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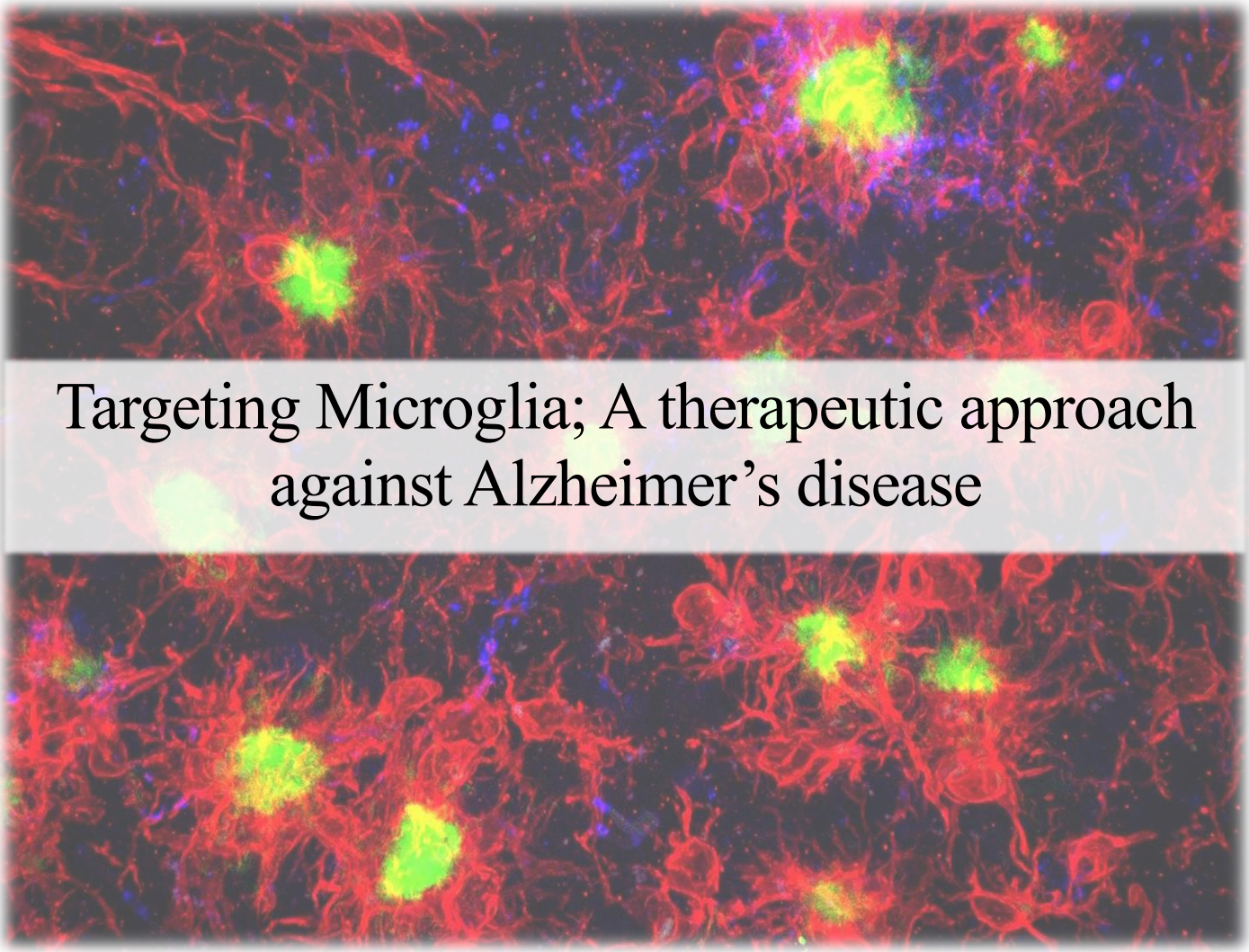

Targeting Microglia; A therapeutic approach against Alzheimer's disease

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Illustration: Aβ plaques (green) surrounded by microglia (red) (Spangenberg et al., 2019)

Abstract

Alzheimer's disease (AD) is responsible for many cases of dementia and is characterized by neuroinflammation. In AD this neuroinflammation is accompanied by increased microglial activation. Since AD treatments are still unavailable, the overall aim of this literature study was to analyze if microglia can be used as a therapeutic target against AD.

Within AD, the formation of amyloid β (Aβ) and neurofibrillary tangles (NFTs) leads to the chronic activation of microglia and the transition into the pro-inflammatory M1 phenotype. Activation of these microglia results in inflammation, leading to neuronal damage. During the progression of AD the anti-inflammatory M2 microglia, which dampen the inflammation response and induce tissue repair and healing, become dysfunctional and are replaced by pro-inflammatory M1 microglia, creating further damage accompanied by cognitive impairments.

Upon age, microglial changes occur as a result of repeated exposure to immune challenging stimuli, leading to the development of a primed phenotype. Activation of this highly sensitive microglia leads to an uncontrolled pro-inflammatory immune response with a decreased capacity of chemotaxis and phagocytosis.

For drug designing, H3k27me3 demethylase Jumonij domain could be used to increase M2 microglia by activation of M2 polarization and inhibition of M1 polarized microglia, preventing further neuronal damage and stimulating tissue repair. Prevention of pro-inflammatory cytokine secretion could be done by targeting microglia specific toll like receptor 4 (TLR 4) and modulation of tumor necrosis factor α receptors (TNFRs) may stimulate neuroprotection and cell survival. Upon age, inhibition of interferon regulatory factor-7 (IRF-7) may prevent development of primed microglia. Next, misfolding Aβ could be corrected by using molecular chaperones to eliminate the stimuli that creates the immune response by microglia.

Finally, it is important to develop microglia related drug that first focus on anti-inflammation to restore damages tissue, followed by maintaining the balance between pro- and anti-inflammation.

Abbreviations

acetylcholine	ACh
acetylcholinesterase	AChE
Alzheimer's Disease	AD
α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid	AMPA
arginase-1	ARG-1
amyloid-β	Aβ
amyloid precursor protein	APP
blood-brain barrier	BBB
butyrylcholinesterase	BChE
cellular inhibitors of apoptosis proteins	cIAPs
central nervous system	CNS
cholinesterase	ChE
cluster of differentiation marker 86	CD86
colony-stimulating factor-1 receptor	CSF-1R
cyclogenase-2	COX-2
cytokine signaling 3	SOCS3
danger-associated molecular patterns	DAMPs
disease-associated microglia	DAM
extracellular micro-vesicles	MVs
familial AD	FAD
glial fibrillary acidic protein	GFAP
granulocyte-macrophage colony-stimulating factor	GM-CSF
including nucleotide-binding oligomerization domain-like	NOD-like
induced nitric oxide species	iNOS
inflammatory zone protein	Fizz1
insulin-like growth factor-1	IGF-1
interferon-γ	IFN- γ
interferon regulatory factor-7	IRF-7
interleukin-6	IL-6
c-jun N-terminal kinase	JNK
lipopolysaccharide	LPS
mitogen activated protein kinase	MAPK
memantine	MEM
microbe-associated molecular patterns	MAMPs
milk fat globule EGF8	MFG-EGF8
myeloid differentiation primary response protein 88	MyD88
nerve growth factor	NGF
neurofibrillary tangles	NFTs
nitric oxide	NO
N-methyl-D-aspartate	NMDA
nuclear factor kappa B	NF-κB
pathogen-associated molecular patterns	PAMPs
peripheral nervous system	PNS
peroxisome proliferator-activated receptor	PPAR
phosphatidyl inositol 3-kinase	PI3K
reactive oxygen species	ROS
reactive oxygen species and nitrogen species	RONS
Rho-associated protein kinase	ROCK
scavenger receptor A1	SR-A1
sporadic AD	SAD
diabetes type 2	T2D
TIR domain-containing adaptor inducing IFN-β	TRIF

toll-like receptor 4	TLR4
TNF receptor-associated factor	TRAF
transforming growth factor β	TGF-β
tumor necrosis factor α	TNF-α
tumor necrosis factor α receptor	TNFR
World Health Organization	WHO

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Part I: Introduction

Worldwide, dementia is a major cause of disability, dependence and death. It is estimated that 44 million people are diagnosed with this neurological disorder. Of all cases, Alzheimer's Disease (AD) is responsible for 50%-70% of dementias (Lane, Hardy, & Schott, 2018, 2018).

AD, a heterogenous neurodegenerative disease, can be divided into sporadic AD (SAD) and familial AD (FAD). SAD, responsible for 95% of the AD related dementia cases, has no hereditary link and can develop in response to environmental factors or genetic factors, such as polymorphisms or mutations in astrocytic and microglial genes, and emotional and mental stress. The other 5% of AD dementia is FAD, generated by mutations in presenilin 1/2 and APP that induce formation of Aβ peptides (Kaur, Sharma, & Deshmukh, 2019). AD is characterized by cortical dementia with loss of memory accompanied by dyspraxia, dysphasia and agnosia. In early stages of the disease memory impairments typically involve the episodic memory. Loss of semantic memory (visual and verbal memory) develops in later stages of the disease and the short-term memory remains until the last stage of AD (Rossor, Fox, Freeborough, & Harvey, 1996).

This neurological pathogenesis is mostly related to a homeostatic disbalance of the central nervous system (CNS), as seen in many other neurological disorders. The CNS, part of the nervous system, consist of the brain and the spinal cord. The branches of nerves from the spinal cord that spread through the body are called the peripheral nervous system (PNS). Within the CNS, homeostasis depends among others on the regulation of the innate immunity (Yang & Zhou, 2019). In healthy individuals, an inflammatory stimuli, such as toxic metabolites, mechanical damage, cell damage, cytokines, or pro-inflammatory mediators that crossed the blood brain barrier (BBB), lead to an innate immune response in the CNS (Subramaniam & Federoff, 2017; Yang & Zhou, 2019). Immune cells, such as microglia and astrocytes, secrete pro-inflammatory cytokines and chemokines, resulting in neuroinflammation. Upon the secretion of these molecules, immune cells restrain further infection and pathogens will be eliminated together with mis-folded proteins and cellular debris (Yang & Zhou, 2019). A side effect of these pro-inflammatory cytokines is that, in high concentrations, they can exert a neurotoxic effect (Subramaniam & Federoff, 2017). After removal of the pathogen, other immune cells secrete anti-inflammatory cytokines to prevent further damage and stimulate healing of brain tissue. A disrupted balance between immune cells that secrete pro- and anti-inflammatory cytokines often leads to an ongoing inflammation of the brain and the development of a neurological disorder (Yang & Zhou, 2019).

In addition to most neurodegenerative diseases, AD is also characterized by these neuroinflammatory changes. Within AD the formation of, among other things, NFTs and Aβ plaques leads to the continued stimulation of the innate immune response (Kuchibhotla et al., 2008; Kumar, Abbas, Fausto, & Aster, 2014; Lane, Hardy, & Schott, 2018). This activation is accompanied by increased microglial activation (Lynch, 2014). Microglia are the primary immune cells of the brain and are important for, among other things, immunological protection and maintaining homeostasis (Nayak, Roth, & McGavern, 2014; Takeuchi, 2013). It is suggested that the increased microglial activation plays an important role during the neuroinflammatory pathology of AD (Moore, Taylor, & Crack, 2019).

Since AD treatments are still unavailable, the overall aim of this literature study is to analyze if microglia can be used as a therapeutic target against AD. In order to answer this question, the focus will lie on the role of microglia in the brain and its relation with AD. Since AD is mostly diagnosed in a later life stage, ageing is also included.

Part II: Microglia and the immune response

Maintaining homeostasis in the brain is essential for normal brain function. It is important to have a balance between the pro-inflammatory and an anti-inflammatory immune response. In case of an antigen, an inflammatory response is necessary for elimination, but negative feedback is essential to inhibit this response after the removal of the antigen, to stimulate healing and protection against further damage (Lynch, 2014; Muffat et al., 2016; Subramaniam & Federoff, 2017). Within the CNS, this process is carried out by glial cells, including microglia, astrocytes and oligodendrocytes (Yang & Zhou, 2019).

II.I. Development and function of microglia

Microglia are highly specialized macrophages of the CNS located in the brain. Here they form the primary immune cells, which are enclosed by the BBB (Lynch, 2014; Muffat et al., 2016; Subramaniam & Federoff, 2017). They have capacity to self-renew and are highly reactive due to their abundance of membrane receptors, and their ability to sample their environment in resting conditions (Lynch, 2014; West, Viengkhou, Campbell, & Hofer, 2019). During early development they migrate from the yolk sac as erythromyeloid precursors/primitive macrophages, prior to further hematopoiesis in the periphery, regions located away from the core regions. They originate from the mesoderm followed by primitive myelopoiesis, where they develop into non-lymphoid leukocytes. After microglial development they have a lifelong residence in a neuro-glial environment. In absence of injury their phenotype is formed during transition stages of nervous system development (neurogenesis, gliogenesis, synaptogenesis and maturation) into a post-mitotic aging tissue, where microglia are not able to undergo mitosis (Muffat et al., 2016).

Microglial functions adapt by interaction with astrocytes, migrating T-cells, neurons, the BBB and blood vessels (Subramaniam & Federoff, 2017). Besides maintaining homeostasis, they are responsible for; antigen presentation, disposal of debris, protection against non-self-antigens and developmental support and protection of neuronal circuit. Besides, they also play a role in inducing neuronal survival, programmed cell death and removal of cellular debris (Nayak et al., 2014).

II.II Neuronal survival and programmed cell death

Neuronal survival and programmed cell death are induced by the secretion of tropic factors, cytokines and chemokines. The release of tropic factors supports the formation of neuronal networks and their survival (Nayak et al., 2014). An example is the release of insulin-like growth factor (IGF-1), that supports survival of layer V cortical neurons during development (Nayak et al., 2014; Ueno et al., 2013). Beside IGF-1 other secreted factors, such as hepatocyte growth factor, platelet-derived growth factor, basic fibroblast growth factor, epidermal growth factor, brain-derived neurotrophic factor and nerve growth factor (NGF), play an important role during neuronal development, function and maintenance during life (Nayak et al., 2014).

In addition to neuronal survival, neuronal cell death is induced by the release of, among others, cathepsin B, glutamate, tumor necrosis factor α (TNF-α) and/or reactive oxygen species and nitrogen species (RONS) (Fricker, Tolkovsky, Borutaite, Coleman, & Brown, 2018). This programmed cell death functions as a homeostatic mechanism, during developmental processes and ageing, maintaining cell populations. It also functions as defense mechanism against damaged cells, as a result of noxious agents or a disease (Elmore, 2007). During this process DNA fragmentation occurs, followed by degradation of nuclear proteins and the cytoskeleton, protein cross-linking and the formation of apoptotic bodies. These bodies then are detected by phagocytic cells by receptor-ligand interaction and are removed by phagocytosis without triggering an inflammatory response (Elmore, 2007). An example of programmed cell death was observed during the development program of the chick eye, where retinal nerve cell apoptosis was induced by NGF (Frade & Barde, 1998; Nayak et al., 2014). After apoptosis of the cell, the remaining cellular debris was removed by microglial phagocytosis without triggering an inflammatory response. Within the CNS this programmed cell death is important, because of the limited ability of adult neurons to proliferate. Furthermore, programmed cell death is essential for removal of faulty neurons as a result of defective differentiation, and neurons with dysfunctional neuronal circuits

(Fricker et al., 2018). Moreover, programmed cell death needs to be strictly regulated, because too much or less programmed cell death may result in disorders, such as developmental defects, neurodegeneration, autoimmune diseases, or cancer (Elmore, 2007). Within the CNS inducing of neuronal survival or programmed cell dead is established by communication between microglia and its microenvironment (Fricker et al., 2018).

II.III Microglia activation and transition

In resting conditions microglia are multiprocessing cells, with constantly motile processes, whereby there are able to continuously scanning the CNS microenvironment (Lynch, 2014). When detecting changes, based on damage, inflammatory factors and/or infection, microglia become activated. This activation is accompanied by transformation into an amoeboid morphology and the differentiation into polarization state M1 or M2 (Lynch, 2014; Nayak et al., 2014; Subramaniam & Federoff, 2017)

Transition polarization state

The transition into the M1 and the M2 polarization state is a balance between the detection and elimination of an antigen, and restoration of the immune response and tissue repair. It is suggested that during this process, microglia may switch from M1 into M2, restoring homeostasis and minimize tissue damage (Orihuela, McPherson, & Harry, 2016; Subramaniam & Federoff, 2017). Previous studies indicate that histone H3k27me3 demethylase Jumonij domain plays an important role for M2 polarization and inhibition of M1 phenotypic microglia (Orihuela et al., 2016; Subramaniam & Federoff, 2017). According to *in vitro* studies M1 phenotypic human monocytes may mature into a M2 phenotype after polarization upon variations in culture condition. Although a shift from M2 into the M1 phenotype may happen, it is regarded rare that M1 state microglia would switch into the M2 state (Orihuela et al., 2016). Despite this, microglia may switch upon stimulation with glatiramer acetate, IL-10, PPAR-γ agonists and beta interferons (Subramaniam & Federoff, 2017). Beside the M1 and M2, different subpopulations may express a mixture of M1/M2 phenotypes (Orihuela et al., 2016; Subramaniam & Federoff, 2017). The balance between the M1 and M2 polarization state is important for maintaining a healthy brain function. Disruptions of this balance may lead to development of neurodegenerative diseases (Subramaniam & Federoff, 2017).

M1 polarization state

Detection of an antigen leads to classical activation of microglia and transformation into the M1 polarization state and is characterized with a pro-inflammatory phenotype. This types of activation is related to the presence of microbe-associated molecular pattern (MAMP), lipopolysaccharide (LPS), interferon-γ (IFN- γ), or granulocyte-macrophage colony-stimulating factor (GM-CSF) (Orihuela et al., 2016; Subramaniam & Federoff, 2017).

Classical activation, induced by these stimuli, is obtained after activation of different pathways, and will be discussed below. First, binding of IFN-γ to its receptors leads to the activation of JAK/STAT signaling pathway, which promotes expression of M1 associated genes and the release of chemokines and cytokines. Second, LPS binds to toll-like receptor 4 (TLR4) and activates through myeloid differentiation primary response protein 88 (MyD88), TIR domain-containing adaptor inducing IFN-β (TRIF) and transcription factors (signal transducer and activator of transcription 5 (STAT5), nuclear factor kappa B (NF-κB) and interferon regulatory factor 1 (IFR-1)) the transition into the M1 polarization state (Subramaniam & Federoff, 2017). Unlike IFN-γ and LPS, the activation upon GM-CSF results in a state characterized by M1 as well as M2 phenotypes.

After M1 polarizations, microglia are able to eliminate the detected antigen by activation of T-cells, triggering an adaptive immune response. This activation is accompanied by the secretion of pro-inflammatory cytokines such as; TNF-α, nitric oxide (NO), interleukin-6 (IL-6), IL-12, IL-17, IL-18, IL-23, IL-1α, IL-1β, and chemokine CCL2. Microglia also present M1 phenotypic markers after polarization; cyclogenase-2 (COX-2), induced nitric oxide species (iNOS), cluster of differentiation marker 86 (CD86) and major histamine complex II (MHC- II), and species, such as prostaglandin E2,

reactive oxygen species (ROS) and reactive nitrogen species (Orihuela et al., 2016; Subramaniam & Federoff, 2017).

M2 polarization state

Upon elimination of the antigen by M1 phenotype microglia, microglia are activated and transform into the M2 phenotype to dampen the immune response. Microglia with a M2 phenotype are anti-inflammatory, have a neuroprotective function and are characterized with a larger cell body compared to M1 phenotypic microglia. They are involved in dampening inflammation, immunoregulation and injury and repair (Subramaniam & Federoff, 2017). The transformation into the M2 polarization state occurs through alternative activation, induced by IL-4, IL-10 and IgG. M2 phenotypic cells can be divided into three groups; M2a, M2b and M2c.

Microglia with the M2a state are induced by IL-4 and IL-13. IL-4 binds to multiple receptors inducing Jak1/Jak3 leading to activation of STAT6 and transcription of M2 a related genes and markers, such as CD206, scavenger receptor class A1 (SR-A1), SR-B1, and suppressor of cytokine signaling 3 (SOCS3), arginase-1 (ARG-1), inflammatory zone protein (Fizz1) and Ym1. The M2a state also leads to the secretion of IL-10, peroxisome proliferator-activated receptor (PPAR) and extracellular matrix proteins. The presence of these markers and the secretion of cytokines stimulates tissue repair and phagocytosis (Orihuela et al., 2016; Subramaniam & Federoff, 2017).

M2b phenotypic microglia are responsible for the recruitment of regulatory T cells and are characterized by fusion of TLRs with Fcγ-receptors after activation of TLRs. Fused TLR and Fcγ receptors creates a binding site for IgG derived from B cells. After activation by IgG, M2b state microglia secrete IL-6, IL-10, IL-1β, TNF-α, and MHCII, CD86 on the cell surface (Subramaniam & Federoff, 2017), resulting in the recruitment of regulatory T cells. Finally, microglia stimulated with glucocorticoid hormone, IL-10 and TGF-β are driven to the M2c phenotype. In this case, IL-10 induces IL-10R1 and IL-10R2 resulting in the activation of the JAK/STAT signaling pathway. Activation of JAK1 leads to the translocation of STAT3 and the migration into the nucleus. In the nucleus, STAT3 inhibits a large group of pro-inflammatory cytokines and markers (IL-10, TGF-β, CD163) associated with M1 activated microglia (Subramaniam & Federoff, 2017).

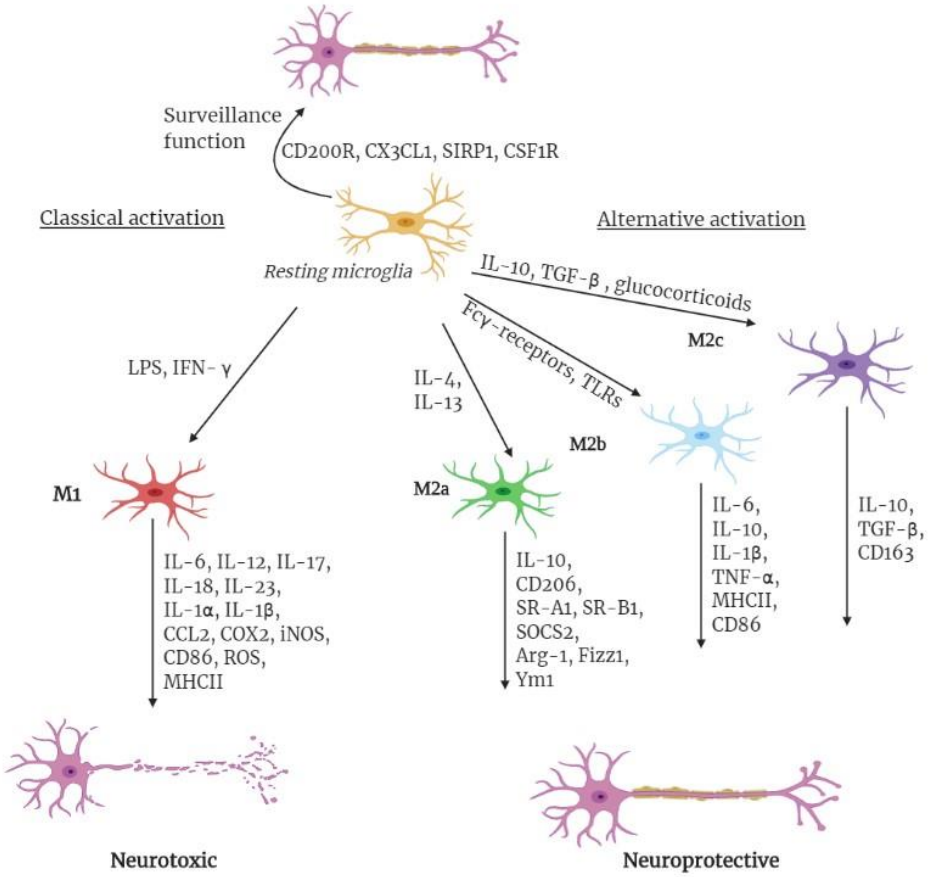

Figure 2 Microglia activation
Schematic overview polarization states and function of activated microglia. Resting microglia perform surveillance of the brain by secretion of factors, such as CD200R, CXCL1, CD172 (SIRP1) and CSF1R. Classical activation stimulated by LPS and/or IFN-γ ensures M1-state microglia leading to neurotoxicity by the release of pro-inflammatory factors. Alternative activation ensues M2-state microglia, that can be subdivided into M2a, M2b, and M2c depending on the stimuli. M2-state microglia acts neuroprotective by the release of a wide range of anti-inflammatory factors.
ARG1, arginase 1; CD, cluster of differentiation; CCL2, chemokine (C-C motif) ligand 2; CSF1R, colony stimulating factor 1 receptor; CXCL, chemokine (C-X-C motif) ligand; COX2, cyclo-oxygenase protein 2; Fizz1, found in inflammatory zone; IFN-γ, interferon γ; IL, interleukin; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide; MHC-II, major histocompatibility complex II; ROS, reactive oxygen species; SR, scavenger receptor; SIRP-1, signal regulatory protein CD172 1; SOC-3, suppressor of cytokine signaling-3 TGF-β, TLR, toll-like receptor; transforming growth factor-β; TNF-α, , tumor necrosis factor-α; Ym1, chitinase-like protein (Subramaniam & Federoff, 2017).
Created with BioRender (Biorender, 2020).

Part III: The role of microglia in Alzheimer's Disease

One of the neurodegenerative diseases accompanied by a disbalance between M1 and M2 microglia is AD. In AD, and mostly all neurodegenerative diseases, microglia changes result in disruption of its normal function and the capacity to recover, leading to brain damage during its pathogenesis (Rossor et al., 1996; Shen, Bao, & Wang, 2018).

III.I Development of AD

Within AD, the formation of NFTs and Aβ plaques contributes to the imbalance between M1 and M2 microglial phenotypes and macroscopic atrophy as a result of neuronal and synaptic loss (Lane, Hardy, & Schott, 2018).

Aβ plaques are extracellular aggregates of abnormal folded Aβ peptides with 40 (Aβ40) or 42 (Aβ42) amino acids and are by-products of amyloid precursor protein (APP) metabolism, located in the neuropil (figure 3). APP functions as receptor for different ligands such as prion protein (PrPc). The Aβ part of APP is located from the extracellular region into the transmembrane domain. APP contains three secretase sites; α, β and γ. APP metabolism starts with cleavage at the α- or β-site, followed by cleavage of the γ-site. Cleavage of the α- site in combination with the γ-site results in the formation of a soluble fragment (non-amyloid pathway) without formation of Aβ. Cleavage within the β- and γ-site leads to the formation of a Aβ40 or Aβ42 monomer (Kumar et al., 2014). These Aβ monomers are sensitive to aggregation. When Aβ aggregates they first form Aβ oligomers, then amyloid fibrils and finally Aβ plaques. Aβ oligomers are toxic and may cause neuronal damage as a result of synaptic dysfunction, cell death and other pathways. They also stimulate kinase activity leading to tau hyperphosphorylation followed by formation of tau aggregation resulting in NFTs (Kumar et al., 2014).

Moreover, Aβ plaques disturbs the calcium homeostasis, leading to an inflammatory reaction and may induce NFT formation (Kuchibhotla et al., 2008; Kumar et al., 2014; Lane, Hardy, & Schott, 2018). NFTs are intracellular aggregations of hyperphosphorylated tau, a microtubule binding protein present in axons (Kumar et al., 2014; Lane, Hardy, & Schott, 2018). After hyperphosphorylation, tau is not able to bind to microtubules, leading to a decrease in neurite outgrowth, axonal transport and synaptic plasticity (Kumar et al., 2014; Morris, Maeda, Vossel, & Mucke, 2011). Formation of these NFTs and Aβ plaques leads to neuroinflammation, playing an important role during the ADs pathophysiology (Kaur et al., 2019).

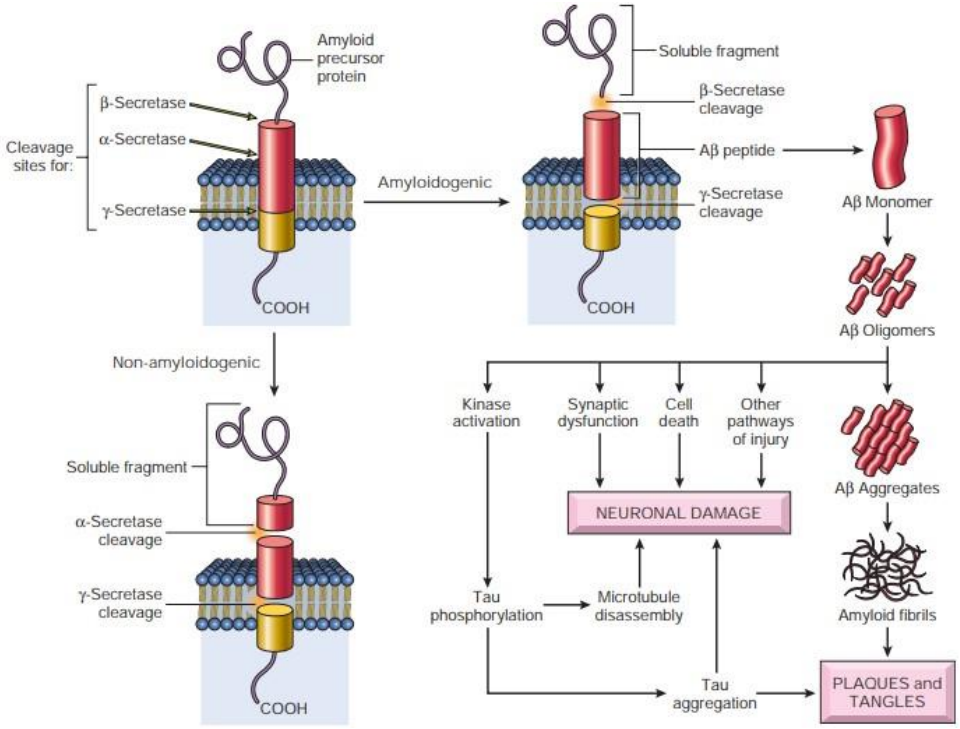

Figure 3 Formation of Aβ plaques and NFTs in AD (Kumar et al., 2014)
Cleavage of amyloid precursor protein by α-, and γ- secretase leads to formation of a soluble harmless fragment (non-amyloidogenic). Formation of Aβ peptides by the cleavage of α-secretase in combination with β-secretase. Aβ peptides form neural toxic aggregated and contribute to the formation of plaques and tangles, a characteristic of AD (Kumar et al., 2014).

III.II Neuroinflammation and Microglia

The inflammation in AD is characterized by chronic activated gliosis enclosing NFTs and Aβ plaques, leading to secretion of pro-inflammatory cytokines, inducing neurodegenerative processes (Kaur et al., 2019). Induction of these neuroinflammatory processes was correlated with AD progression and

cognitive impairments by initiating neurotoxicity and neuronal damage (Kaur et al., 2019; Webers, Heneka, & Gleeson, 2020).

The role of microglia

Detection of NFTs and Aβ plaques leads to microglial activation, inducing pro-inflammatory signaling pathways and reactive gliosis. Despite the fact that the exact function of microglia in AD remains unclear, evidence suggest that microglial activation may in some cases protecting against, and in other cases promote AD (El Khoury & Luster, 2008; Moore et al., 2019; Webers et al., 2020).

In AD, the presence of Aβ peptides leads to the acute activation of microglia (figure 4). Acute activated microglia secrete cytokines stimulating the clearance of Aβ peptides by phagocytosis. After removal of the peptides the immune response is inhibited by negative feedback. When Aβ peptides are produced continuously, as seen in AD, microglia become chronic activated, resulting in sustained secretion of inflammatory cytokines leading to further aggregation of Aβ peptides, failure in Aβ clearance and proliferation of microglia. Ultimately, this chronic activation leads to neurodegeneration and stimulation of AD's pathology (Sarlus & Heneka, 2017).

The activation of microglia is induced by sensing of Aβ peptides through receptors, including nucleotide-binding oligomerization domain-like (NOD-like) receptor and TLRs. TLRs are well studied, and recognize pathogen-associated molecular patterns (PAMPs) and DAMPs. Especially TLR2 and TLR4 are essential for Aβ recognition (Moore et al., 2019). Activation of both TLRs results in secretion of pro-inflammatory cytokines by M1-polarized microglia. The resulting immune responses ensure removal of the damaging stimuli. M2-polarized microglia secrete anti-inflammatory cytokines, such as TNF-α, IL-4 and IL-10, to protect the brain (Shen et al., 2018).

In addition, microglial activation also leads to clearance of Aβ, depending on the soluble oligomeric or fibrillar state of Aβ. Fibrillar Aβ is recognized by a complex of cell surface receptors, including class A/B scavenger receptor, CD47, CD36, CD14, TLR9, TLR6, TLR4, TLR2 and α6β1 integrin. Within this complex, activation of TLRs increases clearance of fibrillar Aβ by phagocytosis. Ablation of TLR2 and TLR4 leads to disturbed recognition of fibrillar Aβ by the complex, resulting in failure of phagocytosis (Moore et al., 2019). In addition to fibrillar Aβ, soluble Aβ is cleared by fluid phase micropinocytosis. During this process soluble Aβ is enclosed by the cellular membrane to form intracellular vesicles. Moreover, it has been shown that excessive deposition of Aβ and NFTs leads to a reduced ability of clearance by activated microglia (Moore et al., 2019). It is suggested that increased levels of, among others, TNF-α, IL-1 and IL-6 results in this failure (Orti-Casan et al., 2019; Shen et al., 2018).

Furthermore, activated microglia also release extracellular micro-vesicles (MVs) by endocytosis. Previous studies showed that MVs function as strong modulators of immunity and inflammation. The secretion of MVs resulted in the stimulation of synaptic activity *in vitro* and *in vivo*. Within AD individuals, MVs levels were elevated and showed a toxic behavior towards neurons and a stimulation of Aβ formation (Moore et al., 2019).

It has also been observed that these activated microglia are involved in neuronal cell death by phagoptosis. Phagoptosis occurs upon the presentation of signal molecules, such as calreticulin, phosphatidylserine, de-sialylated glycoprotein and complement component 1q, and/or loss of signal molecules C47, glycoprotein or neuraminidase. During inflammation, microglia secrete milk fat globule EGF8 (MFG-EGF8). MFG-EGF8 is able to bind phosphatidylserine, stimulating opsonization of neurons. Excessive and chronic neuroinflammation, as with AD, leads to phagoptosis of healthy neurons (Brown & Neher, 2014; Moore et al., 2019).

Within an AD brain, overproduction of pro-inflammatory cytokines is a result of chronic activation of microglia (figure 5) (Orti-Casan et al., 2019). A prolonged elevated secretion of cytokines IL-1α, IL-1β, IL-6 and TNF-α, affects APP processing and accelerate Aβ production, with reduced phagocytosis of Aβ, leading to an increase in the Aβ burden (Kaur et al., 2019; Webers et al., 2020). On the other hand, elevated levels of pro-inflammatory cytokines also result in an increased activity of tau kinases p38-MAPK, CDK5 and GSK-3β. This increased activity results in increased tau hyperphosphorylation, leading to the formation of NFTs (Kaur et al., 2019).

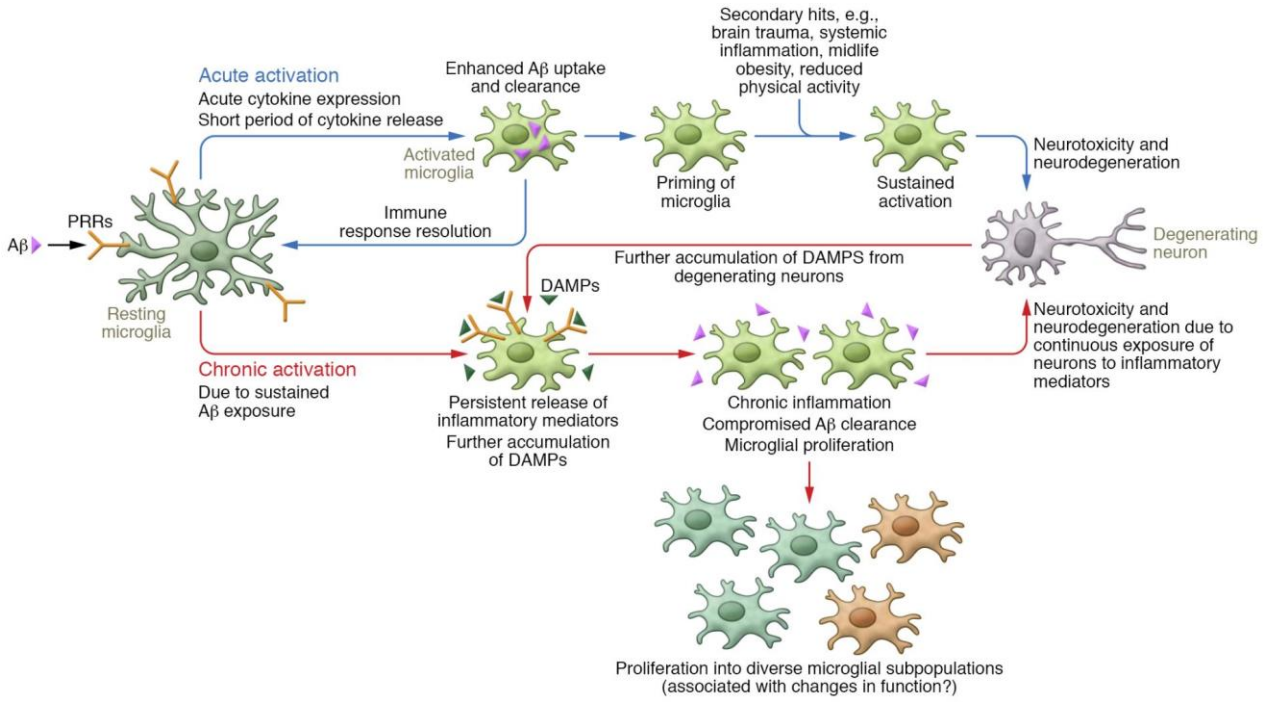

Figure 4 Acute and chronic activation of microglia in AD (Sarlus & Heneka, 2017)
Binding of Aβ results in activation of resting microglia. Acute activated microglia release cytokines stimulating clearance, uptake and phagocytosis of Aβ. Long-term activation induces proliferation and chronic inflammation, leading to neurotoxicity and neurodegeneration. Prolonged microglial activation caused by systemic inflammation, brain trauma, impaired physical activity and obesity, also induces neurotoxicity and neurodegeneration. Released DAMPs from these processes additional activate microglia, resulting in distribution of chronic inflammation and failure of Aβ clearance. Aggregations of Aβ and chronic inflammation are clinical characteristics of AD (Sarlus & Heneka, 2017).

Polarization state microglia in AD

During the pathogenesis of AD, the M1 and M2 microglial phenotypes play different roles. In early stages of AD, clearance of Aβ is related to the M2 phenotype. M2-state microglia show increased expression of ARG1 and Ym1, and increased release of anti-inflammatory cytokines (transforming growth factor β (TGF-β), IL-4, IL-10 and IL-13) and an enhanced phagocytosis (Shen et al., 2018). In later stages of AD, activated M1 microglia become more present. These M1-state microglia are related with increased release of pro-inflammatory cytokines (TNF-α, IL-1, IL-6, IL-12 and IL-18), leading to increased neuronal damage. A study of Kumar et al. showed that increased neurodegeneration in the cortex and hippocampus stimulates a shift from phenotype M2 into M1 (Kumar et al., 2014; Shen et al., 2018).

During AD progression M2 phenotypic microglia levels decrease, relative to the M1 phenotypic microglia (figure 5). This shift may be a result of AD progression, whereas the continuously aggregation of Aβ peptides leads to a chronic activation of microglia over time. M2-state microglia may become dysfunctional and replaced by M1 as a result of this immune response triggered by Aβ plaques (Kumar et al., 2014; Sarlus & Heneka, 2017; Shen et al., 2018). Moreover, it is also suggested that this shift may be a consequence of again, since aged microglia become more vulnerable against immune stimuli (Niraula, Sheridan, & Godbout, 2017).

In addition to M1 and M2 polarized microglia, new microglial phenotypes have been identified. One of these new phenotypes is the disease-associated microglia (DAM), that have a unique functional and transcriptional signature (Shen et al., 2018). DAM is affiliated with phagocytosis, sensory mechanisms and lipid metabolism. It is thought that these microglia may restrict AD progression. Because of the conflicting role of microglia, further research is necessary to determine the exact role of DAM (Shen et al., 2018).

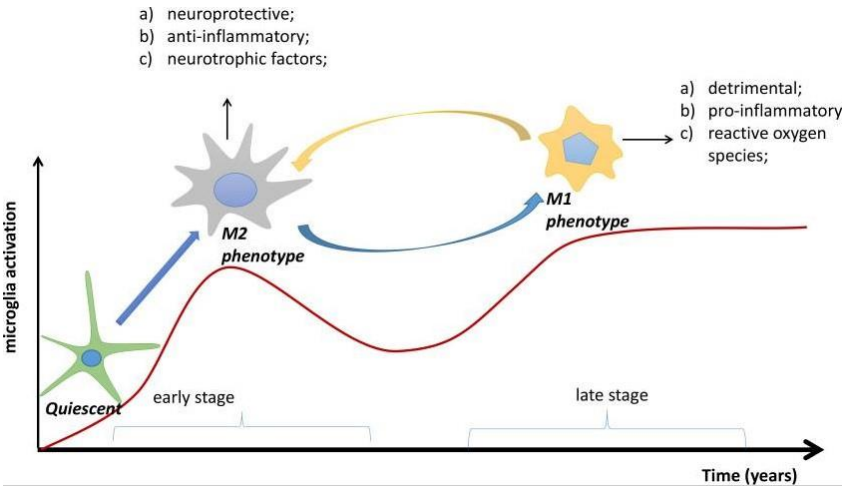

Figure 5 Polarization state microglia in AD (Shen et al., 2018)
Transformation microglial polarization states in AD. Early stage: polarization of M2 phenotypes after activation by aggregations of Aβ plaques. M2 phenotypes are neuroprotective and anti-inflammatory. Late stage: transformation of polarization state M2 into M1, which are pro-inflammatory and detrimental. Red line corresponds for microglial activation (Shen et al., 2018).

III.III Role of microglia in AD development

As described before, microglia contribute to the development of AD and its progression. During this part a summary will be given about the role of microglia in the development and progression of AD.

Before the development of AD, Aβ aggregations may be accidentally formed. When this happens, microglia are acutely activated resulting in the secretion of pro-inflammatory cytokines by M1 microglia, triggering the adaptive immune response for elimination of the plaque. After elimination, the immune response is dampened and tissue repair is induced by M2 activated microglia (Subramaniam & Federoff, 2017).

Within AD, Aβ plaques are continuously formed as a result of, among others, a mutation, leading to chronic activation of microglia. This chronic activation leads to sustained secretion of pro-inflammatory cytokine, that affects APP processing and accelerate Aβ production, with reduced phagocytosis of Aβ, leading to a further increase in the Aβ burden (Kaur et al., 2019; Orti-Casan et al., 2019; Webers et al., 2020). On the other hand, elevated levels of pro-inflammatory cytokines also result in an increased activity of tau kinases leading to the formation of NFTs (Kaur et al., 2019). This creates a vicious circle of microglial activation and, the formation of Aβ plaques and NFTs. Moreover, chronic activation also leads to increased levels of secreted MVs and MFG-EGF8, contributing to neurodegeneration (Brown et al., 2014; Moore et al., 2019).

During AD progression, M2 phenotypic microglia levels decrease, relative to the M1 phenotypic microglia, and M2 microglia may even become dysfunctional and replaced by M1 microglia plaques (Kumar et al., 2014; Sarlus & Heneka, 2017; Shen et al., 2018). This imbalance results in the continuous increase of neuronal damage, because the remaining M2 activated microglia are not able to restore the damage made as a result of the chronic activated M1 microglia (Shen et al., 2018). Ultimately, the brain damage is so severe that is not possible to life.

Besides the presence of Aβ plaques, it is also suggested that age may play an important role in the development and progress of AD, since microglia become more vulnerable against immune stimuli over the years (Niraula et al., 2017).

Part IV: Ageing

Ageing is a natural progressive process, characterized by anatomic chances and function loss accompanied by cognitive impairments (Niraula et al., 2017). Elevated levels of inflammatory signaling are shown during ageing in the CNS and the peripheral system. The inflammation of the CNS is mainly a result of changes in/dysfunction of microglia related to age (Niraula et al., 2017).

IV.I Microglia priming

Within the healthy CNS, resting microglia have a widely branched morphology. After detection of microenvironmental changes, microglia become activated accompanied by obtaining an amoeboid morphology, containing an enlarged cytoplasm (Rawji et al., 2016). During ageing microglia may develop a primed phenotype, as a result of repeated exposure to immune challenging stimuli during life. This phenotype is characterized by an excessive and uncontrolled pro-inflammatory response upon a stimulus, and a dystrophic morphology including, spherical cell body, de-ramified processes, and a fragmented cytoplasm (Niraula et al., 2017; Rawji et al., 2016). In relation to biochemical changes, elevated expression of TLR, IL-1β, MHC-II and a reduction in regulatory molecules CD200R and CX3CR1, telomere shortening and DNA methylation are found in relation to this phenotype (Niraula et al., 2017). Beside phenotypic development upon age, study findings showed that peripheral and central immune changes may induce microglial priming (Niraula et al., 2017). Development of primed microglia can also be a result of chronic psychological stress, epigenetic changes and genomic instabilities (Niraula et al., 2017; Rawji et al., 2016). Microglia with this primed profile are vulnerable to immune stimuli (stress, aging, immune challenge) and are overactive after activation, leading to an excessive pro-inflammatory response with a resistance against regulation (Niraula et al., 2017). In addition to the excessive pro-inflammatory response, microglia also show a decreased capacity of chemotaxis and phagocytosis during ageing.

The development of this aging phenotype may be related to several potential mechanisms. First, communication with microglia by inhibitory ligand-receptor interaction is lost as a result of neuron damage during ageing. Second, during normal ageing misfolding proteins, such as Aβ, accumulate leading to secretion of, elevated levels of, pro-inflammatory cytokines by microglia. Third, increase of TGF-β with age and chronic exposure to it, may deteriorate the capacity to release anti-inflammatory cytokines by microglia (Rawji et al., 2016). Increase of TGF-β is a result of downregulation of IRF-7. IRF-7 plays an important role in switching microglia from an anti-inflammatory into a pro-inflammatory phenotype (Cohen et al., 2014; Rawji et al., 2016).

Moreover, inflammatory signals derived from the periphery may also contribute to the development of primed microglia due to the close interaction between the brain and the immune system (Hoeijmakers, Heinen, van Dam, Lucassen, & Korosi, 2016). Secretion of these signals are detected and interpreted by microglia, leading to the appropriate behavioral and physiological response upon this peripheral infection. Chronic infection of the periphery leads to the continuously activation of microglia and may contributes to the development of the primed phenotype (Norden & Godbout, 2013).

In patients with diabetes type 2 (T2D), low-grade inflammation may drive microglia towards the primed phenotype. The high levels of insulin, as seen in T2D, induce an inflammatory response in the brain, resulting in elevated levels of TNF- α, IL-1β and IL-6. In addition, high insulin levels also contributes to an increase of Aβ in the plasma. It is suggested that increased Aβ may stimulate AD progression, since Aβ needs to compete with insulin for degradation (Gabbouj et al., 2019). As mentioned before Aβ peptides leads to activation of microglia. Chronic activation of microglia by high insulin levels and Aβ peptides may drive microglia into the primed phenotype (Gabbouj et al., 2019; Sarlus & Heneka, 2017).

Besides changes in the central and the peripheral microenvironment during ageing, study results showed that exposure to infection in early-life, especially during brain development, leads to highly sensitive microglia to systemic infections and may contribute to the shift into a priming-like phenotype (Hoeijmakers et al., 2016). In adult rats, experimental results showed, this is seen as an increased activity of microglial pro-inflammatory markers CD11b and MHC- II after early infection (Hoeijmakers et al., 2016).

Finally, the location of injury can also affect the response of ageing microglia. For example, responses to injuries in primarily the grey matter may vary from the injury response in the white matter. In studies, aging rodents showed an increased microglial response towards impact injuries of the thalamus and hippocampus, compared with young rodents (Rawji et al., 2016). However, a decreased microglial response was found in ageing rodents with induced focal demyelination in the white matter (Rawji et al., 2016; Zhao, Li, & Franklin, 2006).

IV.II Aged microglia and AD

In the aged brain, de development of primed microglia may be a result, as well as a cause of neuroinflammation. As mentioned before, primed microglia become highly sensitive upon immune stimuli. As a response to an immune stimuli, primed microglia stimulate a boost in neuroinflammation and further formation of Aβ and tau. This is accompanied by a high decrease in secretion of neurotrophic factors leading to an extreme loss of neurons, with AD as a result (Li, Zong, Cao, Tan, & Tan, 2018).

The cycle that arises between the continuous production of Aβ and the activation of primed microglia, is accompanied by an enormous response leading to further brain damage, and results in the progression of the disease (Angelova & Brown, 2019; Kaur et al., 2019; Niraula et al., 2017; Webers et al., 2020). Study results showed that activation of primed microglia leads to, among others, the increase of ROS production, leading to elevated NO production and decreased levels of glutathione, inducing an pro-inflammatory response. On the other hand, the continuous formation of Aβ plaques leads to the chronic activation of microglia. Since systematic exposure to immune challenging stimuli can drive microglia into the primed phenotype, it is suggested that AD may be partly responsible for the development of these primed microglia (Niraula et al., 2017).

Within AD patients, primed microglia were found near NFTs and Aβ plaques. In relation to Aβ, these microglia have a decreased phagocytic capacity and also secreted elevated levels of IL-1β and TNF-α. Focusing on TNF-α, it is suggested that the elevated levels cause phagocytic failure. Besides this failure, TNF-α is also responsible for regulation of the immune system after secretion. Secreted TNF-α binds to TNFR1, and TNFR2. Binding to TNFR1, located on all tissues and cell types, leads to downstream activation of signaling pathways of, among others, NF-κB, c-jun N-terminal kinase (JNK), p38, and ceramide/sphingomyelinase, inducing inflammation, cell migration, proliferation, necrosis and apoptosis. When binding to TNFR2, present on immune and endothelial cells, interaction with TNF receptor-associated factor (TRAF) 1, TRAF2, cellular inhibitors of apoptosis proteins (cIAPs) and the NF-κB pathway, leads to activation of pro-survival and inflammatory signaling pathways. Moreover, activation of the phosphatidyl inositol 3-kinase (PI3K)/Akt pathway, by TNFR2, stimulates proliferation and survival.

Previous post-mortem studies showed increased levels of TNFR1 and decreased levels of TNFR2 in AD patients. Moreover, a higher probability of binding to TNFR1, compared with binding to TNFR2, is found in AD. The elevated levels of TNFR1 activation results in further neuronal damage, which is exacerbated by the absence of the counterbalance in TNFR2 activation (Orti-Casan et al., 2019).

Part V: Therapeutic treatments AD

Despite the lack of a cure for AD, patients are treated with drugs, controlling the symptoms (Briggs, Kennelly, & O'Neill, 2016). Evidence of therapeutic studies suggest that changes related to the development of AD occurs years before the first symptoms of AD are visible. It may be possible that pharmacological therapy is beneficial before the onset of the first symptoms (Briggs et al., 2016). During this part, two therapies, approved against AD, are discussed. Both in relation to microglia.

V.I Cholinesterase inhibitors

The first therapy, and most recommended is the use of cholinesterase (ChE) inhibitors such as galantamine, rivastigmine and donepezil (Briggs et al., 2016). ChE plays a role in the cholinergic system within the brain. Within a healthy brain ChE subtypes, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are secreted after activation of microglia, astrocytes and oligodendrocytes

(Lane, Potkin, & Enz, 2006). In relation to AD, these ChE were found near Aβ plaques and NFTs. Where it is suggested that they play a role in the formation of these plaques and NFTs (Li, Yang, Chen, & Sun, 2017). Moreover, they also play a role in cognitive loss, due to their role in the cholinergic system (Briggs et al., 2016; Sharma, 2019).

AChE is an enzyme, that plays a critical role during hydrolysis of acetylcholine (ACh) (Li et al., 2017; Sharma, 2019). ACh is one of the most important neurotransmitters and plays a role in learning, memory, attention, sleep, wakefulness and the transfer of sensory information (Briggs et al., 2016; Ferreira-Vieira, Guimaraes, Silva, & Ribeiro, 2016). In relation to AD, a loss of ACh is found during its progression leading to impairment of cognitive functions (Sharma, 2019). Inhibition of AChe by galantamine, rivastigmine and donepezil prevents ACh hydrolysis and increases ACh levels, resulting in improvements of cognitive symptoms in patients with AD. Despite this, AChE inhibitors cannot change the progression of AD and have a short positive effect for 1 up to 3 years (Ferreira-Vieira et al., 2016).

In addition to AChE, BChE, originated from glial cells, functions as a co-regulator of the ACh metabolism (Darvesh, 2016). In healthy individuals, ACh is primarily degraded by BChE. Unless its unclear role, it is reported that there might be a correlation between BChE and detoxification, and drug metabolism (Li et al., 2017). Related to AD, AChE levels decrease with 55-67% and an increase of 120% of BChE is found in relation to normal levels. This indicates that BChE is critical for the hydrolysis of ACh in AD later stages. In AD patients, elevated levels of BChE are found in the limbic structures, such as amygdala and hippocampus and the neocortex, explaining the loss of cognitive and behavior dysfunction (Li et al., 2017). Inhibition of BChE can be performed by the use of ChE inhibitor rivastigmine, preventing the loss of cognitive and behavior dysfunction (Sharma, 2019).

The effects of these inhibitors are limited. Approximately one-third of the patients do not show any improvements, benefits were shown in only one-fifth of all cases and about one-third of the patients cannot handle the medication because of its side effects. Side effects that may occur are muscle cramps, fatigue and gastrointestinal related problems (Briggs et al., 2016).

V.II Memantine

The second therapy is a therapy using memantine (MEM). MEM functions as an antagonist for the non-competitive N-methyl-D-aspartate (NMDA) receptor and dopamine. NMDA receptors are cationic channels regulated by neurotransmitter glutamate. Within the CNS, glutamate plays an important role in the processes of memory and learning. Abnormal elevated levels of released glutamate and concentrations of soluble Aβ, as seen in AD, may lead to the increase of NMDA receptor activity. This leads to increased Ca^{2+} cell influx resulting in impairments of mitochondrial functions. Under normal conditions the mitochondrial permeability transition pore (MPTP), located in the inner mitochondrial membrane, is closed (Ferreira-Vieira et al., 2016; Parks, Murphy, & Liu, 2018). When stimulated with Ca^{2+} this pore opens leading to secretion of cytochrome C, posterior loss of ATP and formation of ROS resulting in excitotoxic death, tau phosphorylation and synaptic dysfunction (Ferreira-Vieira et al., 2016; Pivovarova & Andrews, 2010).

There are two types of NMDA receptors; synaptic and extra synaptic. Synaptic NMDA receptors are located pre- and postsynaptic on neurons. Receptors presynaptic are important in neuronal plasticity and transmission, while receptors postsynaptic control this plasticity. Both synaptic types are important concerning activation of survival and neuronal protective genes (Ferreira-Vieira et al., 2016). The extra synaptic NMDA receptors can be found on dendrites. Activation of these receptors needs high concentrations of glutamate. These receptors control neuronal cell death and neurotoxicity after stimulation with excessive levels of glutamate. Moreover, extra synaptic NMDA receptors play a role in formation of Aβ and hereby contributes to the pathology of AD. Hereby this receptor can been seen as the main target of MEM (Ferreira-Vieira et al., 2016).

Furthermore, NMDA receptors can also be found on the cell surface of microglia. Activation of the receptor may contribute to the secretion of IL-1, TNFα and NO leading to a pro-inflammatory cascade as described before (Liu, Leak, & Hu, 2016).

When using MEM in AD, glutamate cannot bind to the NMDA receptor, leading to a decreased level of NMDA activation. The reduction of Ca^{2+} influx, as a result of this, prevents mitochondrial function impairments and further neuronal loss, and stimulates neuron recovery (Briggs et al., 2016;

Ferreira-Vieira et al., 2016; Pivovarova & Andrews, 2010; Weller & Budson, 2018). Furthermore, it also protects cholinergic neurons against destruction by reducing excitotoxicity (Ferreira-Vieira et al., 2016). According to preclinical data MEM may also inhibit other receptors, such as acetylcholine, nicotine, serotonin and sigma-1 receptor (Ferreira-Vieira et al., 2016). Within AD patients, treatment with MEM improves alertness and memory without changes in AD progression and the development of dementia (Weller & Budson, 2018). This treatment is only useful for patients with a decreased cognitive capacity or when in the mid-stage of AD (Briggs et al., 2016).

Conclusion/discussion

In relation to AD, microglia could be used as a therapeutic target, where alternative therapies may focus on the immunological characteristics and function of microglia. These therapies should focus on preventing further neuronal damage by capitalizing the activation of M2 microglia and the increase of it levels during AD pathogenesis. This could be done by using H3k27me3 demethylase Jumonij domain (Orihuela et al., 2016; Subramaniam & Federoff, 2017). This domain, metylates histone H3K27me3 to H3K27me2/me, leads to an increase in arginase activity when overexpressed. Since arginase leads to polarization into the M2 phenotype, this domain could be useful for manipulating the polarization state (Tang et al., 2014)

Furthermore, a drug could be developed that induces the polarization from the M1 phenotype into the M2 phenotype. Ongoing research showed that the Rho/ROCK pathway and the NF-κβ pathway could be used as a target for this transition. Inhibition of the Rho/ROCK pathway, where Rho plays an important role in the regulation of cellular proliferation and migration, and ROCK is the downstream effector of Rho, may induce a shift from M1 polarized into M2 polarized microglia (Roser, Tonges, & Lingor, 2017; Yao & Zu, 2020). Next, modification of the NF-κβ pathway by, among others, an aryl hydrocarbon receptor antagonist, may prevent microglial activation and stimulation of shifting from the M1 polarization state into M2 (Yao & Zu, 2020).

Moreover, microglia receptors, pathways, and/or cytokines could be blocked to prevent secretions of pro-inflammatory cytokines, preventing neuronal damage. Non-selective inhibition of COX could be used to decrease levels of amyloidogenic Aβ peptides 1-42, lowering the formation of Aβ plaques(Yao & Zu, 2020). Furthermore, inhibition of microglia specific TLR4 could prevent microglial activation MyD88, TRIF and transcription factors leading to transition into the M1 polarization state (Subramaniam & Federoff, 2017). In addition, the microglia specific IL-6R on the cell surface of microglia could be inhibited, preventing further activation and transition into the M1 phenotype by other already polarized M1 microglia (West et al., 2019).

Other receptors that could be used as a target are TNF receptors. Modulation of TNFR2 may stimulate its neuroprotective role and cell survival. Study results showed that TNFR2 agonists protects neurons from oxidative stress induced cell death, seen in AD (Orti-Casan et al., 2019). In relation to AD progression, a combination treatment between inducing TNFR2 and inhibition of TNFR1, preventing inflammation, necrosis and apoptosis, would be ideal. Besides the modulation of these receptors, regulation of its ligand, TNF-α, could lead to a better regulation of the immune response by activation of TNFRs.

Since primed microglia play an important role in progression of AD, a drug could be developed to prevent the transformation into this phenotype. This could be done by inhibition of IRF-7, leading to the avoidance of elevated levels of TGF-β, one of the primed developing stimuli (Cohen et al., 2014; Rawji et al., 2016). Furthermore, the increased Aβ burden, as seen in AD, leads to an enormous pro-inflammatory immune response by these primed microglia, resulting in further neuronal damage (Angelova & Brown, 2019; Kaur et al., 2019; Niraula et al., 2017; Webers et al., 2020). To prevent this damage, selective inhibition of microglia TLRs avoids activation of inflammation cascades.

Additionally, modulation of these primed microglia may be used to correct their function. Current research showed that this modulation could be perform by using monophosphoryl lipid A, a modified LPS stimulating TRIF. Compared to LPS it does not activate TLR4 resulting in activation of the pro-inflammatory MyD88 pathway. Study results showed that the use of this modulator increased the phagocytic capacity alongside a reduced Aβ burden. Also a class of A1 scavenger receptors, pharmacologically upregulated, resulted in an increased Aβ clearance capacity in mouse models (Yao & Zu, 2020b). Moreover, treatments targeting transcriptional regulators which promotes regulation of the microglial phenotype. Peroxisome proliferator-activated receptor γ or IRF-7 are suggested to improve this regulatory phenotype (Yao & Zu, 2020). Next, studies showed that the ageing circulation has negative impact on neurogenesis. Stimulations of the CNS microenvironmental with factors attending in the circulation at young age, may correct these microglial changes (Yao & Zu, 2020).

It is also important to develop microglia related drugs that first focus on anti-inflammation to restore damaged tissue, followed by maintaining the balance between pro- and anti-inflammation. To create this balance, it is also important to prevent continued stimulation of microglia resulting in the

formation of the M1 phenotype, and later the development of the primed phenotype. In AD these stimuli are mostly Aβ plaques. Prevention or removal of these plaques by misfolding Aβ could be done using a therapy with molecular chaperones (Marino Gammazza, Bavisotto, Barone, de Macario, & Macario, 2016). Molecular chaperones can detect and target aggregated or misfolded proteins for degradation, translocation or refolding (Campanella et al., 2018). Since Aβ peptides with 40 or 42 amino acids causes the Aβ plaques in AD, it is important that these aggregates are detected and refolded by chaperones.

Based on the role of microglia during AD pathogenesis and the possible alternative therapies, it can be concluded that microglia might be an important target for the development of a treatment against AD. Microglia based therapies may contribute to dampening ADs progression and restore neuronal damage, if possible. Microglial therapies may prevent cognitive impairments if used in early stages of the disease. Unfortunately, many cases of AD are diagnosed when these impairments starting to become visible. However, the use of microglial based therapies may stabilize the disease.

In addition, it must be taken into account that targeting only microglia will not be enough to fully recover from the disease. AD is a complicated neurological disease accompanied by the influences of different cell types (astrocytes, oligodendrocytes), which may also play an important role in the pathogenesis of AD.

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