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The role of microglia cells in Alzheimer's disease, influence of ageing and a potential therapeutic target

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Abstract

Alzheimer's disease is one of the most common forms of dementia and tremendously decreases the quality of life of millions of patients. The most profound hallmarks of AD are amyloid beta aggregates, tau neurofibrillary tangles, and neuroinflammation. It has been proposed that immunological mechanisms may play a key role in AD pathogenesis. It is thought that ageing influences the functioning of the immune system, and in particular microglial cells, which are the resident macrophages of the brain. These cells also appear to play a role in Alzheimer's disease pathogenesis by causing persistent neuroinflammation, leading to neurodegeneration. As a result of the increasing prevalence of Alzheimer's disease among elderly, the need to develop an effective treatment has also increased. The aim of this essay is therefore to elaborate further on the role of microglial cells in Alzheimer's disease, how is ageing involved and can inflammatory factors be targeted for therapy. The most important aspects of this will be touched upon in this essay, from which it can be concluded that with advanced age, the functioning of microglia becomes impaired, leading to decreased phagocytosis of aggregated proteins and sustained neuroinflammation, contributing to AD pathogenesis. Additionally, the stimulation of the TNF receptor 2, is found to mitigate the neuropathological features of AD, making it a possible therapy for Alzheimer's disease.

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Introduction

Alzheimer‘s Disease (AD) is one of the most common age-related brain disorders known. AD is a particular type of dementia, which is the umbrella term for loss of cognitive functioning, memory, language, reasoning, and other abilities, to such an extent that it strongly influences daily activities and reduces the quality of life (Alzheimer’s Association, 2016). According to the WHO, 55 million people worldwide are suffering from dementia, with AD being the most common (60-70% of all cases) form. Currently, there are no therapies to cure Alzheimer’s disease. The substantial rise in the number of AD patients, and the expectation of 65.7 million cases by 2030, is the reason for the increasing importance of developing a treatment (Prince et al., 2013). Alzheimer‘s Disease can be described as a gradual and progressive neurodegenerative disease, which involves impairment or dying of cells of the nervous system, leading to brain atrophy (Alzheimer’s Association, 2016; Gao and Hong, 2008; Madav, Wairkar & Prabhakar, 2019). In the course of time, the destruction of neurons in the brains of AD patients can affect all parts of the brain, causing that they can no longer perform basic physical functions, and eventually it may even lead to death (Alzheimer’s Association, 2016).

The onset of Alzheimer’s is insidious and often later in life, and as the most consistent risk factor for developing a neurodegenerative disease is increasing age, Alzheimer’s mainly affects the elderly (Przedborski, Vila & Jackson-Lewis, 2003). Cognitive and functional abilities decline gradually, although the rate of decline varies. Disease related symptoms vary between different individuals. The most common symptoms of AD, according to the Alzheimer’s Association (2016), are primarily memory loss, and problems with planning and problem solving. Completion of daily tasks also often becomes difficult, and there is confusion with time or place. In addition, changes in mood and personality, leading to withdrawal from work or social activities, are accompanied by increased anxiety, agitation, and sleep disturbances. As the disease progresses, patients become more dependent on help from others. Diagnosis of the disease is therefore cautious: there is a careful and comprehensive evaluation, with multiple components and experts involved, including: assessment of medical and family history, consultation with family members or other person, to provide input on the functioning of the possible patient, cognitive tests, physical and neurological examination, and blood tests and brain imaging, also to rule out other causes for dementia (Alzheimer’s Association, 2016).

Characteristic features, hallmarks, in the brain of Alzheimer’s patients are senile plaques and neurofibrillary tangles, which are thought to underlie the pathophysiology of Alzheimer’s disease (*Figure 1*) (Madav, Wairkar & Prabhakar, 2019). The senile plaques are composed of aggregates of amyloid beta (Aβ) fragments. Under normal circumstances, Amyloid Precursor Protein (Aβ is a fragment of APP), which is found in neuronal and glial cells, is cleaved by the enzymes α- and γ-secretase (Madav, Wairkar & Prabhakar, 2019). The soluble products of this cleavage ensure improved synaptic plasticity, regulation of excitability of neurons and has

neuroprotective effects (Mattson and Chan, 2003). Under pathological conditions, α-secretase is substituted by β-secretase, creating Aβ monomers, which can form aggregated neurotoxic oligomers of Aβ and eventually β-sheets (Cole & Vassar, 2008). In combination with decreased clearance of Aβ, multiple β-sheets can accumulate and form fibrils on the surface of the brain, which, when they accumulate together, are known as amyloid plaques (Roeters et al., 2017). The role of Aβ aggregation in AD pathology starts with the damaging of mitochondria, causing oxidative stress, and synaptic dysfunction (Fan et al., 2020). The amyloid plaques trigger an immune response by activating microglia and astrocytes (neuroimmune cells), which induce a pro-inflammatory state in the brain (Heneka et al., 2015). This leads to neuronal damage and dysfunction, and apoptosis of neuronal cells (Fan et al., 2020). In addition, oligomers of Aβ are thought to be the most neurotoxic, and their binding to specific receptors leads to impaired learning and memory and decreased cognitive functioning, associated with AD (Fan et al., 2020). The neurofibrillary tangles are caused by oligomerization of tau proteins into entangled structures (Buerger et al., 2006). Under normal circumstances, tau proteins stabilize microtubule structures in neurons (Madav, Wairkar & Prabhakar, 2019). When suffering from Alzheimer's disease, Aβ accumulation and neuroinflammation, which is another hallmark of AD, cause hyperphosphorylation of tau proteins (Arendt, Stieler & Holzer, 2016; Madav, Wairkar & Prabhakar, 2019). The role of tau neurofibrillary tangles (NFTs) in AD pathology is complex and remains to be elucidated more (Fan et al., 2020). However, hyperphosphorylated tau can accumulate, leading to the formation of NFTs. These tangles, which are present inside nerves, are thought to cause defective microtubule functioning, thereby leading to damaging of the neuronal signaling system and impaired functioning of synapses, increased neurotoxicity, and cell dysfunction, which all can be related to neurodegeneration and impaired cognition (Fan et al., 2020).

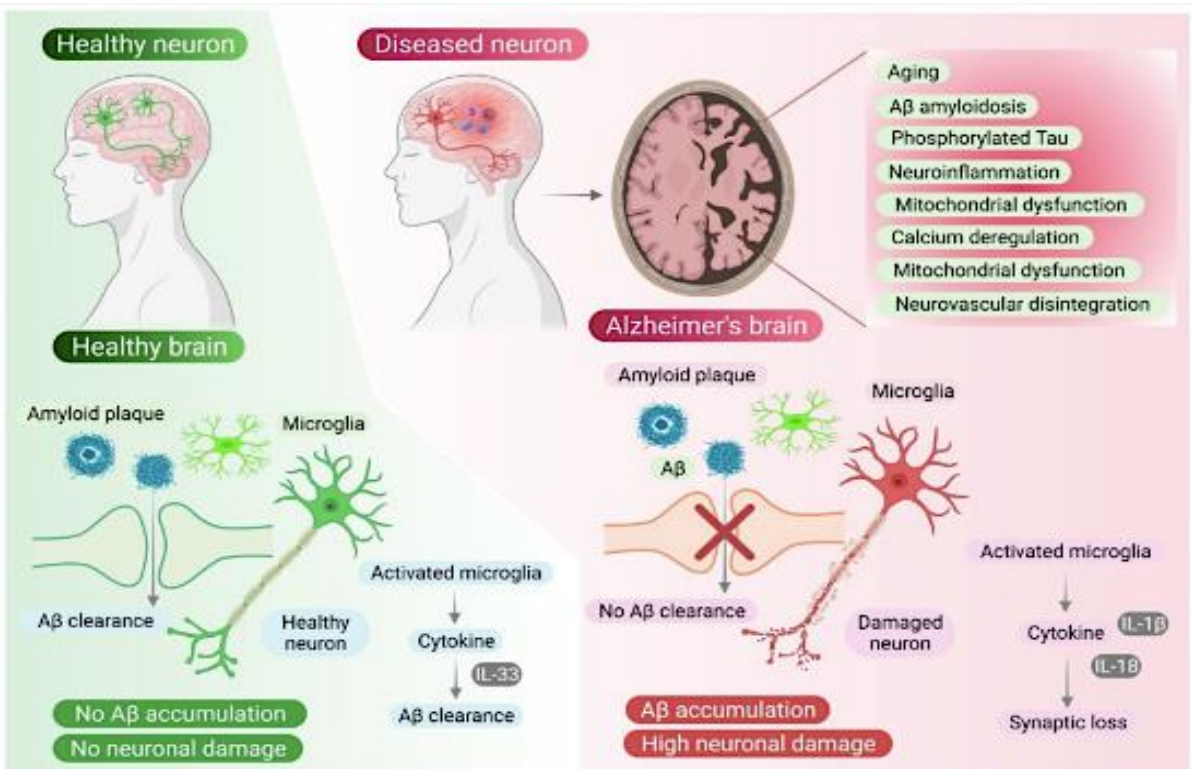

Figure 1. The hallmarks of Alzheimer's disease. The image displays the complex pathophysiology of AD. In a healthy brain, there is no accumulation of Aβ and no neuronal damage. In the Alzheimer's brain, there is Aβ accumulation due to decreased clearance and high neuronal damage, leading AD characteristics, such as plaque formation, hyperphosphorylation of tau, and neuroinflammation (Prasanna et al., 2021).

Immunological mechanisms, especially inflammatory processes, are also involved in the pathogenesis of Alzheimer's disease (Heneka et al., 2015). It has been suggested that neuroinflammation is not only triggered by amyloid plaques and neurofibrillary tangles, but that it itself plays a more causal role in the pathology of AD (Heneka et al., 2015). Misfolded and aggregated proteins bind to receptors on immune cells, triggering an (innate) immune response, accompanied by an overexpression of proinflammatory factors (Heneka et al., 2015). In addition, it has been proposed that ageing and the functioning of the neuroimmune system are related to each other, and possibly participating in the development of Alzheimer's disease (Franco-Bocanegra et al., 2019). There exists evidence that a particular type of neural immune cell, the microglia, is responsible for changes in the brain that occur during ageing, and, in addition, that these immune cells are involved in the development of neurodegenerative diseases, such as Alzheimer's disease (Franco-Bocanegra et al., 2019). Additionally, a particular proinflammatory cytokine called TNFα, which plays a role in neuroinflammation and is a potential therapeutic target, will be focused on (Wang et al., 2015; Ortí-Casañ et al., 2022). Taken all this information together, this leads to my research question: *'What is the role of microglial cells in Alzheimer's disease, how is ageing involved and can inflammatory factors be targeted for therapy?'* This question will be answered by delving deeper in the immune system of the brain, the consequences of ageing, and how this can be related to the development of Alzheimer's disease. Furthermore, the role of microglia and TNF in neurodegeneration and inflammation will be elaborated on, and eventually innovative therapies to treat this tremendous disease will be touched upon.

The neuroimmune system

It is well known that our immune system is critical for survival. The immune system is made up of specialized organs, tissues, and cells, which protect us every day against pathogens to maintain our health. In the brain, the neuroimmune system can be found, which is characterized by its own neuro-specific immune cells, unlike cells of the peripheral immune system (Beardsley & Hauser, 2014). The neuroimmune system protects neurons from damage and additionally mediates healing or pruning of redundant or damaged synapses and neurons (Woodcock et al., 2019). In contrast, excessive neuroinflammation, caused by the immune system, can lead to neuronal cell death and neurodegeneration (Woodcock et al., 2019). Neuronal health and survival are strongly affected by the complex neuroimmune system.

Immune system of the brain

Research has shown that the immune system plays an important role in maintaining a healthy brain. It was shown by Beers et al. (2008) that mice lacking an immune system show increased rate of neurodegeneration in Alzheimer's disease, however, in contrast, recovery of the immune system resulted in slower disease progression. This indicates that something goes wrong in the neuroimmune system during AD, negatively affecting the state of the brain (Beers et al., 2008). One way to protect the brain is through permeable barriers, such as the blood-brain barrier, which protects the brain by regulation of molecular flow and prevention of the infiltration of pathogens and toxins (Kadry, Noorani & Cucullo, 2020). However, in the brain, also immune cells are present to preserve a hazard-free environment. For one thing, the brain hosts its own resident immune cells (Korin et al., 2017). These so-called glial cells are unique for the neuroimmune system and are not found anywhere else in the body (Korin et al., 2017). Furthermore, the meninges are layers in the brain which provide protection from outside the closed environment of the brain. In the fluid between the layers of the meninges, certain infiltrating immune cells can be found during an immune response, such as T cells and macrophages (Korin et al., 2017). These immune cells enter the brain via sinuses, are alert to abnormalities and leave the brain via the lymphatic system (Kwon, 2022). Sensory neurons and immune cells sense and detect harmful stimuli in the brain, and cytokines can regulate and initiate immune responses when needed (Talbot, Foster & Woolf, 2016). In addition, (neuronal) damage can initiate a cascade of inflammatory responses, causing immune cells to express cytokines and chemokines, in turn inducing inflammation of the neuro-system (Talbot, Foster & Woolf, 2016). In this way, the neuroimmune system performs its restorative and protective functions.

Microglia

The most prominent immune cells in the brain are microglia cells (microglia), which comprise 80% of brain immune cells and up to 16% of all cells in the human brain (Franco-Bocanegra et al., 2019; Korin et al., 2017). Microglia are the so-called resident macrophages of the central nervous system and exist in a wide variety of different types (Torres-Platas et al., 2014). Microglia cells are myeloid cells, originating from the yolk sac during development (Tay et al., 2017). They are capable of self-renewal without peripheral hematopoietic input, sustaining their population by continuous turn-over (Réu et al., 2017). From embryonic brain development to adulthood and during ageing, microglia display various functions. During development, microglia play a role in some non-immunological functions, such as shaping and pruning of synaptic architecture, and regulating establishment and maturation of neural networks (Franco-Bocanegra et al., 2019). In the adult brain, microglia mainly fulfill the task of surveillance: maintaining homeostasis and host defense against pathogens and diseases of the CNS (Ransohoff & Khoury, 2016). Microglia perform three main functions: sensing their environment, housekeeping, and protection (Hickman et al., 2018). Microglia cells sense their environment by monitoring changes and potential disturbances in their environment, which is essential to perform their housekeeping and defense functions (Hickman et al., 2018).

Housekeeping tasks include synaptic remodeling, phagocytosis of apoptotic cells or debris, and signaling, which are functions involved in development, maintaining homeostasis, inflammation, and neurodegeneration (Lui et al., 2016; Hickman et al., 2018; Galloway et al., 2019). Further, microglia provide protection against pathogens, but also hazardous self-proteins, such as amyloid beta (Hickman, Allison & El Khoury, 2008). Therefore, microglia express all sorts of immune receptors (Hickman et al., 2013). When these receptors are stimulated, microglia cells can trigger a neuroinflammatory response, accompanied by the secretion of cytokines such as tumor necrosis factor alpha (TNFα) (Hickman, Allison & El Khoury, 2008). However, the potential danger of this is that chronic or persistent neuroinflammation can lead to toxicity, which in turn causes neurodegeneration. In addition, defective functioning of microglia can lead to neurodegeneration (Butler et al., 2021). Microglia cells are thus key players in the neuroimmune system, with great importance for neuronal function and health, however, dysfunctionality of microglia cells can play a role in neurodegenerative diseases (Kim, Jung & Bo-Eun, 2018).

The influence of ageing on the brain and microglial functioning

Throughout the lifespan of humans, molecular and cellular damage accumulates in the body, during the process called ageing. The brain is vulnerable to the changes that occur during ageing, and ageing is therefore one of the greatest risk factors for the development of neurodegenerative disorders (Wrigglesworth et al., 2021). After reaching a certain age, which is also influenced by genetic and environmental factors, the brain begins to shrink (Sowell et al., 2003). This leads to the fact that individuals with an advanced age have more difficulties with complex learning and some cognitive functions (Von Bernhardi et al., 2015). During normal ageing, various changes take place at the cellular level, accumulation of DNA damage, protein mutation and accumulation, increase in oxidative stress and presence of damaging mediators and radicals, and in addition, there can be an imbalance between anti- and pro-inflammatory factors, resulting in mild chronic inflammation in the brain (Von Bernhardi et al., 2015). The imbalance between anti- and pro-inflammatory mediators is caused by a decreased secretion of anti-inflammatory cytokines, such as IL-10 and an increase in pro-inflammatory cytokines such as TNFα (Ye & Johnson, 2001; Lukiw, 2004).

Age-related effects on microglia cells

It is known that the functioning of the immune system weakens during the ageing process (Vitlic, Lord & Phillips, 2014). Correct functioning of microglia is, however, essential for normal functioning of the brain. Ageing does appear to influence the functioning of microglia cells, which in turn affects the brain (*Figure 2*). Microglia cells therefore seem to be involved in the process of brain ageing. During the ageing process, microglia are continuously exposed to foreign organisms or mutations, causing their regulatory function in autophagy to become disrupted (Ott et al., 2016; Choi et al., 2020). Consequently, this may lead to increased

accumulation of proteins and debris (Choi et al., 2020). In addition, it could be suggested that as a consequence, this in turn will lead to neuroinflammation, neuronal death, and eventually even neurodegenerative diseases such as AD (Hickman et al., 2018). In addition, ageing also leads to defective lysosomal degradation, which causes indigestible materials also to accumulate (Nakanishi & Wu, 2009). With age, microglial activation slows down, making them less able to monitor and sense their environment to recognize potential hazards (Hefendehl et al., 2014). Moreover, ageing alters the expression pattern of microglial genes (Holtman et al., 2015). Mutations in these genes can have a major impact on the functioning of microglia (Martin et al., 2017). It has additionally been shown that altered microglial functioning is common in age-related neurodegenerative disorders such as Alzheimer's disease. This indicates that the effect of ageing on microglial functioning does seem to play a role in the pathophysiology of these neurodegenerative disorders, which most often occur at later stages in life (Hefendehl et al., 2014). This makes it difficult to determine whether microglia are still beneficial or play a more harmful role in case of these brain disorders.

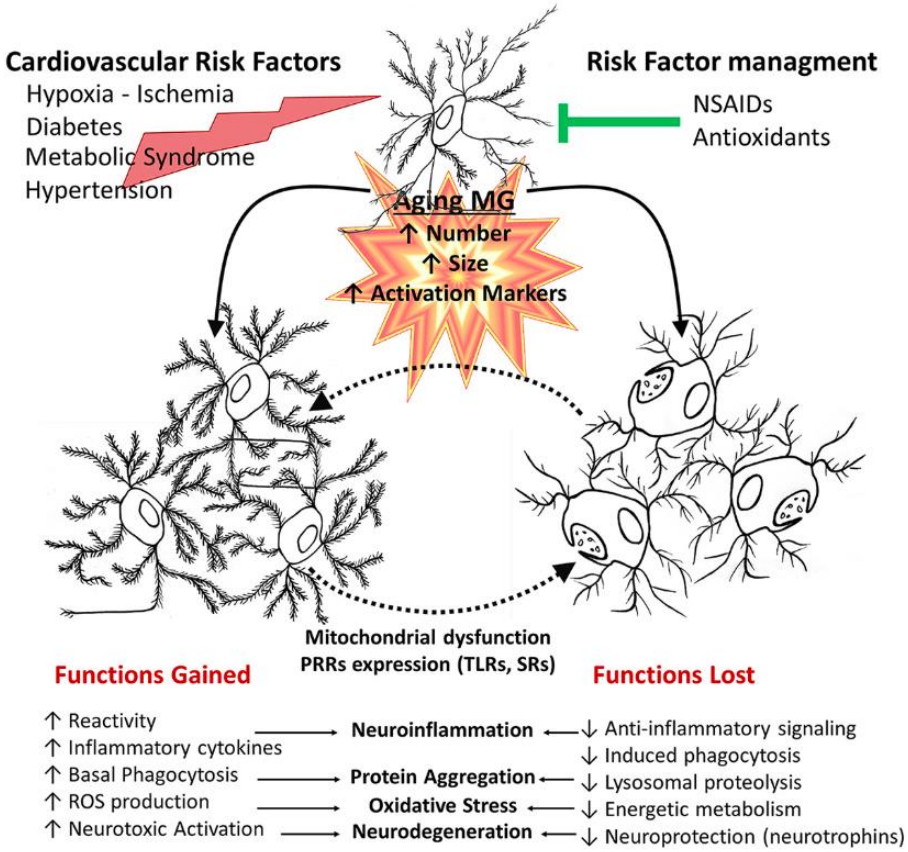

Figure 2. Age-related changes of microglial functioning (Von Bernhardi, Eugenín-von Bernhardi & Eugenín, 2015).

The role of microglia in Alzheimer's disease neurodegeneration

Alzheimer's disease is an age-related, neurodegenerative disorder, and degeneration of nervous tissue can have major impacts on the patient's daily life. Neurodegeneration can be different in its pathophysiology, causing also other types of disorders than AD (Wyss-Coray,

2016). In some cases, it causes memory and cognitive impairment, and in other cases, it can affect a person's ability to correctly perform motor and verbal functions, including breathing (Wyss-Coray, 2016). Neurodegeneration is characterized by the breakdown of neurons, by which they lose their structure and function, accompanied by loss of dendrites, myelin, and other brain matter (Przedborski, Vila & Jackson-Lewis, 2003; Butler et al., 2021). It is suggested that microglia cells are contributors of neurodegeneration, playing a key role in development of neurodegenerative disorders, such as Alzheimer's disease.

The characteristics of Alzheimer's disease, Aβ plaques, hyperphosphorylated tau-containing neurofibrillary tangles, and neuroinflammation, are all involved in degeneration of brain matter. Genetic research first revealed that microglia cells could play a role in neurodegeneration in Alzheimer's disease. Mutations have been found in genes for the expression of certain receptors essential for the initiation phagocytosis, and mutations have also been found in genes essential for carrying out key regulatory processes, such as phagocytic clearance (Lambert et al., 2013; Hsieh et al., 2009). This reduces the ability of microglia cells to take up debris and other potentially hazardous substances. It can therefore be implicated that mutations in these specific genes makes elderly susceptible to the development of Alzheimer's disease, or other neurodegenerative diseases.

As mentioned above, microglia cells can initiate an immune response to hazardous self-proteins. The amyloid beta and tau aggregates found in the brains of Alzheimer's patients can activate microglia cells (Hickman, Allison & El Khoury, 2008). In early AD, activated microglia can bind to toxic Aβ oligomers via cell-surface receptors, which initiates a neuroinflammatory response and ensures a delay of disease progression (Stewart et al., 2010; Hickman, Allison & El Khoury, 2008). And in order to clear the brain from Aβ aggregates, microglia start to phagocytose the ligated oligomers (Heneka et al., 2015). However, inefficient clearance of Aβ seems to play a significant role in Alzheimer's pathogenesis and worsening of the disease (*Figure 3*) (Mawuenyega et al., 2010). As the disease progresses, it appears that microglia cannot prevent accumulation of Aβ (Hickman, Allison & El Khoury, 2008). It has been found that interaction between Aβ and microglia can lead to synapse loss, production of neurotoxins, and excessive secretion of proinflammatory cytokines and TNFα, which can lead to neurodegeneration (Hong et al., 2016; Hickman et al., 2018; Martin et al., 2017). Microglia cells (affected by ageing) lose their ability to recognize and remove Aβ (Kim, Jung & Bo-Eun, 2018), causing a disturbance is the equilibrium between Aβ clearance and Aβ production. Due to decreased expression of Aβ phagocytosis receptors, the phagocytic capacity of microglia is compromised (Hickman, Allison & El Khoury, 2008). It can therefore be suggested that increased accumulation of Aβ is caused by failed phagocytosis of dysfunctional microglia. However, at the same time, the production and secretion of pro-inflammatory cytokines is maintained (Hickman, Allison & El Khoury, 2008). Moreover, it has been indicated that proinflammatory cytokines, released by microglia, may also contribute to accumulation of Aβ (Hickman, Allison & El Khoury, 2008). It has been shown that some proinflammatory cytokines,

including TNFα, can upregulate β-secretase, the enzyme involved in compromised Aβ production, contributing to AD pathology (Yamamoto et al., 2007). In addition, proinflammatory microglia cells, which become activated by interaction with Aβ, can cause increased tau phosphorylation (Lee et al., 2010). This makes activated microglia mediators of tau neurotoxicity (Yoshiyama et al., 2007). Although microglia have a protective role early in the disease, as disease progresses, microglial dysfunction, characterized by impaired Aβ phagocytosis and inducing a proinflammatory cascade, further contributes to worsening of AD pathology (Hickman, Allison & El Khoury, 2008).

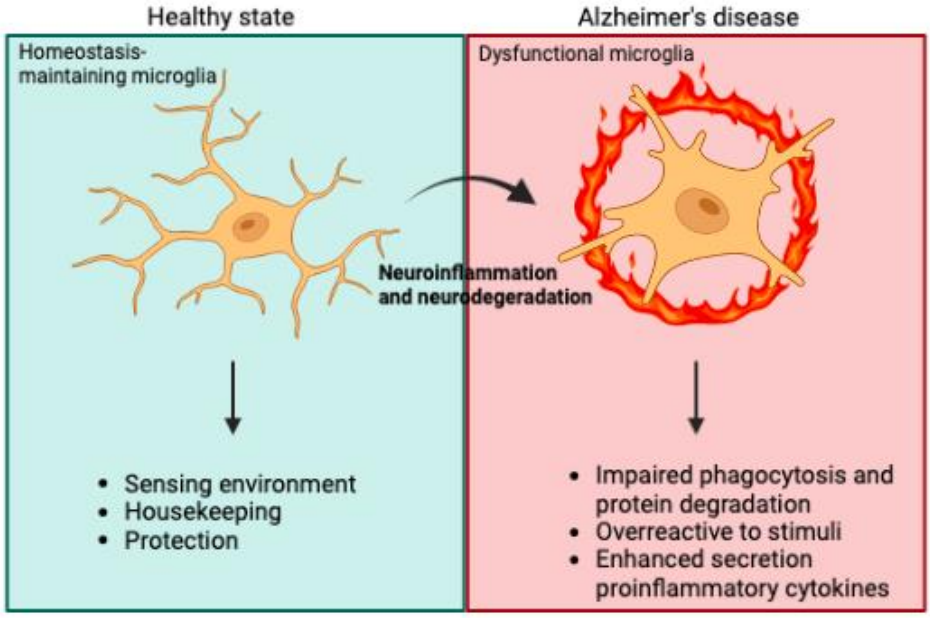

Figure 3. Microglia-mediated neurodegeneration. In a healthy state microglia perform various functions in sensing, housekeeping, and protection. In AD, microglia become dysfunctional, causing impaired phagocytosis (of Aβ) and protein degradation, overactivation, and overexpression of proinflammatory cytokines, inducing neuroinflammation and neurodegeneration. Created with BioRender (2022).

Microglia-mediated neuroinflammation in Alzheimer's disease

Neuroinflammation is, as the name states, inflammation of the nervous tissue, and is most often initiated as mechanism for host defense or in response to brain injury. Neuroinflammation is characterized by a rapid increase of activated glial cells and sustained secretion of cytokines and chemokines (Streit, Mrak & Griffin, 2004). However, neuroinflammation is also thought to be a driver of neurodegeneration (Ramesh, MacLean & Philipp, 2013). Sustained activation of microglia cells causes chronic neuroinflammation, which is associated with neurodegenerative disorders, such as Alzheimer's disease (Heneka et al., 2018). Here, activated microglia cells bind to misfolded and aggregated amyloid beta proteins, triggering a neuroimmune response characterized by the overactivation of microglia

cells and secretion of cytokines and other proinflammatory factors, leading to a profound hallmark of AD: neuroinflammation (Heneka et al., 2018).

Cytokines

Cytokines are signaling proteins produced by a large variety of cells, among which mostly activated immune cells. Cytokines in turn also regulate the activity of (these) immune cells, such as microglia. Moreover, cytokines are key modulators of inflammation. Activated immune cells, or microglia in this case, secrete proinflammatory cytokines upon stimulation, leading to upregulation of inflammatory processes (Zhang & An, 2007). In Alzheimer's disease, accumulation of the Aβ protein is responsible for the neuroinflammatory response. When exposed to Aβ, microglia cells become activated and initiate an immune response, which is characterized by the secretion of proinflammatory cytokines (Patel et al., 2015). The presence of Aβ and exposure to microglia may cause persistent activation of microglia, excessive secretion of proinflammatory cytokines, which eventually will lead to a state of chronic neuroinflammation (Sala et al., 2003). Microglia are therefore a major source of cytokine secretion in AD. Besides the secretion of cytokines, cytokines are in turn also involved in the activation of microglia, creating a vicious circle of inflammatory responses (Heneka et al., 2018).

Other microglia-related mediators in inflammation

In addition to proinflammatory cytokines, also other factors expressed by microglia are involved in inducing and sustaining neuroinflammation. Chemokines, which regulate migration of microglia to proinflammatory sites, provide enhanced neuroinflammation, which allows chemokines to modulate disease progression by exerting effects on microglial positioning and function (Heneka et al., 2018; Kiyota et al., 2009). As mentioned before, microglia also produce neurotoxins when exposed to amyloid beta, this production is stimulated by cytokines. Cytokines thus stimulate the production of compounds such as nitric oxide and hydrogen peroxide, which are toxic to neurons, in turn leading to increased microglial activation and enhanced proinflammatory response (Vodovotz et al., 1996; Jekabsone et al., 2006). In addition, post translational modification of these neurotoxins causes functional and structural damage to the brain by initiating plaque formation and sustained accumulation of proteins, which in turn again plays a role in the enhancement of microglial activation (Kummer et al., 2011).

Tumor Necrosis Factor-α

Tumor necrosis factor alpha (TNFα) is, as mentioned before, a proinflammatory cytokine, and plays a role in the regulation of innate and adaptive immune responses. It has been shown that TNFα is a key player in various neurological diseases, including Alzheimer's disease (Swardfager et al., 2010). TNFα found is to be elevated in the brain of AD patients, and therefore TNFα is thought to be a measure of Alzheimer's disease severity (Wang et al., 2015; Sala et al., 2003). Overexpression of TNFα by activated microglia has been shown to increase

Aβ formation, while it decreases Aβ clearance (Wang et al., 2015). The proinflammatory cytokine additionally contributes to tau hyperphosphorylation (Wang et al., 2015). These consequences of TNFα overexpression, including persistent neuroinflammation for which it is responsible, results in neuronal cell death and enhanced AD pathology (Janelsins et al., 2008). It was additionally shown by Tarkowski et al. (2003) that the risk of AD worsening from mild cognitive impairment to dementia was increased in patients with raised levels of the cytokine. Tumor necrosis factor-α binds to the tumor necrosis factor receptor (TNFR), of which there exist two different types with opposing functions (*Figure 4, left of arrow*). These different receptors each are differentially expressed and regulated, and each have distinct responses upon stimulation (Ortí-Casañ et al., 2022). TNF receptor 1 (TNFR1), to which mainly a soluble form of TNF (sTNF) binds, is responsible for the activation of proinflammatory pathways and is involved in neurodegeneration, while stimulation of TNF receptor 2 (TNFR2), to which mainly the transmembrane form of TNF binds (tmTNF), displays regenerative functions and is involved in neuroprotection (Dong et al., 2015; MacPherson et al., 2017). Since the proinflammatory potential of TNFα plays a significant role in the pathophysiology of neurodegenerative diseases, such as AD, it opens a therapeutic window for the interference with these diseases.

TNF receptor as a potential therapeutic target in Alzheimer's disease

Neuroinflammation is a profound characteristic of Alzheimer's disease and plays a major role in the pathophysiology of this neurodegenerative disorder. Because the number of old people is increasing enormously, there is a great need for an effective therapy. Recently, the possibilities to interfere with neuroinflammation by manipulating certain cytokines as a therapeutic target are being investigated (Dong et al., 2015). As mentioned before, TNFα has been shown to play a significant role in the neuroinflammatory processes associated with AD and has also been found to be elevated in the brains of AD patients (Wang et al., 2015). Treatment in which TNFα is neutralized has been proven to be successful in inflammatory diseases, such as rheumatoid arthritis (Ortí-Casañ et al., 2022). It was therefore tested whether neutralizing of TNFα could have beneficial effects in neurodegenerative diseases. However, treatment of neurodegenerative diseases which included TNFα neutralizing therapy was shown to be questionable or ineffective (Butchart et al., 2015; Ortí-Casañ et al., 2022). This raised the question whether the failure of using neutralizing drugs has something to do with the opposing functions of the TNF receptors, and whether these receptors would be suitable as a target for the treatment of AD.

It has been shown by MacPherson et al. (2017) that inhibition of the soluble form of TNF, which binds to TNF receptor 1, leads to a decrease in amyloid beta plaques, and is able to delay or even prevent neuronal dysfunction in an AD mouse model. In addition, it was demonstrated by Dong et al. (2016) that simultaneously using an antagonist for TNFR1 and an

agonist for TNFR2 counter neuroinflammation and promote neuronal survival in a mouse model for neurodegeneration. In addition, they showed that TNFR2 signaling is essential for neuroprotection, which is the reason why non-selective inhibition of TNF, which inhibits all TNF-signaling, is not effective as a therapy (Dong et al., 2016). Very recently, research by Ortí-Casañ et al. (2022) investigated the protective effect of TNFR2 activation using a TNFR2 agonist in a mouse model for Alzheimer's disease (*Figure 4*). The findings by Ortí-Casañ et al. (2020) demonstrated that use of the TNFR2 agonist NewStar2 reduces plaque formation and decreases expression of BACE-1, which plays a role in the formation and aggregation of toxic Aβ. In addition, they found an increased activity of microglia cells, which displayed increased phagocytosis and clearance of Aβ (Ortí-Casañ et al., 2020). Moreover, it was shown that cognitive functioning was improved (Ortí-Casañ et al., 2020). The findings of this research seem very promising, as the use of NewStar2 seems to improve the neuroprotective function of TNFR2, in an AD mouse model.

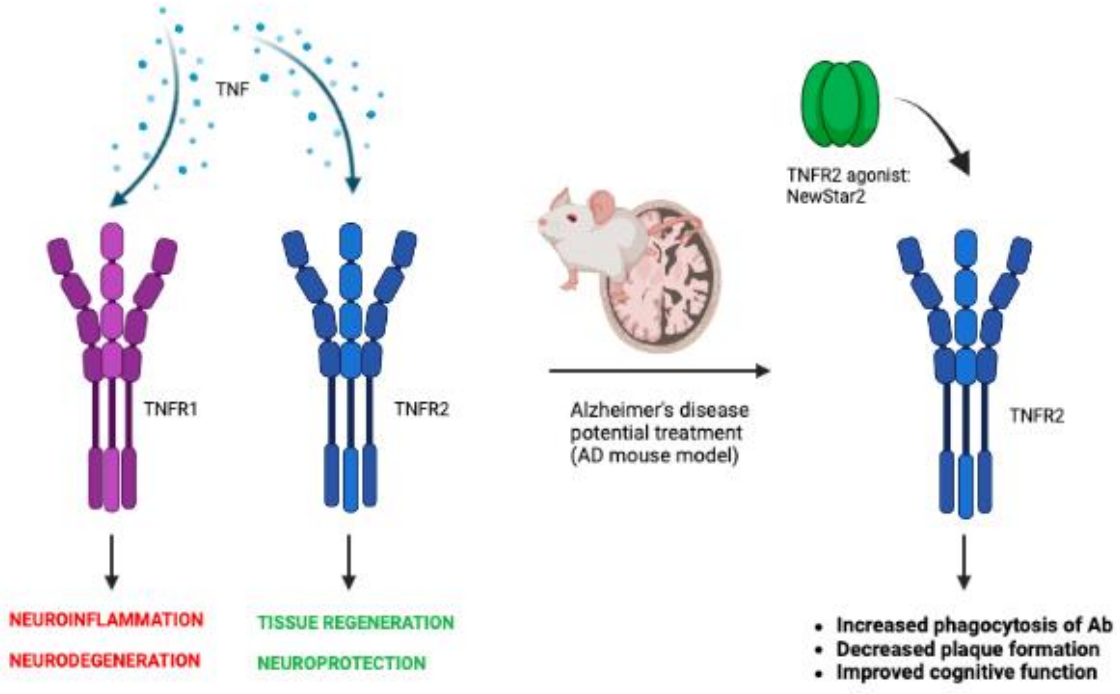

Figure 4. The opposite functions of the TNF receptors and the use of TNFR2 as a therapeutic target. Created with BioRender (2022).

Discussion

Alzheimer's disease is one of most common age-related neurodegenerative disorders known, and the most prominent form of dementia (Alzheimer's Association, 2016). The number of elderly people suffering from AD continues to increase as people reach an older age nowadays (Prince et al., 2013). Alzheimer's disease strongly affects daily life of patients, and reduces their quality of life, due to gradual decline of sensory, cognitive, and motor abilities (Wyss-Coray, 2016). Because of this major burden for Alzheimer's patients, there is a great need for

an effective therapy, and it is therefore a popular area of research. In this essay, the effect of ageing on microglia, and how this influences Alzheimer's disease pathophysiology will be investigated, and additionally how a microglia-associated processes in AD can be targeted to mitigate the consequences of this disease.

Ageing has a major impact on the brain. It is therefore one of the greatest risk factors for the development of neurodegenerative diseases, such as AD (Wrigglesworth et al., 2021). Ageing weakens the functioning of the immune system, and thereby also affects microglia cells. During ageing, the regulatory functions of microglia in phagocytosis and lysosomal degradation become disrupted, and they lose their ability monitor their environment as their activation slows down (Choi et al., 2020; Nakanishi & Wu, 2009; Hefendehl et al., 2014). In addition, ageing causes an increased number of mutations in microglial genes, leading to impairment of their functioning (Holtman et al., 2015; Martin et al., 2017). Altered microglial functioning is common in age-related neurodegenerative disorders such as Alzheimer's disease. This indicates that the effect of ageing on microglial functioning does seem to play a role in the pathophysiology of these neurodegenerative disorders.

Microglia are the most prominent cells of the neuroimmune system, performing a variety of functions in homeostasis and host defense (Hickman et al., 2018). However, microglia are also thought to be drivers of neuroinflammation and neurodegeneration and are additionally found to play a role in AD pathophysiology (Hefendehl et al., 2014; Heneka et al., 2018). In Alzheimer's disease, microglia cells become dysfunctional. They lose their ability to recognize and phagocytose amyloid beta, causing a disturbance in the equilibrium between Aβ production and clearance, which eventually leads to an increase in Aβ accumulation (Hickman, Allison & El Khoury, 2008). Microglia cells, which bind to misfolded and aggregated amyloid beta proteins, trigger a (auto)neuroimmune response characterized by the excessive activation of microglia cells and overexpression of cytokines and other proinflammatory factors, which in turn causes neuroinflammation (Heneka et al., 2018). Consequently, this process leads to neurodegeneration.

A key player in AD-associated neuroinflammation is the proinflammatory cytokine TNFα (Swardfager et al., 2010). This cytokine is found to be elevated in AD patients and is therefore thought to play a significant role in AD pathophysiology (Wang et al., 2015). Recent research has therefore been focusing on targeting this cytokine, or its receptors. As researchers had actively been seeking for an effective therapy to treat Alzheimer's disease, it has been demonstrated that using a TNFR2 agonist stimulates its neuroprotective properties, by decreasing plaque formation and increasing the phagocytic capacity of microglia cells, accompanied with improvement of cognitive functions (Ortí-Casañ et al., 2022). These findings are very promising to use this agonist as a potential treatment for Alzheimer's disease and finally break the endless search to an effective treatment.

The risk of developing Alzheimer's disease increases with age, and ageing is therefore one of the greatest risk factors for the development of neurodegenerative disorders (Wrigglesworth et al., 2021). However, older age does not necessarily cause Alzheimer's disease. For one thing, AD susceptibility may be determined by genetic factors (Lambert et al., 2013). On the one hand there is family history, the chance that an individual will develop Alzheimer's disease is greater if relatives also suffer from the disease. On the other hand, more modification to the DNA, such as mutations, occur during ageing (Holtman et al., 2015). Mutations in certain genes have been shown to increase the risk of AD (Lambert et al., 2013). However, the same mutations do not occur in every individual, causing individuals with a certain mutation to be more vulnerable to development of Alzheimer's disease. Furthermore, it has been demonstrated that ageing weakens the functioning of the immune system, and thereby microglia cells, which causes that these cells are less able to respond to amyloid beta and other pathological features in Alzheimer's disease (Hickman, Allison & El Khoury, 2008).

Recently, researchers have come up with new potential theories behind Alzheimer's disease. For a very long time, the idea of Alzheimer's as a disease in which the formation and accumulation of brain-damaging amyloid beta is seen as the cause for AD (Weaver, 2021). In the search for an appropriate treatment, other options were often ignored (Weaver, 2021). It has been proposed by Meier-Stephenson et al. (2022) that Alzheimer's disease is compatible with a brain-centric disorder of innate immunity. Their vision indicates that amyloid beta, is not an abnormal protein, but can be considered part of the brain's immune system, and that upon brain trauma or infection it plays a role in the innate immunity response (Meier-Stephenson et al., 2022). However, they found that Aβ has difficulties distinguishing between invading bacteria or host brain cells, due to similarities in their membranes, provoking an attack towards host cells, which leads to a chronic, self-perpetuating cycle of microglial activation, pro-inflammatory cytokine release, and tau aggregation, causing progressive loss of brain cell function and dementia (Meier-Stephenson et al., 2022). In addition, also other theories are being proposed, such as the involvement of mitochondria or metals (Mendes et al., 2022; Dusek et al., 2022).

From this essay, it can be concluded that with advanced age, microglial functioning becomes impaired, leading to decreased phagocytosis of aggregated proteins and sustained neuroinflammation, contributing to the development of Alzheimer's disease (*Figure 5*). Dysfunctionality of microglia cells, caused by e.g. age-related mutations, leads to an increased accumulation of harmful proteins, overactivation of microglia and the induction of a neuroinflammatory cascade, causing neuronal death and neurodegeneration. The neuroimmune system thus appears to play a significant role in the pathophysiology of Alzheimer's disease. In addition, neuroinflammation is a key player in the development of AD, which is caused by the secretion of proinflammatory cytokines, such as TNF. However, this also opens a therapeutic window. Stimulation of the TNF receptor 2 is found to mitigate the neuropathological features of AD, making it a possible therapy for Alzheimer's disease. This is

a hopeful conclusion, considering that the mysteries of AD are being unraveled, and that the future for patients of this tremendous disease and their caregivers is promising. It would however be useful to further study whether the mechanism of targeting TNFR2 also applies to humans. In addition, it might be useful for research to focus more on the (neuro)immune system in Alzheimer's disease pathophysiology, since it has been proposed that the disease could be associated with a disorder of the immune system within the brain (Meier-Stephenson et al., 2022). These new insights into Alzheimer's disease provide us with new potential therapeutic targets, leading to, hopefully, effective treatment approaches for this disease.

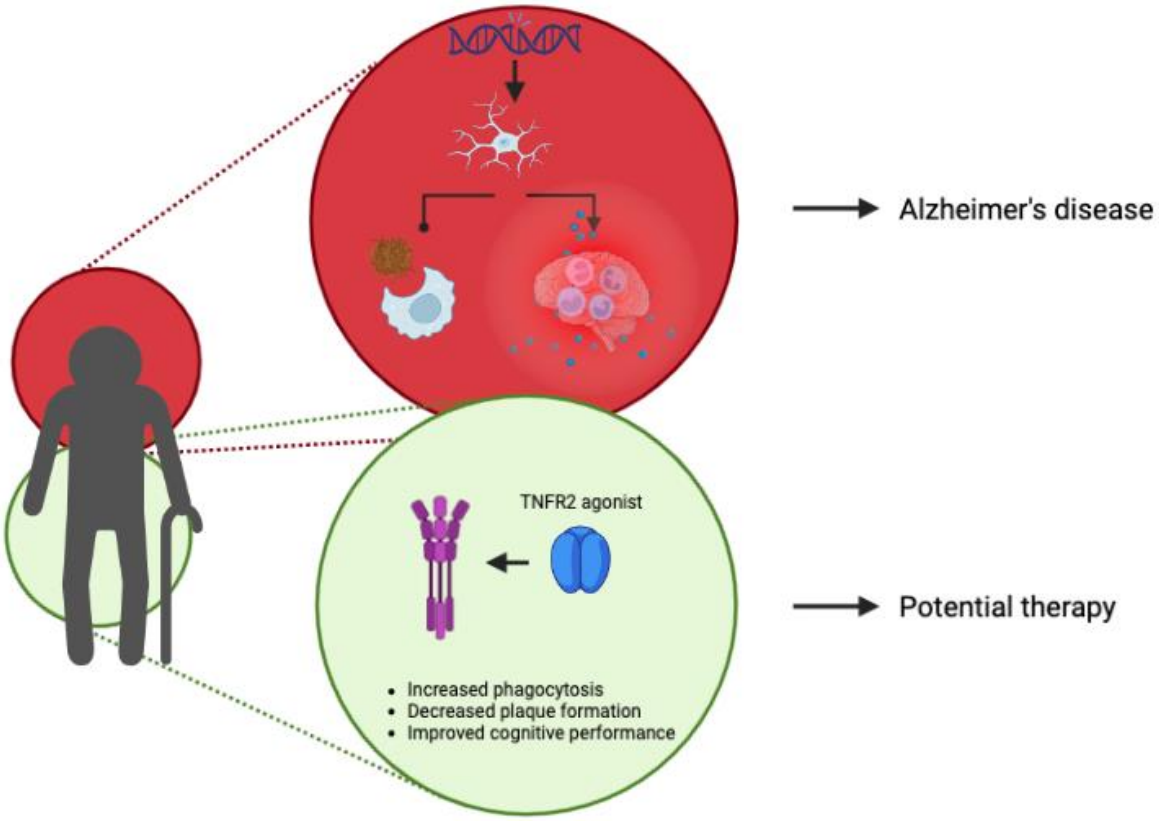

Figure 5. Graphical abstract: "The role of microglia cells in Alzheimer's disease, influence of ageing and potential therapeutic target". Ageing causes mutations in microglial genes, leading to impairment of microglial functioning. Dysfunctional microglia have decreased or impaired phagocytic performance, while pro-inflammatory response is sustained, characterized by overactivation of microglia and persistent secretion of pro-inflammatory cytokines, such as TNF. A TNFR2 agonist can be used to promote neuroprotective effect, making it a potential treatment for AD. Created with BioRender (2022).

References

Alzheimer's Association. (2016). 2016 Alzheimer's disease facts and figures. *Alzheimer's & Dementia*, *12*(4), 459-509.

Arendt, T., Stieler, J. T., & Holzer, M. (2016). Tau and tauopathies. *Brain research bulletin*, *126*, 238-292.

Beardsley, P. M., & Hauser, K. F. (2014). Glial modulators as potential treatments of psychostimulant abuse. *Advances in pharmacology*, *69*, 1-69.

Beers, D. R., Henkel, J. S., Zhao, W., Wang, J., & Appel, S. H. (2008). CD4+ T cells support glial neuroprotection, slow disease progression, and modify glial morphology in an animal model of inherited ALS. *Proceedings of the National Academy of Sciences*, *105*(40), 15558-15563.

Buerger, K., Ewers, M., Pirttilä, T., Zinkowski, R., Alafuzoff, I., Teipel, S. J., ... & Hampel, H. (2006). CSF phosphorylated tau protein correlates with neocortical neurofibrillary pathology in Alzheimer's disease. *Brain*, *129*(11), 3035-3041.

Butchart, J., Brook, L., Hopkins, V., Teeling, J., Püntener, U., Culliford, D., ... & Holmes, C. (2015). Etanercept in Alzheimer disease: A randomized, placebo-controlled, double-blind, phase 2 trial. *Neurology*, *84*(21), 2161-2168.

Butler, C. A., Popescu, A. S., Kitchener, E. J., Allendorf, D. H., Puigdellívol, M., & Brown, G. C. (2021). Microglial phagocytosis of neurons in neurodegeneration, and its regulation. *Journal of neurochemistry*, *158*(3), 621-639.

Choi, I., Zhang, Y., Seegobin, S. P., Pruvost, M., Wang, Q., Purtell, K., ... & Yue, Z. (2020). Microglia clear neuron-released α-synuclein via selective autophagy and prevent neurodegeneration. *Nature communications*, *11*(1), 1-14.

Cole, S. L., & Vassar, R. (2008). The role of amyloid precursor protein processing by BACE1, the β-secretase, in Alzheimer disease pathophysiology. *Journal of Biological Chemistry*, *283*(44), 29621-29625.

Dong, Y., Dekens, D. W., De Deyn, P. P., Naudé, P. J., & Eisel, U. L. (2015). Targeting of tumor necrosis factor alpha receptors as a therapeutic strategy for neurodegenerative disorders. *Antibodies*, *4*(4), 369-408.

Dong, Y., Fischer, R., Naudé, P. J., Maier, O., Nyakas, C., Duffey, M., ... & Eisel, U. L. (2016). Essential protective role of tumor necrosis factor receptor 2 in neurodegeneration. *Proceedings of the National Academy of Sciences*, *113*(43), 12304-12309.

Dusek, P., Hofer, T., Alexander, J., Roos, P. M., & Aaseth, J. O. (2022). Cerebral Iron Deposition in Neurodegeneration. *Biomolecules*, *12*(5), 714.

Fan, L., Mao, C., Hu, X., Zhang, S., Yang, Z., Hu, Z., ... & Xu, Y. (2020). New insights into the pathogenesis of Alzheimer's disease. *Frontiers in Neurology*, *10*, 1312.

Franco-Bocanegra, D. K., McAuley, C., Nicoll, J. A., & Boche, D. (2019). Molecular mechanisms of microglial motility: changes in ageing and Alzheimer's disease. *Cells*, *8*(6), 639.

Galloway, D. A., Phillips, A. E., Owen, D. R., & Moore, C. S. (2019). Phagocytosis in the brain: homeostasis and disease. *Frontiers in Immunology*, *10*, 790.

Gao, H. M., & Hong, J. S. (2008). Why neurodegenerative diseases are progressive: uncontrolled inflammation drives disease progression. *Trends in immunology*, *29*(8), 357-365.

Hefendehl, J. K., Neher, J. J., Sühs, R. B., Kohsaka, S., Skodras, A., & Jucker, M. (2014). Homeostatic and injury-induced microglia behavior in the aging brain. *Aging cell*, *13*(1), 60-69.

Heneka, M. T., Carson, M. J., El Khoury, J., Landreth, G. E., Brosseron, F., Feinstein, D. L., ... & Kummer, M. P. (2015). Neuroinflammation in Alzheimer's disease. *The Lancet Neurology*, *14*(4), 388-405.

Hickman, S. E., Allison, E. K., & El Khoury, J. (2008). Microglial dysfunction and defective β-amyloid clearance pathways in aging Alzheimer's disease mice. *Journal of Neuroscience*, *28*(33), 8354-8360.

Hickman, S. E., Kingery, N. D., Ohsumi, T. K., Borowsky, M. L., Wang, L. C., Means, T. K., & El Khoury, J. (2013). The microglial sensome revealed by direct RNA sequencing. *Nature neuroscience*, *16*(12), 1896-1905.

Hickman, S., Izzy, S., Sen, P., Morsett, L., & El Khoury, J. (2018). Microglia in neurodegeneration. *Nature neuroscience*, *21*(10), 1359-1369.

Holtman, I. R., Raj, D. D., Miller, J. A., Schaafsma, W., Yin, Z., Brouwer, N., ... & Eggen, B. J. (2015). Induction of a common microglia gene expression signature by aging and neurodegenerative conditions: a co-expression meta-analysis. *Acta neuropathologica communications*, *3*(1), 1-18.

Hong, S., Beja-Glasser, V. F., Nfonoyim, B. M., Frouin, A., Li, S., Ramakrishnan, S., ... & Stevens, B. (2016). Complement and microglia mediate early synapse loss in Alzheimer mouse models. *Science*, *352*(6286), 712-716.

Hsieh, C. L., Koike, M., Spusta, S. C., Niemi, E. C., Yenari, M., Nakamura, M. C., & Seaman, W. E. (2009). A role for TREM2 ligands in the phagocytosis of apoptotic neuronal cells by microglia. *Journal of neurochemistry*, *109*(4), 1144-1156.

Janelsins, M. C., Mastrangelo, M. A., Park, K. M., Sudol, K. L., Narrow, W. C., Oddo, S., ... & Bowers, W. J. (2008). Chronic neuron-specific tumor necrosis factor-alpha expression enhances the local inflammatory environment ultimately leading to neuronal death in 3xTg-AD mice. *The American journal of pathology*, *173*(6), 1768-1782.

Jekabsone, A., Mander, P. K., Tickler, A., Sharpe, M., & Brown, G. C. (2006). Fibrillar beta-amyloid peptide Aβ1–40 activates microglial proliferation via stimulating TNF-α release and H2O2 derived from NADPH oxidase: a cell culture study. *Journal of neuroinflammation*, *3*(1), 1-13.

Kadry, H., Noorani, B., & Cucullo, L. (2020). A blood–brain barrier overview on structure, function, impairment, and biomarkers of integrity. *Fluids and Barriers of the CNS*, *17*(1), 1-24.

Kim, Y. S., Jung, H. M., & Yoon, B. E. (2018). Exploring glia to better understand Alzheimer's disease. *Animal cells and systems*, *22*(4), 213-218.

Kiyota, T., Yamamoto, M., Xiong, H., Lambert, M. P., Klein, W. L., Gendelman, H. E., ... & Ikezu, T. (2009). CCL2 accelerates microglia-mediated Aβ oligomer formation and progression of neurocognitive dysfunction. *PloS one*, *4*(7), e6197.

Korin, B., Ben-Shaanan, T. L., Schiller, M., Dubovik, T., Azulay-Debby, H., Boshnak, N. T., ... & Rolls, A. (2017). High-dimensional, single-cell characterization of the brain's immune compartment. *Nature neuroscience*, *20*(9), 1300-1309.

Kummer, M. P., Hermes, M., Delekarte, A., Hammerschmidt, T., Kumar, S., Terwel, D., ... & Heneka, M. T. (2011). Nitration of tyrosine 10 critically enhances amyloid β aggregation and plaque formation. *Neuron*, *71*(5), 833-844.

Kwon, D. (2022). GUARDIANS OF THE BRAIN. *Nature*, *606*(7912), 22-24.

Lambert, J. C., Ibrahim-Verbaas, C. A., Harold, D., Naj, A. C., Sims, R., Bellenguez, C., ... & Nalls, M. A. (2013). Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. *Nature genetics*, *45*(12), 1452-1458.

Lee, D. C., Rizer, J., Selenica, M. L. B., Reid, P., Kraft, C., Johnson, A., ... & Morgan, D. (2010). LPS-induced inflammation exacerbates phospho-tau pathology in rTg4510 mice. *Journal of neuroinflammation*, *7*(1), 1-16.

Lui, H., Zhang, J., Makinson, S. R., Cahill, M. K., Kelley, K. W., Huang, H. Y., ... & Huang, E. J. (2016). Progranulin deficiency promotes circuit-specific synaptic pruning by microglia via complement activation. *Cell*, *165*(4), 921-935.

Lukiw, W. J. (2004). Gene expression profiling in fetal, aged, and Alzheimer hippocampus: a continuum of stress-related signaling. *Neurochemical research*, *29*(6), 1287-1297.

MacPherson, K. P., Sompol, P., Kannarkat, G. T., Chang, J., Sniffen, L., Wildner, M. E., ... & Tansey, M. G. (2017). Peripheral administration of the soluble TNF inhibitor XPro1595 modifies brain immune cell profiles, decreases beta-amyloid plaque load, and rescues impaired long-term potentiation in 5xFAD mice. *Neurobiology of disease*, *102*, 81-95.

Madav, Y., Wairkar, S., & Prabhakar, B. (2019). Recent therapeutic strategies targeting beta amyloid and tauopathies in Alzheimer's disease. *Brain research bulletin*, *146*, 171-184.

Martin, E., Boucher, C., Fontaine, B., & Delarasse, C. (2017). Distinct inflammatory phenotypes of microglia and monocyte-derived macrophages in Alzheimer's disease models: effects of aging and amyloid pathology. *Aging cell*, *16*(1), 27-38.

Mattson, M. P., & Chan, S. L. (2003). Neuronal and glial calcium signaling in Alzheimer's disease. *Cell calcium*, *34*(4-5), 385-397.

Mawuenyega, K. G., Sigurdson, W., Ovod, V., Munsell, L., Kasten, T., Morris, J. C., ... & Bateman, R. J. (2010). Decreased clearance of CNS β-amyloid in Alzheimer's disease. *Science*, *330*(6012), 1774-1774.

Meier-Stephenson, F. S., Meier-Stephenson, V. C., Carter, M. D., Meek, A. R., Wang, Y., Pan, L., ... & Weaver, D. F. (2022). Alzheimer's disease as an autoimmune disorder of innate immunity endogenously modulated by tryptophan metabolites. *Alzheimer's & Dementia: Translational Research & Clinical Interventions*, *8*(1), e12283.

Mendes, D., Peixoto, F., Oliveira, M. M., Andrade, P. B., & Videira, R. A. (2022). Mitochondria research and neurodegenerative diseases: on the track to understanding the biological world of high complexity. *Mitochondrion*.

Nakanishi, H., & Wu, Z. (2009). Microglia-aging: roles of microglial lysosome-and mitochondria-derived reactive oxygen species in brain aging. *Behavioural brain research*, *201*(1), 1-7.

Ortí-Casañ, N., Zuhorn, I. S., Naudé, P. J., De Deyn, P. P., van Schaik, P. E., Wajant, H., & Eisel, U. L. (2022). A TNF receptor 2 agonist ameliorates neuropathology and improves cognition in an Alzheimer's disease mouse model. *Proceedings of the National Academy of Sciences*, *119*(37), e2201137119.

Ott, C., König, J., Höhn, A., Jung, T., & Grune, T. (2016). Macroautophagy is impaired in old murine brain tissue as well as in senescent human fibroblasts. *Redox Biology*, *10*, 266-273.

Patel, N. S., Paris, D., Mathura, V., Quadros, A. N., Crawford, F. C., & Mullan, M. J. (2005). Inflammatory cytokine levels correlate with amyloid load in transgenic mouse models of Alzheimer's disease. *Journal of neuroinflammation*, *2*(1), 1-10.

Prasanna, P., Rathee, S., Rahul, V., Mandal, D., Chandra Goud, M. S., Yadav, P., ... & Jha, S. K. (2021). Microfluidic platforms to unravel mysteries of Alzheimer's Disease: How far have we come?. *Life*, *11*(10), 1022.

Prince, M., Bryce, R., Albanese, E., Wimo, A., Ribeiro, W., & Ferri, C. P. (2013). The global prevalence of dementia: a systematic review and metaanalysis. *Alzheimer's & dementia*, *9*(1), 63-75.

Przedborski, S., Vila, M., & Jackson-Lewis, V. (2003). Series Introduction: Neurodegeneration: What is it and where are we?. *The Journal of clinical investigation*, *111*(1), 3-10.

Ramesh, G., MacLean, A. G., & Philipp, M. T. (2013). Cytokines and chemokines at the crossroads of neuroinflammation, neurodegeneration, and neuropathic pain. *Mediators of inflammation*, *2013*.

Ransohoff, R. M., & El Khoury, J. (2016). Microglia in health and disease. *Cold Spring Harbor perspectives in biology*, *8*(1), a020560.

Réu, P., Khosravi, A., Bernard, S., Mold, J. E., Salehpour, M., Alkass, K., ... & Frisén, J. (2017). The lifespan and turnover of microglia in the human brain. *Cell reports*, *20*(4), 779-784.

Roeters, S. J., Iyer, A., Pletikapić, G., Kogan, V., Subramaniam, V., & Woutersen, S. (2017). Evidence for intramolecular antiparallel beta-sheet structure in alpha-synuclein fibrils from a combination of two-dimensional infrared spectroscopy and atomic force microscopy. *Scientific reports*, *7*(1), 1-11.

Sala, G., Galimberti, G., Canevari, C., Raggi, M. E., Isella, V., Facheris, M., ... & Ferrarese, C. (2003). Peripheral cytokine release in Alzheimer patients: correlation with disease severity. *Neurobiology of aging*, *24*(7), 909-914.

Sowell, E. R., Peterson, B. S., Thompson, P. M., Welcome, S. E., Henkenius, A. L., & Toga, A. W. (2003). Mapping cortical change across the human life span. *Nature neuroscience*, *6*(3), 309-315.

Stewart, C. R., Stuart, L. M., Wilkinson, K., Van Gils, J. M., Deng, J., Halle, A., ... & Moore, K. J. (2010). CD36 ligands promote sterile inflammation through assembly of a Toll-like receptor 4 and 6 heterodimer. *Nature immunology*, *11*(2), 155-161.

Streit, W. J., Mrak, R. E., & Griffin, W. S. T. (2004). Microglia and neuroinflammation: a pathological perspective. *Journal of neuroinflammation*, *1*(1), 1-4.

Swardfager, W., Lanctôt, K., Rothenburg, L., Wong, A., Cappell, J., & Herrmann, N. (2010). A meta-analysis of cytokines in Alzheimer's disease. *Biological psychiatry*, *68*(10), 930-941.

Talbot, S., Foster, S. L., & Woolf, C. J. (2016). Neuroimmunity: physiology and pathology. *Annual review of immunology*, *34*, 421-447.

Tarkowski, E., Andreasen, N., Tarkowski, A., & Blennow, K. (2003). Intrathecal inflammation precedes development of Alzheimer's disease. *Journal of Neurology, Neurosurgery & Psychiatry*, *74*(9), 1200-1205.

Tay, T. L., Mai, D., Dautzenberg, J., Fernandez-Klett, F., Lin, G., Datta, M., ... & Prinz, M. (2017). A new fate mapping system reveals context-dependent random or clonal expansion of microglia. *Nature neuroscience*, *20*(6), 793-803.

Torres-Platas, S. G., Comeau, S., Rachalski, A., Bo, G. D., Cruceanu, C., Turecki, G., ... & Mechawar, N. (2014). Morphometric characterization of microglial phenotypes in human cerebral cortex. *Journal of neuroinflammation*, *11*(1), 1-14.

Vitlic, A., Lord, J. M., & Phillips, A. C. (2014). Stress, ageing and their influence on functional, cellular and molecular aspects of the immune system. *Age*, *36*(3), 1169-1185.

Vodovotz, Y., Lucia, M. S., Flanders, K. C., Chesler, L., Xie, Q. W., Smith, T. W., ... & Sporn, M. B. (1996). Inducible nitric oxide synthase in tangle-bearing neurons of patients with Alzheimer's disease. *The Journal of experimental medicine*, *184*(4), 1425-1433.

Von Bernhardi, R., Eugenín-von Bernhardi, L., & Eugenín, J. (2015). Microglial cell dysregulation in brain aging and neurodegeneration. *Frontiers in aging neuroscience*, *7*, 124.

Wang, W. Y., Tan, M. S., Yu, J. T., & Tan, L. (2015). Role of pro-inflammatory cytokines released from microglia in Alzheimer's disease. *Annals of translational medicine*, *3*(10).

Weaver, D. F. (2021). β-Amyloid is an immunopeptide and Alzheimer's is an autoimmune disease. *Current Alzheimer Research*, *18*(11), 849-857.

Woodcock, E. A., Hillmer, A. T., Mason, G. F., & Cosgrove, K. P. (2019). Imaging biomarkers of the neuroimmune system among substance use disorders: A systematic review. *Complex Psychiatry*, *5*(3), 125-146.

Wrigglesworth, J., Ward, P., Harding, I. H., Nilaweera, D., Wu, Z., Woods, R. L., & Ryan, J. (2021). Factors associated with brain ageing-a systematic review. *BMC neurology*, *21*(1), 1-23.

Wyss-Coray, T. (2016). Ageing, neurodegeneration and brain rejuvenation. *Nature*, *539*(7628), 180-186.

Yamamoto, M., Kiyota, T., Horiba, M., Buescher, J. L., Walsh, S. M., Gendelman, H. E., & Ikezu, T. (2007). Interferon-γ and tumor necrosis factor-α regulate amyloid-β plaque deposition and β-secretase expression in Swedish mutant APP transgenic mice. *The American journal of pathology*, *170*(2), 680-692.

Ye, S. M., & Johnson, R. W. (2001). An age-related decline in interleukin-10 may contribute to the increased expression of interleukin-6 in brain of aged mice. *Neuroimmunomodulation*, *9*(4), 183-192.

Yoshiyama, Y., Higuchi, M., Zhang, B., Huang, S. M., Iwata, N., Saido, T. C., ... & Lee, V. M. Y. (2007). Synapse loss and microglial activation precede tangles in a P301S tauopathy mouse model. *Neuron*, *53*(3), 337-351.

Zhang, J. M., & An, J. (2007). Cytokines, inflammation and pain. *International anesthesiology clinics*, *45*(2), 27.