia stage 1/2
DAP12/TYROBP/KARAP: DNAX Activating Protein of 12kDa
EAAT1/2: Excitatory amino acid transporter 1/2
EOAD: Early-onset Alzheimer's disease
FTH1/FTL: Ferritin heavy chain 1 / Ferritin light chain
GSK3: Glycogen synthase kinase-3
HMGB1: High mobility group box protein 1
hp-tau: Hyperphosphorylated tau
IGF1R: Insulin-like growth factor 1 receptor
IL: Interleukin
IRF/IFN: Interferon regulatory factors; Interferon
ITGAX/Cd11c: Complement component 3 receptor 4 subunit
Kir4.1: Inwardly rectifying potassium channel 4.1
Kv1.3: Voltage-gated potassium channel 1.3
LOAD: Late-onset Alzheimer's disease
LRP1: Low-density lipoprotein receptor-related protein 1
LXR: Liver X receptor
MMP: Matrix metalloprotease
mTORC1: Mechanistic target of rapamycin complex 1
NF-κβ: Nuclear factor kappa-light-chain-enhancer of activated B cells
NFT: Neurofibrillary tangle
NLRP3: NLR family pyrin domain containing 3
NMDA: N-methyl-d-aspartate
OPC/N2 glia: Oligodendrocyte progenitor cell
PS: Phosphatidylserine
p21: Cyclin-dependent kinase inhibitor 1
p38 MAPK: p38 mitogen-activated protein kinases
RAGE: Receptor for advanced glycation end products
RONS: Reactive oxygen and nitrogen species

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SA-β-gal: Senescence-associated beta-galactosidase
SAHF: Senescence-associated heterochromatin foci
SASP: Senescence-associated secretory phenotype
TAM: Tyro3, Axl, and Mer receptor tyrosine kinases
TGF-β2-TGFBR2: Transforming growth factor beta (receptor type) 2
TNF: Tumour necrosis factor
TREM2: Triggering receptor expressed on myeloid cells 2
VPS35: Vacuolar protein sorting ortholog 35

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Introduction

Alzheimer's disease (AD) involves cognitive decline, most prominently manifested as progressive memory loss. Risk factors linked to AD include vascular pathology, declining metabolism, and most dramatically, aging[1–4]. These risk factors contribute especially towards the highly-prevalent (>90% of AD cases) sporadic or late-onset AD (LOAD), and to a lesser extent to the early-onset or inherited, familial AD (EOAD)[3]. In LOAD, synaptic loss is pathologically correlated with neurodegeneration and cognitive decline[1,2]; however, little mechanistic insight is available to drive the development of effective therapeutics. Widely accepted explanations for neuronal loss and tracking LOAD progression have been proposed in the tau hypothesis and amyloid cascade (Box 1). Although exploring each classical AD paradigm has significantly improved progress towards understanding AD, no clinical trial targeting these pathologies has successfully halted AD[3,4]. A recent shift has geared towards understanding AD by focusing on next generation sequencing, aging-related pathology, and glia, or the non-neuronal brain cells. Particularly, many new genetic risk factors including *TREM2, CR1,* and *APOE* have exclusive roles in glia[3]. Some genes mediate increased phagocytic function in these glia, whereas other genes allot glia towards adopting pro-inflammatory states leading to chronic damage and cell death. Recently, the evidence for possible roles of glia and senescence in AD was explored[5,6].

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Box 1: Brief summary of Braak staging and dominant LOAD theories

In LOAD, the amyloid cascade and tau hypotheses are most known for explaining the synaptic loss and neurodegeneration observed over dementia progression:

- Amyloid Cascade: Accumulation of amyloid-beta (Aβ) protein, especially Aβ oligomers, act through multiple pathways to cause neurodegeneration and synaptic loss[2,3]. Misfolded Aβ monomers undergo a slow 'seeding', nucleating phase into damaging Aβ oligomers in AD, which then further aggregate into insoluble Aβ fibrils and diffuse amyloid plaques without hp-tau aggregates[3]. Misfolded hp-tau and Aβ can also destabilize their respective native conformations and spread equivalently as prions[3]. Moreover, both protein pathologies add to each other (Fig. 1)[3]. While the levels of Aβ protein are increased in LOAD patients *vs* controls, diffuse amyloid plaques and insoluble Aβ fibril aggregation do not correlate well with LOAD progression[1,7,8].

- Tau Hypothesis: Upstream enzymes including p38 MAPK and GSK3 phosphorylate multiple residues in tau protein to create hyperphosphorylated tau (hp-tau)[1,3]. Hp-tau starts as soluble monomers and oligomers, but can form higher-complexity aggregates in insoluble fibrils and neurofibrillary tangles (NFTs). Neuritic plaques, which comprise compact amyloid plaques containing both hp-tau and dystrophic neuronal elements[1,3,8], also constitute as hp-tau pathology. Together, all forms of hp-tau aggregation become equivalently classified under "neurofibrillary pathology" or neurodegeneration. This hp-tau pathology correlates well with cognitive impairment and dementia.

- Braak Staging: Although a newer definition of AD involves scoring dementia severity through a framework of Aβ deposition, neurofibrillary pathology, and neurodegeneration[9], postmortem assessments of cognitive decline and correlates of neurodegeneration severity in LOAD have been well tracked through Braak staging[1,7]. Here, hp-tau aggregation and neurofibrillary degeneration is clinically documented into a set of six Braak stages[8]. In stages I and II, initial neurofibrillary pathology spreads from the transentorhinal region into the entorhinal cortex and hippocampus. Stages III and IV involve increased lesions and pathologies in these areas, and novel spread into the temporal lobe and insular cortex. Stages V and VI affect the remaining superior temporal gyrus and neocortex. Finally, Braak staging of hp-tau pathology has been well shown to correlate with LOAD progression, where stage IV marks overt dementia diagnosis (Fig. 2)[1,8,10].

- Hypotheses outside the tau hypothesis and amyloid cascade were postulated, and may focus on other contributors and risk factors associated with LOAD. Although this perspective argues that these factors eventually converge into increased glial senescence burden, comprehensive coverage of these theories is outside the scope of this perspective. Briefly, these other factors include impaired metabolism by a combination of increased insulin resistance, hypertension, obesity, and/or insufficient sleep[3,4]. Vascular dementia, consisting of progressive blood supply blockades, cerebral amyloid angiopathy and Aβ deposition into cerebral blood vessels, likely leads to and/or correlates with LOAD incidence[4]. LOAD pathology is also often co-morbid with pathologies from other diseases such as Parkinson's disease with dementia[1]. Finally, all these different pathologies involve a neuroimmune component; inflammation caused by pro-inflammatory cytokine secretion increases during LOAD, notably as a result of accumulating protein aggregation and declining cell metabolism[2–4].

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Cellular senescence, an irreversible cell arrest caused by replicative senescence or environmental stresses such as DNA damage and oxidative stress[11–13], varies across specific cell type, physiological, and pathological environments. For example, mTORC1, DNA damage (by ATM-NEMO-IKK signalling), and oxidative stress via the p38 MAPK pathway and the NLRP3 inflammasome, can all induce senescence[13–16]. Due to this heterogeneity, no universal markers for senescence are currently available. As later argued, there are also potential senescence subtypes or different senescent cell states, which remain to be characterized. Nevertheless, a uniting, functional feature of senescent cells is that senescence allows affected cells to gain apoptotic resistance[11,14]. This comes at a compensatory expense of losing organelle function. For instance, senescent cells display mitochondrial dysfunction resulting in increased levels of reactive oxygen species[11,17].

Senescent cells present endolysosomal impairment, which results in lysosomal swelling and buildup of different substrates[11]. These substrates can comprise senescence-associated beta-galactosidase (SA-β-gal), ferritin, iron, and lipofuscin accumulation [11,14,18–22]. Other common senescence markers include $p16^{INK4A}$ and p21 upregulation relating to cell cycle arrest, alongside a reorganization of the nuclear lamina in downregulated Lamin B1 and formation of senescence-associated heterochromatin foci (SAHF)[11,14,20]. Senescent cells often functionally adopt a senescence-associated secretory phenotype (SASP), which after being initiated by the cGAS-cGAMP-STING pathway, leads to subsequent release of type I interferons (IFN), pro-inflammatory cytokine, matrix metalloproteases (MMPs), HMGB1 protein, and other secretions that vary across different cell types[11,16,23]. Major transcription factors and proteins involved in SASP regulation include NF-κβ, C/EBP, and HMGB1[13–15].

Although beyond the scope of this perspective's focus on LOAD, senescence plays physiological roles in wound healing, tumour suppression, embryonic development and tissue repair[11]. Senescence is also reliably implicated in aging and accelerated mortality[11,24], where senolytic drugs targeting and removing senescent cells have been shown to increase the lifespan and quality of life in mice[25–28]. Although senescence has been reviewed in terms of potential association with LOAD[5,6,29–31], how senescence relates to LOAD and Braak staging particularly is not well defined. Here, LOAD is uniquely hypothesized as a disease that progresses based on the severity of glial aging and senescence accumulation. While experimental validation is needed, this Perspective pioneers the argumentation of this hypothesis by first explaining LOAD pathology through an aging lens, followed by discussing evidence of glial dysfunction and decline in LOAD. An integration of mechanisms involving AD neurodegeneration and aged glia is then proposed, accounting for Aβ aggregation, hp-tau, and neurodegeneration. Particularly, microglial senescence mechanisms and abundance are posited as a central requirement for Braak staging progression and LOAD.

Part I: Late-onset AD requires aging and glial dysfunction

Aging and oxidative stress are understated risk factors in dominant LOAD paradigms

In all cells, oxygen intake and reactions generate free radicals that accumulate as reactive oxygen and nitrogen species (RONS), such as nitric oxide and superoxide[24]. When RONS accumulation overwhelms antioxidant defenses, RONS irreversibly alter and damage nucleotides, lipids, and proteins in cells. This phenomenon, oxidative stress, progresses with age due to accumulated double-stranded DNA breaks, mitochondrial dysfunction, and declining efficiency of metabolic processes[5,6,11,24]. Oxidative stress propagates pathways that elevate Aβ production, tau hyperphosphorylation, and release of pro-inflammatory cytokines including IL-1 and

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TNF[2,11,16,24,32,33]. Over time, increased pro-inflammatory cytokine production becomes a chronic systemic "inflammation" that increases with aging, and in turn further increases RONS accumulation, leading to exacerbated cell damage and premature death[3,34,35]. Many LOAD risk factors (such as vascular pathology, sleep impairment, and chronic stress) also result in increased RONS levels and chronic, systemic inflammation[2–4]. Furthermore, as later relevant, increased inflammation and the NLRP3 inflammasome can induce local or paracrine senescence via IL-1α activation signaling[15].

Oxidative stress damages all cell types, including neurons. Particularly, RONS inhibit phosphatidylserine (PS) lipid translocases, increase intracellular calcium, deplete ATP, and activate phosphatidylserine scramblases; this "flips" PS from the inner to outer cell membrane, where the exposed outer PS acts as an apoptotic or "eat-me" signal[36–39]. RONS additionally create "oxysterols" by oxidizing membrane cholesterols. Oxysterols have been hypothesized to drive LOAD[40–43], where one such mechanism will be elaborated below in Part II. Additionally, oxidative stress acts via the ATM pathway triggering DNA damage and p38 MAPK signalling to induce senescence[13,14].

Overall, these pathologies engage each other in a shared environment, cause further accumulation of other pathologies, as well as exacerbate the resulting cellular damage and death in a circular relationship (Fig. 1)[2,3,5,6,16,24,32–36,41–44]. Notably, patients must minimally be aged 65 to be potentially diagnosed with LOAD[1,3]. This provides significant time towards accruing aging and increased exposure to oxidative stress, which reflects in increased systemic inflammation, oxysterols, and protein aggregation resulting from RONS interactions[3,40]. What then allows for a healthy, aging cognition against the age-related elevation of traditional AD pathologies in Aβ aggregation, neurofibrillary tau pathology, chronic systemic inflammation, and oxidative stress?

CNS glia lose homeostatic capacity with aging

In the healthy CNS, different glial cell types exist. Here, we will provide discussion of oligodendrocyte progenitor cells (OPCs), astrocytes, and microglia. While reviews have covered their basic roles[45], it should be mentioned that microglia are brain immune macrophages that uniquely originate from the embryonic yolk sac, in contrast to peripheral immune cells that derive from bone marrow; thus, microglia live and function much longer than peripheral immune cells that can infiltrate the CNS[46]. Altogether, glia serve a dizzying array of functions, a subset or "states" of which can be labeled as "homeostatic". Homeostatic glial activities sequester and break down cellular products to preserve optimal nervous system function[45]; this includes degrading products labeled as traditional AD pathologies (Fig. 1). This parenchymal maintenance is achieved by paracrine signaling and endocytosis. Homeostatic glial functions involve trophic factors and secretion of anti-inflammatory cytokines including IL-10 and TGF-β, which minimize cellular stress and pro-inflammatory glial reactions, at least acutely[47–51]. These molecules mediate communication in bi-directional and overlapping loops of glia-neuron and glia-glia interactions, through which glia supporting one another facilitate neuronal activity and plasticity. Microglia, particularly, require OPC-mediated secretions via the TGF-β2-TGFBR2-CX3CR1 signaling axis to sustain their homeostatic functions[48,49]. Astrocytes also release trophic factors and cytokines including IL-3 to reduce pro-inflammatory microglial states[52], and both glia endocytose neuronal, synaptic, and extracellular elements[36,42,53–59]. Some of these elements include the traditional AD pathologies illustrated in Fig. 1[3,45]; for example, microglia phagocytose extracellular Aβ and tau through receptors such as TREM2 and IGF1R[3].

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However, expression of receptors that facilitate clearing of Aβ and tau additionally push homeostatic glia into "pro-inflammatory" states. Examples include the RAGE and LRP1 receptors, both of which activate the NLRP3 inflammasome[3,60–62]. While pro-inflammatory glia provide an effective protection in acute illness and infection, their continuous and exaggerated reactions during aging lead to cellular stress, premature death, and (indirectly) an accumulation of traditional AD pathologies[2,3,5]. The resulting environment gradually lowers homeostatic support for glia and may partially explain a "priming" effect, wherein aged glia exaggerate their pro-inflammatory responses towards smaller amounts of pro-inflammatory cytokines or Aβ[16,63,64]. In parallel, aging and exposure to RONS reduce homeostatic signaling, impairing the TGFβII-Smad pathway in aged microglia[57,58,62]. Aged glia additionally decline in metabolism and endolysosomal systems[6,11,62–64], resulting in reduced phagocytotic capacity and clearing of Aβ as well as hp-tau accumulation. Thus, while homeostatic glial functions protect the brain for most of the adult life, their ability to combat both increasing pro-inflammatory glial responses and traditional AD pathologies wanes over aging and environmental risk factor exposure (Fig. 2).

Glial senescence is a critical contributor to LOAD progression

Due to the processes that occur over aging, especially oxidative stress and concomitant recurrence of AD pathology buildup, cells can become senescent throughout one's lifespan (Fig 3.). In healthy physiology, immune cells that include microglia function to phagocytose and remove senescent cells[65]. However, over one's lifespan, immune cells themselves decline in function and can become senescent. This process, called immunosenescence, is offset by beneficial (epi)genetic variation and the ability of immune cells to proliferate by which healthy daughter cells can phagocytose senescent immune cells[14,65]; however, due to the maximal numbers of mitotic cycles (Hayflick's limit) and exposures to environmental stresses that immune cells can undergo, there is a natural upper limit to the proportion of immune cells that do not become senescent. Thus, as it increases immunosenescence and the cumulative senescent burden of the organism over time, senescence is considered a pathology of aging[11].

Because LOAD requires a significant percentage of one's lifespan to have passed[1,3], it is considered here to represent a disease involving senescence. This view is reinforced by evidence of shortened telomere lengths and increased immunosenescence in LOAD patients[29,30,66,67]. Tissue dysfunction and cognitive impairment in multiple mouse models have also been tied to senescent cell burden in the brain, where removing senescent cells has been shown to lower inflammation and increase the performance in spatial memory tasks[27,68,69]. There is also considerable evidence indicating that the aging brain has relatively higher levels of senescence cell markers compared to younger brains in mice[70], supporting the idea that LOAD progresses in an environment with increased senescent cell burden. Here, based on the previous section explaining how aging predisposes to increases in oxidative stress, Aβ accumulation and hp-tau pathology, an overview is given linking these aging-dependent increases to an induction of senescence among specific glial types. The contributions of these senescent glia to amplifying neurodegeneration and the AD pathology burden is also explored, building off of previous reviews on glial senescence in LOAD[5,6].

First, multiple lines of evidence likely indicate that APP overexpression and subsequent Aβ accumulation is associated with exacerbated OPC loss of function and senescence (Fig. 3). In APP/PS1 mouse models of EOAD, OPCs surrounding amyloid plaques display senescence per immunopositive p21, $p16^{INK4A}$ and SA-β-gal staining[69,71]. OPCs further demonstrate dystrophic shrinkage in cell volume, overall numbers also decrease in APP/PS1 mice, and correlate with decreased myelin levels, likely secondary to reduced oligodendrocyte differentiation and myelin

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generation[71,72]. As senescent OPCs from multiple sclerosis patients demonstrate HMGB1 release and decreased remyelination[73], Aβ-induced senescence in OPCs likely results in decreased myelin production and therefore neuronal support. In LOAD patients, global Aβ accumulation (levels of Aβ protein and not diffuse amyloid plaques) and increased numbers of p21+, senescent OPCs proportionally scale with progressive Braak staging[69]. This suggests that increased senescent OPC burden begins in early Braak stages and precedes the clinical dementia diagnosis, then continues to accumulate in parallel with disease progression into latter Braak stages (Fig. 2). Finally, as Aβ contributes to increased RONS levels in neurons[34], OPCs may potentially become senescent via DNA damage and/or oxidative stress (Fig. 3).

Astrocytes similarly experience an increased senescence in LOAD. Particularly, oxidative stress, Aβ, and hp-tau were all shown to induce human astrocyte senescence[74–76]. These senescent astrocytes exhibit a SASP profile, releasing pro-inflammatory cytokines and MMPs[74–76]. Senescent astrocytes also down-regulate potassium and glutamate transporters Kir4.1, EAAT1, and EAAT2. This results in an increased extracellular glutamate, contributing to neuronal excitotoxicity and death[77], alongside NMDA-receptor signaling and pro-inflammatory behaviour in microglia[78]. Senescent astrocytes positive for $p16^{INK4}$ were additionally found to increase in human brains over the course of aging and be over-represented in frontal cortices from LOAD patients *versus* age-matched controls[74]. Finally, in senescent astrocytes induced by hp-tau uptake, senescent astrocytes display a SASP profile and contribute to cognitive impairment via HMGB1 and activation of the NLRP3 inflammasome[44,75].

Increased senescent OPCs and astrocytes likely exacerbate traditional AD pathology burden and critically contribute to LOAD via multiple effects (Fig. 3): these cells speculatively (in)directly promote all LOAD pathology accumulation (Fig. 1), weaken neuronal support and induce cell death via RONS release and NLRP3 inflammasome activation[3,34,44,61], in addition to increasing pro-inflammatory states in other glia[5,6]. Astrocyte and OPC senescence also induces paracrine glial senescence, potentially through RONS release and NLRP3 activation via IL-1α signalling[5,15,30]. However, as Aβ and/or oxidative stress can trigger OPC and astrocyte senescence without tau[69,71,76], astrocyte and OPC senescence alone does not appear to correlate well with hp-tau spreading and neurofibrillary degeneration in Braak staging. Therefore, an additional player would be required to predict how glial senescence mediates the preclinical to clinical transition in LOAD. One part of this explanation posits that in preclinical LOAD, senescent OPCs and astrocytes speculatively withdraw their homeostatic support and exacerbate AD pathology accumulation; this combined effect would speculatively exhaust and "prime" homeostatic microglia to elicit increased pro-inflammatory reactions and phagocytosis of neurons[5,6,47–49,52,62,63]. Moreover, this priming and phagocytosis would be necessary to induce senescent microglia, which are argued as the final requirement needed to explain the preclinical to clinical LOAD transition, as well as spread of neurofibrillary pathology in NFT and neuritic plaque formation (Fig. 2, Fig. 3).

Part II: Threshold microglial senescence is predicted as the final requirement needed to drive Braak staging

Microglial senescence is driven by phagoptosis of hp-tau-containing neurons

When microglia are primed to phagocytose neurons in LOAD, they likely enter a vicious pathological cycle: multiple lines of evidence suggest that phagocytosis of hp-tau renders microglia senescent. In a P301S tauopathy mouse model, which approximately models AD

 doi:10.20944/preprints202109.0437.v2

pathology through human tau overexpression and subsequent human tau hyperphosphorylation, senescent microglia were identified with positive $p16^{INK4A}$ and SA-β-gal staining[68]. Both extracellular and intracellular hp-tau stress human neurons *in vitro*, induce outer PS exposure, and initiate premature phagocytosis or "phagoptosis" of living neurons causing their death by human microglia[79,80] (Box 2).

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Box 2: Clarifying the changes in microglial priming, ramified morphology, phagoptosis, and neurodegeneration in LOAD

While microglia in homeostatic states often comprise a ramified morphology, this morphological state does not always perform homeostatic functions in humans. Conversely, pro-inflammatory lipopolysaccharide has been shown to induce *in-vitro* a complex, hyper-ramified morphology in human microglia[81]. Pro-inflammatory microglial states are not always equivalent to the "activated" or hypertrophic, amoeboid-shaped microglia that cluster around neuritic plaques in LOAD patients[82–84]; however, amoeboid or hypertrophic morphologies can indicate that human microglia have phagocytosed extracellular materials[83]. This hypertrophic appearance is also observed regardless of the microglia's inflammatory status, as later discussed.

By contrast, in homeostatic conditions, murine microglia were shown to mainly utilize TREM2 in phagocytosing synapses and whole neurons for CNS development, plasticity, and maintenance[39,53]. While homeostatic microglia can phagocytose compartments from dead neurons to optimize the surrounding environment, they can also phagocytose neurons that are stressed but not yet apoptotic. This process is referred to as "phagoptosis"; a premature, non-apoptotic death through microglial phagocytosis[37,38]. Mechanistically, neuronal oxidative stress exposes PS at the outer cellular membrane to act as a ligand. Simultaneously, microglia reacting to pro-inflammatory stimuli produce sialidase that removes or "desialylates" neuronal sialic acids[36–38]. As microglia enter pro-inflammatory states more frequently during aging[39], and sialic acids protect neurons from being phagocytosed[36–38], aging would render neurons more susceptible to phagoptosis. Thus, although microglia in pro-inflammatory states may not directly partake in phagoptosis while secreting pro-inflammatory cytokines, resulting increases of oxidative stress likely "prime" homeostatic microglia to phagoptose neurons. Both the exposure and generation of extracellular and intracellular hp-tau aggregates, respectively, also prompt neurons to expose outer PS[79,80].

Once neuronal PS is sufficiently exposed, extracellular opsonin proteins can coat outer PS and induce phagoptotic death by microglia[36–38]. This process occurs via multiple ligand-receptor pairs that converge into the DAP12/TYROBP/KARAP pathway, stimulating downstream signaling and subsequent phagocytosis through TAM receptor tyrosine kinases in Mer and Axl[37,38]. Specific pathways leading into phagoptosis and DAP12 signaling include TREM2-PS direct binding, or TREM2 binding to an extracellular APOE-PS complex[38,54–56,85]. Complement C1q is another opsonin that coats PS, allowing for subsequent C3b, iC3b complement binding and phagoptosis by CR1/CR3/CR4 complement receptor activation[86]. These complement receptors also trigger downstream DAP12 signaling in microglia[37,38]. A separate pathway alternatively exists where calreticulin opsonin bins to C1q, resulting in the phagoptosis of neurons by microglial LRP1 together with the induction of more microglial, pro-inflammatory responses[3,38].

Of relevance, some homeostatic microglial states in Aβ-overexpressing mice phagoptose neurons and correspondingly exhibit a neurodegeneration/disease-associated microglial phenotype (DAM or MGnD)[87–89]. The transcriptome includes an initial DAM1, involving *APOE* upregulation independent of phagoptosis activation, and a subsequent DAM2, which necessitates TREM2-dependent activation, and further upregulates *APOE* and *ITGAX* relating to CR4 subunit CD11c[88]. Further DAM2 subtypes or states were also identified, with a pro-inflammatory state up-regulating the Kv1.3 channel protein that mediates increased RONS and pro-inflammatory cytokine release[89]. Dark microglia represent another relevant state, which has been observed in mouse models of chronic stress, aging and Aβ pathology, as well as in patients diagnosed with schizophrenia[90,91]. Dark microglia display cellular markers of oxidative stress and lipofuscin presence related to senescence[2,11]. They likely participate in phagoptosis; dark microglia have increased phagocytic inclusions and commonly enwrap processes around shrinking but still viable neuronal elements[90].

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Furthermore, Brelstaff *et al.* recently found that when murine microglial cells were co-cultured *in-vitro* with viable P301S neurons growing intracellular, human hp-tau filaments, these microglia prematurely phagoptosed the neurons then became senescent[92]. These senescent microglia exhibited increased NF-κβ activation, MMP-3 release, and positive SA-β-gal staining; additionally, they displayed an hypo-phagocytic capacity, or poor phagoptosis of P301S neurons containing hp-tau aggregates, together with an aberrant release of insoluble hp-tau aggregates into the local environment[92]. As cultured human microglial cells also phagoptose PS-exposed, P301S neurons containing hp-tau aggregates[57], both human and murine senescent microglia likely release and seed insoluble hp-tau aggregates[93–95]. Furthermore, Brelstaff *et al.* revealed that conditioned media, isolated from murine microglia and P301S neurons, induced senescence in separate murine microglial cultures[92]. This indicates that secretions from senescent microglia, containing hp-tau, can trigger local, paracrine senescence in other microglia. Thus, hp-tau seeding and release by senescent microglia putatively spread *in vivo* to induce a pathological cycle of selective neuronal vulnerability to tau hyperphosphorylation, neuronal phagoptosis, and paracrine senescence.

Microglial priming and senescence in LOAD may be evidenced by single cell transcriptomics

Transcriptomic studies of LOAD patients provide additional evidence that microglia in LOAD display APOE priming and senescence. The largest LOAD microglial sample size for single cell RNA sequencing was examined by Olah *et al.*, where microglial clusters 7, 8, and 4 were found to be particularly enriched in LOAD patients *versus* controls[96]. This indicates that these clusters represent microglial populations associated with LOAD progression. Notably, all three clusters demonstrated upregulated *APOE.* Clusters 4 and 8 further displayed increased ferritin in *FTH1* and *FTL*, while cluster 4 uniquely upregulated multiple *IRF* transcription factors[96]. This in turn suggests that microglial cluster 4 possesses an upregulated type I IFN response to yet unspecified, environmental stimuli. Type I IFN signalling has been implicated in pro-inflammatory microglial responses to Aβ pathology across multiple mouse models[97–99], and is associated with plaque-associated microglia in LOAD patient samples[98]. In the absence of an acute viral infection, activation of the cGAS-cGAMP-STING pathway in senescent cells leads to upregulated type I IFN responses[11,23]. Increased type I IFN response, induced by senescence, may thus further exacerbate pro-inflammatory microglial reactions to Aβ pathology. As senescent cells display increased ferritin indicating iron accumulation[18,19], transcriptomic evidence here suggests that senescent microglia may be enriched in LOAD patients.

Further complementing these results, a microglial population enriched in LOAD patients was separately validated both transcriptionally and by immunohistochemical staining per Nguyen *et al*.[100]. This population was found to increase both ferritin *FTH* and *FTL* expression, associate with neurofibrillary pathology, particularly neuritic plaques, and display signs of dystrophy suggesting senescence. Although dystrophy will be later explained when discussing senescent microglia in LOAD patients, this "dystrophic" microglial population was speculated to be the 'end result' of microglial states responding to Aβ accumulation. These Aβ-responding microglia were also found to upregulate *APOE*[100].

AD pathology accumulation likely mediates microglial priming and senescence in LOAD

Reinforcing the idea of aged microglial priming and senescence in LOAD, increasing oxidative stress accelerates oxysterol production over aging; this oxysterol accumulation is recognized by microglial liver X receptor (LXR)[40–43,101]. Upon successful oxysterol and LXR binding, microglial *APOE* is upregulated[101]. Corroborating this upregulated APOE and "primed" microglial state in

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the context of LOAD, *APOE* upregulation may correspond to DAM1 in mouse models[88,89], and speculatively shift microglia towards the transcriptomic cluster 7 discovered by Olah *et al* [96]. While potential functions remain unelucidated, this may result in extra microglial APOE secretion; as oxysterol generation depletes unoxidized cholesterol, LXR-activated microglia may secrete APOE packaged with cholesterol to aid in myelin, neuronal, and synaptic phagocytosis[102]. Intriguingly, upregulated *APOE3/4* can act as a nuclear receptor in microglia (Fig. 3), further accelerating microglial aging, pro-inflammatory responses, Aβ aggregation, tau hyperphosphorylation, and phagoptosis in *APOE4* variants[103–107]. APOE secretion also leads to APOE-PS binding stressed, desialylated neurons[38]. Combined with the progressive build-up of traditional AD pathology and age-dependent accumulation of senescent OPCs and astrocytes, microglial APOE may (1) likely further pressure neurons to expose outer PS and accumulate tau hyperphosphorylation, and (2) prime particular microglial states to phagotopse neurons (Fig. 3).

Particularly, microglial states expressing TREM2, performing homeostatic functions, and upregulating *APOE* likely bind PS-exposed neurons with hp-tau aggregates. This triggers neuronal and hp-tau phagoptosis through a combination of TREM2, complement receptors, and opsonins[38]. This TREM2-involved phagoptosis likely induces simultaneous senescence and a DAM2(-like) microglial transcriptome[88], which when correlating to human microglia data, may represent the transcriptomic cluster 8 identified by Olah *et al.*[96]. However, it is unknown whether the TREM2-dependent, DAM2 program itself initiates senescence, or if phagoptosing neurons with hp-tau triggers in parallel separate DAM2 and senescence programs. Evidence regarding tau inducing senescence has been reviewed above[68,92], but whether the phagoptosis of stressed neurons devoid of hp-tau aggregates induces microglial senescence remains unclear. Therefore, it can only be currently assumed that a DAM2(-like) microglia state becomes senescent.

The process of hp-tau-induced senescence is still unclear, but hp-tau may inhibit apoptosis acutely as a trade-off by initiating senescence[108]. NFT-containing neurons from both P301L mice and samples of frontotemporal dementia patients displayed increased 'senescence transcriptome scores', associated with an up-regulation of senescence markers such as *CDK2NA*, *TNF*, and *IL-1β*[5,109]. Neurons may moreover induce senescence in response to oxidative stress and DNA damage, as a compensatory trade-off against apoptosis[12,13,108,110]. Selectively vulnerable neurons affected foremost in Braak staging are also often excitatory and undergo NFT formation[111,112], indicating an increase of hp-tau pathology. As hp-tau appears to induce senescence in both microglia and astrocytes[68,75,92], "stressed" neurons exposed to ROS may become senescent by oxidative-stress-induced, tau hyperphosphorylation (Fig. 3). These neurons may then expose PS[79], prior to rendering microglia senescent. These findings also suggest a universal mechanism by which hp-tau pathology, whether via tau hyperphosphorylation, phagoptosis, or local uptake, would act as a senescence inducer in neurons, astrocytes, and microglia (Fig. 3).

Arguing that senescent microglia are dystrophic and hp-tau specific in LOAD patients

While transcriptomics indirectly infer cell function by RNA expression, postmortem LOAD patient brains were found to present dystrophic microglia[10,84,113,114]. Defined morphologically by cytoplasmic spheroids and swollen, beaded process extensions[114], dystrophic microglia associate with and precede NFT pathology following Braak staging[10,84,113,114]. These microglia not only become more abundant with aging, but also significantly increase in number among human hippocampi from differing types of dementias *versus* age-matched controls[115]. Dystrophic microglia were additionally found to contain hp-tau inclusions in aged tree shrews[116].

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In postmortem LOAD patient brains, dystrophic microglia without extreme hypertrophic somas were found to associate with neuritic plaques and neurofibrillary tangles[10], correlate well with overall neurofibrillary burden[117], and feature morphological differences depending on their local environment[100]. Microglia showing dystrophy and an extremely hypertrophic, "amoeboid" appearance have been found to associate with neuritic plaques, in both humans and marmosets[84]. Microglia association with neuritic plaques was particularly observed in the prefrontal cortex of LOAD patients, implicating that hypertrophic and dystrophic microglia form around the preclinical to clinical LOAD transition[84]. This furthermore complements a previous study by Sheng, Mrak, and Griffin (1997), where microglia in LOAD patient parahippocampal cortex were shown to initially transform from a "primed" or ramified appearance to a hypertrophic or amoeboid, phagocytic morphology nearby neuritic plaques[83].

Although microglial senescence remains to be rigorously confirmed with multiple markers in clinical LOAD, it is argued here that in LOAD patients, dystrophic microglia are senescent. While hp-tau internalization and senescence induction in microglia has been reviewed above[92], both neuritic plaques and NFTs contain hp-tau-positive inclusions. Since dystrophic microglia abundance and proximity is correlated well with this neurofibrillary pathology, dystrophic microglia are likely senescent from significant hp-tau exposure. This assumption should be experimentally tested. Furthermore, in a neuron-specific P301S human tauopathy model, dystrophic microglia displayed lysosomal swellings and hp-tau inclusions[118]. This observation of dystrophic microglia containing hp-tau inclusions reinforces the likelihood of dystrophic microglia being senescent; furthermore, microglial dystrophy and endolysosomal impairment was induced in a neuronal VPS35-knockout mouse model[119], while senescent cells display increased lysosomal sizes and decreased lysosomal function[11]. Dystrophic microglia in LOAD patients further present increased APOE and ferritin expression, where increased ferritin retention indicates iron accumulation[100,114–116,120,121]. Notably, senescent cells selectively uptake and accumulate iron[18,19]; microglia are not considered to accumulate iron in homeostatic conditions[115]. Murine and SV40 human microglial cells exposed to iron adopt a dystrophic morphology, accumulate iron, and become hypofunctional in phagocytosing Aβ[122–124], This reinforces dystrophic microglia as likely being senescent. Again, this claim requires additional verification.

Finally, as mentioned, neuritic plaque formation and accumulation is part of neurofibrillary pathology. Thus neuritic plaque abundance correlates well with Braak staging, cognitive decline, and overall LOAD clinical progression[1,7]. Notably, NLRP3 inflammasome staining was also localized proximal to neuritic plaques in LOAD patients[62]. In Aβ-overexpressing 5xFAD mice, early ablation of most microglia states by CSF1R inhibitors reduced neuritic plaque deposition[125]; as employing CSF1R inhibitors at smaller dosages also prevents microglial proliferation and results in decreased microglial senescence in 5xFAD mice[126], it follows that microglial senescence likely accounts for increased neuritic plaque deposition. Altogether, this indicates that dystrophic microglia are not solely due to aging, but likely senescent and specifically involved in LOAD progression. Notably, dystrophic and hypertrophic microglia that associate with neuritic plaques can be named plaque-associated microglia. Here, this term of "plaque-associated" microglia requires more precise definition. In LOAD patients, "plaque-associated" microglia can only be classified in relation to neuritic plaques; human microglia in LOAD patients do not significantly associate with diffuse amyloid plaques[82].

Senescent microglia are predicted to transform morphology, drive neurodegeneration, and form neuritic plaques in LOAD

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From here, we propose that primed microglia observed in LOAD patients initially comprise a ramified or hyper-ramified morphology[81]. After phagoptosing neurons containing hp-tau and becoming newly senescent, microglia likely adopt a state "A" with a dystrophic morphology (Fig. 3). As senescent microglia display a hypophagocytic capacity for hp-tau neurons and have compromised endolysosomal systems[92], here, senescent microglia are accordingly proposed to actively phagocytose, yet fail in degrading further Aβ and hp-tau aggregates[11,55,92]. First, continued microglial senescence and impaired endolysosomal functioning may result in subsequent negligence to support neurons, likely underlying further permitting of NFT formation[8,10,100,113]. This agrees with previous conclusions suggesting that dystrophic microglia precede NFT appearance[10]. Secondly, these now dystrophic microglia likely secrete hp-tau aggregates[92], perhaps via exosomes containing hp-tau[127,128]. As extracellular hp-tau is also taken up by nearby neurons[127,128], while extracellular hp-tau encourages PS exposure in neurons[80], senescent microglia likely sustain premature neurodegeneration by seeding hp-tau aggregates and encouraging local microglial phagoptosis. Senescent microglia could potentially also secrete hp-tau oligomers that are engulfed by local astrocytes, resulting in additional, paracrine senescence via astrocyte-mediated, hp-tau spread[75]. This could further accelerate glial and neuronal senescence burden; furthermore, as aggregated hp-tau activates the NLRP3 inflammasome and increases pro-inflammatory cytokine release[62,94], local inflammation elicited by senescent microglia would likely drive indirect hp-tau aggregation[61,94].

Thirdly, the state "A" dystrophic microglia likely induce paracrine senescence in nearby microglia that are attempting to phagocytose and digest Aβ aggregates. Multiple receptors may facilitate Aβ endocytosis, including a strong TREM2-Aβ affinity binding[129]; as APOE also binds to Aβ oligomers[101,107], providing an alternative TREM2-APOE-Aβ endocytic pathway for actively phagocytosing microglia. Furthermore, "amoeboid" or hypertrophic microglia perform actively-phagocytosing roles in humans beyond inflammation (Box 2). Thus, hypertrophic microglia busily phagocytosing Aβ likely may become senescent via paracrine senescence and hp-tau secretion by state "A" dystrophic microglia; this process is predicted to also induce senescence and dystrophy in these hypertrophic microglia, creating a state "B" dystrophic and hypertrophic microglia (Fig 3.). This can be corroborated with the microglial "activation" seen in LOAD patients[82–84,100].

While non-fibrillar, Aβ secretion may be useful in forming protective diffuse plaques, state "B" senescent microglia are predicted to fail in degrading these endocytosed products; instead, they putatively secrete Aβ-hp-tau aggregates[1,60,93–95,130]. Due to the likely senescence-inducing nature of hp-tau, microglial secretion of Aβ-hp-tau aggregates is speculatively specific to senescent microglia and critically accelerates buildup and spread of both Aβ and hp-tau[58–60,94,131]. Fourthly, secreted Aβ-hp-tau aggregates with dystrophic neurites then are predicted to aggregate into neuritic plaques with involvement of microglial TAM receptors[58,82,83]. Fifthly, as plaque-associated or state "B" senescent microglia continue to seed hp-tau[95], secreted hp-tau and Aβ aggregates are likely and continuously being uptaken by nearby microglia to attempt contain AD pathology spread within a localized region. Albeit, this compensatory response likely recruits additional nearby microglia to become senescent, leading to further neuritic plaques and potentially causing a dynamic response in recruiting other microglia. The "plaque-associated" microglial clustering seen in LOAD patients is predicted to emerge as a result of this likely positive feedback loop, particularly in paracrine senescence, further aggregate seeding, and creation of neuritic plaques by several state "B" senescent microglia[82–84,100]. Furthermore, this overwhelming concentration of Aβ and hp-tau seeding and pathology in neuritic plaques would cause local "clustering" of more senescent astrocytes and OPCs (Fig. 2).

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Senescent microglia are thus putatively accountable for neuritic plaque formation and indirect NFT formation, critical Aβ, hp-tau, and pro-inflammatory cytokine pathology beyond threshold tolerance, among localized brain regions corresponding to Braak staging, and overall LOAD progression[1,8,61,62,82,83,94,132]. Furthermore, state "B" senescent microglia are predicted to respond to overwhelming Aβ aggregation with an increased type I IFN response[23,97–99], leading to pro-inflammatory cytokine secretion and *Kv1.3* expression exacerbating local inflammation[122,133–135]. These exhausted, senescent microglia may also correspond to the transcriptomic cluster 4 per Olah *et al.*[96], featuring increased *FTH1* and *FTL* expression implicating iron accumulation and senescence[18,19,100,115,121], *APOE* upregulation, and *IRF* transcription factors expression corresponding to an increased type I IFN response[23,97–99]. In mouse models with Aβ pathology, this microglial stage may correspond to a pro-inflammatory, TREM2-dependent DAM2 state observed in response to Aβ pathology[89,136]. Notably, TREM2 in hypertrophic and/or dystrophic microglial states may be concomitantly downregulated due to pro-inflammatory exposure[137].

Additionally, microglia can proliferate in response to accumulated Aβ pathology[59]. Over the course of aging, this further accelerates homeostatic microglial transition towards senescence through telomere shortening[5,6,31,126,138]. This may independently generate senescent microglia that exacerbate Aβ pathology, local inflammation, and hp-tau secretion; thus, indirectly accelerating the conversion of senescent microglia through phagoptosing of Aβ-stressed neurons containing hp-tau. Alternatively, these senescent microglia may instead accelerate hp-tau seeding and spreading. However, while Aβ aggregation, DNA damage and other triggers may contribute to microglial senescence via alternate routes, such as replicative senescence or oxidative stress[31,126], there is an absence of tau pathology and lesser abundance of senescent microglia in APP-overexpressing mouse models[68,92,126]. Although experimental validation is needed, this suggests that microglial phagoptosis and senescence involving the uptake of hp-tau is the most specific and plausible, major contribution towards neurofibrillary pathology and LOAD progression[139].

Finally, although senescence among the microglial population likely occurs over one's lifespan, it is the overwhelming buildup and decreased clearance of senescent microglia in the aging brain that putatively underlies LOAD progression. Particularly, although immune cells that include microglia function to phagocytose and remove senescent cells[65], immunosenescence in immune cells also builds up over the span of aging and likely predisposes an environment for increased glial senescence burden[5,29,67] (Fig. 2). Because microglia are immune cells, microglial senescence would arguably act as both (i) a threshold requirement for Braak staging progression and LOAD, and (ii) a final failed form of immunosenescence.

In LOAD, microglial clustering, presumed formation of neuritic plaques and extreme hypertrophy among the prefrontal cortex emerges together with the clinical presentation around Braak stages III-IV[1,8]. Thus, it is predicted that senescent microglia actively accelerate hp-tau seeding and build-up of neuritic plaques that tip preclinical LOAD into clinical progression[130]. Furthermore, the exacerbated, resulting localized concentration and gradual accumulation of Aβ and neurofibrillary pathology determines clinical LOAD progression[132], increasing synaptic loss, pro-inflammatory glial responses, and overall glial and paracrine senescence (Fig. 2).

Discussion

The current hypothesis posits that when aged individuals present threshold senescent glial cell accumulation, they transition from healthy cognition into mild cognitive impairment and LOAD diagnosis (Fig. 2). This likely occurs through senescent glia maintaining a poor physiological environment for local cells causing death, paracrine senescence, and exacerbated buildup of AD

pathologies leading to sustained neurodegeneration. Glial senescence accumulation is causally set by AD pathologies, aging-induced physiological decline and initial glial senescence induction, but also subsequent paracrine senescence (Fig. 1, 3). The hypothesis also posits that threshold glial senescence represents a central consideration in understanding and treating LOAD. While this hypothesis has not yet been empirically tested, it yields further understanding regarding LOAD and proposes many testable components[140]. And crucially, all speculation and predictions offered here should be experimentally assessed for validation or rejection.

Its frameworks are compatible with current field paradigms regarding the amyloid cascade, tau, and synaptic loss hypotheses, and likely numerous other theories that have helped provide better knowledge of LOAD. It can also be expanded upon, as this perspective has not covered endothelial cell senescence and blood-brain barrier leakage contribution[141], other LOAD risk factors, and the potential role of senescent glia in mixed dementias and co-morbidities. These other topics deserve future and independent reviews. Other peripheral immune cells, particularly those in the adaptive immune system in lymphocytes *versus* innate immune microglia, may also become senescent and contribute to LOAD. This could occur via increased systemic inflammation, and/or infiltration and senescence-mediated exacerbation of Aβ/hp-tau seeding[29,67,142]. However, there is currently insufficient evidence that peripheral immune cells infiltrate the brain, become senescent, and directly participate in Braak staging and LOAD compared to microglia.

This framework may further apply to EOAD, with one main exception: if glial and particularly microglial senescence are responsible for clinical AD progression, mutations responsible for EOAD may involve quicker microglial senescence through exhaustion. Particularly, with the TREM2 R47H variant, microglia may have relatively decreased metabolic capacity to clear out quicker Aβ and consequent hp-tau buildup in EOAD[31,143]. Conjecturally, mutations could make it harder for other immune cells to clear out senescent microglial accumulation over aging[5]. Dark microglia may also be found to be senescent, provided that dark microglia likely phagoptose neurons and show other associated ultrastructural features[90].

Additionally, some explanations made against this hypothesis can be made. Senescent microglia were concluded as absent from the inferior parietal cortex of LOAD patients classified under Braak stages I – VI[69]. Particularly, plaque-associated microglia in APP-PS1 mice were immunonegative for p21, and thus concluded to be non-senescent. As this could contradict the current hypothesis, an alternative explanation is given here. Foremost, as there is no available information yet about the abundance of tau hyperphosphorylation in this model, a lack of senescent microglia may have resulted from a lack of hp-tau pathology. Furthermore, although p21 has been used a senescence marker, p21 was shown to be transiently expressed after senescence induction in fibroblasts[144]; it is currently unknown how long p21 induction in murine senescent microglia persists[27]. It is thus recommended to confirm or reject the current framework by assessing microglial senescence in LOAD patients using multiple methods, such as lipofuscin accumulation, iron and/or SA-β-gal staining, and a more constitutively-upregulated p16[5,11,126,144]. Moreover, the morphological and molecular signatures of senescent glia, which could also be heterogeneous across spatio-temporal contexts, should be further characterized in LOAD.

Secondly, although precise quantifications of senescent glia remain to be provided in LOAD patients, it could be argued that senescent glia numbers are too low to account for LOAD progression. This hypothesis disagrees with this viewpoint, instead arguing that even lowest estimates of senescent glia percentage in a whole brain population are likely damaging enough to sustain a local, neurodegenerative environment that underlies Braak staging. The contributions of glial senescence in mouse models, alongside dystrophic and senescent microglia correlation and

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association to neurofibrillary pathology in LOAD patients, arguably involves too much evidence and support in microglial senescence accounting for at least some aspects of LOAD progression. Third, inhibiting microglial proliferation through smaller CSF1R inhibitor dosages was shown to inhibit senescent microglia abundance through reduced Iba1/SA-β-gal fluorescence in 5xFAD mice. CSF1R inhibition did not remove Iba1/SA-β-gal positive cells completely at 7.5 months of age; while this can be interpreted towards other factors, the 5xFAD model also features hp-tau pathology at this timepoint[145]. Thus, the remaining senescent microglia after CSF1R inhibition may have become senescent through hp-tau engulfment or endocytosis. Finally, hp-tau spread and paracrine senescence may have a greater proponent accounted for by senescent astrocytes and neurons[75,128]; however, the current associations and overt evidence presented regarding dystrophic, senescent microglia has stronger support in the hypothesis's favour.

More broadly, tauopathies including chronic traumatic encephalopathy may represent a favourable target for treating senescent microglia and administrating therapeutics. Although beyond this perspective, LOAD pathology is also often co-morbid with other dementias such as vascular dementia and Parkinson's disease with dementia[1]. Thus, solutions that target senescent glia in LOAD will likely help managing or treating symptoms in these other diseases; co-morbid pathologies may also have independent mechanisms inducing glial senescence. Furthermore, data from tau PET studies indicated that tau seeding occurs locally from Braak stages III+[132]. As this tau propagation is correlated with increased microglial activity[146], this reinforces the idea of paracrine glial senescence via spreading hp-tau. This may thus act as a central mechanism to target for promoting health along the aging trajectory, by preventing mild cognitive impairment and age-related, neurodegenerative diseases.

Finally, if the current framework is correct, increasing glial senescence can potentially account for why previous therapies have not worked; these medicines have not targeted senescent glia, and thus likely have not halted AD pathology accumulation. A specific solution towards treating LOAD is encouraged here through senolytics, or drugs that selectively kill senescent cells (Fig. 4)[25]. As detailed, successful senolytic administration is expected to treat the accelerated, traditional AD pathology burden caused by senescent glia; this should allow renewal and recovery from remaining brain parenchyma, and for subsequent plasticity to extend or sustain an improved quality of life. While senolytics can or should be paired with other AD treatments for clinical trials, several senolytic experiments have demonstrated usefulness in preventing cognitive impairment in tauopathy, APP/PS1, and aged non-transgenic mouse models[27,68,69,109]. The senolytics dasatinib and quercetin have also successfully eliminated senescent cells in a clinical trial for patients with diabetic kidney disease[147], purporting hope for treating LOAD as an aging disease of glial senescence.

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Author contributions
V.L. led the article conceptualization, manuscript writing, and figure creation; V.L., L.M., and M.E.T. critically discussed the content and contributed to editing and revising the manuscript.

Competing interests
The authors declare no competing interests.

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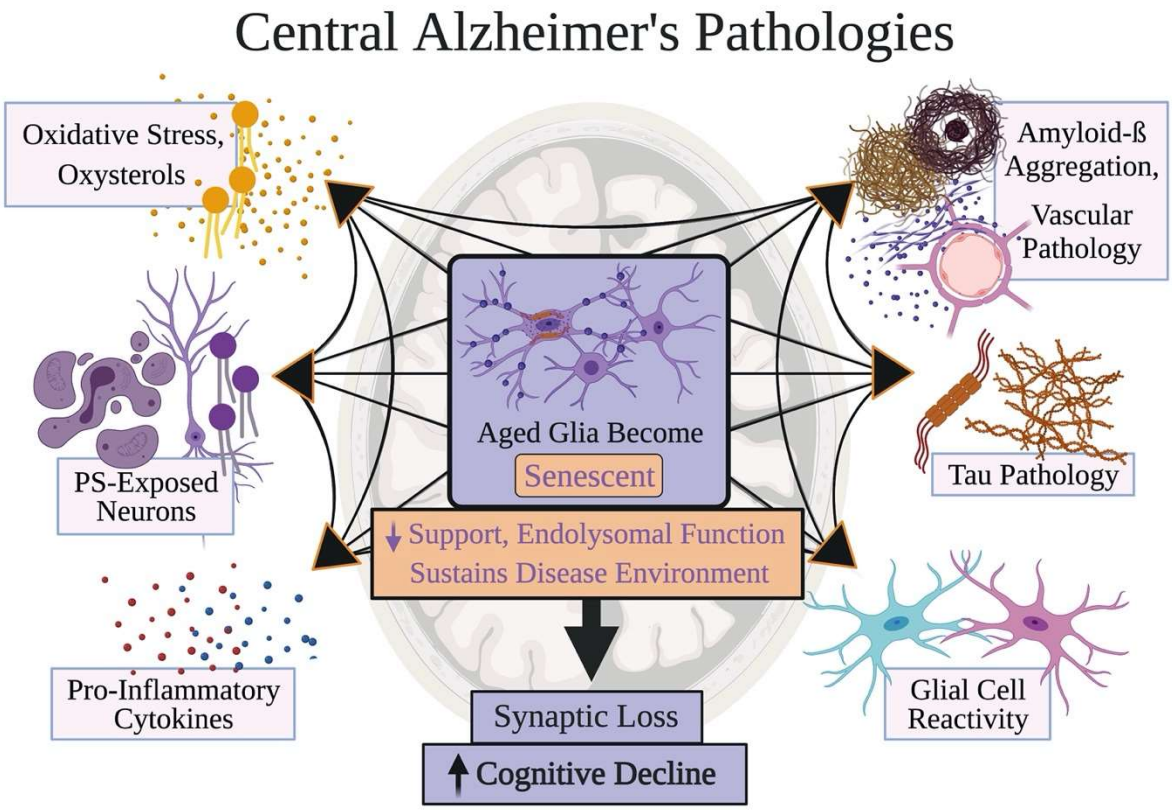

Fig. 1: Central Alzheimer's Pathologies. Risk factors for late-onset Alzheimer's disease (LOAD) end up adding to at least one of six main pathologies indicated in each corner, where each main pathology eventually adds to and increases the burden of other main LOAD pathology. These pathologies converge to ultimately drive improper neuronal support, synaptic loss, and death, resulting in neurodegeneration clinically corresponding to cognitive decline. Centrally, overwhelming levels of these six LOAD pathologies would also lead to threshold glial senescence. Glial senescence is briefly highlighted as dysfunctional glia phenotypes with impaired endolysosomal function and support for nearby cells. As argued later, glial senescence likely further enhances all six pathologies and sustains a disease environment favouring and irreversibly inducing further neurodegeneration; it is predicted that threshold glial senescence may differentiate a cognitively healthy individual from one diagnosed with LOAD.

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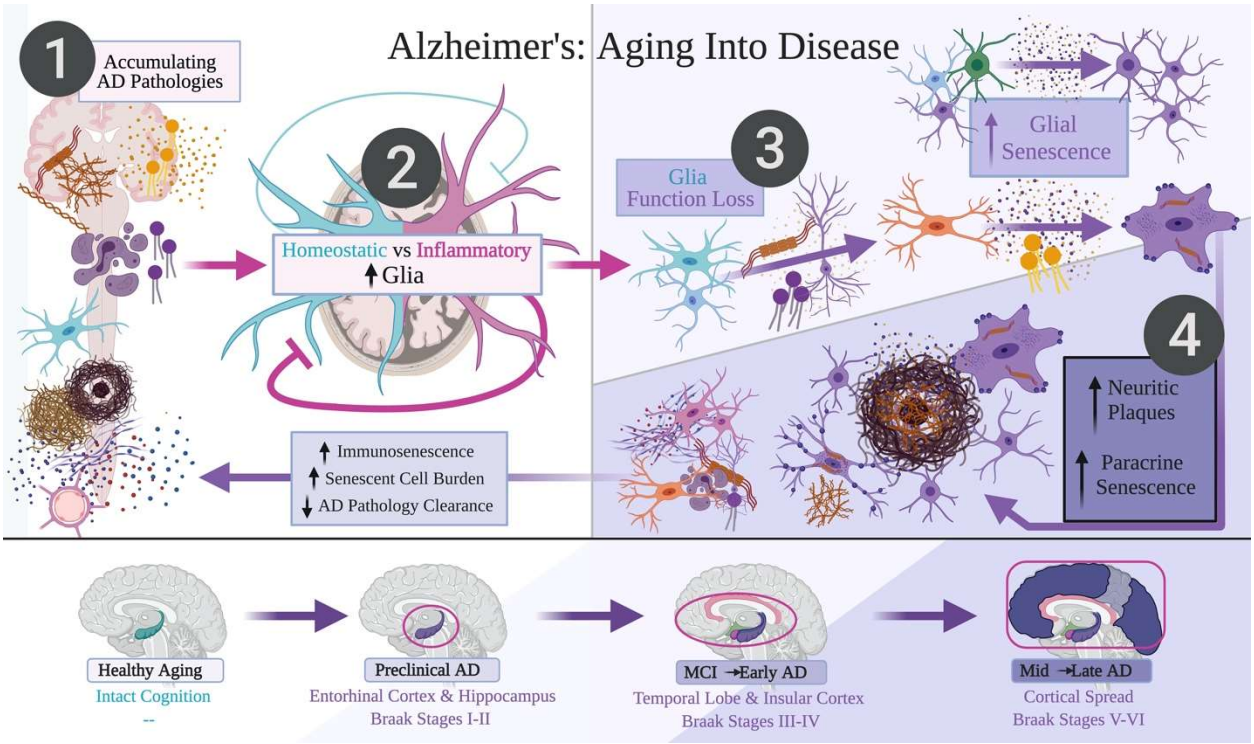

Fig. 2: Alzheimer's: Aging Into Disease. A LOAD framework is proposed from healthy aging to late-stage AD, presenting the testable hypothesis that glial senescence accumulation directly corresponds to clinical LOAD progression. (1) Oxidative stress, amyloid-beta (Aβ), neurofibrillary pathology in hyper-phosphorylated tau (hp-tau), and chronic inflammation are predicted to progressively accrue throughout aging as by-products of central nervous system function and metabolism. (2) Main LOAD pathology levels are enhanced by inflammatory glial states, and are sufficiently cleared by glia performing homeostatic roles. However, homeostatic glial functions decline throughout aging and decrease proficiency in containing LOAD pathology. (3) In preclinical AD, increasing proportions of aged glia interact with LOAD pathology to become senescent. This includes oligodendrocyte progenitor cells and astrocytes. Microglia performing homeostatic roles become senescent after engulfing neurons containing hp-tau, and then become incompetent in breaking down further phagocytosed hp-tau and Aβ aggregates.
(4) Senescent microglia instead secrete hp-tau to induce paracrine senescence in other microglia, especially those actively engulfing Aβ; failure to suddenly degrade both hp-tau and Aβ likely induces secretion of Aβ and hp-tau aggregates that coalesce into neuritic amyloid plaques. These neuritic plaques correspond to Braak staging and clinical LOAD progression. Finally, this combination of senescent glia, reduced homeostatic glia support, and non-senescent, inflammatory glial states is predicted to drive neurodegeneration and cognitive decline in LOAD. Finally, while senescent glia burden is cleared out by immune cells, increasing immunosenescence over aging by genetic and environmental circumstances likely slowly declines; thus, senescent glia burden is predicted to ultimately differentiate healthy cognition from mild cognitive impairment and LOAD. Purple colouring denotes more severe senescent glia burden and Braak staging progression in LOAD. Circled brain regions in the bottom diagram correspond to text (e.g., entorhinal cortex and hippocampus corresponding to the circled region in preclinical AD).

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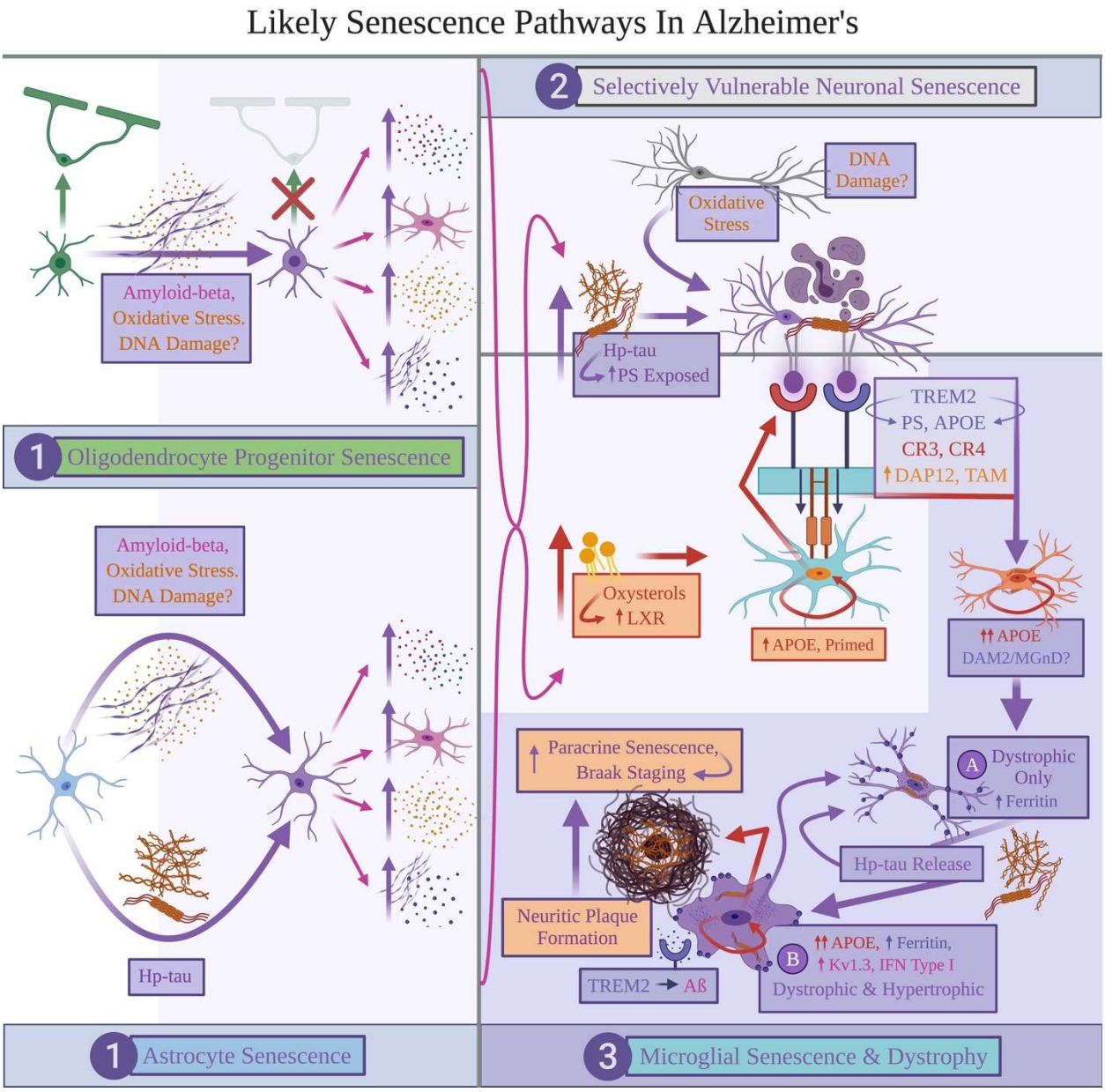

Fig. 3: Likely Senescence Pathways In Alzheimer's. (1) Oligodendrocyte progenitor cells (OPCs) and astrocytes likely both react with amyloid-beta (Aβ) to become senescent. Oxidative stress and DNA damage may also contribute to Aβ–induced senescence. Senescent OPCs and astrocytes are predicted to indirectly increase pro-inflammatory microglial states, oxidative stress, and Aβ accumulation. (2) Oxidative stress, inflammation, and possibly DNA damage likely induce tau hyperphosphorylation in neurons, which may also render these neurons senescent. Oxidative stress and hyperphosphorylated tau (hp-tau) aggregation also induce neurons to translocate and expose outer phosphatidylserine (PS).

(3) These factors speculatively favour conditions that induce overwhelming microglial senescence in LOAD. Aβ pathology contributes to oxidative stress that oxidizes cholesterol to form oxysterols; these oxysterols then bind with liver-X-receptor (LXR) expressed by microglia in homeostatic states. This binding results in upregulated apolipoprotein E (APOE) expression, which

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increases microglial-mediated Aβ phagocytosis and degradation. However, oxidative stress also primes APOE-upregulated microglia to bind to neuronal PS using various receptors, and perform "phagoptosis" to phagocytose and prematurely kill PS-exposed neurons. Engulfing hyperphosphorylated tau (hp-tau) turns microglia senescent and hypofunctional, rendering them unable to properly phagocytose LOAD pathology. These subtype or state "A" senescent microglia are proposed to display dystrophic morphology, accumulate ferritin, secrete soluble hp-tau rendering paracrine senescence in nearby glia, and precede neurofibrillary tangle formation in neurons.

Local microglia attempting to actively phagocytose Aβ are predicted to be caught in this paracrine senescence, becoming subtype or state "B" senescent microglia that take on a simultaneous hypertrophic or "amoeboid" appearance with dystrophy. These hypertrophic, dystrophic, and senescent microglia likely secrete failed, phagocytosed aggregates that form neuritic plaques and further induce paracrine glial senescence; as decreasing homeostatic microglia remain available to degrade Aβ, Aβ aggregation continuously accumulates and likely initiates a type I interferon response, *APOE* and *KCNA3* upregulation in state "B" senescent microglia.

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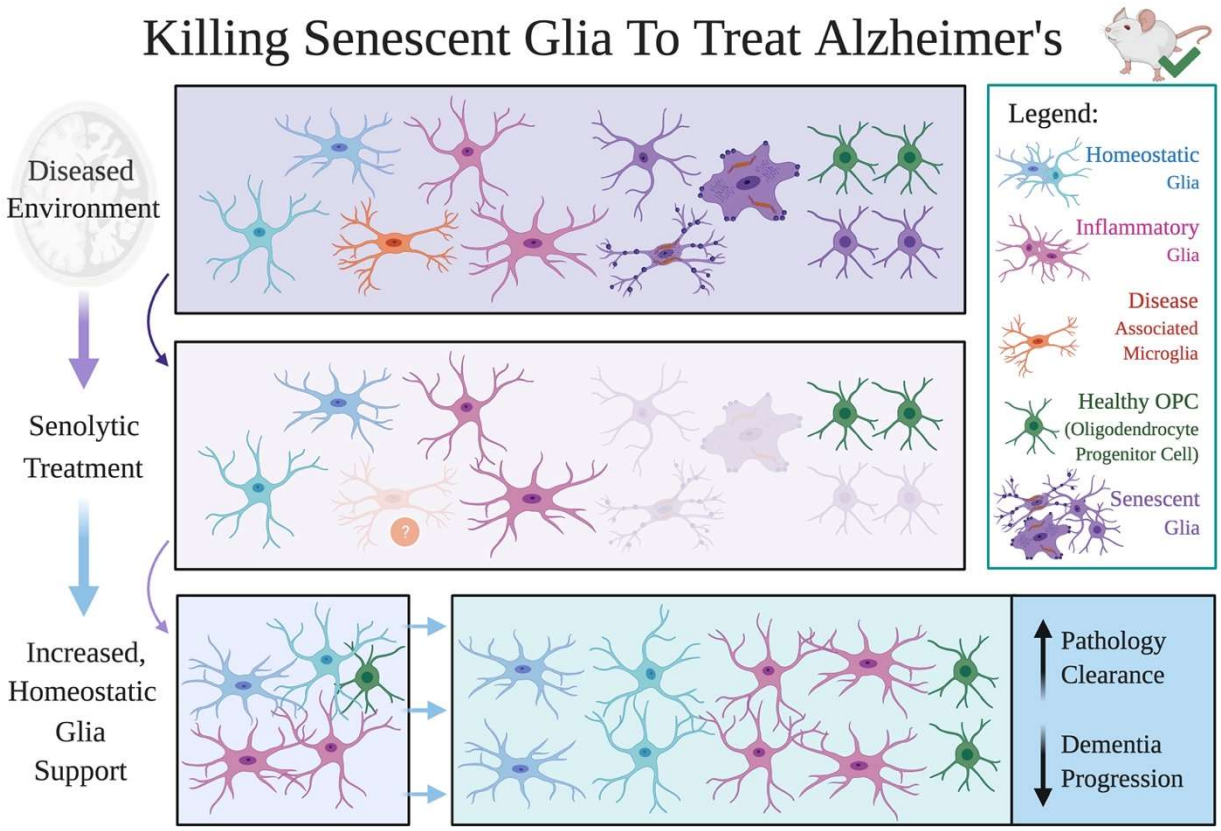

Fig. 4: Killing Senescent Glia To Treat Alzheimer's. Senolytics are drugs that selectively kill senescent cells, and have shown to reduce cognitive decline in disease mouse models. If senescent glia cause irreversible synaptic loss and accumulation of main LOAD pathologies, senolytics are expected to provide treatment for AD. Remaining glial populations can repopulate and renew homeostatic function to effectively clear out and reduce main LOAD pathologies, without senescent glia detrimentally exerting a gradient of LOAD pathology accumulation. Resulting plasticity should hopefully return the aging brain back to a healthy cognition or preclinical LOAD state, or at least halt further LOAD progression. Future treatments can also be utilized in combination with senolytics. This would allow better restoring of glial homeostatic functions, and minimize damage accrued by pro-inflammatory glial states and other LOAD pathologies. It is unknown if disease-associated microglial phenotypes are targeted by senolytics.

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