# The changing microglial immune response: Cause or consequence of Alzheimer's Disease?

F.W. Wijnhorst (S3748316)
Bachelor Thesis Biology – Behaviour & Neurosciences
Faculty of Science and Engineering
Supervised by prof. dr. U.L.M. Eisel and L.M. Jorna
University of Groningen
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# Contents

## Abstract

Alzheimer's Disease (AD) is a debilitating, neurodegenerative disease currently affecting more than 55 million people. The pathology is characterized by neuronal cell loss, Amyloid$\beta$ (A$\beta$) plaque formation, and hyperphosphorylated tau aggregates. Neuroinflammation and specifically microglia are thought to play a critical role in the onset and development of AD. Microglia represent the innate immune cells of the brain that regulate neuroinflammation through the secretion of cytokines and chemokines. Age is the most important risk factor for AD. Microglia show signs of senescence and as microglia are tightly involved in AD, it is possible that the aging of microglia affects the onset and progress of AD. This review will analyze how the phenotype of microglia changes during aging, the role of microglia in AD, and whether the changing phenotype is a cause or consequence of AD. Microglia are most likely subjected to the direct effects of aging and microglia are closely involved in the most important hallmarks of AD. Despite the difficulty of appointing a primary cause of AD, it is very well possible that aging microglia are critical in the onset of AD, and microglial aging could be a cause of the development of AD. Targeting the aging of microglia presents a promising therapeutic strategy in developing a treatment for this – as of yet – untreatable disease.

## Introduction

Alzheimer's Disease (AD) is a neurodegenerative disease with great impact on a patient's life. Currently, over 55 million people suffer from AD and this number will increase to an estimated 78 million cases in 2030 (Gauthier et al. 2021). It is the most common cause of dementia, accounting for 60-80% of the cases, and leads to severe impairment of cognitive functioning (*2022 Alzheimer's Disease Facts and Figures* 2022). Patients often suffer from difficulties in spatial memory, processing of language, and decision-making. Characteristics of AD are the formation of Amyloid$\beta$ (A$\beta$) plaques, depositions of neurofibrillary tangles (NFTs), and neuronal cell loss in the hippocampus. At present, the drugs for AD only function to relieve some of the symptoms, but the progression of the disease cannot be stopped, and the development of an effective treatment still requires extensive research.

Neuroinflammation currently is a topic of interest within the field of AD. Neuroinflammation is the immune response of the brain to injury and infection, and is regulated by cytokines and chemokines. Pro-inflammatory cytokines include interleukin-1$\beta$ (IL-1$\beta$), interleukin-6 (IL-6), Tumour Necrosis Factor-$\alpha$ (TNF), and interferon-$\gamma$ (IFN), while anti-inflammatory cytokines include Transforming Growth Factor-$\beta$ (TGF) and interleukin-10 (IL-10) (Behzadi 2020). Neuroinflammation is regulated by glial cells and primarily by microglia and astrocytes (Barcia et al. 2016). Microglia represent the innate immune system of the brain and take up 10-15% of the glial cells in the brain (Eng-Ang et al. 2012). When stimulated by markers of injury or pathogens, these cells become activated, alter their morphology, and secrete cytokines and chemokines to induce an inflammatory response (Cristovão et al. 2014). This neuroinflammation then protects against neurodegeneration and provides neuroprotection.

However, in recent years the possibly adverse role of neuroinflammation in the development of neurodegenerative diseases, including AD, Parkinson's Disease, and Multiple Sclerosis, has gotten more attention. Especially the role of microglia appears to be critical in the development of AD and could be an effective therapeutic strategy target for developing a treatment. The brains of AD patients show increased pro-inflammatory cytokine levels and microglia are activated by A$\beta$ (Onyango et al. 2021). Moreover, A$\beta$-associated microglia show an altered morphology, and activated microglia are thought to contribute to A$\beta$ accumulation (Edler et al. 2018, Cai et al. 2014).

Age is the most prominent risk factor for the development of AD. Microglia are, in contrast to neurons, capable of self-renewal, but also show signs of cellular senescence (Santambrogio et al. 2001, Luo et al. 2010). The above facts combined lead to the hypothesis that the aging of microglia directly contributes to the development of AD and present microglia as a possible therapeutic target to prevent AD. Studies using animal models are promising, but much of the mechanism and the actual cause of AD remain unknown. This review will elaborate on the aging of microglia, the development of AD, and microglial activity in AD. Because a thorough understanding of the disease, its development, and progress are crucial for finding a treatment, and aging microglia are suggested to play a critical role in all of those, this review will discuss the question: *How does the phenotype of microglia change during aging and is this a cause or consequence of Alzheimer's Disease?*

# Alzheimer's Disease

## Hallmarks of Alzheimer's Disease

In 1907, Alois Alzheimer was the first to document the neurodegenerative disease that has been named after him (Stelzmann et al. 1995). The details he described of the patient's case are still relevant today and count as the most important hallmarks of AD. Apart from the typical memory loss and change in personality in later stages, Alzheimer also described the presence of neurofibrils, which currently are still used for the pathological diagnosis (DeTure et al. 2019).

These neurofibrils, characteristic of the pathology of AD, are now recognized as the depositions of Amyloid$\beta$ (A$\beta$) plaques and neurofibrillary tau tangles. A$\beta$ derives from the Amyloid Precursor Protein (APP) and is formed when APP is cleaved by both a $\beta$-secretase and $\gamma$-secretase at the N- and C-terminal of A$\beta$, respectively. The 4 kDa A$\beta$ peptide then remains either associated with the plasma membrane or is secreted into the extracellular space, where it can form aggregates like oligomers, protofibrils, amyloid fibrils, and plaques (Chen et al. 2017). A$\beta$ depositions are thought to induce apoptosis in neurons and promote the production of reactive oxygen species (ROS) (Forloni 1996). The amyloid cascade hypothesis states that the A$\beta$ depositions in the brain are a direct cause of the development of AD. Although it is evident that A$\beta$ is strongly involved in AD, there is an ongoing discussion on whether there is a causative relation, and little is known about the mechanism through which A$\beta$ would lead to AD. Clinical trials that target A$\beta$ show that clearing A$\beta$ is insufficient to improve AD-related symptoms and suggest another primary factor as a cause of AD (Castellani et al. 2019).

More strongly correlated with the degree of cognitive dysfunction in AD is the density of neurofibrillary tangles (NFTs). NFTs are filamentous aggregates of tau, a microtubule-associated protein. Tau protein has an important function in the stabilization of microtubules and thereby the stabilization of growing axons. The protein can be modified post translationally by phosphorylation, which decreases its efficiency (Brion 1998). Hyperphosphorylation of tau, as seen in AD, leads to the intracellular aggregation into insoluble NFTs, which survive neuronal cell death and remain in the extracellular space. The tau aggregates spread from the entorhinal cortex (EC), where the NFTs first appear, to the hippocampus and the neocortex (Braak et al. 1991). This happens in a predictable manner which is divided into Braak stages I to VI, with stage VI representing fully developed AD with tau pathology spread into the neocortex (Braak et al. 1991). Dysfunctional regulation of tau phosphorylation is thought to contribute to the development of AD by causing neuronal loss (Hernández et al. 2007).

The hippocampus is a critical brain area involved in AD. The A$\beta$ plaques, NFTs, and neuronal cell loss are all found in the hippocampus (Chu 2012). The hippocampus is a region involved in learning and memory, and neurodegeneration in this region has a severe impact on cognitive functioning. Early symptoms include short-term memory loss, difficulties in decision-making, and finding the right words. As the disease progresses, the cognitive functioning declines to a stage where a patient no longer recognizes relatives, shows changes in personality and behavior, and requires help with basic activities (Martins et al. 2019).

Ever since the first documentation of AD, research into the disease has made great de-

velopments and has become thorough and diverging. New insights provide new therapeutic strategy targets and hopeful perspectives for finding an effective treatment, which so far has not been found. Currently, more than 140 agents are tested as possible treatment for AD, with the targets ranging from the A$\beta$ cascade to epigenetic factors and neuroinflammation (Cummings et al. 2022). One branch of Alzheimer's research focuses on the involvement of the immune system and neuroinflammation in the disease, in which microglia play a critical role.

## Alzheimer's Disease and neuroinflammation

In recent years it has become increasingly clear that neuroinflammation plays a critical role in the development of AD and there is abundant evidence to support this. For example, factors that are known to increase the risk of developing AD are also associated with inflammation. There are multiple polymorphisms of genes associated with inflammation and the development of AD (Bis et al. 2020), and also dietary factors like obesity and alcohol consumption are both associated with neuroinflammation and an increased risk for AD (Armstrong 2019, Collins et al. 2014, Aguilar-Valles et al. 2015, Anstey et al. 2011).

Neuroinflammation is the immune response of the central nervous system to infection or injury, induced by microglia and astrocytes that interact with neurons. Key in neuroinflammation is the secretion of pro- and anti-inflammatory cytokines and chemokines that enable a well-regulated response (Yang et al. 2018). A prominent pathway that induces neuroinflammation is the Nuclear Factor-$\kappa$B (NF-$\kappa$B) pathway. NF-$\kappa$B is a transcription factor of inflammatory and immune genes and is thereby a crucial player in the regulation of neuroinflammation (Barnes 1997). Activation of the NF-$\kappa$B pathway is associated with AD pathology and targeting this pathway appears to have positive effects on AD-related symptoms (Jha et al. 2019). For instance, NF-$\kappa$B inhibitors are shown to reduce the production of A$\beta$ (Paris et al. 2007).

It makes sense that neuroinflammatory processes are activated in AD in response to the neurotoxic deposits and neurodegeneration. It is likely that neuroinflammation is involved in the pathogenesis of AD (Akiyama et al. 2000), but it is still uncertain whether neuroinflammation is capable of directly causing the onset of AD. In 1998, Hauss-Wegrzyniak et al. provided compelling evidence for the causative relation between neuroinflammation and AD. They induced a state of chronic neuroinflammation in the brains of young rats and established the effects on Alzheimer's-related symptoms. After confirming the activated state of microglia in the hippocampus, they found a significant loss of hippocampal CA3 pyramidal neurons as well as an impairment of spatial memory, two symptoms typically related to AD (Hauss-Wegrzyniak et al. 1998). Moreover, chronic stimulation of the immune system of wild-type mice induces the accumulation of APP, altered tau phosphorylation along with depositions of tau aggregates, and impairment of the working memory (Krstic et al. 2012).

Remarkably, however, despite the critical role of neuroinflammation in AD, treatments with anti-inflammatory agents do not always improve the symptoms. Studies into the effect of inflammatory inhibitors show diverging results. The Rotterdam Study, an epidemiological study, looked into the association between the use of Non-Steroid Anti-Inflammatory Drugs (NSAIDs) and the risk of developing AD in almost 7.000 subjects. They conclude that long-

term use of NSAIDs actually could protect against AD (in 't Veld et al. 2001). However, there may be some biases of influence that are hard to eliminate. For example, there could be socioeconomic factors that affect both cognitive impairment and NSAID use (Aisen 2002). In contrast with the results of the Rotterdam Study, multiple clinical trials looking at different NSAIDs could not provide results in line with that conclusion (Aisen 2002). Overall, the application of NSAIDs as prevention for or treatment of AD is still quite controversial due to conflicting study results and the harmful effects of chronic exposure (Jordan et al. 2020).

As microglia represent the immune cells of the brain and function as the main regulators of neuroinflammation, they play a central role in the progress of AD. The involvement of microglia in AD has been investigated extensively over the past years and research has provided great insights. Still, the particular role of microglia in the onset of AD remains a question. Answering this question could provide new therapeutic strategy targets for finding an effective treatment for AD.

## Alzheimer's Disease and microglia

To make accurate hypotheses on the role of microglia in neuroinflammation and the development of AD, it is necessary to review the functioning of microglia in the AD brain.

To start, microglia seem to be involved in different stages of A$\beta$ plaque formation. As immune cells of the brain, they become active and produce ROS and TNF in response to the binding of A$\beta$ (El Khoury et al. 1998). A$\beta$ promotes inflammation through the NF–$\kappa$B pathway (Rubio-Perez et al. 2012). However, despite the activated state of microglia, accumulation of A$\beta$ plaques continues to take place. It is suggested that the microglia eventually fail to properly remove the A$\beta$ depositions as a result of the pro-inflammatory response that seems to reduce the phagocytic properties of microglia via the NF–$\kappa$B pathway (Koenigsknecht-Talboo et al. 2005). TNF was shown to reduce the expression of genes that are involved in the binding and phagocytosis of A$\beta$ (Hickman et al. 2008). So, in the early stages, microglia will activate in order to remove the neurotoxic depositions but will eventually become dysfunctional with decreased binding and degrading of A$\beta$ (Hickman et al. 2008). Research into the effects of the microglial response to A$\beta$ deposition shows that the pro-inflammatory cytokines TNF and IFN both cause an upregulation of $\beta$-secretase expression, while TNF, IFN, and IL-1$\beta$ increase the activity of $\gamma$-secretase (Yamamoto et al. 2007, Liao et al. 2004). These effects combined could lead to an increase in the cleavage of APP into A$\beta$. Additionally, IL-1$\beta$, a pro-inflammatory cytokine secreted by microglia, increased APP mRNA in neuronal cells (Forloni et al. 1992). This would suggest that microglia are not only involved in the processing of A$\beta$ depositions in the brain but also play a role in the production of these depositions. The cytokines secreted by microglia appear to contribute to A$\beta$ plaque formation.

Microglia also play an interesting role in the other hallmark of AD, the NFTs. The spreading of the NFTs from the EC to the hippocampus and the neocortex appears to be regulated by microglia through exosome secretion, the secretion of extracellular vesicles (Asai et al. 2015). Microglia therefore also contribute to the progression of AD by regulating the spread of the neurotoxic NFTs. Additionally, as with the A$\beta$ plaques, microglia show an immune response to tau by removing both soluble and insoluble tau aggregates from the

extracellular space (Bolós et al. 2016). However, microglia are shown to contribute to tau hyperphosphorylation as well. Maphis et al. (2015) provide proof of the prominent role of microglia in tau pathology. Amongst other things, they established that the activated microglia in response to human tau (hTau) taken from transgenic, hTau-expressing mice brains are sufficient to induce tau pathology in wild-type mice brains (Maphis et al. 2015). So, microglia are not only involved in the removal of extracellular tau, but also in the process of tau pathology.

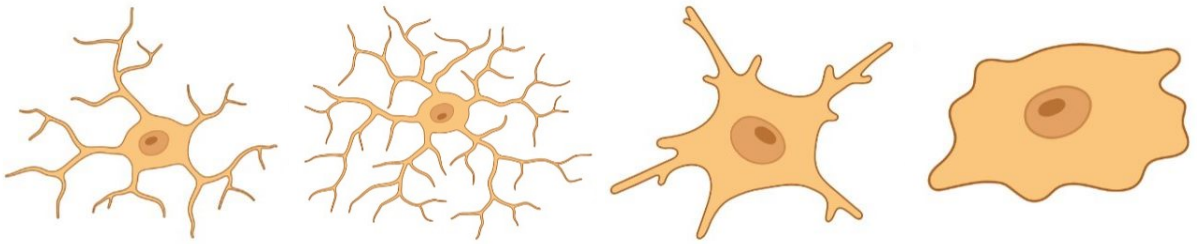
In AD, two of the major genetic risk factors are the $\epsilon 4$ variant of the APOE gene and the R47H variant of the triggering receptor expressed on myeloid cells 2 (TREM2) gene (Perea et al. 2020). APOE is a gene that encodes for apolipoprotein E (ApoE). ApoE is a protein involved in the transport of lipids like cholesterol and is secreted by astrocytes and microglia (Mahley 1988). The APOE-$\epsilon 4$ allele alters the microglial response to A$\beta$ by causing an increase in the secretion of cytokines, including IL-1$\beta$ and TNF (Rodriguez et al. 2014, Lanfranco et al. 2021). TREM2 is a gene that encodes a transmembrane protein and is expressed by microglia. The receptor protein binds glycoproteins and lipids, like ApoE. It is a critical protein in AD and is involved in the adhesion of microglia to A$\beta$ plaques, the formation of A$\beta$ depositions, tau pathology, and neurodegeneration (Qin et al. 2021). Moreover, because TREM2 has been reported to have anti-inflammatory effects, Jonsson et al. (2013) suggest that the R47H allele possibly causes an increased risk of AD due to an excessive inflammatory response. Because these are the most prominent genetic risk factors for the development of AD and these factors are shown to affect microglial functioning, it is possible that microglial dysfunction is a major contributor to the progress of AD.

These data show the critical role of microglia in AD. Microglia are involved in the two most important hallmarks of AD, the A$\beta$ plaques and the NFTs, and the major genetic risk factors for AD are genes encoding proteins crucial in the functioning of microglia. The microglial response to A$\beta$ and NFTs is shown to contribute to AD pathology and it is possible that microglial dysfunction as a result of the APOE-$\epsilon 4$ and R47H alleles is what causes the increased risk of the development of AD.

## Aging microglia

Microglia are the innate immune cells of the central nervous system and take care of homeostasis and recovery after injury in the brain. They can phagocytose invading microorganisms, induce inflammation, and promote neurogenesis. Microglia precursor cells appear in the developing rat brain as soon as day 11 of the embryonic stage and differentiate until postnatal day 17 (Eng-Ang et al. 2012). Throughout adulthood, microglia show plasticity and changes in phenotype (Santambrogio et al. 2001). However, immunosenescence, as seen in macrophages of the peripheral immune system (Ginaldi et al. 2001), possibly also affects microglial functioning in the brain directly as we age (Luo et al. 2010).

Activated microglia are mediators of neuroinflammation and the first indication that the brain's immune system is active. As mentioned before, neuroinflammation is the immune response of the brain to injury and infections, and protects against misfolded proteins, cell debris, and pathogens (Yang et al. 2018). When stimulated, microglia secrete pro-inflammatory cytokines, like Interleukin-1$\beta$ (IL-1$\beta$) and Tumour Necrosis Factor-$\alpha$ (TNF),



(a) Ramified stage (b) Hyperramified stage (c) Bushy stage (d) Amoeboid stage

Figure 1: Morphology of microglia in 4 different stages: Microglia in the ramified stage (1a) survey the environment to react to injury. In response to injury, microglia become hyperramified (1b) and reorientate to the site of injure. The hyperramified stage is characterized by extended branching and an increase in the secretion of pro-inflammatory cytokines. Continued stimulation leads to the development into the bushy stage (1c). In this stage, microglia have enlarged cell bodies and thickened branches. Eventually microglia will develop into the amoeboid stage (1d), associated with a rounded morphology, limited to no branching, and maximal cytokine secretion.

and chemokines, which in turn promote downstream processes including neurodegeneration, phagocytosis, neurogenesis, neuroprotection, and more (Shabab et al. 2017). These reactions then signal back to the microglia which leads to the secretion of both pro- and anti-inflammatory compounds, ensuring a precisely regulated process that prevents an excessive, harmful response (Yang et al. 2018).

Microglia typically show 4 distinct morphologies. The ramified stage (Fig. 1a) is characterized by relatively small cell bodies and long branches. In this stage, microglia survey the environment to detect injury. When stimulated, the microglia shift to the hyperramified stage (Fig. 1b) with extended branching, the secretion of pro-inflammatory cytokines, and reorientation towards the site of injury (Beynon et al. 2012). Through further pro-inflammatory stimulation, microglia evolve into the so-called 'bushy' state (Fig 1c) with enlarged cell bodies and thickened branches. Eventually, microglia will develop into the amoeboid stage (Fig. 1d). In the amoeboid stage, microglia have rounded cell bodies with limited to no branching. The amoeboid stage is associated with an active state along with maximal pro-inflammatory cytokine secretion, free radicals, and microglial apoptosis (Crews et al. 2016). Figure 1 shows the shape of microglia in all four stages.

The aging of the brain has been a topic of great interest in the past decades. Particularly, the possibly intrinsic aging of microglia and its relation to neurodegenerative diseases has been investigated more and more in recent years, however much remains unknown. For example, it is still uncertain whether the changing phenotype is directly or indirectly affected by aging. There are many hypotheses on the aging of microglia and how this contributes to the pathological aging of the brain. One prominent hypothesis states that aged microglia show an increased inflammatory response caused by cellular senescence which contributes to a mild chronic state of inflammation (Harry 2013). Evidence in favor of this hypothesis includes the established increase in pro-inflammatory cytokine Interleukin-6 and a decrease in anti-inflammatory cytokine Interleukin-10 levels in the brain of aged mice (Ye et al. 2001). In addition to the altered cytokine secretion profile, aged microglia also show a change in the expression of major histocompatibility complex II (MHC-II) and complement

receptor 3 (CD11b), two inflammatory markers. Both markers are receptors presented on the microglial surface and their expression increases during aging (Norden et al. 2013). The upregulated expression of MHC-II is also associated with an abnormal, rounded morphology of microglia (Perry et al. 1993). Moreover, in a human autopsy research, Bachstetter et al. (2017) show that the hippocampus and cortex of aged individuals show more rod-shaped microglia compared to younger subjects. Rod-shaped microglia were famously described first by Frans Nissl in 1899 and are narrow cells with planar branches (Taylor et al. 2014). They are thought to be an additional, intermediate stage between the ramified and amoeboid microglial stage (Au et al. 2017). This would suggest that the rod-shaped microglia in aged brains are in a more primed stage and can transform into the activated stage more easily. In line with the hypothesis that aged microglia cause a state of mild chronic inflammation, all these results imply that the microglia in aged brains are constantly in a slightly activated state.

Another hypothesis is the microglial dysfunction hypothesis. This hypothesis states that microglia become dystrophic as a consequence of aging, thereby degenerating and losing their neuroprotective capacities, which in turn contributes to the development of AD (Streit 2004). Dystrophic microglia are characterized by de-ramification, the retraction of branches into short, twisted branches, and cytoplasmic fragmentation (Streit, Sammons, et al. 2004). These dystrophic microglia appear to be more prevalent in the brains of AD patients compared to healthily aged brains (Shahidehpour et al. 2021). The microglial dysfunction hypothesis circumvents the idea that microglial activation becomes neurotoxic, as assumed by the mild chronic inflammation hypothesis. This idea is somewhat questionable from an evolutionary viewpoint because a vital organ like the brain should not be vulnerable to possibly harmful resident cells (Biber et al. 2014). This, and the fact that treatment with anti-inflammatory agents does not improve neurodegeneration (Streit, Braak, et al. 2009) presents data in favor of the microglial dysfunction hypothesis of microglial aging as opposed to the mild chronic inflammation hypothesis. The microglial dysfunction hypothesis is extremely interesting and could have a great influence on the way neuroscientists approach the topic of aging microglia and AD. However, this theory is not as explored as the mild chronic inflammation hypothesis and requires more research to develop.

There are many reports and hypotheses on the changing phenotype of microglia in aging. However, the question and discussion remain on whether these changes are direct or indirect effects of aging. Aging is defined as 'the progressive accumulation of diverse deleterious changes in cells and tissues with advancing age that increase the risk of disease and death', and is attributed to the presence of free radicals in the Free Radical Theory of Aging (Harman 2003). Interestingly, Nakanishi et al. (2009) provide evidence showing that there is age-dependent lysosomal and autophagic dysfunction in microglia. This, in turn, causes the accumulation of mitochondria that hyper-generate ROS in microglia due to an extremely slow mitochondrial turnover. The increase in ROS causes excessive inflammation through activation of NF–$\kappa$B, a transcription factor for inflammatory and immune genes (Nakanishi et al. 2009). This would suggest that the changing phenotype of microglia is a direct and intrinsic effect of aging and that this contributes to mild chronic inflammation. Another factor directly associated with aging is telomere shortening. It is even implied as the sole mechanism of aging, suggested to still happen in absence of oxidative stress, and without

telomere shortening, aging is expected to cease (Mikhelson et al. 2010). In microglia, telomere shortening has been established, as well as its involvement in dementia (Flanary et al. 2007), also suggesting a direct effect of aging on microglia.

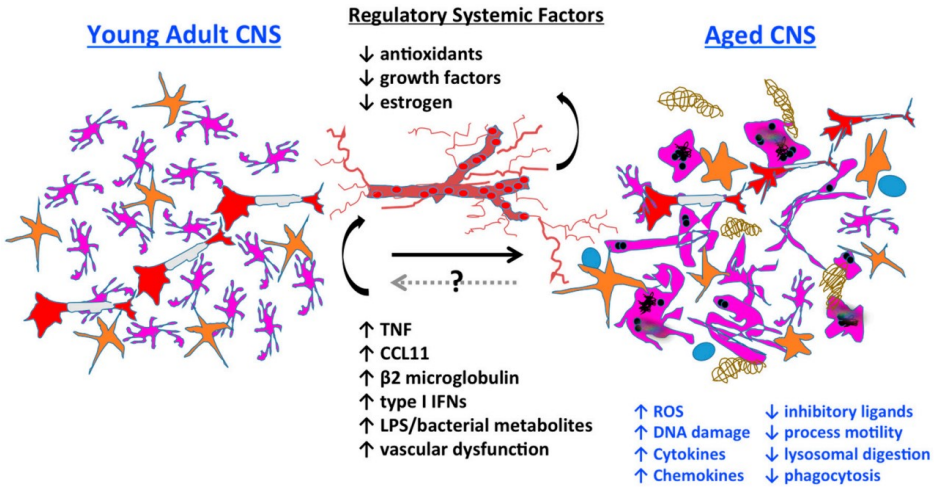



Figure 2: The changes seen in the brain's environment and microglia during aging: The regulatory systemic factors show changes in the brain's environment during aging, which affect the phenotype of microglia. There is a decrease in anti-aging factors and an increase in pro-aging factors. Aged microglia show an increase in ROS production, DNA damage, and cytokine and chemokine secretion combined with a decrease in inhibitory ligand secretion, process motility, lysosomal digestion, and phagocytosis. Source: Koellhoffer et al. 2017

Figure 2 by Koellhoffer et al. (2017) shows some of the processes happening to microglia (in pink) in aging. It shows the morphological changes, the de-ramification into round cells, as well as the changes in certain compound levels and processes. The regulatory system factors are all factors thought to contribute to the aging of microglia (Koellhoffer et al. 2017). These factors include a decrease in antioxidants, growth factors, and estrogen, and an increase in pro-inflammatory compounds TNF, IFN, and lipopolysaccharide. Anti-oxidants, growth factors, and estrogen are known to be anti-aging compounds. Antioxidants prevent or slow down the damaging effects of free radicals (Shalaby 2019), estrogens counteract the effects of oxidants (Thakur 1999), and growth factors promote regeneration of injured neurons (Ball et al. 2002). A decline in these factors contributes to aging. The regulatory system factors that are increased in aging are pro-aging compounds. Pro-inflammatory cytokines are shown to contribute to aging (Salvioli et al. 2006). CCL11, a chemokine, and $\beta$2 microglobulin, a component of MHC-I, both show increased levels in aging leading to a decline in neurogenesis and an increase in cognitive impairment (Villeda et al. 2011, Smith et al. 2015). Lastly, vascular dysfunction is also increasing with age and is thought to contribute to A$\beta$ accumulation (Janota et al. 2016). The effects of aging on microglia are shown in blue. There is an increase in ROS production, DNA damage, cytokine secretion, and chemokine secretion. This goes along with a decrease in the secreation of inhibitory ligands, process motility, lysosomal digestion, and phagocytosis. This suggests that aged microglia show both a dysfunctional phenotype and a phenotype associated with chronic inflammation. All factors combined are thought to lead to diminished immune surveillance and a decrease in debris clearance, which in turn leads to plaque formation, depicted as

brown loops (Koellhoffer et al. 2017).

Considering the data on the changing phenotype of microglia, it is likely that microglia suffer directly from aging. The aging of microglia is likely to have major effects on the cognitive functioning of aged brains and pathological aging. The two hypotheses on how the aging of microglia contributes to cognitive decline, the mild chronic inflammation hypothesis and the microglial dysfunction hypothesis, are both supported by scientific research but do require a more detailed understanding of the actual mechanisms. It is important to note that the two hypotheses are not mutually exclusive and it is very well possible that there is actually an interplay of both systems. Streit et al. (2014) suggest that microglia are both directly and indirectly affected by aging. Directly by the presence of free radicals, causing microglial dysfunction, and indirectly by the effects of free radicals on neurons, causing a mild chronic neuroinflammatory response of microglia (Streit, Xue, et al. 2014). This theory combines the two hypotheses and could explain the different phenotypes of microglia in aged brains, namely the more activated together with the dystrophic phenotypes.

### Aging microglia and Alzheimer's Disease

Research into the effect of aging microglia on AD reveals that microglia show an age-dependent decline in the ability to phagocytose A$\beta$ plaques (Floden et al. 2011). Induction of a senescence-like phenotype in microglia leads to an increase in A$\beta$ due to dysfunctional autophagy (Angelova et al. 2018). Moreover, immunohistochemistry shows the association of dystrophic microglia to tau aggregates, and analysis of microglial morphology at different Braak stages suggests that microglial dystrophy precedes tau pathologies (Streit, Braak, et al. 2009). Rodriguez-Callejas et al. show that dystrophic microglia in brains of aged marmosets contain hyperphosphorylated tau, while activated microglia do not, supporting the hypothesis that microglial dysfunction in aging contributes to AD. Lastly, telomere shortening as seen in aging microglia is more prominent in AD, with shorter telomeres and a larger quantity of short telomeres in microglia of AD patients compared to microglia of a non-demented subject (Flanary et al. 2007). These data suggest a relation between microglial senescence and AD. A possible mechanism through which the aging of microglia contributes to the onset of AD is the epigenetic modification of IL-1$\beta$ by SIRT1. Aging microglia show a decrease in the expression of SIRT1 (Cho et al. 2015), which is a deacetylase involved in aging and capable of inhibiting NF-$\kappa$B (Fusco et al. 2012). This age-dependent decrease in SIRT1 expression is linked to elevated IL-1$\beta$ transcription through downregulation of DNA methyltransferases as well as increased memory deficits in hTau-expressing mice (Cho et al. 2015). These results show a causative role of aging microglia in cognitive decline and, possibly, the progress of AD.

The data known on the aging of microglia and the involvement of microglia in AD present the aging of microglia as a therapeutic strategy target to prevent or treat AD. Indeed, selective removal of senescent microglia prevents tau hyperphosphorylation, neurodegeneration, and a decrease in short-term memory in mice (Bussian et al. 2018). Research into cell senescence has led to the development of so-called senolytics, agents that specifically remove senescent cells. Two examples of senolytic drugs are dasatinib and quercetin, two compounds that induce the apoptosis of senescent cells (Zhu et al. 2015). Currently, there is a pilot clini-

cal trial being executed using human subjects that suffer from mild AD and are treated with a combination of dasatinib and quercetin (Gonzales et al. 2021). Additionally, the impairment in A$\beta$ plaque phagocytosis appears to be reversible by young microglia (Daria et al. 2017). These studies emphasize the role of aging microglia in AD and show the promising perspectives of targeting senescent cells in developing a treatment. Although these studies are promising, little is known about the precise relation between aging microglia and AD. Scientific research that focuses specifically on the effect of aging microglia in the development of AD lacks, while this could be an intriguing branch within AD research that could bring important new insights into the onset and progression of the disease.

## Discussion

This review aimed to establish the effects of aging on microglia and whether this is a cause or consequence of Alzheimer's Disease. From the collected data it can be concluded that microglia have a crucial role in the development and progress of AD, in the early and later stages. It is highly likely that the aging of microglia is one of the causes of AD, because microglia are directly affected by aging, aging microglia are shown to be involved in the pathogenis of the main hallmarks of AD, and targeting aging microglia appears to be successful in improving AD-related symptoms.

Microglia show an altered phenotype as a consequence of aging, displaying a difference in morphology as well as cytokine secretion profile and membrane receptor expression. Some microglia in the aged brain appear to be in a chronically activated state, or primed state, while others show a dystrophic phenotype with de-ramification and cytoplasmic fragmentation. These phenotypes are associated with the mild chronic inflammation hypothesis and the microglial dysfunction hypothesis, respectively. The established effects of ROS on microglia and the documented telomere shortening suggest that microglia are subjected to direct effects of aging (Aging microglia).

The fact that microglia have a critical role in AD is evident. They are proven to be involved in two of the most important hallmarks of AD, namely the A$\beta$ plaque depositions and the NFTs. In both cases, microglia are suggested to be involved in the production as well as the removal of the lesions. Moreover, genetic risk factors like the $\epsilon$4 allele of APOE and the R47H variant of TREM2 affect the function of microglia (Alzheimer's Disease and microglia).

A significant amount of data supports the idea that the aging of microglia is a direct cause of AD. For instance, chronic stimulation of the immune system can induce AD-related symptoms like neuronal cell loss, APP accumulation, tau hyperphosphorylation, and impairment of spatial and working memory (Alzheimer's Disease and neuroinflammation). Moreover, pro-inflammatory cytokines increase APP expression, $\beta$-secretase expression, and $\gamma$-secretase activity (Alzheimer's Disease and microglia). This, in combination with the fact that aged microglia show an increase in pro-inflammatory cytokine secretion supports a causative relation between microglial aging and A$\beta$ plaque formation. Also, tau phosphorylation is proven to be increased by activated microglia transferred into wild-type mice brains (Alzheimer's Disease and microglia). Again, combined with the chronic inflammation seen in aged microglia, this adds to the idea that aged microglia are a cause of AD. Research into the specific

relation between aged microglia and AD shows that aged microglia become less efficient in A$\beta$ phagocytosis and the presence of dystrophic microglia, as seen in aging, precedes tau pathology (Aging microglia and Alzheimer's Disease). Lastly, remarkable evidence in favor of this hypothesis is studies that target the aging of microglia as a treatment for AD. Removal of senescent microglia improves AD-related symptoms, like tau hyperphosphorylation, neurodegeneration, and cognitive impairment, while replacement of aged microglia with young microglia reverses the decline in A$\beta$ phagocytosis (Aging microglia and Alzheimer's Disease).

These insights imply a critical role of aging microglia in the onset of AD, but there are only a few studies that look into the specific relation between aging microglia and the development of AD. Moreover, whereas the mild chronic inflammation hypothesis has been widely explored, the microglial dysfunction hypothesis has received less attention. However, as treatments with NSAIDs do not show the expected result in line with the mild chronic inflammation hypothesis and there is various evidence in favor of the microglial dysfunction hypothesis, the latter could be an interesting new approach to aging microglia. Thus, future research should focus on dystrophic microglia and their influence on the onset of AD. For example, it would be interesting to establish the effects of senolytic agents on A$\beta$ plaque formation or the effect of targeting the NF-$\kappa$B pathway, through which ROS is expected to contribute to microglial aging, on the changing phenotype of microglia and thereby AD.

In general, it is often difficult to conclude whether biological processes are a cause or consequence of other processes, due to the complexity of nature and the innumerable factors involved. The same goes for the aging of microglia and AD. However, establishing that it is very well possible that aging microglia are causally related to the onset of AD could be of great significance to developing an effective treatment for this debilitating disease, which so far has been unsuccessful. Although data suggest that aging microglia play a crucial role, it is difficult to identify the aging of microglia as the direct cause of Alzheimer's Disease. For that, extensive research is still required, if it would be possible at all to appoint one single cause of AD. Concluding, since the aging of microglia is likely to be at least one of the major factors in the development of AD, targeting this process could be a promising strategy to prevent and treat this disease, with major impacts on society.

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