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Immune Activation in Alzheimer Disease

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Abstract

Alzheimer disease (AD) is the most common neurodegenerative disease, and with no efficient curative treatment available, its medical, social, and economic burdens are expected to dramatically increase. AD is historically characterized by amyloid β (Aβ) plaques and tau neurofibrillary tangles, but over the last 25 years chronic immune activation has been identified as an important factor contributing to AD pathogenesis. In this article, we review recent and important advances in our understanding of the significance of immune activation in the development of AD. We describe how brain-resident macrophages, the microglia, are able to detect Aβ species and be activated, as well as the consequences of activated microglia in AD pathogenesis. We discuss transcriptional changes of microglia in AD, their unique heterogeneity in humans, and emerging strategies to study human microglia. Finally, we expose, beyond Aβ and microglia, the role of peripheral signals and different cell types in immune activation.

1. INTRODUCTION

Alzheimer disease (AD) is the leading dementia-causing neurodegenerative disease worldwide, directly affecting approximately 50 million people, and this number is expected to increase threefold by 2050 (1). Indirectly, AD also heavily affects patients' families, as well as health care and social systems. Clinically, AD is characterized by progressive cognitive impairment, memory decline, sleep disorders, mood change, and loss of activities of daily living. Molecularly, the classical markers of AD are neuronal accumulation of abnormally hyperphosphorylated tau (p-tau) protein, extracellular accumulation of amyloid beta (Aβ) into senile plaques, synaptic defects such as synapse loss, and neuronal death leading to extensive brain atrophy. One of the first and most well-known paradigms to explain the development of the pathology was the amyloid cascade hypothesis, according to which Aβ acts upstream and is the cause of the defects found in AD (2). Decades of clinical trials based mostly on this hypothesis have led to the development of several therapies that slow down the cognitive decline of patients but not to a definitive cure, thus underlining the importance of better understanding the disease to find new treatments. Accumulating evidence highlights and describes the involvement of neuroinflammation in the pathophysiology of AD (3). For example, while most AD cases (sporadic and with late-onset) result from a complex interaction between genetic and environmental factors, immune response pathways are a major class of risk factors identified by genome-wide association studies (GWAS) (4).

Neuroinflammation is a physiological response to a stimulus, such as infection, brain injury, stress, or aging, via the activation of brain-resident innate immune macrophages, the microglia, and astrocytes (5). When the initial stimulus is gone, the inflammatory response resolves, but in AD, this neuroinflammation is maintained over time, hence becoming chronic. Chronic neuroinflammation is pathological, as the sustained release of proinflammatory molecules, such as complement factors and cytokines, leads to synaptic damages, neurodegeneration, and ultimately to cognitive deficits (6). Microglia play a complex role in AD, since they participate in the clearance of Aβ but are also the predominant cells that chronically secrete these neurotoxic molecules.

As a first line of defense, microglia constantly scan and survey the brain environment and can detect the pathological triggers owing to their pattern recognition receptors, which recognize damage- or pathogen-associated molecular patterns (DAMPs/PAMPs). Aβ depositions are recognized by these receptors and initiate the activation of microglia, which in turn secrete various proinflammatory molecules, such as cytokines and complement components, hence amplifying the inflammation. Chronic neuroinflammation has recently been under intense scrutiny, as it is involved in various phenotypes characterizing AD, such as the development and spread of tau pathology, neurotoxic astrocyte activation, and neuron dysfunctions and death.

This article provides an overview of the most recent knowledge on the activation mechanisms of microglia in AD and the pleiotropic effects of activated microglia in the disease, opening the way to new therapeutic strategies. Given the importance of microglia, new emerging in vitro and in vivo models to analyze these cells in a more relevant and complex environment have been developed and are described here. Finally, the impact of the microbiota, peripheral immune activation, and nonmicroglia brain cells, such as infiltrating immune cells and astrocytes, on neuroinflammation is also reported.

2. MICROGLIA ACTIVATION IN ALZHEIMER DISEASE

2.1. CD33

The CD33 gene encodes sialic acid–binding immunoglobulin-like lectin 3 (Siglec3), a transmembrane glycoprotein expressed on the surface of myeloid progenitor cells, mature monocytes, and macrophages. GWAS have identified CD33 as an important genetic locus associated with

late-onset AD (7). The rs3865444^{C} AD risk allele, also called the major allele, located in the promoter region is characterized by elevated monocyte cell surface expression of CD33 but reduced $A\beta_{1–42}$ uptake (8). In humans, mutant carriers showed an increase in activated microglia, but amyloid pathology was increased too (9). In vitro, CD33 seemed to inhibit $A\beta_{1–42}$ uptake and clearance, and conversely, CD33 knockout in APP/PS1 or 5xFAD mice reduced amyloid pathology (8, 10). APP/PS1 is a mouse model with amyloid deposition beginning at 6 months of age, gliosis, and cognitive decline at around 9 months, based on the overexpression of two familial mutations (APP Swedish and PS1dE9). 5xFAD is an amyloid pathology model and is significantly more extreme than the APP/PS1 model, as it results from the overexpression of three familial mutations in the *APP* gene (Swedish, Florida, and London mutations) and two in the *PSEN1* gene (M146L and L286V), resulting in accelerated plaque formation, strong neuroinflammation, cognitive deficits, and neuronal loss (absent in the APP/PS1 model). We refer the reader to comprehensive reviews that describe and compare AD mouse models (11, 12) and to Alzforum (**https://www.alzforum.org/research-models/alzheimers-disease**). A recent GWAS reported that elevated CD33 mRNA and protein levels in blood, as well as CD33 expression on peripheral immune cells, were associated with an elevated risk of AD but that AD does not seem to be the cause of these increases (13). These results support an increase in expression rather than a decrease in degradation of CD33 in AD, but they also support the idea that CD33 can be a peripheral biomarker and a relevant therapeutic target. Indeed, CD33 gene therapy knockdown in the APP/PS1 mouse brain reduced neuroinflammation and Aβ accumulation (14). CD33 can be spliced into a long isoform (CD33M) or a short isoform (CD33m) devoid of the sialic acid–binding site. One AD protective allele, the minor T allele of rs12459419 located in exon 2, led to enhanced exon 2 skipping and thus to increased CD33m levels (15). Whereas the CD33M variant inhibits Aβ phagocytosis by microglia, CD33m is a gain-of-function variant that boosts Aβ phagocytosis (16, 17). CD33 acts upstream of, and opposite to, triggering receptor expressed on myeloid cells 2 (TREM2), suggesting that reduced microglial amyloid clearance could be rescued by therapeutic inhibition of CD33 or activation of TREM2 (10).

2.2. TREM2/DAP12 Complex and Signaling

TREM2 is a risk factor for late-onset sporadic AD (18, 19). In the APP/PS1 model, TREM2 knockout or the expression of the loss-of-function AD variant R47H caused a loss of plaque-associated microglia (PAM), increased neuritic dystrophy, and facilitated the seeding and spreading of neuritic plaque tau aggregates (20). In 2017, via single-cell RNA-sequencing (scRNA-seq) transcriptomic analysis of immune cells from the 5xFAD mouse brain, Keren-Shaul et al. (21) identified a protective and AD-specific microglia subtype: disease-associated microglia (DAM). DAM were found around plaques of this model and acquire their identity in two steps: The first step is TREM2-independent upregulation of Tyrobp and Apoe and downregulation of microglia checkpoint genes such as *Cx3cr1*; the second step occurs via a TREM2-dependent increase in lipid metabolism and phagocytosis. When activated, TREM2 signals through its associated immunoreceptor tyrosine-based activation motif (ITAM)-containing adapter protein DAP12, which recruits and transmits intracellular signals through the protein tyrosine kinase SYK (22). The R47H variant is unable to activate microglia via SYK (23), a loss of function that likely contributes to the observed increased risk. In addition, soluble TREM2 (a marker of TREM2 microglial activity) levels are elevated in the cerebrospinal fluid (CSF) of subjects with pathological p-tau and total tau levels, even in the predementia stages, which is associated with a reduced increase in amyloid PET and reduced tau PET, reinforcing the idea of a protective effect of TREM2 in AD (24, 25). One transcription factor that binds to the promoter of the TREM2 gene and promotes its transcription is Yin Yang 1 (YY1), with YY1 and TREM2 levels reduced in the APP/PS45 mouse

brain (26). This double transgenic model originates from crossing APP23 mice (overexpressing the familial Swedish mutation) with PS45 mice (overexpressing the familial G384A *PSEN1* gene mutation) and develops neuritic plaques in the neocortex and hippocampus at 6–8 weeks of age, as well as memory deficits.

2.3. TAM Receptors

Tyrosine-based activation motif (TAM) receptor tyrosine kinases (Tyro3, Axl, MerTK) are involved in phagocytosis engagement and inhibition of inflammation. In normal conditions, human and mouse microglia express a high level of the TAM receptor tyrosine kinase MerTK and low levels of Axl but do not express Tyro3. In vivo, exposure to Aβ plaques resulted in upregulation of Axl and MerTK receptors in PAM, which correlates with the presence of plaque-associated pan-TAM ligand growth arrest specific 6 (Gas6) and the TAM receptor cofactor phosphatidylserine. $Axl^{-/-}/MerTK^{-/-}$ microglia failed to detect, envelop, proliferate, activate, and phagocytose Aβ plaques, which consequently led to the development of fewer dense-core plaques but more diffuse plaques and more deposition around blood vessels (27). These results demonstrate that the TAM system is profoundly involved in the detection and phagocytosis of Aβ plaques by microglia. Activation of the TAM receptors, in turn, resulted in compaction of diffuse plaques into dense-core plaques and protection from cerebral amyloid angiopathy (27). The soluble TAM receptors sAXL and sTyro3, markers of receptor activation, were elevated in the CSF of subjective cognitive decline subjects with elevated levels of tau or neurodegeneration markers and were positively correlated with protection from brain atrophy and cognitive decline (25). Similarly, Gas6 levels were also significantly elevated in AD patients' CSF and inversely correlated with disease duration and a decrease in the mini-mental state examination (MMSE) score (28). Progressive increase in Gas6 mRNA levels in the frontal cortex along with AD progression, correlating with a decrease in the MMSE score, was also reported (29). Altogether, these results support a protective role for the TAM system early in AD. However, a recent study found a situation in which increasing Gas6 levels become detrimental. The overexpression of Gas6 in hippocampal neurons of old APP/PS1 male mice resulted in fewer but bigger amyloid plaques, increased microglial expression of proinflammatory CCL2 and CXCL13, and worsening performance at the contextual fear conditioning test (30). This finding might be due to aberrantly high Gas6 levels and localization, as even in AD, Gas6 is certainly not highly expressed by all neurons at all times and the protein is localized mainly around the plaques. This result could also indicate that Gas6 has a beneficial action within only a specific concentration range.

2.4. CD36/TLR4/TLR6

The class B scavenger receptor CD36 can recognize Aβ and trigger the formation of a CD36-TLR4-TLR6 heterotrimer, leading to microglia activation. Indeed, the TLR4-TLR6 signal through MyD88 to activate NF-κB ultimately leads to microglial secretion of nitric oxide, reactive oxygen species (ROS), and IL-1β (31). The transmembrane domain of TLR4 and TLR6 is crucial for their dimerization, and blocking this interaction inhibits microglia activation and neuronal death (32). TREM2 promoted microglial Aβ phagocytosis by upregulating CD36 via the transcription factor CCAAT/enhancer-binding protein alpha (C/EBPα) (33). In macrophages in vitro, CD36 is pivotal to the uptake of soluble Aβ, and the activation of the NLRP3 inflammasome, following Aβ-induced lysosomal damage and leakage (34).

2.5. RAGE

The receptor for advanced glycation end products (RAGE) is an Aβ receptor that activates microglia (NF-κB activation, TNF-α production, and chemotaxis). Moreover, its expression was

increased in microglia, neurons, and cerebral vessels of the AD brain (35). Pharmacologically blocking the binding of Aβ on RAGE decreased in vivo TNF-α, IL-1β, and IL-6 levels in the cortex and hippocampus, as well as the amyloid burden (36). More recently, further details about the role of RAGE in Aβ-mediated microglia activation were uncovered. The RAGE–TXNIP (thioredoxin-interacting protein) axis induced the transport of Aβ from the cell surface to mitochondria, where it caused mitochondrial dysfunction and led to enhanced mitochondrial fission and depolarization, and increased ROS production. Ultimately, mitochondrial dysfunctions caused activation of the NLRP3 inflammasome, cleavage of gasdermin D, and secretion of cytokines (37). Moreover, RAGE is also a tau oligomer receptor and is involved in tau uptake and modulation of IL-1β secretion (38).

2.6. CD40/CD40L

$A\beta_{1–42}$ and $A\beta_{1–40}$ induce the expression of the receptor CD40 in primary microglia and on microglia of APPswe transgenic mice. This mouse model overexpresses the familial AD Swedish double mutation (KM670/671NL), developing Aβ deposition beginning at 6 months of age, with strong gliosis and neuronal loss around plaques, as well as cognitive decline even 3 months prior to amyloid deposition. Adding CD40 ligand (CD40L) to Aβ synergistically increases TNF-α production and neuronal injury. Consequently, APPswe/CD40L$^{-/-}$ mice displayed reduced microglia and astroglia activation but also reduced amyloid burden and tau phosphorylation (39, 40). In the APP/PS1 mouse model, use of an anti-CD40L antibody was sufficient to reduce amyloid pathology (40). In addition, APP/PS1 mice deficient for the receptor CD40 also displayed reduced microgliosis, astrogliosis, and amyloid pathology, demonstrating the participation of CD40 signaling in AD (41). In the 5xFAD mouse model, CD40 levels were increased in the cortex and hippocampus, and in vitro, overexpression of CD40 led to the activation of the NF-κB signaling pathway and to more tau aggregation (42).

2.7. Microglia Receptor Recycling via LC3-Associated Endocytosis

After binding to Aβ, microglia receptors are internalized in the cytoplasm through endocytosis, where Aβ can be degraded and the receptor can be recycled back to the plasma membrane for a new uptake of Aβ. A newly identified process, microglial LC3-associated endocytosis (LANDO), was found to have a protective role against Aβ-mediated neuroinflammation. In microglia, ATG5 and Rubicon recruit LC3 to clathrin^{+}, Rab5^{+}, and Aβ-containing endosomes (43). Crucially, LANDO participates in recycling internalized Aβ receptors such as TREM2, TLR4, and CD36 back to the plasma membrane, more specifically, returning receptors to the plasma membrane after their internalization in endosomes. 5xFAD mice lacking LANDO but not autophagy in microglia displayed enhanced proinflammatory microglia activation, Aβ, and p-tau levels, together with increased neurodegeneration and memory impairments (43). A striking result was that deletion of the WD domain of the autophagy protein Atg16L in wild-type mice was sufficient to cause endogenous and spontaneous Aβ deposition, neuroinflammation, tau hyperphosphorylation, and neurodegeneration (44). The WD domain is necessary for LC3 lipidation at single membranes and, consequently, for LANDO-dependent recycling of microglia TREM2, TLR4, and CD36 Aβ receptors (44). In this new model of spontaneous AD, neuroinflammation seemed to be the key to the establishment and progression of the pathology, as treatment with the MCC950 inflammasome inhibitor reduced tau hyperphosphorylation and neurodegeneration but not amyloid pathology (44). This result once again supports the idea that Aβ pathology lies upstream and that neuroinflammation is a link between Aβ and tau pathology. Additionally, LANDO might also be an important regulator of Aβ-induced inflammation in the human brain, as Atg16L, ATG5, and Rubicon were downregulated in AD patients (44).

2.8. NLRP3 Inflammasome

Ligation of a pattern recognition receptor and initiation of the gene transcription of cytokine proforms and the autoinhibited NLRP3 are the initial steps of NLRP3 inflammasome activation by Aβ fibrils, which are recognized as a danger signal by the microglia. Subsequent steps of inflammasome licensing such as structural damage of lysosomes followed by the release of the lysosomal protease cathepsin B (CatB) are needed for the maturation and activation of the NLRP3 inflammasome (45). The NLRP3 inflammasome is strongly activated in AD and likely contributes to disease pathogenesis, as APP/PS1/NLRP3$^{-/-}$ mice were protected from caspase-1 cleavage, excessive IL-1β secretion, amyloid deposition, synaptic dysfunction, and spatial memory deficits (46). Activation of the NLRP3 inflammasome in microglia also mediates fibrillar Aβ-induced tau pathology in neurons via secretion of IL-1β and regulation of tau kinases and phosphatases (47). Tau monomers and oligomers act as strong DAMPs and can activate the NLRP3–ASC inflammasome and cause IL-1β secretion (47). Like Aβ, aggregated tau seeds are taken up by microglia and directed to lysosomes, where they cause structural damage and subsequent CatB release, leading to NLRP3–ASC inflammasome activation (47, 48). Hence, pharmacological treatment of tau mutant mice with the NLRP3 inhibitor MCC950 abolished the seeding of exogenous tau (48).

2.9. NLRP1, NLRC4, and AIM2 Inflammasomes

Although much less documented, other inflammasomes appear to also contribute to AD progression. Unlike NLRP3, which is highly expressed in microglia, NLRP1 is expressed mainly in neurons. Four nonsynonymous polymorphisms of NLRP1 have been identified as risk factors for AD (49). In APP/PS1 mice in particular, NLRP1 is upregulated and its silencing could reduce neuronal pyroptosis and cognitive defects (50). NLRP1-positive neurons are enriched in AD brains and the NLRP1 inflammasome can activate caspase-1 and subsequently caspase-6, thus participating in neuronal stress and the $A\beta_{42}$ ratio increase (51). Moreover, as J20 mice [a model that overexpresses the Swedish and Indiana (V717F) familial AD mutations in the *APP* gene, causing amyloid deposition beginning at 5–7 months of age followed by gliosis, neuronal loss, and memory deficits] in an *Nlrp1*-, *Casp1*-, or *Casp6*-null background improved in their neuroinflammation, amyloidogenesis, and cognitive impairments, the proteins coded by these three genes became new promising therapeutic targets for AD (52). NLRC4 mRNA and protein levels were increased in neocortical brain samples of AD patients (53). Absent in melanoma 2 (AIM2) expression was increased in the brain of AD patients and APP/PS1 mice (54). AIM2 expression was also increased in the 5xFAD mouse brain, and its ablation could reduce Aβ deposition and the Iba1 microglia signal area (55). AIM2 knockout also resulted in increased IL-6 and IL-18 levels and in a failure to rescue cognitive dysfunctions (55), suggesting that different types of inflammasomes could be involved in separate aspects of the disease.

2.10. ASC Specks

Following the NLRP3 activation, the adaptor protein apoptosis-associated speck-like protein containing a C-terminal caspase recruitment domain (ASC) is recruited to form ASC helical fibrils specks, which recruit and activate caspase-1 thereafter. Ultimately, persistent inflammasome activation or hyperinflammation results in pyroptosis, an inflammatory cell death, and consequently in the extracellular release of ASC specks, which eventually spreads inflammasome activation to neighboring cells (56, 57). Exposure to ASC specks stimulates the release of both matrix-metalloproteinase 10 (MMP10) and MMP3 by primary microglia, of which MMP10 can then propagate the neuroinflammation to adjacent microglia by stimulating their release of proinflammatory IL-6, TNF-α, and CXL1 (58). Importantly, ASC specks released into the extracellular

space also rapidly bound to both $A\beta_{1-42}$ and $A\beta_{1-40}$, acting as a cross-seed and accelerating amyloid oligomerization and aggregation. In vivo, even at an early stage, ASC specks can be detected in the core of Aβ plaques. Injecting APP/PS1 brain homogenates or ASC specks into the APP/PS1 mouse brain increases the number of Aβ deposits and total area of Aβ, an effect absent in injected APP/PS1/ASC$^{-/-}$ mice, which already constitutively display improved memory performance (59). Coapplication of anti-ASC antibody with the APP/PS1 brain homogenates in APP/PS1 mice blocked the spread of Aβ pathology, demonstrating that NLRP3 inflammasome activation participates in the seeding and spreading of Aβ pathology through ASC speck release and could thus represent a novel therapeutic target. Moreover, ASC deficiency also inhibited tau pathology in tauP301S (PS19) mice (48). This mouse model of tauopathy is due to the expression of the human Tau protein with the P301S mutation that causes early-onset frontotemporal dementia in humans. This model displays microgliosis at 3 months of age, neurofibrillary tangle-like inclusions at 6 months, and neurodegeneration at 8 months, but no amyloid pathology. Additionally, ASC might be considered and used as an AD biomarker, as its levels were elevated in the serum of AD patients, and even more so in patients with mild cognitive impairment, suggesting that it might even be an early biomarker (60).

2.11. Caspase-1

Ultimately, caspase-1 activity is the effector of inflammasome activation. Caspase-1 deletion in APP/PS1 or J20 mice lowered Aβ levels and improved synaptic function and neurobehavioral deficits (46, 61). Treatment of symptomatic J20 AD mice with the nontoxic blood-brain barrier (BBB)-permeable small-molecule caspase-1 inhibitor VX-765 prevents Aβ accumulation, inflammation, synapse loss, and memory deficits (61). The effects of VX-765 on memory function are reversible, as cessation of treatment led to reappearance of the deficits. Presymptomatic treatment of J20 mice delayed microglia activation and memory deficits (62). Selective inhibition of caspase-1 ameliorated spatial learning and memory impairments and reduced tau hyperphosphorylation in the brain of accelerated aging (SAMP8) mice at early stages (63).

2.12. Metabolic Reprogramming

Nonactivated microglia rely mostly on mitochondrial respiration for their energy production. Monomeric Aβ (mAβ), oligomeric Aβ (oAβ), or fibrillary Aβ (fAβ) exposure provokes acute microglia activation, characterized by rapid metabolic reprogramming from oxidative phosphorylation (OXPHOS) to aerobic glycolysis (Warburg effect) via the upregulation of the mammalian target of rapamycin (mTOR)–hypoxia-inducible factor-1α (HIF-1α) pathway. Glycolysis generates less ATP per unit of glucose metabolized than does OXPHOS but metabolizes glucose more quickly, which fits the sudden increase in energy demand by activating microglia. Accordingly, inhibiting glycolysis in mice treated with $A\beta_{1-42}$ reduced release of proinflammatory cytokines in the hippocampus (64). However, chronic exposure to Aβ led to a downregulation of mTOR–HIF-1α signaling, ultimately decreasing TNF-α release and phagocytic activity (65). This state of immune tolerance in AD is not irreversible, as a metabolic boost (via treatment with inflammatory mTOR-activating IFN-γ) administered systematically in 5xFAD mice still bolstered microglia activation and recruitment to Aβ plaques and ultimately their phagocytosis (65). Defective mTOR signaling has another deleterious consequence: exacerbated autophagy. TREM2 promotes homeostasis via mTOR signaling, but in 5xFAD/Trem2$^{-/-}$ mice, microglia featured decreased mTOR activation and thus less expression of genes encoding translation initiation factors, ribosomal proteins, glucose transporters, glycolytic enzymes, and HIF-1α. The resulting cellular stress resulted in aberrant autophagy, which was insufficient to avoid apoptosis. Yet energy supplementation with

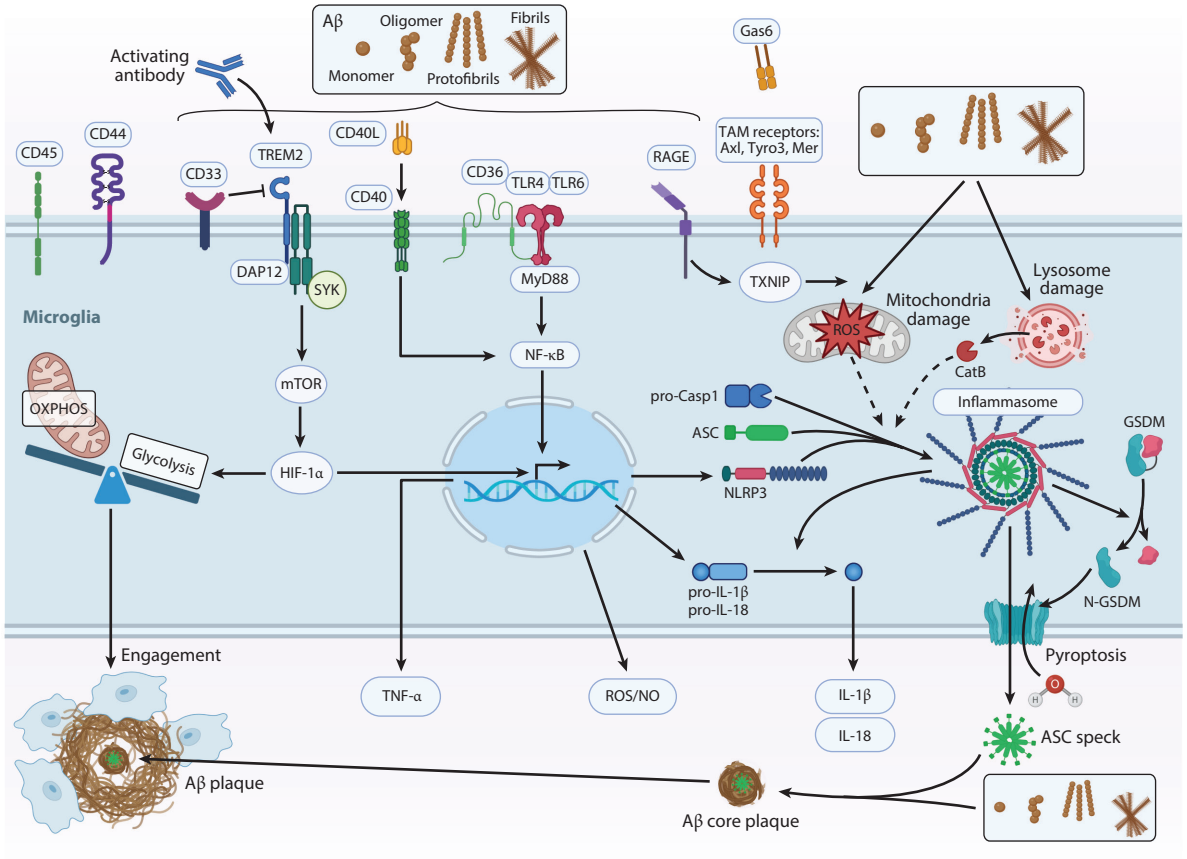

Figure 1

Microglia activation in AD. Various Aβ species can be detected and can activate microglia via several cell receptors (CD33, TREM2, CD40, CD36/TLR4/6, and RAGE). TREM2, through DAP12/SYK, activates the mTOR–HIF-1α signaling pathway, leading to secretion of TNF-α and a boost of glycolysis. CD40 and CD36/TLR4/6 end up activating the NF-κB transcription factor, resulting in the expression of NLRP3, pro-IL-1β, and pro-IL-18, as well as the secretion of ROS/NOS. Internalized Aβ damages mitochondria and lysosomes, leading to the release of ROS and CatB acting as the activation signal for the formation of the NLRP3 inflammasome. Inflammasome activation leads to the activation of pro-caspase-1; cleavage of pro-IL-1β, pro-IL-18, and GSDM; and release of ASC specks. N-GSDM can form pores in the plasma membrane, causing the pyroptotic death of microglia. Extracellular ASC specks can cross-seed with Aβ to accelerate its aggregation into plaques. Abbreviations: Aβ, amyloid beta; AD, Alzheimer disease; ASC, apoptosis-associated speck-like protein containing a C-terminal caspase recruitment domain; CatB, cathepsin B; Gas6, growth arrest specific 6; GSDM, gasdermin D; HIF-1α, hypoxia-inducible factor-1α; mTOR, mammalian target of rapamycin; N-GSDM, N-terminal GSDM; NO, nitric oxide; OXPHOS, oxidative phosphorylation; RAGE, receptor for advanced glycation end products; ROS, reactive oxygen species; TAM, tyrosine-based activation motif; TLR, Toll-like receptor; TXNIP, thioredoxin-interacting protein.

cyclocreatine rescued defective mTOR signaling (66). Use of a TREM2-activating antibody was sufficient to rescue mTOR signaling and boost both glycolysis and OXPHOS, thus restoring microglia activation, proliferation, and phagocytosis (67). Similarly, treating APP/PS1 mice with capsaicin, through its TRPV1 receptor, reduced the OXPHOS and glycolysis impairments, decreased the expression of IL-6 and TNF-α, but increased the expression of anti-inflammatory IL-10 (68). Whereas acute Aβ exposure provokes a shift toward glycolysis, chronic exposure downregulates both OXPHOS and aerobic glycolysis (**Figure 1**).

2.13. Microglia Transcriptional Changes in Alzheimer Disease

In 2017, scRNA-seq of brain CD45^{+} immune cells from 5xFAD mice allowed for the discovery of DAM, a new subtype of microglia specific to AD and other neurodegenerative diseases (21). Primarily during the early phase of the disease, microglia begin to switch from a homeostatic state to DAM through a first transition step that is *Trem2* independent and involves downregulating the expression of several homeostatic genes and upregulating *Apoe*. The second step to reach the full activation of DAM occurs primarily during advanced stages and is *Trem2* dependent (21). DAM are localized in close proximity to Aβ plaques and are actively phagocytic, suggesting that DAM might be a beneficial microglia subtype with the potential to restrict AD pathology. But this discovery did not answer whether DAM are beneficial in the long term or whether they are beneficial only during the early stages, and because the initial stimuli fail to be eliminated, DAM turn toxic during the later stages. This issue was later clarified with the finding of two subpopulations of microglia within DAM: proinflammatory DAM (piDAM) and anti-inflammatory DAM (aiDAM) (69). piDAM arise earlier, are characterized by higher expression of CD44, CD45, and $K_v1.3$ channels, and are regulated by NF-κB, while aiDAM are characterized by CXCR4 expression and regulated by LXRα/β (69). Treatments that inhibit piDAM or that promote aiDAM resulted in Aβ clearance (69). These results suggest that not all DAM are detrimental and that it would be promising to design a treatment that specifically tips the balance toward aiDAM in AD. Changes induced specifically in microglia close to Aβ plaques were also analyzed by spatial transcriptomics assays to measure in situ hundreds of small brain subdomains in $App^{NL\text{-}G\text{-}F}$ mice (70). Unlike APP/PS1 or 5xFAD mice, this mouse model relies not on overexpression but on the knock-in of a humanized *APP* gene with three familial mutations [Swedish, Arctic (E693G), and Iberian (I716F)], leading to cortical Aβ depositions, by 2 months of age, with gliosis and synapse loss around plaques but not neuronal loss. The plaque-induced genes are mainly microglial and also astrocytic, and they include the gene encoding C1qa and APOE (70). The mechanisms that control microglial gene expression and how they switch to a pathological state in AD are also a crucial point that remains to be better described. The transcription factor C/EBPβ is a pivotal master regulator of the microglia proinflammation state and downstream neurodegeneration. In the absence of a stimulus, C/EBPβ is kept in check by the ubiquitin ligase COP1 through the promotion of its proteasomal degradation (71). But upon immune challenge, like lipopolysaccharide (LPS), COP1 is inactivated, allowing C/EBPβ to promote proinflammatory gene expression. COP1-depleted microglia also display neurotoxic properties that are C1q dependent (71). A recent multiomics analysis of human AD brains found that the levels of C/EBPβ protein increased and the levels of COP1 decreased (72), suggesting that a lift of COP1 inhibition might participate in AD pathology. It is worth noting that gut dysbiosis has been associated with activation of C/EBPβ in the AD mouse brain, with antibiotic treatment alleviating the pathology (73).

2.14. Microglial Transcriptome Evolutionary Divergences

Recent scRNA-seq analysis comparison of microglia gene expression in 10 evolutionarily distinct species revealed the existence of a conserved core microglial transcriptional program across all mammals (74). Nevertheless, human microglia displayed several unique properties, such as a significantly higher heterogeneity encompassing multiple subtypes, compared with other species, including nonhuman primates (74). The differences between human microglia and their counterparts in other species were more pronounced when looking at the expression of AD-linked (and Parkinson disease–linked) genes. Importantly, mouse microglia displayed low or no expression of many AD GWAS risk factors, and of the genes with similar expression profiles, some showed significant differences in terms of amino acid sequence similarity (74–76).

2.15. Human Microglia Transcriptomic Heterogeneity in Alzheimer Disease

As detailed above, a significant body of evidence from mouse studies describes the phenotypic alterations of microglia in AD and multiple other neurodegenerative disorders by scRNA-seq (21, 77, 78). In the past few years, several single-nucleus RNA-sequencing (snRNA-seq) studies of microglia in human AD brains have been performed and have yielded variable, sometimes contradictory, observations as to whether an equivalent microglia activation profile is present in the human brain (79–82). The first studies reported a small number of genes differentially expressed in the AD brain. Mathys et al. (80) reported 22 genes upregulated in microglia from AD patients (of which only 5 overlapped with the DAM signature). Grubman et al. (79) reported only 8 genes upregulated in AD, whereas Zhou et al. (81) found only 4 genes upregulated in an snRNA-seq study of AD TREM2 variants. This last study also identified only 11 DAM genes enriched in AD patients compared with controls. Del-Aguila et al. (83) analyzed single-nucleus transcriptomes from three AD patients and were unable to recapitulate an activation signature. However, the most recent and larger studies did show that human microglia display a transcriptomic change that correlates with different AD pathologies. Gerrits et al. (82) sequenced 482,472 nuclei from nondemented control brains and AD brains containing only Aβ plaques or both Aβ plaques and tau pathology and showed that microglia can acquire distinct transcriptomic states in AD of either a more phagocytic (*ITGAX*, *SPP1*) or proinflammatory (*IL1B*, *NFKB1*) nature. Of note, there are several limitations when working with postmortem tissue, including both biological (e.g., having a snapshot of late pathological stages) and technical [e.g., lower intrinsic resolution of single-nucleus technologies compared with single-cell technologies (84) and potential artifacts related to tissue preservation and postmortem intervals (85)].

3. ACTIVATED MICROGLIA IN ALZHEIMER DISEASE PATHOGENESIS

3.1. Microglia Aβ Uptake and Clearance

Microglia participate in the degradation of extracellular Aβ species by secreting proteinases (insulin-degrading enzyme, epinephrine, and metalloprotease-9). But they can also directly interact with different extracellular Aβ species through a variety of receptors mentioned and described above: CD33, TREM2/DAP12, TAMs, CD36, TLR4, TLR6, RAGE, and CD40. These interactions, as well as the amyloid internalization via this receptor-mediated uptake, damage mitochondria and lysosomes and activate microglia through various pathways, such as the NFκB, metabolic reprogramming, and inflammasome activation pathways. But Aβ uptake by microglia does not simply trigger a proinflammatory response; it also participates in the clearance of Aβ, the initial inflammatory stimulus. Indeed, microglia can phagocytose Aβ species and degrade them in lysosomes, and this degradation system, together with the enzymatic degradation and glymphatic clearance of Aβ, works to prevent Aβ plaque formation in the early stages of AD in APP/PS1 mice (86). Nevertheless, the efficacy of the clearing systems gradually diminishes over time, leading to increasing Aβ aggregation, while microglia are still proinflammatory and cause neurotoxicity. This is evidenced by the fact that depletion of microglia after amyloid pathology does not affect amyloid burden but reduces spine and neuronal loss (87). Hence, maintaining the clearing capacity of microglia over time appears to be a promising treatment strategy. Coculturing organotypic brain slices from old APP/PS1 mice with those from young wild-type mice, or just with the young conditioned media, reversed the phagocytic defects of microglia and reduced amyloid deposition (88). Additionally, one mechanism that participates in the decline of microglia degradation capacities is lysosomal acidification defects, but experiments on reacidification of microglia lysosomes have already provided promising results to reverse neurodegeneration (89).

3.2. Microglia Turnover and Proliferation

The in vivo microglia life span and proliferation rate were also open questions that could have been answered by following single, labeled microglia by multiphoton microscopy over time. In nonpathological conditions, neocortical microglia were long-lasting, with a median lifetime above 15 months (90). Proliferation of microglia was low and compensated for the few dead microglia, such that the number of microglia remained stable over the lifetime of the mice (90). In the APP/PS1 model of amyloid pathology, however, non-PAM died only slightly more in the model mice than in the wild-type mice but had a proliferation rate that was three times higher. The rates of death and proliferation of PAM were almost identical, but because the newly generated microglia in plaque-free areas were recruited toward nearby plaques, the number of microglia covering plaques increased over time (90).

3.3. Plaque Engagement Kinetics

The development of in vivo multiphoton microscopy enabled researchers to study the kinetics of plaque growth and the reaction of microglia inside the mouse brain. The results showed that plaques formed surprisingly quickly in the APP/PS1 or PDAPP model (an amyloid pathology model overexpressing APP with the familial AD Indiana mutation, resulting in Aβ deposits at 6–9 months of age and gliosis, dystrophic neurons, and synapse loss) over 24 h and that after this period, they did not change their size, independently of their initial diameter (91). After plaque formation, microglia were attracted to the plaque within 1 day, not to clear the plaque but more likely to restrict its growth (91). In the following days, neuritic dystrophies were increasingly detectable (91). Another article reported that in the APP/PS1 model, microglia rapidly extended their processes to cover the plaque surface within hours (92). Approximately three new microglia per month were recruited around the plaque, independently of the plaque volume, until a threshold was reached, dependent on the volume. Hence, smaller plaques were covered by fewer microglia and displayed modest growth, and larger plaques were covered by more microglia and diminished in size over time (92). These results were obtained with mice, but in regard to the kinetics of plaque formation in the human brain, plaques do not significantly increase in size over the course of the disease (93).

3.4. Tau Spreading and Tau-Mediated Neurodegeneration

One early event in AD is the spread of misfolded abnormally phosphorylated tau protein from the entorhinal cortex to the hippocampal area. Microglia positively correlate with this tau pathology, and this relationship was evidenced by the fact that depletion of microglia inhibited tau propagation in a mouse model of tau propagation (94). Mechanistically, it was shown that microglia participate in tau spreading by secreting exosomes containing p-tau oligomers that could be transferred to neurons, such that inhibition of exosome synthesis reduced tau spreading (94). Genetic ablation of bridging integrator 1 (*Bin1*), a strong locus associated with late-onset AD, led to decreased secretion of tau-enriched extracellular vesicles in vitro and to decreased tau spreading in a male PS19 mouse model of tauopathy (95). Additionally, TREM2 suppressed microglia-mediated tau spreading, as TREM2 knockout promoted tau trafficking after microglia uptake and its exosomal secretion, resulting in the secretion of exosomes that contain more tau and that have increased tau-seeding capacities (96). Crucially, microglia in AD appeared to be the driving actor responsible for Aβ-induced tau pathology, as TREM2 knockout mice featured increased tau accumulation and spreading and atrophy, but only in the presence of amyloid pathology (97). Recently, scRNA-seq of brain parenchyma $CD45^+$ immune cells revealed that mice with tauopathy, but not mice with amyloidopathy, presented an enrichment of specific DAM, with higher expression of IFN-γ, CD11c,

MHC-II, and cytokines (98). Once again, microglia depletion in the P301S/apolipoprotein E isoform 4 (APOE4) (TE4) mouse model, a PS19 model with a knock-in of human APOE4, rescued tau-mediated hippocampal atrophy and increased ventricular volume (98).

3.5. Neurotoxic Activation of Astrocytes

Chronic neuroinflammation causes neurotoxicity in AD, but microglia are not the only cell type involved in the neuroinflammation process and its downstream toxicity. Astrocytes, the most abundant cell type of the central nervous system (CNS), are also implicated, especially after switching to a reactive state. Astrocytes share with microglia numerous immune molecules and pathways to detect, internalize, and participate in the clearance of Aβ. For instance, astrocytes also express some scavenger receptors, TLRs, RAGE receptors, and TREM2 at their surface to detect Aβ (99). Microglia can induce the A1 astrocytic state via the secretion of three cytokines, IL-1α, TNF-α, and complement component 1, q subcomponent (C1q), that are necessary and sufficient for this transition (100). Indeed, conditioned media of microglia treated with oAβ was enough to induce A1 astrocytes through enhanced glycolysis (101). Importantly, A1 astrocytes lost their homeostatic functions and caused the death of neurons and oligodendrocytes through caspase-2/3-dependent apoptosis via the secretion of a soluble toxin, in addition to IL-1α, TNF-α, and C1q. In the AD brain, between 30% and 60% of astrocytes residing in the hippocampus or prefrontal cortex were A1 astrocytes, suggesting that activated microglia can drive neurodegeneration indirectly through secreting cytokines that shift astrocytes toward an A1 phenotype. Similarly, TNF-α, via the STAT3 pathway, caused astrocytes to transition into inflammatory reactive GBP2^{+} (guanylate binding protein 2) astrocytes, characterized by secretion of α1-antichymotrypsin driving BBB disruption, a symptom also present in AD (102). Targeting the transition toward A1 astrocytes seems to be a relevant strategy. The use of brain-penetrant glucagon-like peptide-1 receptor (GLP-1R) agonist NLY01 in 5xFAD and 3xTgAD mice prevented microglia-mediated A1 reactive astrocyte conversion, rescuing neuronal cell death and memory impairments (103). 3xTgAD mice overexpress the APP Swedish and tau P301L mutations and have a knock-in of the PSEN1 M146V mutation, displaying progressive Aβ and tau pathologies (absent in 5xFAD mice), as well as early neuroinflammation and cognitive deficits.

3.6. Neuronal Cell Death

Microglia are not only indirectly neurotoxic but also directly neurotoxic (**Figure 2**). In vivo multiphoton microscopy in the 3xTgAD model revealed that microglia were recruited to neurons before neuron elimination and that the fractalkine/CX3CL1 expressed by injured neurons and its unique receptor, the microglial CX3CR1, were involved in this AD neuron loss (104). This fact was supported by the observation that *Cx3cr1* knockout rescued neuron loss in 3xTgAD mice (104).

3.7. Inhibition of Neuronal Activity

In AD, abnormal neuronal hyperactivity is a major early neuronal dysfunction (105). A link between this hyperexcitability and the abnormally active microglia was suggested by Badimon et al. (106), who showed that, physiologically, the local release of ATP at the synapse of active neurons acted as a microglial chemoattractant through its detection by the microglial purinergic receptor P2RY12. This led to the directional branch extension toward active synaptic boutons. Additionally, this extracellular ATP is converted into AMP by the microglia ectoenzyme CD39 and is further converted into a suppressor of neuronal activity, adenosine. Consequently, microglia depletion or inhibition of the P2RY12-mediated ATP response or microglial CD39-mediated conversion of

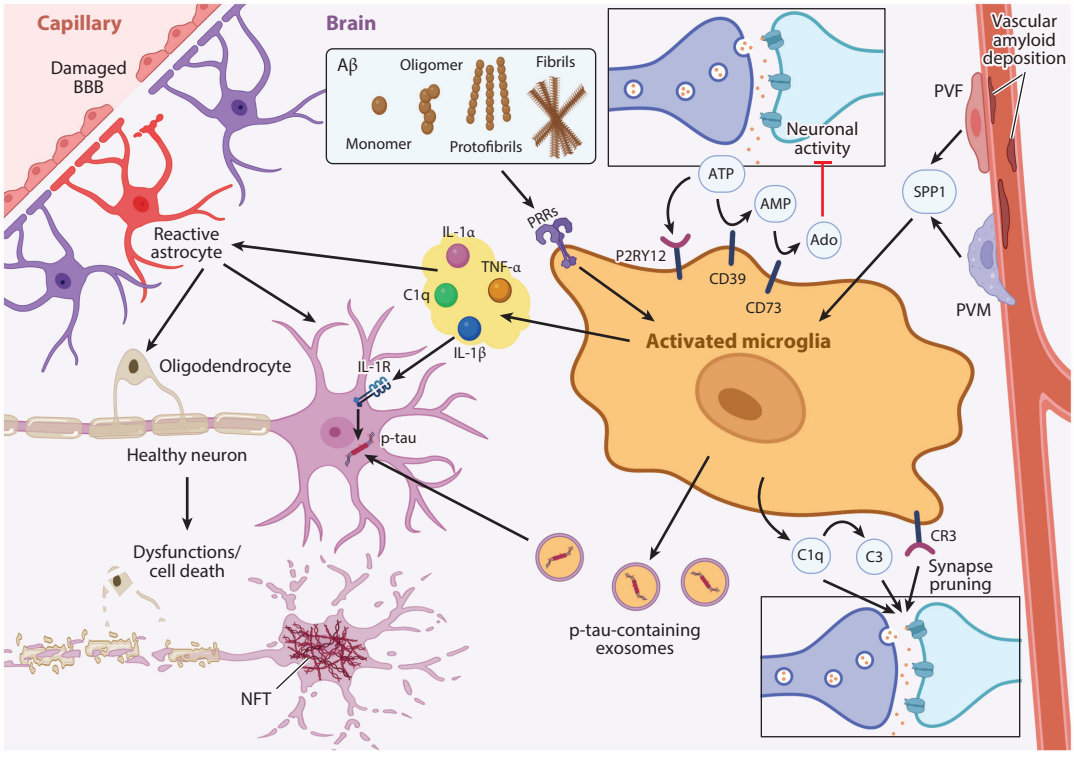

Figure 2

Activated microglia in AD. Exposure to Aβ species or SPP1 secreted by PVFs and PVMs in response to vascular amyloid deposition can activate microglia. Microglia can also be attracted to the ATP present at active synapses by detecting it via the P2RY12 receptor. ATP can be converted into AMP via CD39 and further down into the neuronal activity inhibitor Ado via CD73. Activated microglia secrete inflammatory factors such as IL-1α, TNF-α, and C1q that can switch astrocytes to a reactive state in which they secrete toxins causing neuron and oligodendrocyte apoptosis and BBB damage. Additionally, activated microglia participate in tau spreading by secreting exosomes containing p-tau that can be internalized by surrounding neurons. Microglia-secreted IL-1β is recognized by neuronal IL-1R, and through the regulation of tau kinases, it also participates in the hyperphosphorylation of tau and the formation of NFTs. C1q and the downstream complement cascade protein C3 can decorate synapses, causing their CR3-dependent elimination by microglia. Abbreviations: Aβ, amyloid beta; AD, Alzheimer disease; Ado, adenosine; BBB, blood-brain barrier; C1q, complement component 1, q subcomponent; CR3, complement receptor 3; NFT, neurofibrillary tangle; p-tau, hyperphosphorylated tau; PRR, pattern recognition receptor; PVF, perivascular fibroblast; PVM, perivascular macrophage; SPP1, secreted phosphoprotein 1.

ATP into AMP induced exaggerated neuronal activity and seizures (106). As the expression of the genes encoding P2RY12 and CD39 was decreased in the APP/PS1 and 5xFAD models, one could expect that in AD microglia have a defective physiological negative response to neuronal activity, leading to the increased neuron excitability in AD (106).

3.8. Complement System

As discussed above, microglia-secreted C1q participates in the Aβ-induced activation of neurotoxic A1 astrocytes (100, 101). But early microglia activation in AD has also been linked to early synapse loss. At the preplaque stage, Aβ-soluble aggregates cause resident microglia to inappropriately engulf and eliminate synapses through activation of the complement system. In murine models of cerebral amyloid deposition, before Aβ plaque detection, soluble oAβ induced the deposition of C1q, the initiating protein of the classical complement cascade, and of its downstream

effector C3, resulting in synapse decoration, an event that causes complement receptor 3 (CR3)-dependent synapse loss (107). oAβ injection alone failed to cause synapse loss in brains lacking C1q, and microglia had an increased expression of *C1qa*, suggesting that oAβ-induced synapse loss is dependent on C1q secreted by microglia. Globally, the inhibition of the complement factors C1q and C3 or the microglial complement receptor CR3 reduced the number of synapse-phagocytosing microglia and, consequently, early synapse loss (107). All in all, prior to Aβ deposition, oAβ challenge causes the complement cascade and microglia to synergistically cause synapse loss. In AD patient frontal cortices, microglia were located mostly around Aβ plaques and were found to have strong C1qa immunoreactivity (108). These results suggest that inhibiting the complement system may protect from early synapse loss observed during AD. In P301S mice hippocampi, it was uncovered that microglia preferentially engulfed inhibitory synapses but that astrocytes also engulfed excitatory synapses in a C1q-dependent fashion (109). C1q depletion was beneficial because it reduced microglial and astrocytic synapse engulfment, but depletion of TREM2 in an amyloid and TauPS2APP mouse model (a triple transgenic model that combines Aβ and tau pathologies beginning at 4 months of age via the expression of the Swedish APP, Tau P301L, and PSEN2 N141I mutations) impaired microglial synapse pruning, whereas the astrocytes could compensate for this impairment (109). This finding demonstrates that to rescue the synapse density in AD, blocking only the microglial pruning would not be sufficient, whereas blocking the complement activation would be more relevant. Accordingly, pharmacological inhibition of the C1q complement cascade reduced engulfment of glutamatergic synapses by microglia and cognitive capacities in a rat model of AD (110). CNS-associated macrophages can also modulate synapse pruning by microglia and contribute to AD synapse loss. In the amyloid $App^{NF\text{-}L}$ mouse model, at an early stage, without senile plaques but with vascular oAβ deposition, perivascular macrophages and perivascular fibroblasts had an elevated level of secreted phosphoprotein 1 (SPP1/osteopontin). This factor caused microglia to activate C1q and engulf synapses (111). SPP1 treatment alone was sufficient to induce synapse engulfment by $Spp1^{KO/KO}$ primary microglia, and the presence of oAβ failed to provoke C1q activation or synapse engulfment in $Spp1^{KO/KO}$ mice, suggesting that oAβ acts upstream of perivascular SPP1. In AD patients, secreted SPP1 levels are elevated in the CSF and plasma (112, 113). Microglial TREM2 can inhibit the classical complement cascade and resulting synapse loss by binding to C1q (114). In AD brains, expression of C3 and C3aR1 has been correlated with cognitive decline and Braak stages (115). The pharmacological or genetic inactivation of C3aR1 in tau mouse models rescued various parameters, such as neuroinflammation, tau hyperphosphorylation, neurodegeneration, and behavioral defects (115, 116). These results highlight the important role of the activation of the C3–C3aR1 network and support the idea to target C3aR1 to treat tauopathies. A similar situation for the C5a–C5aR1 network was also reported, since the levels of C5a in serum increased with AD severity and correlated positively with the levels of other proinflammatory factors and negatively with cognitive functions, which also raise the question of its use as a blood biomarker (117). As for the interest in targeting C5a in AD, genetic or pharmacological inactivation of C3aR1 in AD mouse models resulted in neuroprotection and reduction of cognitive deficits via anti-inflammatory effects (118, 119). Three AD risk factors identified via GWAS are complement genes: *CLU* (inhibitor of the complement cascade), *CR1* (receptor for complement factor C3b and regulator of the complement cascade), and *CS1* (component of the C1 complex) (120). The complement cascade, generating C1 complex to C9, culminates in the formation of membrane attack complex (MAC) made of C5b-9, and it is worth noting that one missense variant in the MAC component *C7* gene, which causes the protein to lose its inhibitory effect on excitatory transmission, was also associated with AD (121). The use of an anti-C7 antibody in $App^{NL\text{-}G\text{-}F}$ mice, where MAC formation increases with age and severity, reduced synapse loss near plaques, suggesting that MAC can directly damage synapses (122).

4. EMERGING APPROACHES FOR THE STUDY OF HUMAN MICROGLIA IN ALZHEIMER DISEASE

4.1. In Vitro Human Microglia Platforms

Primary microglia can be isolated from surgical resections or autopsy, although from a limited number of and from specific cohorts of subjects. Alternatively, in an effort to tackle the genetic and transcriptomic uniqueness of human microglia, especially in relation to disease, multiple protocols for microglia differentiation from induced pluripotent stem cells (iPSCs) have been generated over the last decade (see 123 for a detailed review), including mimicking the developmental ontogeny of microglia and producing primitive yolk sac progenitors (124, 125). In addition, a novel iPSC-derived microglia-like cell based on the inducible expression of six transcription factors (PU.1, MAFB, CEBPα, CEBPβ, IRF-5, and IRF-8) across two independent loci has been developed. The induction of these factors and the additional supplementation with exogenous growth factors (GM-CSF, M-CSF, IL-34, and TEG-β) generate microglia-like cells in approximately 8 days (126). Although these strategies have yielded relevant insights into microglial disease–related processes, it has been extensively shown that outside the brain environment, microglia undergo rapid and profound transcriptomic changes (76, 127, 128) that would limit their ability to recapitulate brain-native phenotypes and functions (75, 76, 129), as well as their interactions with other cells in their environment.

Although more elaborate, several 3D platforms culture iPSC-derived microglia in a multicellular environment. A mixed-species system has shown that while astrocytes and neurons are important to induce a homeostatic transcriptional state in microglia, microglia are in turn essential for astrocytes to respond to inflammatory challenges (130). The generation of immunocompetent brain organoids (i.e., containing microglia) has been somehow more complicated, as in most cases there is poor microglia integration and survival. However, a recent system has been developed where cortical organoids are seeded with stem cells that overexpress the master transcription factor PU.1 in an inducible manner (131). In this context, human iPSC-derived microglia display a heterogeneous transcriptomic profile and can respond to acute treatments with soluble Aβ aggregates (131). Although these 3D platforms can provide useful insights into cellular interactions, they also present limitations when aiming to elucidate the role of microglia in AD and other age-related disorders, as they do not robustly display many appropriate hallmarks of disease (e.g., Aβ plaques, tau tangles, TDP-43 aggregates).

4.2. Microglia Humanized Models

While organoids provide an approximation of the brain during developmental stages, a complementary approach has been developed that consists of the in vivo xenotransplantation of human microglia into a fully developed mouse brain. The first reports of robust iPSC-derived microglia transplantation were provided by Mancuso et al. (76) and Hasselman et al. (75) and then by Xu et al. (132). These strategies have been refined over time to reliably produce microglial precursor cells with high efficiency and achieve consistent replacements of up to 80% of the total brain volume (125). Importantly, these models require two critical manipulations of the mouse background, as the hosts need to be immunodeficient (generally *Rag2*$^{-/-}$) and express the human version of colony-stimulating factor 1 (CSF1) (due to cross-species incompatibilities of the murine CSF1 with the human CSF1 receptor) (133). Transplanted microglia display classical morphological features and density across multiple brain regions and survey the brain parenchyma via highly motile processes (75, 76). Transplanted cells can also differentiate into other tissue-resident macrophages, including perivascular macrophages and border macrophages (76). A few weeks after transplantation, microglia mimic the transcriptomic (76) and proteomic (134) profiles of primary human cells

and retain expression of AD risk genes (76). The main advantage of these hybrid systems is that they can be extended to multiple disease backgrounds. Recent in-depth scRNA-seq characterization of human microglia transplanted into the *App*$^{NL\text{-}G\text{-}F}$ mouse brain revealed that whereas these cells can adopt a DAM profile, they display a pronounced antigen-presenting HLA response in the proximity of Aβ plaques, as well as a proinflammatory cytokine/chemokine response to soluble Aβ aggregates, which seem to be absent in mouse systems (135). Remarkably, these human-specific responses are also found in single-nucleus sequencing from AD brains (135).

In parallel, a new complementary model has been developed to study human microglia within a relevant physiological, vascularized environment: immunocompetent organoids in vitro and their subsequent engraftment into the brain of immunocompromised mice. The human microglia within the transplanted organoids also present transcriptomic characteristics similar to those of primary human cells, can surveil their environment, and can react to local injuries and inflammatory challenges (136). These transplantation models are highly attractive, as cells can be genetically manipulated to model AD risk factors (135, 137) while they retain human-specific aspects of microglial biology and develop in a brain environment that manifests relevant disease hallmarks. However, they all share the important caveat that adaptive immunity is absent. This appears highly relevant in view of recent data highlighting the role of T cells in tau-induced neurodegeneration (98) (**Figure 3**). Future efforts should be dedicated to integrating both human adaptive and innate immune aspects into the same model system.

5. NON-Aβ MODULATORS OF NEUROINFLAMMATION

5.1. Microbiota

Compared with control TE4 mice, a PS19 mouse model with a knock-in of human APOE4 living in germ-free (GF) conditions showed beneficial decreased late tau phosphorylation, glial activation, hippocampal atrophy, and ventricle enlargement (138). The GF improvement was, however, lost after microbiota recolonization by a control mouse microbiome. Even though less efficient than GF conditions and only transient, the antibiotics cocktail in control mice reduced glial activation and brain atrophy (138). The benefits of GF conditions and antibiotics seemed to be mediated by reduced production of short-chain fatty acids (SCFAs) by the microbiota, as SCFA supplementation resulted in the concomitant observation of increased tau phosphorylation and gliosis in the hippocampus of old GF mice. Transplantation of AD patient gut microbiota into APP/PS1 mice activated the intestinal NLRP3 inflammasome and resulted in intestinal inflammatory factors such as IL-1β, IL-18, and TNF-α that can reach the CNS through the circulatory system, causing the activation of microglia (139). The secreted SCFAs might again be the cause, since on their own they can promote the expression of intestinal NLRP3 and inflammatory factors (IL-18, IL-6, and TNF-α) (140).

5.2. Peripheral Immune Stimulation

The brain is not a strictly isolated immune organ, meaning that neuroinflammation is affected not only by the challenges present in the brain but also by the peripheral immune system. Lopez-Rodriguez et al. (141) demonstrated that in the APP/PS1 model, microglia and astrocytes are in a stressed state in which they will overreact to secondary peripheral inflammatory stimulation. Following peripheral LPS application, PAM produced exaggerated IL-1β, which sparks plaque-associated astrocytes to overproduce chemokines and IL-6, but also disrupted gamma rhythms. Specifically in AD patients who died with infection, a correlated increase in IL-1β and IL-6 was observed, compared with AD patients without infection (141). By using two-photon laser microscopy, it was possible to study the changes in microglia after peripheral

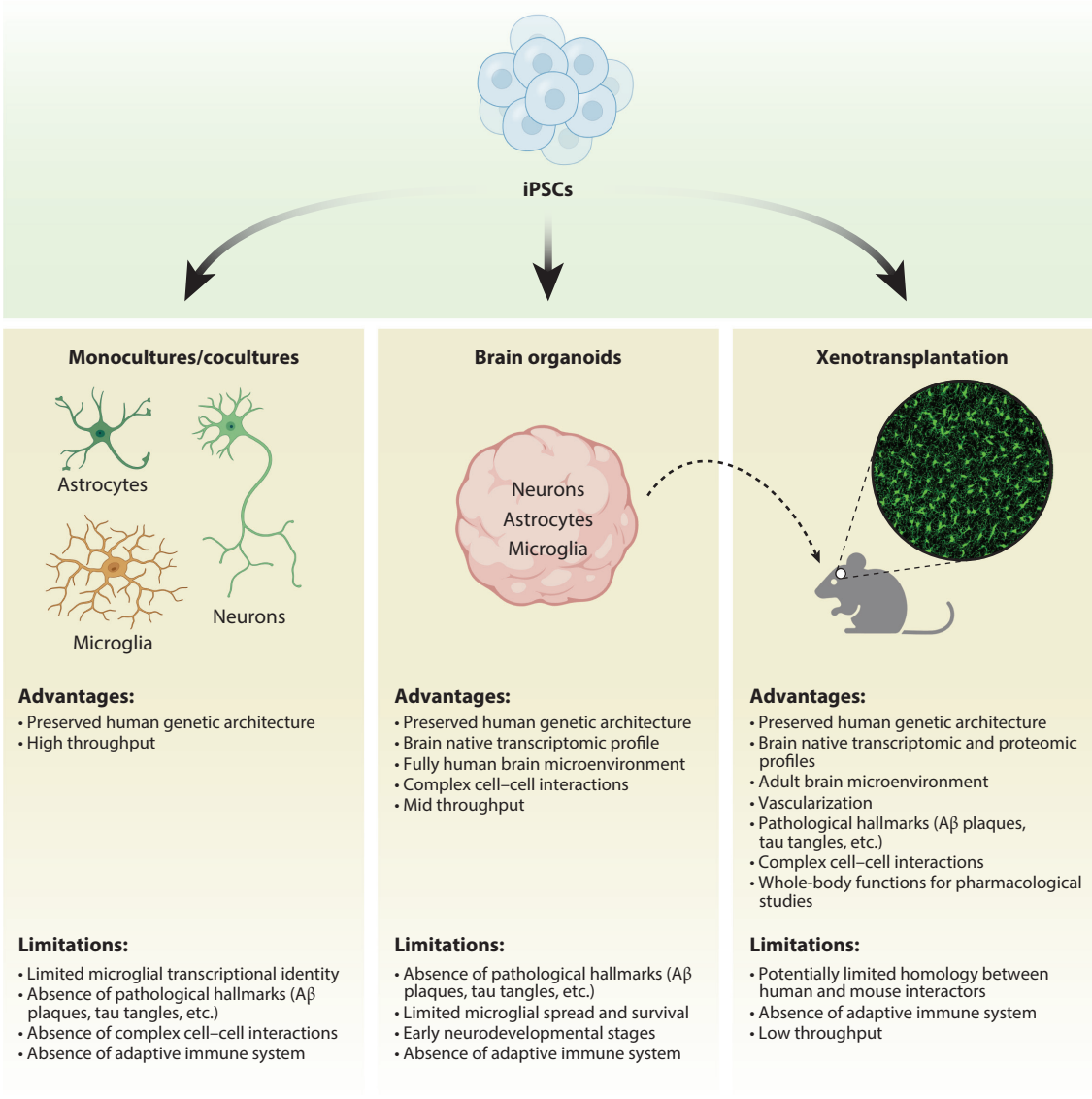

Figure 3

Emerging humanized microglia approaches. Human induced pluripotent stem cells (iPSCs) can be differentiated into microglia, astrocytes, or neurons to be cultivated as 2D monocultures or cocultures. These differentiated human cell types can also be combined to generate 3D organoids. Both human iPSC-derived microglia and human brain organoids can be xenotransplanted into a fully developed brain of an adult mouse model. The advantages and limitations of each approach are listed.

LPS administration in APP/PS1 mice. Within 24 h after LPS administration, microglia transiently changed their morphology to a more activated shape with fewer and shorter branches, a change that resolved 9 days later (142). Unfortunately, LPS challenge additionally reduced Aβ uptake by microglia and caused myeloid cells to infiltrate the brain, effects that are dependent on the NLRP3 inflammasome (142). In AD patients, systemic infections increased brain cytokine levels, hypoperfusion, and BBB leakage, independently of the Aβ levels (143). Systemic inflammation

might be an interesting therapeutic target, as the use of the immunomodulator and antioxidant dimethyl fumarate suppressed brain-derived inflammatory cytokines and long-term memory deficits in wild-type mice following peripheral LPS challenge (144). Compared with wild-type mice, 5xFAD mice have higher basal IL-1β levels in their blood and brain, and after LPS-induced peripheral inflammation, these levels increase even more in the AD mouse model (145). Selectively inhibiting gasdermin D–dependent pyroptosis in the periphery with disulfiram prevented not only increases in IL-1β levels but also microglia activation (145).

5.3. Central Impact of Peripheral Immune Cells

Tauopathy mouse models but not amyloid mouse models have a strong increase in infiltrating T cells in their parenchyma, with increased $CD4^+$ but predominantly $CD8^+$ subtypes (98). $CD8^+$ T cells in tauopathic mouse brain have particularly enhanced IFN-γ expression. T cell depletion or anti-IFN-γ treatment led to reduced microglia activation, tau phosphorylation, and brain atrophy, suggesting that in tauopathic brains, IFN-γ secreted by $CD8^+$ infiltrated T cells contributes to tau pathology and neurodegeneration through the increase in microglial inflammation (98). In human AD brains, a relationship between tau pathology development and T cell infiltration has been demonstrated. The infiltration of $CD3^+$ T cells in parenchyma progresses with the tau Braak stages, when the Aβ level stays the same (98). The exact causes of T cell infiltration are not completely elucidated, but in the absence of IL-17RA, an interleukin upregulated in the late stages of AD, APP/PS1 mice have decreased infiltration of $CD8^+$ T cells and $Gr1^+/CD11b^+$ myeloid cells, which could be due to decreased production of myeloid cell– and T cell–attracting chemokines CXCL1 and CXCL9/10 by glial cells (146). However, in contradiction to this result, it was suggested that chronically activated microglia limit $CD3^+/CD8^+$ T cell infiltration, as microglia ablation in APP/PS1 mice increased their infiltration (147).

Contrary to injection of effector T cells, intravenous injection of regulatory T cells (Tregs) ($CD4^+$, $CD25^+$, $Foxp3^+$) into 3xTgAD mice was beneficial in terms of reducing splenocytic IL-2, IL-6, and IFN-γ production and improving spatial memory (148). Conversely, amplification of Tregs with peripheral low-dose IL-2 treatment increased numbers of PAM and restored cognitive functions in APP/PS1 mice (149). But Tregs also decreased the amount of C3-positive astrocytes and favored A2-like phenotypes (150).

5.4. Meningeal Lymphatics and Drainage Pathways

Meningeal lymphatic drainage is a potent mechanism by which the brain deals with Aβ accumulation. Modulating this mechanism also affected the efficacy of one important therapeutic strategy that similarly aims to clear Aβ: the anti-Aβ monoclonal antibodies. Ablation of meningeal lymphatics in 5xFAD mice increased microgliosis and Aβ deposition and impeded the effects of anti-Aβ immunotherapy (151). By contrast, viral delivery of vascular endothelial growth factor C to grow the lymphatic vasculature and boost lymphatic drainage reduced microgliosis and Aβ burden and showed a synergistic effect when combined with the anti-Aβ antibody (151).

5.5. Astrocytes

The metabolism of astrocytes is blatantly different from that of microglia, as astrocytes rely primarily on glycolysis. In fact, astrocytes have only a few mitochondria that have a low capacity for OXPHOS, yet OXPHOS in astrocytes is pivotal for brain lipid homeostasis by degrading fatty acids. Indeed, in mitochondria, fatty acids are degraded by β-oxidation to fuel tricarboxylic acid (TCA) to fuel OXPHOS. The specific inhibition of OXPHOS in astrocytes in wild-type mice was enough to cause AD-like synaptic and memory impairments (152). OXPHOS-deficient

astrocytes accumulate lipid droplets and adopt a reactive state, with upregulation of TNF-α, IL-6, and IL-3. Importantly, these astrocytes could directly activate microglia by secreting IL-3 that will bind to microglial IL-3Rα (152). Observation of 5xFAD mouse brains revealed early impaired fatty acid degradation in astrocytes shortly after Aβ accumulation and subsequent accumulation of lipid droplets in astrocytes, suggesting that early Aβ challenge might initiate lipid metabolism defects in astrocytes (152). Indeed, chronic treatment of iPSC-derived astrocytes with sonicated $A\beta_{42}$ fibrils led to Aβ accumulation, mitochondrial deficits, accumulation of lipid droplets close to mitochondria, and reduced OXPHOS. In reaction, astrocytes temporally compensated for this by increasing glycolysis and also by increasing the peroxisomal fatty acid β-oxidation (153). Furthermore, APOE4 could also lower fatty acid oxidation and cause lipid droplet accumulation in astrocytes (154). Nevertheless, the reactive astrocytes should not simply be categorized as detrimental and seen as a target to eliminate, as removing reactive astrocytes in an amyloid mouse model aggravated the levels of proinflammatory cytokines and synaptic and memory deficits (155). Caution should be taken because only the proliferating astrocytes were ablated in this study, whereas OXPHOS-deficient astrocytes, for instance, can activate without an increase in proliferation (152). Using snRNA-seq in the 5xFAD model, Habib et al. (156) observed a unique astrocytic state, named disease-associated astrocytes, that mirrored DAM, which were also identified in 5xFAD mice. These disease-associated astrocytes appeared before cognitive decline and increased only with disease progression. Moreover, disease-associated astrocyte-like cells were also found in old wild-type mice and were enriched in human AD patients (156).

5.6. Neuronal Gamma Frequencies

AD has been recently characterized by a disorder of high-frequency neuronal activity. In AD, synaptic dysfunction was linked to perturbation of oscillatory activity. Transcranial magnetic stimulation and electroencephalography (TMS-EEG) could detect reduced frontal gamma activity (40 Hz) in AD patients, and the more pronounced the reduction was, the worse the synaptic plasticity dysfunctions and cognitive decline were at the following evaluation (157). These results are in favor of the use of this low-cost noninvasive procedure to measure frontal gamma activity in patients, which could be considered a new biomarker, especially since in the AD mouse model, gamma oscillations decreased before the onset of plaque formation and cognitive decline (158). Regarding the origins of the oscillatory perturbation, an acute application of IL-1β to hippocampal slices triggered a disruption of gamma oscillations in slices from old APP/PS1 mice but not in wild-type slices (141). This finding suggests that inflammation participates in gamma perturbations and that chronic inflammation in AD might render the immune system hypersensitive, such that an acute inflammation will make it overreact and disrupt the frequency oscillation. Several reports unraveled the benefits of gamma frequency stimulation to rescue the perturbation of oscillatory activity and several AD brain dysfunctions. With a noninvasive light flickering at 40 Hz that provokes 40-Hz oscillations in the visual cortex, it was possible to enhance microglia Aβ uptake and reduce soluble and insoluble $A\beta_{1–40}$ and $A\beta_{1–42}$ levels, plaque load, and p-tau in amyloid or tau mouse models (158). Similar results could also be obtained in the auditory cortex after a 40-Hz auditory tone stimulation, with noticeable memory improvement (159). A combination of visual and auditory gamma stimulation was beneficial not only in these areas; it could also reduce amyloid burden in the medial prefrontal cortex (159). Mechanistically, it was suggested that transauricular vagal nerve stimulation at 40 Hz could reduce amyloid load in the hippocampus by inhibiting the P2X7R/NLRP3/caspase-1 signaling pathway, thus resulting in lower levels of IL-1β and IL-18 in the hippocampus (160).

5.7. Microglia Senescence

The main risk factor for AD is aging, and it is known that aging by itself has a deleterious impact on microglia functions. In old mice, the levels of the lipid messenger prostaglandin E2 are increased in the brain and the expression of its receptor EP2 is increased in microglia (161). This signaling drove the storage of glucose into glycogen, causing decreased glucose flux and mitochondrial OXPHOS. In old wild-type mice, this bioenergetic impairment was reversed by inhibiting EP2 signaling in microglia, which resulted in decreased age-related inflammation and cognitive impairments, the latter of which was also ameliorated by peripheral myeloid EP2 signaling inhibition (161).

Senescence-associated microglia (SAM) are characterized by reduced proliferation, increased expression of the cyclin-dependent kinase inhibitor *Cdkn1a*/$p21^{Cip1}$, dystrophic morphologies with reduced morphological complexity, and an altered inflammatory secretome (162). Autophagy activity declines with age and this could promote SAM development and AD progression. In fact, DAM from 5xFAD mice have increased autophagy, whereas autophagy-deficient microglia displayed a senescent phenotype that prevents them from proliferating and engaging with Aβ plaques (163). Removing SAM with senolytic drugs boosted the recruitment of additional microglia around plaques and the compactness of the plaques (163). However, early and excessive microglia proliferation in APP/PS1 mice led to the generation of senescent DAM, characterized by increased β-galactose activity, specific transcriptional signatures, and shortened telomeres (164). Inhibiting early microglia proliferation with a CSF1 receptor inhibitor could reduce microglial senescence and ameliorate Aβ pathology (164). Of interest, microglia in postmortem AD brains also displayed a senescence-associated secretory pattern characteristic of senescent microglia (164). Preventing microglia proliferation with another CSF1 receptor inhibitor was also beneficial in the P301S murine tau model, as it limited microglial expansion, reduced the expression of IL-1β and TNF-α, and reduced tau-induced neurodegeneration (165).

5.8. Genetic Risk Factors

APOE4 was the first identified risk factor and it is still the strongest genetic risk factor for late-onset AD. Risk increases with the isoform number (E2 < E3 < E4), culminating with APOE4 homozygote individuals having an increased risk for AD 15 times higher than that of APOE3 homozygote individuals. APOE is a lipid transporter and isoforms modify the lipid-binding properties and receptor binding affinity, with APOE4 preferentially binding to lower-density lipoproteins but APOE3 and APOE2 to high-density lipoproteins. APOE is expressed mainly by astrocytes but also by microglia. APOE also binds to Aβ and can be recognized by the microglial TREM2 receptor (166). APOE3, but not APOE4, induced faster microglial recruitment toward injected Aβ, facilitated Aβ uptake, and ameliorated Aβ effects on cognition (167). The reduced Aβ uptake by APOE4-expressing microglia was even more accentuated by TREM2 deletion, showing that APOE and TREM2 interact (167). In the P301S tau mouse model, APOE4 expression exacerbated tau-linked inflammation and neurodegeneration (168), whereas TREM2 knockout reduced neurodegeneration (169). Surprisingly, however, the ablation of TREM2 in TE4 mice exacerbated neurodegeneration and tau pathology compared with those in control TE4 mice (170). This finding demonstrates that in the presence of APOE4, tau-induced TREM2-independent microgliosis is sufficient to promote tau-mediated neurodegeneration (170). This explains why the total removal of microglia in TE4 mice successfully blocked tau-dependent neurodegeneration (171).

After the identification of APOE4 risk factors in 1993, the field waited several decades for the rise of GWAS of extensively large populations to identify more new risk variants. Of the 85 currently identified risk loci, approximately half contain myeloid candidate causal genes (4, 172).

Even though more work is needed to decipher the precise functional impacts of these variants and the way they contribute to increase the risk of developing AD, the contribution of microglia to late-onset AD is undeniable. The variants associated with these enriched immune-related functions include CD33, TREM2, CR1, INPP5D, APOE, BIN1, API1, MS4A, ABCA7, CLU, MAF, PICALM, and PLCG2.

6. CONCLUDING REMARKS

Because of their various pattern recognition receptors, microglia can detect Aβ accumulation in the brain, leading to their activation. Inflammatory activation of microglia causes transcriptional changes, metabolic modifications, buildup of NLRP3 inflammasomes, and release of inflammation mediators, such as ASC specks, cytokines/chemokines, or complement cascade molecules (**Figure 1**). Transcriptomic studies of microglia have highlighted the evolutionary specificity and heterogeneity of the human microglia transcriptomic profile. Other peripheral and central players can modulate neuroinflammation either indirectly by acting on microglia or directly by participating in inflammatory cascades and buildup of further danger-associated molecular patterns. Once activated, microglia will, among other functions, proliferate and engage with amyloid deposits but also activate surrounding astrocytes in a neurotoxic state; participate in tau spreading; and affect neurons by pruning their synapses, inhibiting their activity, or even causing their death (**Figure 2**). To get a closer study of human microglia in a relevant environment, several approaches have recently been developed, such as primary human microglia in vitro culture and 3D human organoids and their xenotransplantation into murine brains (**Figure 3**). Although they are promising therapeutic targets, the mechanisms of neuroinflammation in AD need to be better deciphered, as neuroinflammation on the whole involves an important and intricate amount of dynamic signaling pathways and cell types at different locations and stages of the disease. Moreover, one must always remember that it is the overactivated and chronic inflammation that is detrimental, so the goal is not to completely inhibit neuroinflammation but rather to control it so that the response does not overreact and become chronic. Any modulation of microglia, however, must also take account of possible effects on the peripheral immune system as long as such a therapy cannot be restricted to the brain. Because increasing data point toward significant differences between murine and human microglia, efforts should be made to ensure that immune pathways of therapeutic interest are being tested for their identical roles and functions between species.

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