

The engagement of microglia in tau-targeted immunotherapy in Alzheimer's disease

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Abstract. Alzheimer's disease (AD) is an age-related neurodegenerative disease characterized by progressive memory decline, histopathological lesions such as amyloid β plaques and neurofibrillary tangles, and neuroinflammation driven by glial cells. Microglia, the innate immune cells of the brain, dynamically survey their environment for signs of infection and cell damage. Although our understanding of microglia and their modes of activation has expanded in recent years, their role in AD is still not completely understood. Broad range of microglia phenotypes, from neuroinflammatory to neuroprotective, found in neurodegenerative diseases make their role difficult to discern. In this review, we summarize activities of microglia in healthy and AD brains and their possible role during immunotherapy targeted against pathological tau proteins.

Key words: Alzheimer's disease — Microglia — Neuroinflammation — Tau immunotherapy

Abbreviations: A β , amyloid β ; AD, Alzheimer's disease; APP, β -amyloid precursor protein; CNS, central nervous system; DAM, disease associated microglia; eTau, extracellular tau; Fc γ R, Fc gamma receptor; GSK3 β , glycogen synthase kinase 3 β ; IFN γ , interferon gamma; IgG, immunoglobulin G; IL, interleukin; ITAM, immunoreceptor tyrosine activating motifs; ITIM, immunoreceptor tyrosine inhibitory motifs; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; NFT, neurofibrillary tangles; PET, positron emission tomography; PHF, paired helical filaments; PRR, pattern recognition receptors; ROS, reactive oxygen species; SRA, scavenger receptor A; TLR, toll-like receptors; TNF α , tumour necrosis factor alpha; TREM2, triggering receptor expressed in myeloid cells 2; TSPO, mitochondrial translocator protein; UPS, ubiquitin-proteasome system.

Introduction

Alzheimer's disease (AD) is the most prevalent type of dementia with proteinopathy being the key pathogenic trigger. Two main pathological hallmarks of AD are the accumulation of extracellular amyloid β plaques (A β) and intracellular

neurofibrillary tangles (NFTs) (Prince et al. 2015). Numerous studies point out the significant contribution of neuroinflammation in AD pathology. Analysis of AD brains revealed the presence of activated microglia and neuroinflammation in the brain tissues affected by neurodegeneration (Sheffield et al. 2000; Gebicke-Haerter 2001; Ishizawa and Dickson 2001; Gerhard et al. 2006; Cosenza-Nashat et al. 2009; Keren-Shaul et al. 2017). Microglia are the innate immune cells of the central nervous system (CNS) that help maintain homeostasis of the brain, promote learning and memory, and respond to various pathogenic stimuli in different

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brain areas throughout the lifespan (Colonna and Butovsky 2017; Lenz and Nelson 2018). The role of microglia in the AD neuropathology is not fully understood. It is assumed that their activation might be beneficial at the beginning of the disease, but it seems to turn rather detrimental in later stages (Jay et al. 2015, 2017; Jiang et al. 2016; Bemiller et al. 2017; Leyns et al. 2017). Microglia mediate a phagocytic clearance of pathologic protein aggregates from the brain (Luo et al. 2015; Rivera-Escalera et al. 2019), while chronic activation of microglia shifts their phenotype towards more pro-inflammatory, which together with gradual deterioration of phagocytic activity promote neuroinflammation and neurotoxicity (Keren-Shaul et al. 2017).

For decades, the amyloid hypothesis has dominated research on AD. Amyloid hypothesis is based on the principle of serial causality, where the primary pathogenic trigger is the accumulated A β that initiates and drives subsequent tau hyperphosphorylation, as well as other clinical and histopathological features of AD (Hardy and Higgins 1992). Since the formulation of amyloid cascade hypothesis over 30 years ago, number of alternative hypotheses emerged. Tau hypothesis postulates that aberrant accumulation of hyperphosphorylated tau aggregates precedes A β pathology (Braak and Braak 1997; Braak and Del Tredici 2011) and that the development and localisation of tau pathology closely correlate with other measures of neurodegenerative disease progression including neuronal dysfunction and death, synaptic loss, and cognitive decline (Arriagada et al. 1992; Giannakopoulos et al. 2003; Small and Duff 2008; Polydoro et al. 2009). In addition, tau pathology progresses along interconnected neuroanatomical pathways in a disease-specific pattern (Braak and Braak 1995; Braak et al. 2011). Both hypotheses are not mutually exclusive, as common upstream drivers, such as GSK3 β (glycogen synthase kinase 3 β), a key enzyme in tau phosphorylation and APP (β -amyloid precursor protein) metabolism, may cause parallel elevation in A β and tau hyperphosphorylation through independent mechanisms (Phiel et al. 2003; Liu et al. 2006).

The role of microglia in the central nervous system under normal and pathological conditions

Healthy brain microglia

Origin and functions

As was shown in mice, microglia arise from extra-embryonic yolk sac myeloid progenitors in early embryonic development (embryonic day 7.5), as opposed to other tissue-resident macrophages that are derived from fetal hematopoietic stem cells (Alliot et al. 1999; Ginhoux et al. 2010; Sheng et al. 2015). In humans, the microglial popula-

tion density ranges from 0.5% to 16.6% of the total cells in the brain (Mittelbronn et al. 2001). Microglial progenitors enter the CNS before blood-brain barrier is formed and their population is primarily maintained through self-renewal of CNS-resident microglia (Ajami et al. 2007). However, small degree of renewal is achieved through infiltrating bone marrow-derived monocytes, especially under pathological conditions (Varvel et al. 2016). This self-sustaining feature of microglia makes them particularly sensitive to local disturbances. Primary function of microglia is to survey their microenvironment, scavenge cell debris and damaged brain cells, eliminate dysfunctional or less active synapses (synaptic pruning), modulate synaptic transmission by paracrine signaling, and to provide trophic support for neurons (Wes et al. 2016). Disrupted synaptic pruning negatively affects learning processes, and eventually may contribute to the synaptic loss observed in AD (Chung et al. 2015). Increasing body of evidence suggests that microglia derived from different CNS compartments display distinct functions and phenotypes (Olah et al. 2018, 2020; Swanson et al. 2020). We have shown that neonatal murine microglia derived from brain cortex exhibit more anti-inflammatory phenotype and promote neurogenesis, while spinal cord-derived microglia are prone to be more pro-inflammatory in nature (Murgoci et al. 2020). As microglia are mitotically-active cells, Reu and colleagues analysed microglial turnover in the human brain. Microglia were shown to renew at a relatively slow pace, with an estimated median rate of 28% *per year* and have an average life span of 4.2 years (Reu et al. 2017).

Microglia phenotypes

Classical dogma of both resident macrophages and microglia is that they display two main phenotypes, “activated” (or M1) phenotype and “resting” (or M2) phenotype. M1 phenotype is also known as a pro-inflammatory state, in which secretion of cytokines interleukin-1 β (IL1 β), interleukin-6 (IL6), interleukin-12 (IL12), interleukin-18 (IL18), tumour necrosis factor- α (TNF α) dominates. On the other hand, M2 state is characterized by elevated phagocytic activity without production of toxic nitric oxide (NO) and by the expression of anti-inflammatory cytokines interleukin-4 (IL4), interleukin-10 (IL10), interleukin-13 (IL13) and transforming growth factor- β (TGF β) (Goerdt and Orfanos 1999; Mantovani et al. 2002; Koenigsknecht-Talboo and Landreth 2005; Zelcer et al. 2007). We now understand, that microglial phenotypes are far more heterogenic and overlapping than originally thought, and that “resting” microglia are in fact motile and constantly surveying their environment, thus helping maintain brain homeostasis (Wes et al. 2016). Dynamic change of microglia polarization depends on multiple factors and accumulating evidence point at the role of mitochondria in this transi-

tion (Ferber et al. 2010; Pozzo et al. 2019; Ren et al. 2020). Ferber et al. (2010) for example reported that some aspects of alternative M2 microglial activation induced by IL4 depend on functional mitochondria. Because this alternative activation is implicated in dampening of inflammation, mitochondrial dysfunction in microglia might contribute to the detrimental effects of neuroinflammation on neurodegeneration. Additionally, mitochondrial translocator protein (TSPO), located on the outer mitochondrial membrane, has been thought to have a homeostatic function in neuroinflammatory conditions. To counteract the pro-inflammatory state of microglia, activated TSPO reduces production of reactive oxygen species (ROS) and stimulates neurosteroidogenesis (Pozzo et al. 2019). Upon aging, microglia typically exhibit a change in morphology including thickening, de-ramification and hypertrophy of the cell body. In addition to morphological changes, increased expression of surface markers CD11b, CD68, CD11c and F4/80 was reported in the mouse brains (Hart et al. 2012). Age-related morphological changes in murine microglia are in accordance with those in human microglia. It was shown that microglial processes shorten with age, exhibit less branching and reduced arborized area (Davies et al. 2017). Moreover, Keren-Shaul and colleagues described and characterized a new type of microglia in the brain cortex with the aid of single-cell RNA-sequencing, called disease associated microglia – DAM (Keren-Shaul et al. 2017) (see next chapter for more details).

Antibody effector function

Microglia express Fc gamma receptors (FcγR) I, IIb, III and IV which are involved in the internalisation of antigen-antibody immune complexes destined for intracellular degradation. The constant region (Fc) of immunoglobulin G (IgG) antibody protruding from the immune complexes is recognized by surface FcγR, leading to their internalization by microglia. FcγR clustering on the cell surface is required for the phosphorylation of cytoplasmic motifs on the receptor. Mice express three classes of activating IgG receptors FcγR1, FcγR3 and FcγR4. Binding of antibodies to activating FcγR results in phosphorylation of immunoreceptor tyrosine activating motifs (ITAMs) and down-stream activation pathways activating the cell. Low-affinity inhibitory receptors FcγR IIb contain immunoreceptor tyrosine inhibitory motifs (ITIM) in their intracellular domain. Phosphorylation of ITIM results in initiating inhibitory signalling pathways (Fuller et al. 2014). With increasing age, murine microglia exhibit elevated expression of FcγR1 (Hart et al. 2012). Microglia also express toll-like receptors (TLR), pattern recognition receptors (PRR) and scavenger receptor A (SRA) which mediate phagocytosis of non-antibody bound ligands (Olson and Miller 2004; Zhang et al. 2014).

Microglia in Alzheimer's disease

Microglia actively help maintain brain homeostasis and functions in the prodromal or early stages of AD. However, under conditions of chronic neuroinflammation, influenced by numerous environmental and/or genetic factors, the pathogenesis of AD may start to develop. Main cell types involved in neuroinflammatory processes in brain are microglia and astrocytes, but capillary endothelial cells and infiltrating blood cell can also contribute to neuroinflammation, especially when the blood brain barrier functions are compromised (Montagne et al. 2015; Varvel et al. 2016). Activated microglia have been shown to colocalize with deposits of NFTs and Aβ plaques in human brains (Cras et al. 1991; Overmyer et al. 1999; Sheffield et al. 2000; Keren-Shaul et al. 2017). Although microglia are more activated in later stages of tangle formation, their phagocytic activity is getting compromised (Bolos et al. 2016).

Primed microglia and immune memory

In an ageing brain, microglia are prone to become hypersensitive to repeated exposure to inflammatory stimuli, a phenomenon known as “priming”. This means that primed microglia have a lower threshold for activation and can become harmful upon further stimulation. Microglial priming was first described in the brains of mice with prion disease, as diseased mice showed an elevated microglial inflammatory response after systemic administration of lipopolysaccharide (LPS) or polyinosinic:polycytidylic acid (poly I:C) (Cunningham et al. 2005). Microglial priming is generally considered maladaptive and detrimental. Molecular pathways involved in the mechanism of microglial priming remain only partially elucidated. Up-regulation of *Il1b*, *Mhc2* gene expression and reduction of *Cx3cr1-Cx3cl1* signalling cascade was previously reported (Field et al. 2010; Holtman et al. 2015b). Priming of microglia may be a consequence of cellular aging and environmental factors inducing e.g. ischaemia, traumatic brain injury (Leng and Edison 2021), or chronic low-grade systemic inflammation, as can be seen in atherosclerosis, type 2 diabetes mellitus, obesity, high fat diet (Casserly and Topol 2004; Balakrishnan et al. 2005; Donath and Shoelson 2011; Drake et al. 2011; Butler et al. 2020). Interestingly, emerging evidence points toward epigenetic regulation of inflammatory pathways implicated in microglial priming. Matt et al. (2016) found that expression of DNA methylating enzymes is decreased in aged murine microglia, and that decreased methylation in the promoter region of *Il1b* gene results in increased intracellular production of IL1. In another study, specific inhibition of histone deacetylases 1 and 2 resulted in reduced LPS-mediated expression of cytokines in BV2 murine microglia (Durham et al. 2017). Several investigators

propose that microglial priming is a form of innate immune memory, as it exhibits similarities with trained immunity of peripheral macrophages. After the activating stimulus of microglia subdues, a long-lasting epigenetic memory of the stimuli may remain in the form of histone post-translational modification, specifically by H3K4me1 marks located at defined latent enhancer sites in the microglial genome. Upon re-encounter of the stimuli, these latent enhancers may play role in the augmented inflammatory response (Haley et al. 2019; Neher and Cunningham 2019).

Disease-associated microglia (DAM)

DAM are recently identified subset of microglia found at sites of neurodegeneration. During the onset of neurodegenerative disease, microglia evolve to DAM through a two-step mechanism, (i) TREM2-independent and (ii) TREM2-dependent. This transition is associated with significant changes in gene expression. The first step in DAM activation, TREM2-independent, is associated with down-regulation of several important microglia homeostatic genes, including those for purinergic receptors *P2ry12/P2ry13*, *Cx3cr1*, *Tmem119* and up-regulation of *ApoE*, TREM2 adaptor *Tyrobp*, and *B2m* gene expression. The second phase of DAM activation, TREM2-dependent, involves up-regulation of multiple genes such as *Cst7*, *Ctsd*, *Lpl*, and *Trem2*, which are involved predominately in lysosomal/phagocytic pathways, endocytosis, lipid metabolism. These results support the observation that deficiency in TREM2 in the late stage of AD in a mouse model exacerbated disease, led to microglial dysfunction and apoptosis (Keren-Shaul et al. 2017). Other research groups similarly reported microglial subpopulation with disease-specific expression profile (Holtman et al. 2015a; Krasemann et al. 2017; Kang et al. 2018). A precise identification of molecular mediators that trigger the DAM phenotype might offer interesting and plausible approach for future neuroprotective therapies.

TREM2

The TREM2 gene (triggering receptor expressed in myeloid cells 2) encodes a single-pass transmembrane receptor expressed by microglia, that induces phagocytosis, promotes survival and modulates inflammatory signalling pathways (Ulland and Colonna 2018). TREM2 was shown to act as a microglial sensor that recognises anionic and zwitterionic lipids exposed on the cell surface of neurons damaged by A β pathology (Wang et al. 2015). TREM2 is considered as a high-risk genetic factor for AD. The rs75932628 (R47H) polymorphism of TREM2 was associated with up to three-fold increased risk of nonfamilial AD (Jonsson et al. 2013). Homozygous loss-of-function mutations in TREM2 cause a severe, rare form of dementia with bone cystic le-

sions known as Nasu-Hakola disease (Bianchin et al. 2004). Based on their findings, Gratuze and colleagues proposed that the role of TREM2 depended on A β pathology and the stage of the disease (Gratuze et al. 2020). At early phases of the disease, the R47H variant reduces microgliosis around senile plaques, thereby increasing their number, and also promotes tau propagation. However, in advanced stages of the disease, when tau pathology is prominent, this variant attenuates tau-dependent synapse loss by reducing microglial phagocytosis.

Mitochondrial dysfunction

Mitochondrial dysfunction is critically involved in AD pathology. AD-associated chronic exposure to pathogenic stimuli directly alters metabolic processes in the brain (Brooks et al. 2007; Kapogiannis and Mattson 2011; Mastroeni et al. 2017; Croteau et al. 2018). Large body of evidence suggests that pathological forms of tau negatively affect mitochondria, including (i) mitochondrial transport (Schulz et al. 2012; Shahpasand et al. 2012), (ii) morphology (DuBoff et al. 2012; Amadoro et al. 2014), and (iii) bioenergetics (David et al. 2005; Rhein et al. 2009; Quintanilla et al. 2014). Studies on transgenic mice with P301L mutant tau revealed, that complex I of mitochondrial respiratory chain is especially sensitive to abnormal tau (David et al. 2005; Rhein et al. 2009), and reversely, mitochondrial stress *per se* was shown to promote hyperphosphorylation of tau in a mouse model of oxidative stress that lacks superoxide dismutase 2 (SOD2) (Melov et al. 2007). Optimal calcium concentration in mitochondria is inevitable for normal function. Xie et al. (2017) showed that reduction of excessive calcium uptake by mitochondria prevented apoptosis of A β -treated BV2 microglia-like cells and primary mouse microglia. Some authors suggest that modulating microglial metabolism might be an interesting new approach for treating AD. In this regard, Baik and colleagues observed that acute exposure to A β triggers metabolic reprogramming of microglia from oxidative phosphorylation to glycolysis, which is mTOR-dependent. However, chronic exposure to A β , represented by a mouse model of AD (5XFAD mice), was associated with metabolic defect, which were restored by IFN γ treatment (Baik et al. 2019). Additional studies are needed to elucidate the role of tau pathology in mitochondrial metabolic (dys) functions in microglia.

Senescence

With increasing age, microglia undergo replicative senescence, and are subjected to changes in telomere length, proliferation rate and morphology (Flanary and Streit 2004; Miller and Streit 2007). Bussian and colleagues investigated the role of senescent glial cells in the onset and progression

of tauopathy. To test this, authors used animal model of tauopathy, PS19 transgenic mice overexpressing mutant human tau in neurons. These mice accumulated senescent microglia and astrocytes, as indicated by the expression of markers of senescence, such as *p16*, *p19*, *p21*, *Pai1* and *Casp8*. Clearance of these senescent cells *via* transgenic approach (INK-ATTAC mice) or pharmacological intervention (senolytic agent ABT263) prevented gliosis, hyperphosphorylation of both soluble and insoluble tau and cortical/hippocampal neurodegeneration (Bussian et al. 2018). This approach might provide a new therapeutic challenge for the treatment of tauopathies.

Neuroinflammation

Mediators

Neuroinflammation is defined as an activation of the resident immune cells of brain in reaction to injury, disease or infection, with an aim of rapid localization and elimination of pathogen. Typical mediators of neuroinflammatory responses in brain are pro-inflammatory cytokines (e.g. IL1 β , IL6, TNF α), chemokines (e.g. CCL2, CCL5, CXCL1), small molecule messengers (e.g. nitric oxide-NO, prostaglandins) and ROS. Most of these inflammatory signals are being propagated *via* activated microglia, to a lesser extent by astrocytes (Norden et al. 2016). Whether neuroinflammation is a cause, a contributor, or a consequence of tau pathology is subject to extensive debate. Accumulating evidence points towards chronic neuroinflammation as a crucial factor implicated in the pathogenesis of neurodegenerative diseases (Heneka et al. 2015).

Mechanisms of action

Mechanistically, we and others proved that misfolded truncated tau aggregates are potent inducers of neuroinflammation (Kovac et al. 2011; Morales et al. 2013). We have demonstrated that primary rat microglia increased production of NO and cytokines IL1 β , IL6 and TNF α upon exposure to misfolded truncated tau through up-regulated gene expression of MAP-kinases (*Jnk*, *Erk1*, *p38b*) and transcription factors *Ap1*, *Nfkb* (Kovac et al. 2011). Similarly, Morales and colleagues (Morales et al. 2013) observed that aggregated tau oligomers and fibrils induced morphological changes and generation of pro-inflammatory IL6 and NO in rat microglia. Hippocampal synapse loss and robust microglial activation preceded the formation of NFTs in P301S transgenic mice overexpressing mutant human tau, thereby linking early microgliosis to the progression of tauopathy (Yoshiyama et al. 2007). On the other hand, activated microglia were shown to further exacerbate tau

pathology, thus forming a vicious cycle. Production of pro-inflammatory cytokines, such as IL1, by activated microglia leads to an increase in tau phosphorylation and cytotoxicity in neurons *via* the p38 MAP kinase pathway (Sheng et al. 2001; Li et al. 2003). Similar results were obtained after cocultivation of hippocampal neurons with activated astrocytes which led to an increase of NO production and consequently hyperphosphorylation of tau (Quintanilla et al. 2004). Another pro-inflammatory cytokine IL6 was found to promote CDK5 activity, one of the major kinases phosphorylating tau, *via* a MAPK-p38 dependent pathway (Saez et al. 2004).

Evidence from human brain

Post mortem analyses of brain tissue in humans suffering from various forms of tauopathies, such as AD, frontotemporal dementia and corticobasal degeneration revealed the presence of activated microglia in brain tissues affected by neurodegeneration (Gebicke-Haerter 2001; Ishizawa and Dickson 2001; Gerhard et al. 2006). Several studies used *in vivo* PET imaging to investigate neuroinflammation in patients with dementia. Most used PET ligands were targeting TSPO, a marker of activated microglia (Cosenza-Nashat et al. 2009). Study by Cagnin et al. (2001), revealed that cortical areas with high TSPO tracer binding demonstrated the highest rate of atrophy and glucose hypometabolism over 12–24 months follow-up period. Statistically significant differences in inflammation between control and dementia patients were reported and associated with severity of dementia, as indicated by cognitive tests scores. Regions such as the frontal, temporal, parietal, cingulate cortices and hippocampus demonstrated highest correlation of inflammation with cognitive deficits (Versijpt et al. 2003; Edison et al. 2008; Yokokura et al. 2011).

These findings prove a connection between neuroinflammation and processes responsible for neurodegeneration and indicate that long-term activation of immune cells of the CNS can play a pathogenic role in AD and other types of tauopathies.

Tau clearance mechanisms

Microglia have been shown to be able to internalise tau protein aggregates both *in vitro* and *in vivo* (Luo et al. 2015; Bolos et al. 2016). Surprisingly, it was shown that peripheral macrophages can phagocytose and degrade tau oligomers after LPS stimulation more rapidly than either rat primary microglia or immortalized BV2 microglial cell line (Majerova et al. 2014). Once tau is internalised, its fate is to either undergo degradation *via* (i) ubiquitin-proteasome system (UPS) or (ii) autophagy-lysosomal pathway.

Ubiquitin-proteasome system

Under physiological conditions, tau can be degraded by the 20S proteasome in an ubiquitin-independent manner (Grune et al. 2010). Exposure to irreversible proteasome inhibitor lactacystin blocked tau degradation in human neuroblastoma cell line SH-SY5Y (David et al. 2002). However, an ubiquitin-dependent pathway of tau degradation has also been described. In general, misfolded proteins must be refolded by molecular chaperones (e.g. HSC70, HSP70, HSP90) or targeted for degradation by the UPS to prevent aggregation and cytotoxicity. Hsc70-interacting protein (CHIP) acts as an ubiquitin protein ligase facilitating ubiquitination and degradation of abnormal, hyperphosphorylated tau. Sahara et al. investigated, whether the lack of CHIP may be involved in NFT formation. In human AD brains, the protein expression of CHIP was up-regulated, but the amount of PHF-tau (paired helical filament-tau) inversely correlated with the CHIP protein level, suggesting that increases in CHIP may protect against NFT formation in the early stages of AD (Sahara et al. 2005). A reduction in proteasome peptidase activities has been reported in the short-interval *post mortem* brains of AD patients (Keller et al. 2000; Lopez Salon et al. 2000). Incubation of isolated proteasomes with PHFs derived from AD brains resulted in a marked reduction in the proteasomal activity (Keck et al. 2003).

Autophagy-lysosomal pathway

Autophagy significantly contributes to tau protein degradation. Treatment of rat hippocampal slice cultures with chloroquine, a lysosomal inhibitor, led to an accumulation of intracellular phosphorylated tau (Bendiske and Bahr 2003). One group has demonstrated, that transfected MEF cell line deficient in ATG5 (autophagy protein) and expressing mutant P301L tau, had significantly attenuated response to starvation-induced autophagy of tau inclusions (Wong et al. 2008). Autophagy receptor proteins such as p62 (Ramesh Babu et al. 2008), NDP52 (Jo et al. 2014), NBR1 (D'Agostino et al. 2011), optineurin (Xu et al. 2019) have been implicated to facilitate tau clearance. Methylene blue has also been shown to be a potent inducer of autophagy. *In vivo* application of methylene blue to JNPL3 mice with tauopathy resulted in reduction of phosphorylated tau and insoluble fraction of tau in hippocampus (Congdon et al. 2012). Microglia are not the only brain cells capable of phagocytosis, astrocytes also exert a low degree of phagocytic activity. In a study by Martini-Stoica et al. (2018), enhanced expression and activity of transcription factor EB (TFEB) was found in both human brains affected with dementia and transgenic PS19 tauopathy mice. TFEB is a master regulator of lysosomal biogenesis and autophagy. In response to tau

pathology, astroglial overexpression of TFEB in mice resulted in reduced tau spreading *in vivo*.

The role of microglia in tau immunotherapy

Immunotherapy comprises two approaches, active or passive. In active immunotherapy, a pathogenic antigen is being administered which stimulates the adaptive immune system to elicit a long-lasting immune response. Activated B-lymphocytes undergo the maturation process and produce antibodies with high affinity to the injected antigen. The long-lasting immune response is ensured by generating memory B- and T-cells. In passive immunization, a specific antibody targeted at a pathogen is being directly administered, without inducing any adaptive immune responses. Passive immunization offers a transient form of acquired immunity which can bypass the concerns arising from active immunization, if patients cannot develop their own antibodies. It reduces the risk of permanent immunological adverse effects given by short half-life of the administered antibodies, but also offers the possibility of quantitative modulation of the therapy and for highly specific targeting epitope (Congdon and Sigurdsson 2018).

To date, A β immunotherapies for the treatment of AD have been largely unsuccessful, indicating that treatment may need to be administered earlier in the course of neurodegenerative disease, even before cognitive symptoms start to appear. Recent approval of Biogen's anti-amyloid antibody Aduhelm (formerly known as aducanumab) by FDA (The United States Food and Drug Administration), although heavily criticised within scientific community for the lack of significant evidence, will inevitably revive the interest in immunotherapy against misfolded proteins in AD (<https://www.alzforum.org/news/series/fallout-continues-after-aducanumab-approval>).

Although it is believed that patients at earlier stages of AD might still benefit from this approach, tau immunotherapies might offer a better solution since tau pathology is tightly associated with clinical symptoms and disease progression (Congdon and Sigurdsson 2018). Majority of antibodies that are currently being developed in clinical research as anti-tau immunotherapeutics have an IgG4 isotype, which results in limited effector function due to weak binding of IgG4 to Fc γ Rs (for details see Table 1). This approach eliminates potential unfavourable effect of microglia activation but at the same time desirable tau clearing properties. In summary, tau-targeting vaccines recognize different tau species and have been suggested to act through various mechanisms: by preventing tau aggregation and cell-to-cell spreading (Theunis et al. 2013; Albayram et al. 2017; Courade et al. 2018; Novak et al. 2018; Albert et al. 2019; Weisova et al. 2019), by facilitating tau uptake by microglia (Zilkova et al.

2020) and endo-lysosomal degradation of tau in microglia (Andersson et al. 2019) or proteasomal degradation of tau seeds in neurons (McEwan et al. 2017).

Antibody-mediated tau clearance might alleviate tau pathology

Compelling body of evidence suggest that microglia engulf and degrade tau aggregates more efficiently in a complex with anti-tau antibody, which is an important underlying mechanism of action for the tau-targeted immunotherapy research. Various *in vitro* studies demonstrated how anti-tau antibodies significantly potentiate uptake and degradation

of pathological tau *via* an Fc-dependent manner in BV2 microglia-like cells (Funk et al. 2015), primary mouse microglia (Luo et al. 2015; Andersson et al. 2019) or primary human microglia (Zilkova et al. 2020). Fc effector function is essential for the antibody-enhanced internalization and degradation of tau by microglia (Fig. 1). Tau-targeted immunotherapies with effector function may effectively suppress tau pathology by inducing tau clearance mechanisms. Selected vaccines, AADvac1 and Lu AF87908, which are being tested in human clinical trials for treatment of AD, have been reported to have this effector function in preclinical research. On the other hand, the rationale for vaccines RO 7105705 and ABBV-8E12 with reduced effector function is

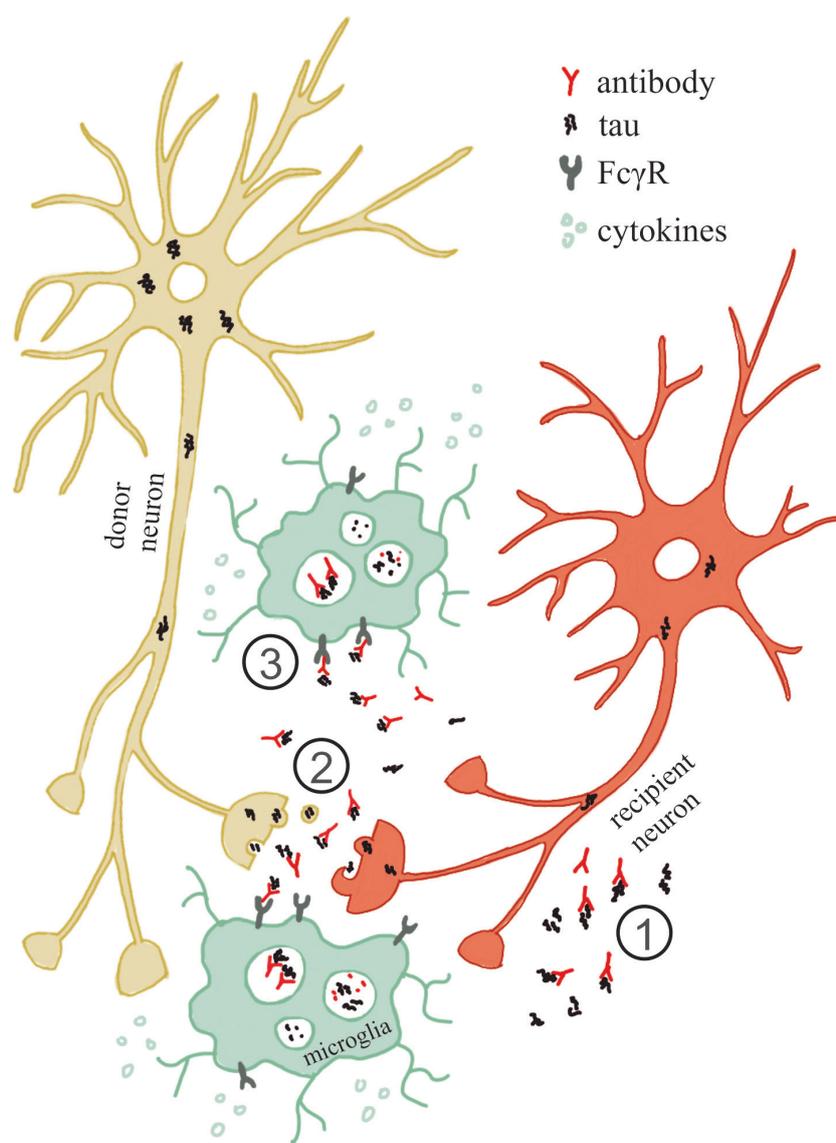


Figure 1. Mechanism of action of anti-tau therapeutic antibody: (1) tau-tau interaction inhibition, (2) tau spreading inhibition, (3) promotion of tau phagocytosis by microglia.

Table 1. Summary of ongoing and completed tau-targeted immunotherapies for dementia

Name of antibody	Company	Type, isotype	Tau targeting epitope	Mechanism of action	The role of microglia	Outcome of development
<i>Active immunization</i>						
AADvac1	Axon Neuroscience	KDNIKH-VPGGGS peptide linked to KLH	aa294-305	blocking the aggregation of tauons / spread of tau pathology, stimulation of antibody-dependent removal by microglia (Kontsekova et al. 2014a; 2014b; Zilkova et al. 2020)	full effector IgG1 anti-tau antibody induced tau uptake by microglia but did not promote pro-inflammatory activity (Zilkova et al. 2020)	completed 2-year phase 2 trial in patients with mild AD (NCT02579252)
ACI-35	AC Immune, Janssen	liposomal anti-pS396/pS404 Tau vaccine	aa393-408	reduction of soluble phosphorylated tau (Tau pS396/pS404) and insoluble aggregated tau levels (Theunis et al. 2013)	no signs of inflammation/microgliosis in the brain of vaccinated Tau.P301L mice (Theunis et al. 2013)	ongoing phase Ib/2a with re-designed version ACI-35.030 in subjects with early AD (NCT04445831)
<i>Passive immunization</i>						
LY303560 / Zagotenemab	Eli Lilly	undisclosed	aa7-9; 313-322	neutralization of tau aggregates	undisclosed	phase 2 trial in participants with early AD is ongoing (NCT03518073)
RO 7105705 / Semorinemab	AC Immune, Genentech	IgG4	N-terminus, extracellular Tau	blocking of cell-to-cell spread of tauopathy (Lee et al. 2016)	effectorless antibody suppressed aberrant microglial activation (Lee et al. 2016)	phase 2 trial did not meet primary efficacy endpoint in subjects with moderate AD (NCT03289143); another phase 2 study (NCT03828747) in subjects with probable/moderate AD is ongoing
BIIB092 / Gosuranemab	Biogen	IgG4	N-terminally fragmented forms of extracellular Tau	blocking of tau seeding	effectorless antibody	phase 2 trial in subjects MCI and mild AD is ongoing (NCT03352557); discontinued phase 2 trial in patients with PSP (NCT03068468)
ABBV-8E12 / C2N 8E12 / Tilavonemab	Abbvie	IgG4	aa25-30, extracellular Tau	inhibition of cell-to-cell spread of tauopathy (Yanamandra et al. 2015)	effectorless antibody; reduced microglial activation in brains of P301S mice (Yanamandra et al. 2013)	phase 2 trial extended for long-term safety follow-up of subjects with early AD (NCT02880956); discontinued phase 2 trial in patients with PSP (NCT02494024)
BIIB076 / NI-105	Biogen	IgG1	undisclosed	uptake by neurons, anti-aggregation	undisclosed	undisclosed results of phase 1 trial (NCT03056729)
UCB0107 / Bepranemab	UCB Biopharma	IgG4	aa235-250	blocking of intercellular spreading of tauopathy / tau seeding (Courade et al. 2018; Albert et al. 2019)	undisclosed	phase 1 trials are ongoing (NCT03605082 and NCT03464227)

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Table 1. (continued)

Name of antibody	Company	Type, isotype	Tau targeting epitope	Mechanism of action	The role of microglia	Outcome of development
JNJ-63733657	Johnson & Johnson	undisclosed	central region, exact epitope undisclosed	targeting seeding-capable tau	undisclosed	two separate phase 1 trials are ongoing (NCT03375697, NCT03689153)
Lu AF87908	H. Lundbeck A/S	IgG1	aa386-408, pS396, pS404	blocking of tau seeding; tau clearance by microglia (Rosenqvist et al. 2018; Andersson et al. 2019)	IgG1 antibody mediated internalization and clearance of pathological insoluble tau by primary mouse microglia (Andersson et al. 2019)	phase 1 trial is ongoing (NCT04149860)
PNT001	Pinteon Therapeutics	undisclosed	cis-pT231 Tau	neutralization and disruption of highly neurotoxic cis-pT231 tau form spread (Albayram et al. 2017)	undisclosed	phase 1 trial is ongoing (NCT04096287)

AD, Alzheimer's disease; MCI, mild cognitive impairment; PSP, progressive supranuclear palsy.

to avoid potentially harmful microglial activation (Novak et al. 2018). Further details of ongoing clinical trials of tau-targeting immunotherapies are described in Table 1.

AADvac1

AADvac1 is one of the only two active anti-tau vaccines that have been in clinical development to date. The aim of AADvac1 active immunization is to induce antibodies that block the spread of tau pathology by immobilizing “tauons” and opsonizing them for removal by the immune system, specifically by microglia (Kontsekova et al. 2014a, 2014b; Novak et al. 2017). Selection of mouse monoclonal antibody DC8E8 preceded AADVac1 vaccine development. Preclinical research has demonstrated that DC8E8 antibody is not internalized by neurons, but it effectively blocked neuronal uptake of pathological tau seeds, which is an important mechanism to prevent spreading of tau pathology (Weisova et al. 2019). DC8E8 and its humanized version AX004, promoted phagocytosis of oligomerized truncated tau_{151-391/4R} by human primary microglia isolated from post-mortem brains with dementia via Fcγ-dependent mechanisms, whereas antibody-tau complexes did not exacerbate pro-inflammatory activity of post-mortem isolated human microglia from AD and non-AD brains (Zilkova et al. 2020). Moreover, successfully completed phase 1 and 2 of AADvac1 clinical trials confirmed safety and effectivity of tau immunotherapy with AADvac1 vaccine in subjects with early/mild AD (see Table 1 for further details) and showed promising results by improving the biomarkers of neurodegeneration and tau pathology (Novak et al. 2021).

Lu AF87908

Andersson and colleagues demonstrated that FcγR binding is necessary for IgG1 antibody-mediated internalization of pathological insoluble tau by primary mouse microglia (Andersson et al. 2019). Consequent clearance of tau was prevented by pharmacological inhibition of lysosomal acidification (chloroquine/bafilomycin A1). Inhibition of ubiquitin-proteasomal degradation seemed not to abolish the clearance of antibody-bound tau in microglia. This pS396-tau binding antibody reduced seeding of human tau derived from AD brain *in vitro* in mouse cortical neurons and *in vivo* in transgenic rTg4510 mouse model (Rosenqvist et al. 2018), indicating that it neutralizes seed-prone pathological tau. Humanized version of pS396-tau antibody, Lu AF 87908, is being currently investigated in phase 1 clinical trial for safety and tolerability profile (Table 1).

RO 7105705

RO 7105705 is a passive vaccine containing anti-tau IgG4 humanized antibody that targets extracellular tau (eTau) on

the N-terminus on all six human tau isoforms, regardless of its monomeric/oligomeric or phosphorylation status. In preclinical research, effectorless antibody directed against the phospho-tau epitope effectively prevented accumulation of tau in neuronal culture without inducing microglial activation and pro-inflammatory cytokine release (Lee et al. 2016). RO 7105705 vaccine is currently in the phase 2b trial testing in moderate AD, first phase 2 study did not meet primary endpoint in subjects with prodromal to mild AD (Table 1).

Tilavonemab/C2N 8E12/ABBV-8E12

8E12 is a humanized IgG4 antibody with an aim to target aggregated, pathological eTau's epitope aa25-30 on N-terminus. This antibody acts in extracellular space and blocks pathological tau cell-to-cell spreading *in vitro* (Kfoury et al. 2012). Furthermore, preclinical research in transgenic P301S mice expressing human mutant tau demonstrated that 8E12 reduces microglial activation, neurofibrillary pathology, brain atrophy and deficits in the conditioned fear response (Yanamandra et al. 2013; Yanamandra et al. 2015). Effects of 8E12 immunotherapy are currently being investigated in phase 2 clinical trial in people with early AD (Table 1).

Summary and conclusion

Based on the observations from ongoing tau immunotherapies, all anti-tau therapeutic antibodies are aimed to bind abnormal tau proteins, eliminate their toxic functions, prevent their neuronal internalization and/or block intercellular spreading of pathogenic aggregated tau. These antibody properties are independent of the effector function of the antibody. Assuming that an effective therapeutic antibody should eliminate pathological tau proteins from the diseased brain, the effector function of such antibody becomes desirable. Our preclinical data with therapeutic anti-tau antibody AX004 indicate that IgG1 antibodies (with effector function) are more effective in facilitating the uptake of extracellular abnormal tau by adult human microglia than the IgG4 isotype (Zilkova et al. 2020). Moreover, no safety signals have been observed throughout the course of the AADvac1 phase 1 and phase 2 clinical trials, although AADvac1 vaccination generated predominantly IgG1 antibody response in AD patients (Novak et al. 2018). Our data suggest that IgG1 isotype is better suited for therapeutic development.

Microglia are considered the key players in the pathogenesis of AD and other tauopathies and their activation might be both beneficial and detrimental for the surrounding cells. Microglia displayed only modest phagocytic capacity for pathologic extracellular tau oligomers and this can be further affected by aging and tau pathology progression. Current re-

sults from tau immunotherapy show that phagocytic potential of microglia can be accelerated by assistance of therapeutic anti-tau antibodies and indicate the important contribution of microglia to the clearance of tau pathology from AD brains.

Future directions

These findings highlight an important role for microglia in AD progression in both positive and negative ways. It appears that with adequate response to AD immunotherapy, microglia could serve as beneficial effectors of therapeutic antibodies, since they are the primary cell type in the brain to mediate Fc receptor-facilitated antibody effector function. If the potential beneficial effects of microglia in AD immunotherapy are meant to outweigh the risk of overly-activated harmful microglia, further work needs to address: (1) how to reprogram diseased microglia to promote their homeostatic functions, (2) how to enhance microglial clearance of tau and A β aggregates, (3) the complex microglial interaction with neighboring cells in diseased areas of the brain, and (4) the better understanding and functional characterization of diverse populations of microglia in the brain. Further research is also needed to elucidate the mechanistic pathways involved in metabolic de-regulation in microglia, and how modulation of these immuno-metabolic impairments in microglia may ultimately benefit the patients.

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