



OPEN ACCESS

EDITED BY

Jacob Raber,
Oregon Health and Science University,
United States

REVIEWED BY

Stergios Tsartsalis,
Hôpitaux universitaires de Genève (HUG),
Switzerland

*CORRESPONDENCE

Marie-Ève Tremblay,
✉ evetremblay@uvic.ca

SPECIALTY SECTION

This article was submitted to
Neuropharmacology,
a section of the journal
Frontiers in Pharmacology

RECEIVED 16 December 2022

ACCEPTED 03 February 2023

PUBLISHED 09 February 2023

CITATION

McKee CG, Hoffos M, Vecchiarelli HA and
Tremblay M-È (2023), Microglia: A
pharmacological target for the treatment
of age-related cognitive decline and
Alzheimer's disease.
Front. Pharmacol. 14:1125982.
doi: 10.3389/fphar.2023.1125982

COPYRIGHT

© 2023 McKee, Hoffos, Vecchiarelli and
Tremblay. This is an open-access article
distributed under the terms of the
[Creative Commons Attribution License
\(CC BY\)](https://creativecommons.org/licenses/by/4.0/). The use, distribution or
reproduction in other forums is
permitted, provided the original author(s)
and the copyright owner(s) are credited
and that the original publication in this
journal is cited, in accordance with
accepted academic practice. No use,
distribution or reproduction is permitted
which does not comply with these terms.

Microglia: A pharmacological target for the treatment of age-related cognitive decline and Alzheimer's disease

Chloe G. McKee^{1,2}, Madison Hoffos^{1,2}, Haley A. Vecchiarelli¹ and Marie-Ève Tremblay^{1,3,4,5,6,7,8*}

¹Division of Medical Sciences, University of Victoria, Victoria, BC, Canada, ²Department of Biology, University of Victoria, Victoria, BC, Canada, ³Département de Médecine Moléculaire, Université Laval, Québec City, QC, Canada, ⁴Axe Neurosciences, Centre de Recherche du CHU de Québec, Université Laval, Québec City, QC, Canada, ⁵Neurology and Neurosurgery Department, McGill University, Montreal, QC, Canada, ⁶Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, BC, Canada, ⁷Centre for Advanced Materials and Related Technology (CAMTEC), University of Victoria, Victoria, BC, Canada, ⁸Institute for Aging and Lifelong Health, University of Victoria, Victoria, BC, Canada

As individuals age, microglia, the resident immune cells of the central nervous system (CNS), become less effective at preserving brain circuits. Increases in microglial inflammatory activity are thought to contribute to age-related declines in cognitive functions and to transitions toward mild cognitive impairment (MCI) and Alzheimer's disease (AD). As microglia possess receptors for communicating with the CNS environment, pharmacological therapies targeting these pathways hold potential for promoting homeostatic microglial functions within the aging CNS. Preclinical and early phase clinical trials investigating the therapeutic effects of pharmacological agents acting on microglia, including reactive oxygen species, TREM2, fractalkine signaling, the complement cascade, and the NLRP3 inflammasome, are currently underway; however, important questions remain unanswered. Current challenges include target selectivity, as many of the signaling pathways are expressed in other cell types. Furthermore, microglia are a heterogenous cell population with transcriptomic, proteomic, and microscopy studies revealing distinct microglial states, whose activities and abundance shift across the lifespan. For example, homeostatic microglia can transform into pathological states characterized by markers of oxidative stress. Selective pharmacological targeting aimed at limiting transitions to pathological states or promoting homeostatic or protective states, could help to avoid potentially harmful off-target effects on beneficial states or other cell types. In this mini-review we cover current microglial pathways of interest for the prevention and treatment of age-related cognitive decline and CNS disorders of aging focusing on MCI and AD. We also discuss the heterogeneity of microglia described in these conditions and how pharmacological agents could target specific microglial states.

KEYWORDS

microglia, microglial diversity, cognitive aging, mild cognitive impairment, Alzheimer's disease, pharmacology

Introduction

Microglia, the resident immune cell of the central nervous system (CNS), play important roles across health and disease. They defend the CNS against infection and injury (Napoli and Neumann, 2009), contribute to neurogenesis (Sierra et al., 2014), refine synaptic connections (Paolicelli et al., 2011; Tremblay, 2011), and support neurons and other glial cells (Hansson and Rönnbäck, 2003). As microglia perform essential functions, their dysfunction is implicated in nearly all neurological pathologies, including the age-related neurodegenerative condition mild cognitive impairment (MCI), which can transition into Alzheimer's disease (AD) (Tay et al., 2018). Genome-wide association studies (GWAS) found microglia to most strongly express 60% of single nucleotide polymorphisms (SNPs) associated with AD risk (Zhang et al., 2014; McQuade and Blurton-Jones, 2019). Microglial functions are also affected during normal aging, which describes a process of gradual decline in cognitive functions including reasoning, memory, and processing speed (Harada et al., 2013). Microglia are highly sensitive to their surrounding environment and respond by modulating their morphology and activity (Carvalho-Paulo et al., 2021). Microglia possess various receptors to sense their microenvironment that also regulate their functions (Song and Suk, 2017; Šimončíčová et al., 2022), holding potential for the pharmacological treatment and prevention of MCI and AD.

During aging, the CNS environment becomes more inflammatory, triggering microglial reactivity (Norden et al., 2015), dystrophy (Streit et al., 2004), dysfunction (Mosher and Wyss-Coray, 2014), and senescence (Angelova and Brown, 2019). Microglial reactivity alters the balance between pro- and anti-inflammatory cytokine release, which can impair glial and neuronal functions leading to tissue damage. Notable age-related changes in microglia include their increased production of reactive oxygen species (ROS) and deficits in lysosomal digestion (Nakanishi and Wu, 2009). This compromises their maintenance of homeostasis, notably *via* reduced phagocytosis of apoptotic cells, aggregated proteins such as amyloid-beta (A β), and myelin debris (Harry, 2013; Rawji et al., 2016; Koellhoffer et al., 2017; Gabandé-Rodríguez et al., 2020). However, microglial phagocytosis is also involved in synaptic loss, a major correlate of cognitive decline hypothesized to be involved in the initiation and acceleration of AD (Terry et al., 1991; Scheff et al., 2006; Herrup, 2015). Thus, pharmacological approaches modulating microglial phagocytosis should consider cargo specificity. Classical pharmacological approaches, such as minocycline, focused on preventing microglial reactivity (Šimončíčová et al., 2022) and have limited efficacy likely because they compromise beneficial microglial functions. New therapeutic approaches should promote beneficial microglial functions while preventing or reversing detrimental ones. Considering that microglia are highly heterogeneous, as notably identified by transcriptomic and microscopy studies (Table 1) (Paolicelli et al., 2022), such a goal could be accomplished by targeting specific microglial states which become more abundant with aging and AD pathology.

In this mini-review we cover current microglial pathways which have shown potential for the prevention or treatment of AD and slowing of cognitive aging. We also discuss how pharmacological agents could target specific microglial states to maintain protective functions or

prevent detrimental ones during aging (Figure 1). Challenges surrounding microglial pharmacological targeting still remain, such as getting therapeutics across the blood-brain barrier (BBB) (Gabathuler, 2010) and avoiding off-target effects on other CNS cells that express many of the same receptors of interest as microglia.

Reactive oxygen species

ROS are molecules containing unpaired electrons formed by the partial reduction of oxygen within the mitochondrial electron transport chain (Guo et al., 2013). Under physiological conditions, ROS act as important signaling molecules (Ray et al., 2012); however, their levels increase during aging and in AD (Brieger et al., 2012; Manoharan et al., 2016). When ROS overwhelm antioxidant defense mechanisms, they can damage macromolecules and organelles, such as mitochondria (Guo et al., 2013), where disrupted cristae membranes are a marker of oxidative stress (Picca et al., 2020). Dark microglia (DM), a pathology associated microglial state (Table 1) found in mouse models of aging and AD pathology, and aging human post-mortem samples, often contain mitochondria with swollen and disrupted membranes (Bisht et al., 2016; St-Pierre et al., 2022a).

Lipid-droplet accumulating microglia (LDAM) which increase during aging in mice and humans, produce high levels of ROS and proinflammatory cytokines [e.g., interleukin (IL)-1 β , IL-6, and tumor necrosis factor (TNF) α] and have deficits in phagocytosis (Ye et al., 2017). Promoting microglial autophagy, responsible for transporting cytoplasmic organelles to lysosomes for degradation, through *in vitro* treatment with rapamycin, a Food and Drug Administration-approved immunosuppressant with anti-cognitive aging potential (Blagosklonny, 2019), decreased ROS and pro-inflammatory cytokine production (Ye et al., 2017). Thus, increasing microglial autophagy may limit lipid droplet buildup and transitions from homeostatic microglia to LDAM.

Increased signaling through the lipid messenger prostaglandin E2 (PGE2), a downstream component of the cyclooxygenase-2 pathway involved in inflammation was reported in aged mice (Minhas et al., 2021). This pathway converts glucose to glycogen, while reducing glucose flux through glycolysis and the Krebs cycle, thereby decreasing ATP production. Pharmacological inhibition of PGE2 in the periphery restored microglial mitochondrial morphology, ATP production, and long-term potentiation within the hippocampus to young adult levels, while resolving spatial memory deficits (Minhas et al., 2021). Furthermore, long-term treatment with drugs that inhibit cyclooxygenases, key enzymes involved in the production of PGE2, such as non-steroidal anti-inflammatory drugs (NSAIDs), delayed AD onset in pilot clinical trials and decreased microglial reactivity in rodent models of AD pathology (Ferencik et al., 2001; Krause and Müller, 2010).

Studies in models of A β pathology including 5XFAD (Baik et al., 2019) and APP/PS1 mice (McIntosh et al., 2019; Guillot-Sestier et al., 2021), have shown that microglia surrounding A β plaques shift from oxidative phosphorylation (OXPHOS) to the less efficient glycolytic metabolism. This reduces ATP production, decreases microglial phagocytosis, and leads to increased A β accumulation (McIntosh et al., 2019). Additionally, transitions to glycolytic metabolism caused microglia to become more iron retentive

TABLE 1 Microglial states associated with normal cognitive aging and age-related neurodegenerative diseases which are discussed in our mini-review.

Microglial state	Species and model identified in	Method of identification	Genetic markers	Protein markers	Beneficial or detrimental?	Potential pharmacological approaches	Relevant studies
Dark (DM)	Mouse: <i>Cx3Cr1</i> KO (3 Mo), APP-PS1 (14 Mo, 20 Mo), C57BL/6J (3 Mo, 14 Mo, 20 Mo) Human post-mortem: aged (81 years), middle-aged (45 years), AD cases	EM	Not yet investigated to our best knowledge	CD11B ↑ IBA1 ↓ 4D4 + TREM2 + P2RY12 – CX3CR1 –	Dependent on context Detrimental when over phagocytosing synapses, beneficial when clearing Aβ plaques ↑ iron storage suggesting dysfunctional iron metabolism, possibly contributing to electron-dense appearance	Fractalkine supplementation Inhibition of the cyclooxygenase pathway to limit ROS production Antibodies targeting complement cascade receptors or proteins	Bisht et al. (2016) St-Pierre et al. (2022b) El Hajj et al. (2019)
Lipid-droplet-accumulating (LDAM)	Mouse: <i>Grn</i> KO (9–10 Mo), <i>Wrrn</i> ^{Δhel/Δhel} (14 Mo), C57BL/6J (14 Mo, 20 Mo), CBA/CaJ (20 Mo)	EM Transcriptomics Cytokine measurements on isolated microglia	<i>Cat</i> ↑ <i>Kl</i> ↑ <i>Ppp1cb</i> ↑ <i>Rap1b</i> ↑ <i>Plin3</i> ↑ <i>Ady</i> ↑ <i>Clec7a</i> ↓	CCL3 ↑ CX3CL10 ↑ IL-6 ↑	Detrimental Defective in phagocytosis leading to an accumulation of debris, produce ↑ levels of ROS and pro-inflammatory cytokines	Inhibition of the cyclooxygenase pathway to ↓ ROS levels	Marschallinger et al. (2020) Hui et al. (2018) Tremblay et al. (2012)
Dystrophic/senescent	Mouse: C57BL/6J (24 Mo) Human post-mortem: various ages (10–100), AD cases	Transcriptomics LM	<i>ApoE</i> ↑ <i>B2m</i> ↑ <i>Cst7</i> ↑ <i>Fth1</i> ↑ <i>Cd11c</i> ↑ <i>Lpl</i> ↑ <i>Cx3cr1</i> ↓ <i>P2ry12</i> ↓ <i>Tmem119</i> ↓	Ferritin	Detrimental ↓ ability to efficiently migrate to sites of injury, phagocytose debris, and survey the parenchyma ↑ iron storage, suggesting dysfunctional iron metabolism possibly contributing to ↑ oxidative stress	Inhibition of the cyclooxygenase pathway to ↓ ROS production	Streit et al. (2004) Streit et al. (2009) Shahidehpour et al. (2021) Lopes et al. (2008) Zhang et al. (2022)
White matter associated (WAM)	Mouse: C57BL/6J (18–20 Mo)	Transcriptomics LM EM	<i>ApoE</i> ↑ <i>Trem2</i> ↑ <i>Cst7</i> ↑ <i>Clec7a</i> ↑ <i>Bm2</i> ↑ <i>Lyz2</i> ↑ <i>P2ry12</i> ↓ <i>P2ry13</i> ↓ <i>Csfr1</i> ↓ <i>Cx3cr1</i> ↓ <i>Tmem119</i> ↓	Not yet investigated to our best knowledge	Beneficial Potential to clear degenerated myelin that accumulates in the white matter during aging and neurodegenerative disease	Agonizing antibodies that ↑ TREM2 signaling Antibodies that block the shedding of soluble TREM2	Safaiyan et al. (2021)
Disease associated (DAM)	Mouse: 5XFAD (1 Mo, 3 Mo, 6 Mo, 8 Mo) Human post-mortem: AD cases	Transcriptomics LM	<i>Cx3cr1</i> ↓ <i>P2ry12/13</i> ↓ <i>Tmem119</i> ↓ <i>ApoE</i> ↑ <i>Trem2</i> ↑ <i>Cst7</i> ↑ <i>Lpl</i> ↑ <i>Clec7a</i> ↑	Not yet investigated to our best knowledge	Beneficial Potential to restrict neurodegeneration by phagocytosing debris and Aβ plaques	Agonizing antibodies that increase TREM2 Antibodies that block the shedding of soluble TREM2	Keren-Shaul et al. (2017)

Abbreviations: CX3CR1 KO, fractalkine signaling deficient mice where the fractalkine receptor, CX3CR1 is mutated; APP-PS1, amyloid-β (Aβ) based mouse model of Alzheimer's disease (AD) pathology; C57BL/6J, wild-type mice, known to undergo age-related loss of audition; *Grn* KO, mouse model of frontotemporal dementia pathology where exons of the granulin (*Grn*) allele are deleted; *Wrrn*^{Δhel/Δhel}, mouse model of Werner's syndrome pathology where the helicase domain of the murine WRN orthologue are deleted; CBA/CaJ, wild-type mice, known to undergo age-related loss of vision; 5XFAD, Aβ based mouse model of AD pathology; months (Mo); electron microscopy (EM); light microscopy (LM); reactive oxygen species (ROS); triggering receptor expressed on myeloid cells 2 (TREM2).

(McIntosh et al., 2019), a known trigger of ROS production (Picca et al., 2020). Increased ferritin immunoreactivity was reported in dystrophic and senescent microglia in human post-mortem samples from patients with and without dementia (Lopes et al., 2008). Although more research is needed, iron retention could contribute to the electron-dense appearance of DM. Overall, these findings suggest that reducing oxidative stress and maintaining microglial mitochondrial OXPHOS could help promote transitions from DM and LDAM to homeostatic states, while possibly limiting cognitive decline during aging.

Complement cascade

The complement cascade is an innate immune system pathway that detects and removes pathogens and dying cells (Ricklin et al., 2010). Within the CNS, neurons, astrocytes, and primarily microglia produce the complement protein C1q (Kouser et al., 2015; Fonseca et al., 2017; Asano et al., 2020). When C1q is deposited onto synapses it acts as an “eat me signal” targeting them for phagocytosis (Hong et al., 2016b) or trogocytosis (Lim and Ruthazer, 2021).

C1q expression is consistently upregulated with age in the mouse and human CNS, especially in the hippocampus (Stephan et al., 2013), and may contribute to the synaptic loss observed during normal aging (Terry et al., 1991; Jackson et al., 2019). Furthermore, A β fibrils produce complement proteins in various mouse models of AD pathology (Rogers et al., 1992; Bradt et al., 1998). Knocking out the C3 convertase, located downstream of C1q, is protective against age-dependent synapse and neuron loss within the hippocampus, and results in improved memory and spatial learning in aged wild-type (Shi et al., 2015) and APP/PS1 (Shi et al., 2017) mice. Pharmacological approaches such as small molecule inhibitors and monoclonal antibodies have demonstrated some success in mouse models of AD pathology. Oral administration of PMX205, an antagonist of C5aR, a receptor which triggers microglial phagocytosis, significantly reduced A β load in Tg2576 mice (model of A β pathology), together with A β load and hyperphosphorylated Tau in 3xTg mice (model of A β /Tau pathology), compared to untreated controls (Fonseca et al., 2009). In Tau-P301S mice (model of Tau pathology), injection of a C1q-blocking antibody into the hippocampus reduced synaptic markers by 50% within microglial lysosomes, suggesting decreased synaptic phagocytosis (Dejanovic et al., 2018). The broad spectrum humanized monoclonal antibody against C1q, ANX005, is currently undergoing Phase II trials, while its murine precursor ANXM-1 inhibited the complement cascade *in vivo* in wild-type mice centrally receiving A β oligomers (Hong et al., 2016a).

In terms of targeting microglial states, DM highly express C3R in their processes surrounding synapses and A β (Bisht et al., 2016). Inhibition of the complement cascade could help lessen the pathological synapse loss associated with DM; however, challenges include a lack of specificity as neurons and astrocytes, among other cells, also express complement components (Barnum, 1995).

NLRP3 inflammasome

The NLRP3 inflammasome is a pattern recognition receptor expressed by many cell types (Holbrook et al., 2021), including

microglia (Nayak et al., 2014), that recognizes homeostasis-altering molecular patterns (HAMPs), danger-associated molecular patterns (DAMPs), and pathogen-associated molecular patterns (PAMPs) (Kelley et al., 2019). HAMPs arise from cellular imbalances such as endoplasmic reticulum and Golgi stress (Liston and Masters, 2017), PAMPs are produced by microbial pathogens, and DAMPs by uric acid crystals (Liston and Masters, 2017) and ROS (Kelley et al., 2019), among other signals released during endogenous cell death.

When activated, NLRP3 induces caspase-1-mediated cleavage of IL-18 and IL-1 β (Kelley et al., 2019). IL-1 β which is elevated in the cerebrospinal fluid (CSF) and brain of patients with AD is considered toxic to microglia (Blevins et al., 2022) by triggering increased oxidative stress and apoptosis (Liu and Quan, 2018). Caspase-1 is also associated with inflammatory and cytoprotective responses that include cell death. Heneka et al. (2013) identified increased caspase-1 expression as an indication that NLRP3 may induce neurodegeneration, in patients with AD and APP/PS1 mice. APP/PS1 mice deficient in NLRP3 or caspase-1 displayed reduced spatial memory loss and IL-1 β activation, suggesting beneficial outcomes of targeting caspase-1, or its upstream effectors, in AD.

In terms of targeting, VX-765, a small molecule caspase-1 inhibitor that is BBB permeable and approved for clinical trials, reversed impairment of episodic and spatial memory in J20 APP^{SwInd} mice (model of A β pathology) (Sri et al., 2019), while preventing brain inflammation and A β build-up (Flores et al., 2018). Another small molecule caspase-1 inhibitor, MCC950, inhibits NLRP3 assembly, and IL-1 β and A β build-up, while increasing microglial phagocytosis of A β (Dempsey et al., 2017). These changes were associated with improved working and recognition memory in APP/PS1 mice (Dempsey et al., 2017). MCC950 also prevented Tau pathology in Tau-P301S mice (Stancu et al., 2019).

Antagonizing antibodies that block the assembly of NLRP3 successfully modified disease outcomes in mouse models of AD pathology. Apoptosis-associated speck-like protein containing a CARD (ASC) is produced by microglia and is a central adaptor protein of NLRP3 that binds to A β and aids in the formation of A β oligomers (Venegas et al., 2017). Venegas et al. (2017) reported that an anti-ASC antibody prevented A β from accumulating in APP/PS1 mice. de Rivero Vaccari et al. (2022) also demonstrated that IC100, an anti-ASC antibody, which is BBB permeable and binds ASC filaments, blocks IL-1 β production in human blood cell inflammasome assays. *In vivo* testing of IC100 is warranted to determine its therapeutic potential in treating AD.

In a rat model of AD pathology, systemic injection of mefenamic acid, a NSAID, was found to prevent memory deficits (Daniels et al., 2016). Similar behavioral results and decreased microglial reactivity and IL-1 β expression, with IL-1 β being a product of the NLRP3 signaling pathway, were observed in 3xTg mice centrally receiving mefenamic acid (Daniels et al., 2016). Thus far, animal models testing NLRP3 pathway inhibitors suggest that such treatments may have pharmacological relevance in reducing cognitive aging and AD pathology within patients.

Fractalkine signaling

Fractalkine signaling is one of the most important communication pathways between neurons and microglia (Mecca

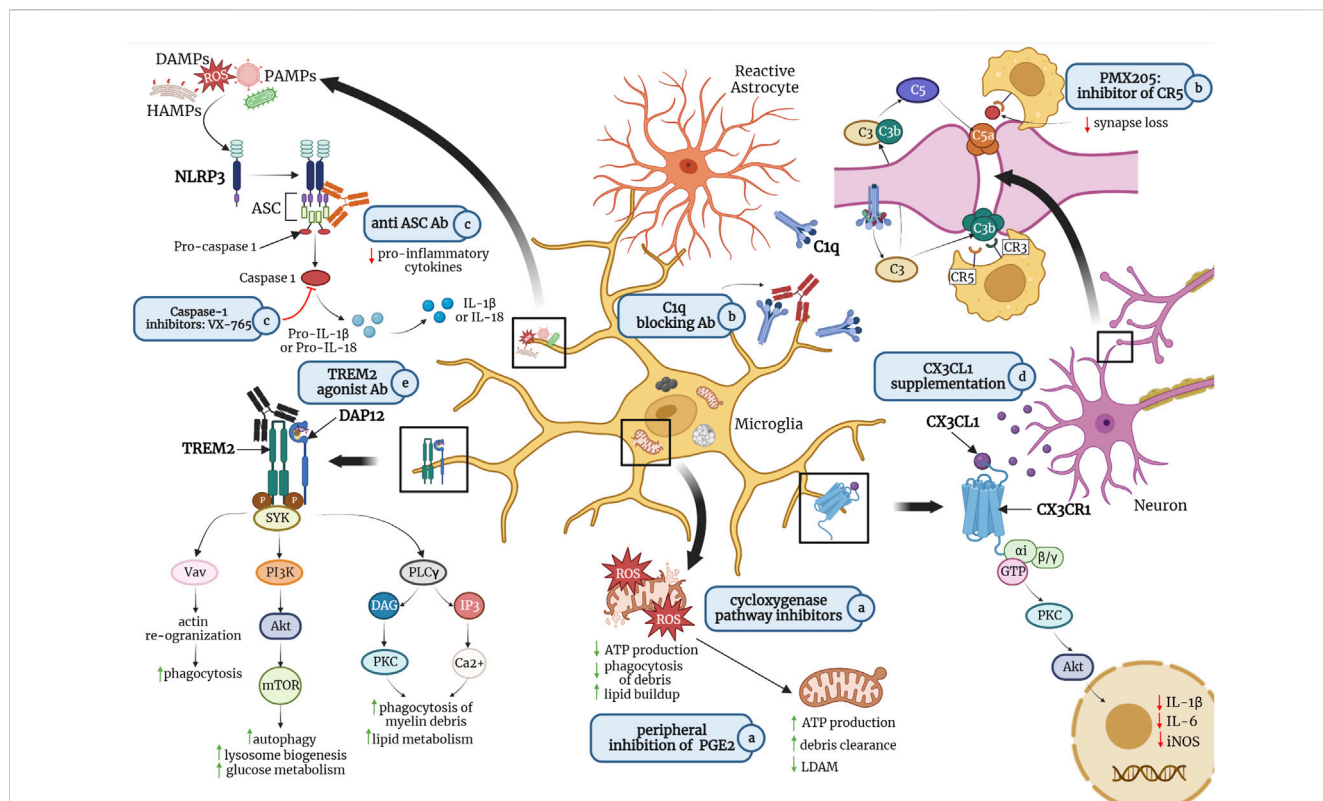


FIGURE 1

Potential pharmacological treatments for promoting homeostatic microglial states and preventing or reversing detrimental states include a) peripheral inhibition of lipid messenger prostaglandin E2 (PGE2) or cyclooxygenase inhibitors such as non-steroidal anti-inflammatory drugs (NSAIDs) to maintain mitochondrial morphology, thereby increasing adenosine triphosphate (ATP) production and debris clearance; b) antibodies against C1q, the initiating protein in the complement cascade, or small molecule inhibitors of complement receptor 5 (CR5), such as PMX205 to reduce microglia-mediated phagocytosis of synapses; c) caspase-1 inhibitors or antibodies against apoptosis-associated speck-like protein containing a CARD (ASC), a central adaptor protein of the NLR family pyrin domain containing 3 (NLRP3) inflammasome, to inhibit the production of pro-inflammatory cytokines such as interleukin (IL)-1β and IL-18; d) fractalkine (CX3CL1) supplementation to reduce the production of pro-inflammatory cytokines; and e) agonist antibodies targeting triggering receptor expressed on myeloid cells (TREM2) to promote microglial phagocytosis of myelin debris and amyloid-β (Aβ) plaques, as well as glucose metabolism. Abbreviations: ROS, reactive oxygen species; LDAM, lipid-droplet accumulating microglia; CX3CR1, fractalkine receptor; PKC, protein kinase C; Akt, protein kinase B; iNOS, inducible nitric oxide synthase; IL-6, interleukin 6; HAMPs, homeostasis-altering molecular patterns; DAMPs, danger-associated molecular patterns; PAMPs, pathogen-associated molecular patterns; C3, C3 convertase; C5, C5 convertase; C5a, complement component C5a (an anaphylatoxin); CR5, complement receptor five (binds to C5a); DAP12, DNAX-activating protein of 12 kDa; SYK, spleen tyrosine kinase; Vav, a guanine nucleotide exchange factor (GEF) for small G proteins of the Rho family; PI3K, phosphatidylinositol-3 kinase; mTOR, mammalian target of rapamycin; PLCγ, phospholipase C gamma; DAG, diacylglycerol; IP3, inositol trisphosphate. Figure is not drawn to scale. Created in Biorender.

et al., 2018; Angelopoulou et al., 2020). Neurons primarily release the chemokine fractalkine (CX3CL1) which binds to the fractalkine receptor (CX3CR1), expressed primarily by microglia (Pawelec et al., 2020), although this is not universal in the CNS (Hatori et al., 2002). Fractalkine signaling is commonly viewed as an “OFF” switch that maintains microglia in a homeostatic state, by downregulating their production of IL-1β (Bachstetter et al., 2011). CX3CL1 expression decreases across the lifespan, being lower in middle-aged and further in aged *versus* young adult rats (Lyons et al., 2009; Bachstetter et al., 2011). Furthermore, human patients with AD compared to MCI and healthy controls have significantly less CX3CL1 in their CSF (Perea et al., 2018).

Targeting of the fractalkine pathway has demonstrated some success. Aged rats centrally treated with recombinant CX3CL1 exhibited increased hippocampal neurogenesis (Bachstetter et al., 2011),

suggesting the potential of such treatments to decrease memory impairment in aging. Treatment with exogenous CX3CL1 attenuated microglial reactivity, by decreasing the expression of major histocompatibility complex class II and CD40 in hippocampal sections from aged rats (Lyons et al., 2009). Targeting the fractalkine pathway to treat AD has been more complex. CX3CR1 deficiency in APPS1 and R1.40 mice, models of Aβ pathology, resulted in reduced Aβ deposition potentially through enhanced microglial Aβ phagocytosis (Lee et al., 2010). mRNA expression of TNFα decreased while IL-1β increased in CX3CR1 deficient APP/PS1 mice. Contrastingly, CX3CR1 deficiency in the hAPP J20 mouse model of Aβ pathology did not significantly affect Aβ deposition and instead upregulated TNFα and IL-6 (Cho et al., 2011). Furthermore, CX3CR1 deficiency exacerbated Tau phosphorylation and worsened learning and memory in these mice, suggesting increased cognitive impairment. Similar results were observed in hTau mice, a

model of tauopathy, where CX3CR1 deficiency increased tau phosphorylation and aggregation, and the expression of CD68, a marker of phagocytosis notably expressed by microglia (Bhaskar et al., 2010). Further work on simultaneously reducing A β deposition and tau pathology *via* targeting of the fractalkine pathway, combined with other targets, is warranted.

In terms of microglial diversity, CX3CR1 deficient young adult mice display a significantly greater density of DM in the hippocampus compared to age-matched WT controls (Bisht et al., 2016). This suggests that fractalkine supplementation may help to prevent increases in DM known to become abundant during aging and AD pathology. Although this supplementation holds potential as a clinical treatment, for cognitive aging especially, it will be necessary to limit its off-target effects on other cells and prevent potentially deleterious outcomes on tau pathology.

TREM2

TREM2 is an immunoglobulin superfamily receptor, expressed most prominently in microglia within the CNS (Turnbull et al., 2006). The binding of TREM2 ligands including A β , apolipoproteins, phospholipids, and lipopolysaccharides (Kober and Brett, 2017) activates DNAX-activating protein of 12 kDa (DAP12), TREM2's binding partner, increasing microglial migration, lipid metabolism, autophagy, and phagocytosis, notably of myelin (Mazaheri et al., 2017; McQuade et al., 2020; Qu and Li, 2021) and synapses (Filipello et al., 2018). GWAS revealed TREM2 SNPs to increase AD risk by 2–4 times (McQuade and Blurton-Jones, 2019), in addition to accelerating cognitive decline (Rajagopalan et al., 2013; Replogle et al., 2015). Knocking out TREM2 in PS2APP mice, a model of A β pathology, impairs microglia-mediated compaction of A β into dense plaques (Meilandt et al., 2020).

Activation of the program that leads to disease-associated microglia (DAM) and white-matter associated microglia (WAM), neuroprotective states found in AD and aging, respectively, requires TREM2 signaling. Pharmacological approaches aimed at increasing TREM2 signaling and promoting transitions to DAM hold potential for treating AD. Wang et al. (2020) found that the anti-human TREM2 agonistic antibody, AL002c, decreased filamentous plaques and neurite dystrophy in 5XFAD mice carrying the R47H TREM2 variant associated with AD risk. The clinical variant of AL002c, AL002, was found to be safe and well tolerated in a phase I clinical trial (NCT03635047) (Wang et al., 2020). Other antibody approaches include the monoclonal antibody, 4D9, which promotes TREM2 signaling by blocking shedding of soluble TREM2 (Schlepckow et al., 2020). 4D9 increased microglial phagocytosis of myelin debris and A β *in vitro*, while reducing amyloidogenesis in the APP-NL-G-F knock-in mouse model of A β pathology (Schlepckow et al., 2020). Increasing TREM2 signaling has the potential to limit the accumulation of lipid droplets and myelin debris which are thought to contribute to LDAM emergence (Marschallinger et al., 2020).

Although modulation of the TREM2 pathway could promote homeostatic microglial states, consideration of disease stage may be important. Work in Psen1 mice, a model of A β pathology, found that knockdown of TREM2 in the early to middle stages of AD was beneficial as it inhibited microglial phagocytosis of synapses, while

this same approach in middle to late stages was detrimental by impairing microglial phagocytosis of A β (Sheng et al., 2019). Future work targeting TREM2 will need to balance promoting microglial phagocytosis of myelin debris and A β , while limiting synapse loss, perhaps in combination with other microglial targets.

Conclusion

Microglia are a heterogenous population whose morphology and activity shifts across the lifespan and between health and disease contexts (Grabert et al., 2016; Hammond et al., 2019). Although more research is needed to elucidate the functions of different microglial states, the diversity of this cell population holds selective pharmacotherapeutic potential for the prevention and treatment of cognitive aging, MCI, and AD. It will be important to identify differences in expressed receptors and signaling pathways, but also specific activities such as phagocytosis, metabolism, and inflammatory mediator production between microglial states (Šimončičová et al., 2022). This could allow for the design of drugs or antibody-mediated therapies that target specific microglial states, while preserving others and their critical functions.

However, several challenges remain. None of the receptors and associated pathways discussed herein or elsewhere (Rock and Peterson, 2006; Song and Suk, 2017; Šimončičová et al., 2022) are microglia-specific. Outstanding obstacles include getting therapeutics across the BBB and translation of the findings from animal models of AD pathology to human clinical trials (Shineman et al., 2011; Banik et al., 2015). Translation issues may be explained by genetic and epigenetic variations across patient profiles (Neuner et al., 2019), differences between human and murine microglia (Abels et al., 2021; Yvanka de Soysa et al., 2022), and the current inability of animal models to capture the complex pathology underlying AD including environmental risk factors (Bales, 2012; Onos et al., 2016). As the field evolves to tackle such issues, exploring microglial diversity will be an essential step to design more selective and effective therapeutics for treating and preventing AD and for slowing the overall cognitive aging process.

Author contributions

CGM wrote the introduction, the sections on reactive oxygen species, the complement cascade, fractalkine signaling, and TREM2, as well as the conclusion. MH wrote the section on the NLRP3 inflammasome. CGM conceived the figure and table. CGM, HAV, and M-ET edited the manuscript while HAV and MT provided supervisory guidance. All authors contributed to the article and approved the submitted version.

Funding

CGM is supported by a Jamie Cassels Undergraduate Research Award (JCURA) from the University of Victoria

and a Boehm Family Award for Excellence in Science. HAV is supported by a fellowship from the Canadian Institutes of Health Research (CIHR) and is a Michael Smith Health Research BC Research Trainee. M-ET holds a Canada Research Chair (Tier 2) in *Neurobiology of Aging and Cognition*.

Acknowledgments

We acknowledge with respect the Lekwungen peoples on whose traditional territory the University of Victoria stands, and the Songhees, Esquimalt and WSÁNEĆ peoples whose historical relationships with the land continue to this day.

References

- Abels, E. R., Nieland, L., Hickman, S., Broekman, M. L. D., El Khoury, J., and Maas, S. L. N. (2021). Comparative analysis identifies similarities between the human and murine microglial sensomes. *Int. J. Mol. Sci.* 22, 1495. doi:10.3390/ijms22031495
- Angelopoulou, E., Paudel, Y. N., Shaikh, M. F., and Piperi, C. (2020). Fractalkine (CX3CL1) signaling and neuroinflammation in Parkinson's disease: Potential clinical and therapeutic implications. *Pharmacol. Res.* 158, 104930. doi:10.1016/j.phrs.2020.104930
- Angelova, D. M., and Brown, D. R. (2019). Microglia and the aging brain: Are senescent microglia the key to neurodegeneration? *J. Neurochem.* 151, 676–688. doi:10.1111/jnc.14860
- Asano, S., Hayashi, Y., Iwata, K., Okada-Ogawa, A., Hitomi, S., Shibuta, I., et al. (2020). Microglia–astrocyte communication via C1q contributes to orofacial neuropathic pain associated with infraorbital nerve injury. *Int. J. Mol. Sci.* 21, 6834. doi:10.3390/ijms21186834
- Bachstetter, A. D., Morganti, J. M., Jernberg, J., Schlunk, A., Mitchell, S. H., Brewster, K. W., et al. (2011). Fractalkine and CX3CR1 regulate hippocampal neurogenesis in adult and aged rats. *Neurobiol. Aging* 32, 2030–2044. doi:10.1016/j.neurobiolaging.2009.11.022
- Baik, S. H., Kang, S., Lee, W., Choi, H., Chung, S., Kim, J.-I., et al. (2019). A breakdown in metabolic reprogramming causes microglia dysfunction in Alzheimer's disease. *Cell Metab.* 30, 493–507. doi:10.1016/j.cmet.2019.06.005
- Bales, K. R. (2012). The value and limitations of transgenic mouse models used in drug discovery for Alzheimer's disease: An update. *Expert Opin. Drug Discov.* 7, 281–297. doi:10.1517/17460441.2012.666234
- Banik, A., Brown, R. E., Bamburg, J., Lahiri, D. K., Khurana, D., Friedland, R. P., et al. (2015). Translation of pre-clinical studies into successful clinical trials for Alzheimer's disease: What are the roadblocks and how can they be overcome? *J. Alzheimer's Dis.* 47, 815–843. doi:10.3233/JAD-150136
- Barnum, S. R. (1995). Complement biosynthesis in the central nervous system. *Crit. Rev. Oral Biol. Med.* 6, 132–146. doi:10.1177/10454411950060020301
- Bhaskar, K., Konerth, M., Kokiko-Cochran, O. N., Cardona, A., Ransohoff, R. M., and Lamb, B. T. (2010). Regulation of tau pathology by the microglial fractalkine receptor. *Neuron* 68, 19–31. doi:10.1016/j.neuron.2010.08.023
- Bisht, K., Sharma, K. P., Lecours, C., Gabriela Sánchez, M., El Hajj, H., Milior, G., et al. (2016). Dark microglia: A new phenotype predominantly associated with pathological states. *Glia* 64, 826–839. doi:10.1002/glia.22966
- Blagosklonny, M. V. (2019). Rapamycin for longevity: Opinion article. *Aging (Albany NY)* 11, 8048–8067. doi:10.18632/aging.102355
- Blevins, H. M., Xu, Y., Biby, S., and Zhang, S. (2022). The NLRP3 inflammasome pathway: A review of mechanisms and inhibitors for the treatment of inflammatory diseases. *Front. Aging Neurosci.* 14, 879021. doi:10.3389/fnagi.2022.879021
- Bradt, B. M., Kolb, W. P., and Cooper, N. R. (1998). *Complement-dependent proinflammatory properties of the Alzheimer's disease*. γ -Peptide. 8.
- Brieger, K., Schiavone, S., Miller Jr., and Krause, K. (2012). Reactive oxygen species: From health to disease. *Swiss Med. Wkly.* 142, w13659. doi:10.4414/smww.2012.13659
- Carvalho-Paulo, D., Bento Torres Neto, J., Filho, C. S., de Oliveira, T. C. G., de Sousa, A. A., dos Reis, R. R., et al. (2021). Microglial morphology across distantly related species: Phylogenetic, environmental and age influences on microglia reactivity and surveillance states. *Frontiers in Immunology* 12. Available at: <https://www.frontiersin.org/articles/10.3389/fimmu.2021.683026> (Accessed November 7, 2022).
- Cho, S.-H., Sun, B., Zhou, Y., Kauppinen, T. M., Halabisky, B., Wes, P., et al. (2011). CX3CR1 protein signaling modulates microglial activation and protects against plaque-

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

independent cognitive deficits in a mouse model of alzheimer disease. *J. Biol. Chem.* 286, 32713–32722. doi:10.1074/jbc.M111.254268

Daniels, M. J. D., Rivers-Auty, J., Schilling, T., Spencer, N. G., Watremez, W., Fasolino, V., et al. (2016). Fenamate NSAIDs inhibit the NLRP3 inflammasome and protect against Alzheimer's disease in rodent models. *Nat. Commun.* 7, 12504. doi:10.1038/ncomms12504

de Rivero Vaccari, J. P., Mim, C., Hadad, R., Cyr, B., Stefansdottir, T. A., and Keane, R. W. (2022). Mechanism of action of IC 100, a humanized IgG4 monoclonal antibody targeting apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC). *Transl. Res.* 251, 27–40. doi:10.1016/j.trsl.2022.06.016

Dejanovic, B., Huntley, M. A., De Mazière, A., Meilandt, W. J., Wu, T., Srinivasan, K., et al. (2018). Changes in the synaptic proteome in tauopathy and rescue of tau-induced synapse loss by C1q antibodies. *Neuron* 100, 1322–1336. doi:10.1016/j.neuron.2018.10.014

Dempsey, C., Rubio Araza, A., Bryson, K. J., Finucane, O., Larkin, C., Mills, E. L., et al. (2017). Inhibiting the NLRP3 inflammasome with MCC950 promotes non-phlogistic clearance of amyloid- β and cognitive function in APP/PS1 mice. *Brain, Behav. Immun.* 61, 306–316. doi:10.1016/j.bbi.2016.12.014

El Hajj, H., Savage, J. C., Bisht, K., Parent, M., Vallières, L., Rivest, S., et al. (2019). Ultrastructural evidence of microglial heterogeneity in Alzheimer's disease amyloid pathology. *J. Neuroinflammation* 16, 87. doi:10.1186/s12974-019-1473-9

Ferencik, M., Novak, M., Rovensky, J., and Rybar, I. (2001). Alzheimer's disease, inflammation and non-steroidal anti-inflammatory drugs. *Bratisl. Lek. Listy* 102, 123–132.

Filipello, F., Morini, R., Corradini, I., Zerbi, V., Canzi, A., Michalski, B., et al. (2018). The microglial innate immune receptor TREM2 is required for synapse elimination and normal brain connectivity. *Immunity* 48, 979–991. doi:10.1016/j.immuni.2018.04.016

Flores, J., Noël, A., Foveau, B., Lynham, J., Lecrux, C., and LeBlanc, A. C. (2018). Caspase-1 inhibition alleviates cognitive impairment and neuropathology in an Alzheimer's disease mouse model. *Nat. Commun.* 9, 3916. doi:10.1038/s41467-018-06449-x

Fonseca, M. I., Ager, R. R., Chu, S.-H., Yazan, O., Sanderson, S. D., LaFerla, F. M., et al. (2009). Treatment with a C5aR antagonist decreases pathology and enhances behavioral performance in murine models of Alzheimer's disease. *J. Immunol.* 183, 1375–1383. doi:10.4049/jimmunol.0901005

Fonseca, M. I., Chu, S.-H., Hernandez, M. X., Fang, M. J., Modarresi, L., Selvan, P., et al. (2017). Cell-specific deletion of C1qa identifies microglia as the dominant source of C1q in mouse brain. *J. Neuroinflammation* 14, 48. doi:10.1186/s12974-017-0814-9

Gabandé-Rodríguez, E., Keane, L., and Capasso, M. (2020). Microglial phagocytosis in aging and Alzheimer's disease. *J. Neurosci. Res.* 98, 284–298. doi:10.1002/jnr.24419

Gabathuler, R. (2010). Approaches to transport therapeutic drugs across the blood–brain barrier to treat brain diseases. *Neurobiol. Dis.* 37, 48–57. doi:10.1016/j.nbd.2009.07.028

Grabert, K., Michoel, T., Karavolos, M. H., Clohisey, S., Baillie, J. K., Stevens, M. P., et al. (2016). Microglial brain region–dependent diversity and selective regional sensitivities to aging. *Nat. Neurosci.* 19, 504–516. doi:10.1038/nn.4222

Guillot-Sestier, M.-V., Araiz, A. R., Mela, V., Gaban, A. S., O'Neill, E., Joshi, L., et al. (2021). Microglial metabolism is a pivotal factor in sexual dimorphism in Alzheimer's disease. *Commun. Biol.* 4, 711. doi:10.1038/s42003-021-02259-y

Guo, C., Sun, L., Chen, X., and Zhang, D. (2013). *Oxidative stress, mitochondrial damage and neurodegenerative diseases****O. Neural Regeneration Research, 12.

Hammond, T. R., Dufort, C., Dissing-Olesen, L., Giera, S., Young, A., Wysoker, A., et al. (2019). Single-cell RNA sequencing of microglia throughout the mouse lifespan

- and in the injured brain reveals complex cell-state changes. *Immunity* 50, 253–271. doi:10.1016/j.immuni.2018.11.004
- Hansson, E., and Rönnebeck, L. (2003). Glial neuronal signaling in the central nervous system. *FASEB J.* 17, 341–348. doi:10.1096/fj.02-0429rev
- Harada, C. N., Natelson Love, M. C., and Triebel, K. L. (2013). Normal cognitive aging. *Clin. Geriatric Med.* 29, 737–752. doi:10.1016/j.cger.2013.07.002
- Harry, G. J. (2013). Microglia during development and aging. *Pharmacol. Ther.* 139, 313–326. doi:10.1016/j.pharmthera.2013.04.013
- Hatori, K., Nagai, A., Heisel, R., Ryu, J. K., and Kim, S. U. (2002). Fractalkine and fractalkine receptors in human neurons and glial cells. *J. Neurosci. Res.* 69, 418–426. doi:10.1002/jnr.10304
- Heneka, M. T., Kummer, M. P., Stutz, A., Delekate, A., Schwartz, S., Vieira-Saecker, A., et al. (2013). NLRP3 is activated in Alzheimer's disease and contributes to pathology in APP/PS1 mice. *Nature* 493, 674–678. doi:10.1038/nature11729
- Herrup, K. (2015). The case for rejecting the amyloid cascade hypothesis. *Nat. Neurosci.* 18, 794–799. doi:10.1038/nn.4017
- Holbrook, J. A., Jarosz-Griffiths, H. H., Caseley, E., Lara-Reyna, S., Poulter, J. A., Williams-Gray, C. H., et al. (2021). Neurodegenerative disease and the NLRP3 inflammasome. *Front. Pharmacol.* 12, 643254. doi:10.3389/fphar.2021.643254
- Hong, S., Beja-Glasser, V. F., Nfonoyim, B. M., Frouin, A., Li, S., Ramakrishnan, S., et al. (2016a). Complement and microglia mediate early synapse loss in Alzheimer mouse models. *Science* 352, 712–716. doi:10.1126/science.aad8373
- Hong, S., Dissing-Olesen, L., and Stevens, B. (2016b). New insights on the role of microglia in synaptic pruning in health and disease. *Curr. Opin. Neurobiol.* 36, 128–134. doi:10.1016/j.conb.2015.12.004
- Hui, C. W., St-Pierre, M.-K., Detuncq, J., Aumailley, L., Dubois, M.-J., Couture, V., et al. (2018). Nonfunctional mutant Wrn protein leads to neurological deficits, neuronal stress, microglial alteration, and immune imbalance in a mouse model of Werner syndrome. *Brain, Behav. Immun.* 73, 450–469. doi:10.1016/j.bbi.2018.06.007
- Jackson, J., Jambirina, E., Li, J., Marston, H., Menzies, F., Phillips, K., et al. (2019). Targeting the synapse in Alzheimer's disease. *Front. Neurosci.* 13, 735. doi:10.3389/fnins.2019.00735
- Kelley, N., Jeltama, D., Duan, Y., and He, Y. (2019). The NLRP3 inflammasome: An overview of mechanisms of activation and regulation. *IJMS* 20, 3328. doi:10.3390/ijms20133328
- Keren-Shaul, H., Spinrad, A., Weiner, A., Matcovitch-Natan, O., Dvir-Szternfeld, R., Ulland, T. K., et al. (2017). A unique microglia type associated with restricting development of Alzheimer's disease. *Cell* 169, 1276–1290. doi:10.1016/j.cell.2017.05.018
- Kober, D. L., and Brett, T. J. (2017). TREM2-Ligand interactions in health and disease. *J. Mol. Biol.* 429, 1607–1629. doi:10.1016/j.jmb.2017.04.004
- Koellhoffer, E., McCullough, L., and Ritzel, R. (2017). Old maids: Aging and its impact on microglia function. *IJMS* 18, 769. doi:10.3390/ijms18040769
- Kouser, L., Madhukaran, S. P., Shastri, A., Saraon, A., Ferluga, J., Al-Mozaini, M., et al. (2015). Emerging and novel functions of complement protein C1q. *Frontiers in Immunology* 6. Available at: <https://www.frontiersin.org/articles/10.3389/fimmu.2015.00317> (Accessed November 11, 2022).
- Krause, D. L., and Müller, N. (2010). Neuroinflammation, microglia and implications for anti-inflammatory treatment in Alzheimer's disease. *Int. J. Alzheimer's Dis.* 2010, e732806. doi:10.4061/2010/732806
- Lee, S., Varvel, N. H., Konerth, M. E., Xu, G., Cardona, A. E., Ransohoff, R. M., et al. (2010). CX3CR1 deficiency alters microglial activation and reduces beta-amyloid deposition in two Alzheimer's disease mouse models. *Am. J. Pathology* 177, 2549–2562. doi:10.2353/ajpath.2010.100265
- Lim, T. K., and Ruthazer, E. S. (2021). Microglial trogocytosis and the complement system regulate axonal pruning *in vivo*. *eLife* 10, e62167. doi:10.7554/eLife.62167
- Liston, A., and Masters, S. L. (2017). Homeostasis-altering molecular processes as mechanisms of inflammasome activation. *Nat. Rev. Immunol.* 17, 208–214. doi:10.1038/nri.2016.151
- Liu, X., and Quan, N. (2018). Microglia and CNS interleukin-1: Beyond immunological concepts. *Frontiers in Neurology* 9. Available at: <https://www.frontiersin.org/articles/10.3389/fneur.2018.00008> (Accessed December 12, 2022).
- Lopes, K. O., Sparks, D. L., and Streit, W. J. (2008). Microglial dystrophy in the aged and Alzheimer's disease brain is associated with ferritin immunoreactivity. *Glia* 56, 1048–1060. doi:10.1002/glia.20678
- Lyons, A., Lynch, A. M., Downer, E. J., Hanley, R., O'Sullivan, J. B., Smith, A., et al. (2009). Fractalkine-induced activation of the phosphatidylinositol-3 kinase pathway attenuates microglial activation *in vivo* and *in vitro*. *J. Neurochem.* 110, 1547–1556. doi:10.1111/j.1471-4159.2009.06253.x
- Manoharan, S., Guillemin, G. J., Abiramasundari, R. S., Essa, M. M., Akbar, M., and Akbar, M. D. (2016). The role of reactive oxygen species in the pathogenesis of Alzheimer's disease, Parkinson's disease, and huntington's disease: A mini review. *Oxidative Med. Cell. Longev.* 2016, 8590578–8590615. doi:10.1155/2016/8590578
- Marschallinger, J., Iram, T., Zardeneta, M., Lee, S. E., Lehallier, B., Haney, M. S., et al. (2020). Lipid-droplet-accumulating microglia represent a dysfunctional and proinflammatory state in the aging brain. *Nat. Neurosci.* 23, 194–208. doi:10.1038/s41593-019-0566-1
- Mazaheri, F., Snaidero, N., Kleinberger, G., Madore, C., Daria, A., Werner, G., et al. (2017). TREM2 deficiency impairs chemotaxis and microglial responses to neuronal injury. *EMBO Rep.* 18, 1186–1198. doi:10.15252/embr.201743922
- McIntosh, A., Mela, V., Harty, C., Minogue, A. M., Costello, D. A., Kerskens, C., et al. (2019). Iron accumulation in microglia triggers a cascade of events that leads to altered metabolism and compromised function in APP/PS1 mice. *Brain Pathol.* 29, 606–621. doi:10.1111/bpa.12704
- McQuade, A., and Blurton-Jones, M. (2019). Microglia in Alzheimer's disease: Exploring how genetics and phenotype influence risk. *J. Mol. Biol.* 431, 1805–1817. doi:10.1016/j.jmb.2019.01.045
- McQuade, A., Kang, Y. J., Hasselmann, J., Jairaman, A., Sotelo, A., Coburn, M., et al. (2020). Gene expression and functional deficits underlie TREM2-knockout microglia responses in human models of Alzheimer's disease. *Nat. Commun.* 11, 5370. doi:10.1038/s41467-020-19227-5
- Mecca, C., Giambanco, I., Donato, R., and Arcuri, C. (2018). Microglia and aging: The role of the TREM2-DAP12 and cx3cl1-cx3cr1 axes. *IJMS* 19, 318. doi:10.3390/ijms19010318
- Meilandt, W. J., Ngu, H., Gogineni, A., Lalehzadeh, G., Lee, S.-H., Srinivasan, K., et al. (2020). Trem2 deletion reduces late-stage amyloid plaque accumulation, elevates the aβ42:aβ40 ratio, and exacerbates axonal dystrophy and dendritic spine loss in the PS2APP Alzheimer's mouse model. *J. Neurosci.* 40, 1956–1974. doi:10.1523/JNEUROSCI.1871-19.2019
- Minhas, P. S., Latif-Hernandez, A., McReynolds, M. R., Durairaj, A. S., Wang, Q., Rubin, A., et al. (2021). Restoring metabolism of myeloid cells reverses cognitive decline in ageing. *Nature* 590, 122–128. doi:10.1038/s41586-020-03160-0
- Mosher, K. I., and Wyss-Coray, T. (2014). Microglial dysfunction in brain aging and Alzheimer's disease. *Biochem. Pharmacol.* 88, 594–604. doi:10.1016/j.bcp.2014.01.008
- Nakanishi, H., and Wu, Z. (2009). Microglia-aging: Roles of microglial lysosome- and mitochondria-derived reactive oxygen species in brain aging. *Behav. Brain Res.* 201, 1–7. doi:10.1016/j.bbr.2009.02.001
- Napoli, I., and Neumann, H. (2009). Microglial clearance function in health and disease. *Neuroscience* 158, 1030–1038. doi:10.1016/j.neuroscience.2008.06.046
- Nayak, D., Roth, T. L., and McGavern, D. B. (2014). Microglia development and function. *Annu. Rev. Immunol.* 32, 367–402. doi:10.1146/annurev-immunol-032713-120240
- Neuner, S. M., Heuer, S. E., Huentelman, M. J., O'Connell, K. M. S., and Kaczorowski, C. C. (2019). Harnessing genetic complexity to enhance translatability of Alzheimer's disease mouse models: A path toward precision medicine. *Neuron* 101, 399–411. doi:10.1016/j.neuron.2018.11.040
- Norden, D. M., Muccigrosso, M. M., and Godbout, J. P. (2015). Microglial priming and enhanced reactivity to secondary insult in aging, and traumatic CNS injury, and neurodegenerative disease. *Neuropharmacology* 96, 29–41. doi:10.1016/j.neuropharm.2014.10.028
- Onos, K. D., Sukoff Rizzo, S. J., Howell, G. R., and Sasner, M. (2016). Toward more predictive genetic mouse models of Alzheimer's disease. *Brain Res. Bull.* 122, 1–11. doi:10.1016/j.brainresbull.2015.12.003
- Paolicelli, R. C., Bolasco, G., Pagani, F., Maggi, L., Scianni, M., Panzanelli, P., et al. (2011). Synaptic pruning by microglia is necessary for normal brain development. *Science* 333, 1456–1458. doi:10.1126/science.1202529
- Paolicelli, R. C., Sierra, A., Stevens, B., Tremblay, M.-E., Aguzzi, A., Ajami, B., et al. (2022). Microglia states and nomenclature: A field at its crossroads. *Neuron* 110, 3458–3483. doi:10.1016/j.neuron.2022.10.020
- Pawelec, P., Ziemka-Nalecz, M., Sypecka, J., and Zaleska, T. (2020). The impact of the cx3cl1/cx3cr1 Axis in neurological disorders. *Cells* 9, 2277. doi:10.3390/cells9102277
- Perea, J. R., Lleó, A., Alcolea, D., Fortea, J., Ávila, J., and Bolós, M. (2018). Decreased CX3CL1 levels in the cerebrospinal fluid of patients with Alzheimer's disease. *Front. Neurosci.* 12, 609. doi:10.3389/fnins.2018.00609
- Picca, A., Calvani, R., Coelho-Junior, H. J., Landi, F., Bernabei, R., and Marzetti, E. (2020). Mitochondrial dysfunction, oxidative stress, and neuroinflammation: Intertwined roads to neurodegeneration. *Antioxidants* 9, 647. doi:10.3390/antiox9080647
- Qu, W., and Li, L. (2021). Microglial TREM2 at the intersection of brain aging and Alzheimer's disease. *Neuroscientist*, 107385842110407. doi:10.1177/10738584211040786
- Rajagopalan, P., Hibar, D. P., and Thompson, P. M. (2013). TREM2 and neurodegenerative disease. *N. Engl. J. Med.* 369, 1565–1567. doi:10.1056/NEJMc1306509
- Rawji, K. S., Mishra, M. K., Michaels, N. J., Rivest, S., Stys, P. K., and Yong, V. W. (2016). Immunosenscence of microglia and macrophages: Impact on the ageing central nervous system. *Brain* 139, 653–661. doi:10.1093/brain/awv395
- Ray, P. D., Huang, B.-W., and Tsuji, Y. (2012). Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. *Cell. Signal.* 24, 981–990. doi:10.1016/j.cellsig.2012.01.008

- Replogle, J. M., Chan, G., White, C. C., Raj, T., Winn, P. A., Evans, D. A., et al. (2015). A TREM1 variant alters the accumulation of Alzheimer-related amyloid pathology. *Ann. Neurology* 77, 469–477. doi:10.1002/ana.24337
- Ricklin, D., Hajshengallis, G., Yang, K., and Lambris, J. D. (2010). Complement: A key system for immune surveillance and homeostasis. *Nat. Immunol.* 11, 785–797. doi:10.1038/ni.1923
- Rock, R. B., and Peterson, P. K. (2006). Microglia as a pharmacological target in infectious and inflammatory diseases of the brain. *Jrnl NeuroImmune Pharm.* 1, 117–126. doi:10.1007/s11481-006-9012-8
- Rogers, J., Styren, S. D., Civin, W. H., Brachova, L., Wardi, P., and Lieberburg, I. (1992). Complement activation by β -amyloid in Alzheimer disease. *Proc. Natl. Acad. Sci. U. S. A.* 5.
- Safaiyan, S., Besson-Girard, S., Kaya, T., Cantuti-Castelvetri, L., Liu, L., Ji, H., et al. (2021). White matter aging drives microglial diversity. *Neuron* 109, 1100–1117.e10. doi:10.1016/j.neuron.2021.01.027
- Scheff, S. W., Price, D. A., Schmitt, F. A., and Mufson, E. J. (2006). Hippocampal synaptic loss in early Alzheimer's disease and mild cognitive impairment. *Neurobiol. Aging* 27, 1372–1384. doi:10.1016/j.neurobiolaging.2005.09.012
- Schlepckow, K., Monroe, K. M., Kleinberger, G., Cantuti-Castelvetri, L., Parhizkar, S., Xia, D., et al. (2020). Enhancing protective microglial activities with a dual function TREM 2 antibody to the stalk region. *EMBO Mol. Med.* 12, e11227. doi:10.15252/emmm.201911227
- Shahidehpour, R. K., Higdon, R. E., Crawford, N. G., Neltner, J. H., Ighodaro, E. T., Patel, E., et al. (2021). Dystrophic microglia are associated with neurodegenerative disease and not healthy aging in the human brain. *Neurobiol. Aging* 99, 19–27. doi:10.1016/j.neurobiolaging.2020.12.003
- Sheng, L., Chen, M., Cai, K., Song, Y., Yu, D., Zhang, H., et al. (2019). Microglial Trem2 induces synaptic impairment at early stage and prevents amyloidosis at late stage in APP/PS1 mice. *FASEB J.* 33, 10425–10442. doi:10.1096/fj.201900527R
- Shi, Q., Chowdhury, S., Ma, R., Le, K. X., Hong, S., Caldarone, B. J., et al. (2017). Complement C3 deficiency protects against neurodegeneration in aged plaque-rich APP/PS1 mice. *Sci. Transl. Med.* 9, eaaf6295. doi:10.1126/scitranslmed.aaf6295
- Shi, Q., Colodner, K. J., Matousek, S. B., Merry, K., Hong, S., Kenison, J. E., et al. (2015). Complement C3-deficient mice fail to display age-related hippocampal decline. *J. Neurosci.* 35, 13029–13042. doi:10.1523/JNEUROSCI.1698-15.2015
- Shineman, D. W., Basi, G. S., Bizon, J. L., Colton, C. A., Greenberg, B. D., Hollister, B. A., et al. (2011). Accelerating drug discovery for Alzheimer's disease: Best practices for preclinical animal studies. *Alzheimer's Res. Ther.* 3, 28. doi:10.1186/alzrt90
- Sierra, A., Beccari, S., Diaz-Aparicio, I., Encinas, J. M., Comeau, S., and Tremblay, M.-È. (2014). Surveillance, phagocytosis, and inflammation: How never-resting microglia influence adult hippocampal neurogenesis. *Neural Plast.* 2014, 610343–610415. doi:10.1155/2014/610343
- Šimončičová, E., Gonçalves de Andrade, E., Vecchiarelli, H. A., Awogbindin, I. O., Delage, C. I., and Tremblay, M.-È. (2022). Present and future of microglial pharmacology. *Trends Pharmacol. Sci.* 43, 669–685. doi:10.1016/j.tips.2021.11.006
- Song, G. J., and Suk, K. (2017). Pharmacological modulation of functional phenotypes of microglia in neurodegenerative diseases. *Front. Aging Neurosci.* 9, 139. doi:10.3389/fnagi.2017.00139
- Sri, S., Pegasiou, C.-M., Cave, C. A., Hough, K., Wood, N., Gomez-Nicola, D., et al. (2019). Emergence of synaptic and cognitive impairment in a mature-onset APP mouse model of Alzheimer's disease. *Acta Neuropathol. Commun.* 7, 25. doi:10.1186/s40478-019-0670-1
- St-Pierre, M.-K., Carrier, M., Gonzalez Ibanez, F., Simoncicova, E., Wallman, M.-J., Vallières, L., et al. (2022a). Dark microglia are present in human post-mortem brain samples and display ultrastructural and metabolic alterations during aging in a mouse model of amyloid deposition.
- St-Pierre, M.-K., Carrier, M., González Ibáñez, F., Šimončičová, E., Wallman, M.-J., Vallières, L., et al. (2022b). Ultrastructural characterization of dark microglia during aging in a mouse model of Alzheimer's disease pathology and in human post-mortem brain samples. *J. Neuroinflammation* 19, 235. doi:10.1186/s12974-022-02595-8
- Stancu, I.-C., Cremers, N., Vanrusselt, H., Couturier, J., Vanoosthuysen, A., Kessels, S., et al. (2019). Aggregated Tau activates NLRP3-ASC inflammasome exacerbating exogenously seeded and non-exogenously seeded Tau pathology in vivo. *Acta Neuropathol.* 137, 599–617. doi:10.1007/s00401-018-01957-y
- Streit, W. J., Braak, H., Xue, Q.-S., and Bechmann, I. (2009). Dystrophic (senescent) rather than activated microglial cells are associated with tau pathology and likely precede neurodegeneration in Alzheimer's disease. *Acta Neuropathol.* 118, 475–485. doi:10.1007/s00401-009-0556-6
- Streit, W. J., Sammons, N. W., Kuhns, A. J., and Sparks, D. L. (2004). Dystrophic microglia in the aging human brain. *Glia* 45, 208–212. doi:10.1002/glia.10319
- Tay, T. L., Béchade, C., D'Andrea, I., St-Pierre, M.-K., Henry, M. S., Roumier, A., et al. (2018). Microglia gone rogue: Impacts on psychiatric disorders across the lifespan. *Front. Mol. Neurosci.* 10, 421. doi:10.3389/fnmol.2017.00421
- Terry, R. D., Masliah, E., Salmon, D., Butters, N., DeTeresa, R., Hill, R., et al. (1991). Physical basis of cognitive alterations in Alzheimer's Disease: Synapse loss is the major correlate of cognitive impairment. *Ann. Neurology Official J. Am. Neurological Assoc. Child Neurology Soc.* 30, 572–580. doi:10.1002/ana.410300410
- Tremblay, M.-È. (2011). The role of microglia at synapses in the healthy CNS: Novel insights from recent imaging studies. *Neuron Glia Biol.* 7, 67–76. doi:10.1017/S1740925X12000038
- Tremblay, M.-È., Zettel, M. L., Ison, J. R., Allen, P. D., and Majewska, A. K. (2012). Effects of aging and sensory loss on glial cells in mouse visual and auditory cortices. *Glia* 60, 541–558. doi:10.1002/glia.22287
- Turnbull, I. R., Gilfillan, S., Cella, M., Aoshi, T., Miller, M., Piccio, L., et al. (2006). Cutting edge: TREM-2 attenuates macrophage activation. *J. Immunol.* 177, 3520–3524. doi:10.4049/jimmunol.177.6.3520
- Venegas, C., Kumar, S., Franklin, B. S., Dierkes, T., Brinkschulte, R., Tejera, D., et al. (2017). Microglia-derived ASC specks cross-seed amyloid- β in Alzheimer's disease. *Nature* 552, 355–361. doi:10.1038/nature25158
- Wang, S., Mustafa, M., Yuede, C. M., Salazar, S. V., Kong, P., Long, H., et al. (2020). Anti-human TREM2 induces microglia proliferation and reduces pathology in an Alzheimer's disease model. *J. Exp. Med.* 217, e20200785. doi:10.1084/jem.20200785
- Ye, J., Jiang, Z., Chen, X., Liu, M., Li, J., and Liu, N. (2017). The role of autophagy in pro-inflammatory responses of microglia activation via mitochondrial reactive oxygen species in vitro. *J. Neurochem.* 142, 215–230. doi:10.1111/jnc.14042
- Yvanka de Soysa, T., Therrien, M., Walker, A. C., and Stevens, B. (2022). Redefining microglia states: Lessons and limits of human and mouse models to study microglia states in neurodegenerative diseases. *Seminars Immunol.* 60, 101651. doi:10.1016/j.smim.2022.101651
- Zhang, X., Pearsall, V. M., Carver, C. M., Atkinson, E. J., Clarkson, B. D. S., Grund, E. M., et al. (2022). Rejuvenation of the aged brain immune cell landscape in mice through p16-positive senescent cell clearance. *Nat. Commun.* 13, 5671. doi:10.1038/s41467-022-33226-8
- Zhang, Y., Chen, K., Sloan, S. A., Bennett, M. L., Scholze, A. R., O'Keefe, S., et al. (2014). An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. *J. Neurosci.* 34, 11929–11947. doi:10.1523/JNEUROSCI.1860-14.2014