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REVIEW

The role of innate immune responses and neuroinflammation in amyloid accumulation and progression of Alzheimer's disease

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Abstract

Alzheimer's disease (AD) is characterized by amyloid beta (Aβ) accumulation, tau pathology and neuroinflammation. Recently, there has been considerable interest in the role of neuroinflammation in directly contributing to the progression of AD. Studies in mice and humans have identified a role for microglial cells, the resident innate immune cells of the central nervous system, in AD. Activated microglia are a key hallmark of the disease and the secretion of proinflammatory cytokines by microglia may result in a positive feedback loop between neurons and microglia, resulting in ongoing low-grade inflammation. Traditionally, the pathways of Aβ production and neuroinflammation have been considered independently; however, recent studies suggest that these processes may converge to promote the pathology associated with AD. Here we review the importance of inflammation and microglia in AD development and effects of inflammatory responses on cellular pathways of neurons, including Aβ generation.

INTRODUCTION

Alzheimer's disease (AD) was first identified in 1906 by Alois Alzheimer who described the characteristic memory loss and confusion, as well as other psychological symptoms, in a 51-year-old female patient. In the same patient's brain, Alzheimer identified plaque formation, neurofibrillary pathology, tangles, astrogliosis and neuronal loss.[1] AD is now recognized as the most common neurodegenerative disease and is characterized by initial short-term memory loss followed by subsequent severe deficits attributed to neuronal loss.[2] An estimate suggested that 46.8 million people globally were living with dementia in 2015, with this number expected to reach 131.5 million by 2050.[3]

AD is characterized by widespread neuronal degeneration, synaptic loss affecting mainly the hippocampus and cortex, resulting in diffuse brain atrophy.[4] Accumulation and deposition of amyloid-beta (Aβ) peptides and neurofibrillary tangles were long considered the sole major hallmarks of AD. However, more recently neuroinflammation has emerged as a third hallmark of the disease.[5]

Genome-wide association studies of late-onset AD (LOAD) have identified genetic risk factors, which can be divided into distinct functional classes. Notably, immune responses and immune-related pathways represent one of the major classes of genetic risk factors for LOAD (Table 1). Moreover, microglia, which are resident innate immune cells in the brain, have been identified as a central player in disease pathogenesis.[6] An interaction between the products of activated microglia and neurons could result in a positive feedback loop to establish an ongoing chronic inflammatory condition.[7]

This review will highlight the importance of neuroinflammation in the development of AD, and the advances arising from the integration of multiple disciplines investigating this disease, namely neurobiology, immunology, biochemistry, cell biology and genetics. The potential of inflammatory responses to

Table 1. Summary of selected genetic risk factors associated with late-onset Alzheimer's disease.

Functional class	Risk gene
Innate immune response	Triggering receptor expressed in myeloid cells 2 (*TREM2*)[67] Ephrin type-A receptor 1 precursor (*EPHA1*)[79] Complement receptor 1 (*CR1*)[79] SLP adaptor and CSK interacting membrane protein (*SCIMP*)[79] *CD33*[89] *HLA-DRB1*[79] Inositol polyphosphate-5-phosphatase D (*INPPD5*)[79] ABI family member 3 (*ABI3*)[79] Membrane spanning 4A (*MS4A*)[80] Cytokine-dependent hematopoietic cell linker (*CLNK*)[79] Cas scaffolding protein family member 4 (*CASS4*)[79]
Cholesterol metabolism	Apolipoprotein E (*APOE*)[113] Enoyl-CoA hydratase domain containing 3 (*ECHDC3*)[79] Clusterin (*CLU*)[81] ATP binding cassette subfamily A member 7 (*ABCA7*)[80] Sortilin protein-related receptor (*SORL1*)[80]
Endocytosis	Sortilin protein-related receptor (*SORL1*)[80] Bridging integrator 1 (*BIN1*)[80] Sorting nexin 3 (*SNX3*)[80] Phosphatidylinositol binding clathrin assembly protein (*PICALM*)[80] CD2-associated protein (CD2AP)[80]
Amyloid-beta precursor protein processing	A disintegrin and metalloproteinase with thrombospondin motifs 4 (ADAMTS4)[79] A disintegrin and metalloproteinase domain-containing protein 10 (*ADAM10*)[79] Aph-1 homolog B (*APH1B*)[79]

perturb the cellular pathways of neurons, including the generation of Aβ, will also be considered.

AMYLOID PRECURSOR PROTEIN

The human amyloid-beta precursor protein (APP) gene was first identified in 1987[8] and although one of the most studied human proteins, the function of APP remains poorly defined. A number of putative physiological functions have been assigned to APP, such as regulation of neurite outgrowth, cell adhesion, synaptogenesis and cell survival.[9] APP, a type 1 membrane protein, is synthesized in the endoplasmic reticulum and transported to endosomes and/or the cell surface via the secretory pathway.[10-12] Post-translational modifications of newly synthesized APP such as glycosylation, phosphorylation and sulfation occur during transit through the Golgi.[13] Proteolytic processing of APP occurs at multiple locations in the cell and results in a variety of peptide products, including the pathogenic Aβ peptides, which are subsequently exported from the cell.[14] APP is located on chromosome 21 and exists in many different isoforms because of alternative splicing of the nascent transcript.[15] APP695 is the major neuronal isoform.

β-SECRETASE FAMILY

The generation of Aβ occurs from the processing of APP via membrane-bound proteases called secretases. Three secretases, α, β and γ, are involved in the processing of APP; β and γ secretases have a role in mediating the amyloidogenic cleavage of APP, whereas α-secretase prevents Aβ generation by cleaving APP within the Aβ domain.[16]

BACE1 (beta-site APP cleaving enzyme 1) is the major β secretase responsible for amyloidogenic cleavage of APP in the brain.[17] BACE1 is a 501-residue type 1 transmembrane protease and belongs to the pepsin family of aspartyl proteases with optimal activity at acidic pH.[18,19] The protease is synthesized as a larger precursor, proBACE1, which is modified by glycosylation and cleaved by a furin-like endoprotease in the *trans*-Golgi network to generate mature BACE1. Like other aspartic proteases, BACE1 precursor is a zymogen and is synthesized in the endoplasmic reticulum. The maturation of BACE1 increases the catalytic activity of the enzyme over that of immature BACE1.[18] An increased localization of BACE1 within the endoplasmic reticulum (which will represent the zymogenic nonactive form) reduces generation of Aβ, whereas intracellular

trafficking of BACE1 to the more acidic endosomes enhances Aβ production.[18]

Mature BACE1 recycles between the plasma membrane and early and recycling endosomes.[10] The recycling is regulated by a dileucine motif within the sequence, DISLL, of the cytoplasmic tail at residues 496–500.[10] BACE1 can be phosphorylated on Ser498 within this DISLL. The phosphorylation of this motif regulates BACE1 recycling between the cell surface and endosomal compartments. There are two mechanisms for transport of BACE1 from early endosomes to recycling endosomes. A slow, sorting nexin 4-mediated, pathway that can transport both nonphosphorylated and phosphorylated BACE1, and a fast, sorting signal-mediated, pathway dependent on the phosphorylation status of the DISLL motif of BACE1.[10] Golgi-localized γ-ear containing Arf binding protein 1 recognizes the phosphorylated DISLL motif of BACE1 and promotes cargo transport to the recycling endosomes. Perturbation of the signal sorting pathway by either mutation of the Ser in the DISLL motif or silencing Golgi-localized γ-ear containing Arf binding protein 1 or retromer results in about 30% reduction in the rate of transport of BACE1 to the recycling endosomes. The phosphorylated DISLL-mediated trafficking of BACE1 is biologically relevant as elimination of the fast transport of BACE1 from early endosomes to recycling endosomes results in a threefold increase in Aβ production.[10]

The analysis of $BACE1^{-/-}$ mice also provides substantial evidence for a crucial role of BACE1 in AD.[20] Aβ generation, amyloid pathology and cognitive deficiencies are abrogated when $BACE1^{-/-}$ mice are crossed to APP transgenics.[18] In AD, BACE1 activity is elevated. BACE activity in AD increased by 63% in the temporal cortex and 13% in the frontal cortex, but not in the cerebellum.[17] As BACE1 is the only β secretase in the brain, BACE1 has been considered a therapeutic target for lowering cerebral Aβ levels in AD.[18] However, an array of problems and challenges have arisen associated with the development of a therapeutic BACE1 inhibitor. The issues include the ability to cross the blood–brain barrier and the realization that there are a large number of physiological substrates for BACE1, and inhibition of BACE1 activity will result in off-target effects.[21] Notably, BACE1 knockout mice have revealed defects in synaptic function and behavioral problems.[16]

AMYLOIDOGENIC AND NONAMYLOIDOGENIC PROCESSING OF APP

APP undergoes complex, sequential proteolytic processing in the central nervous system (CNS) via two major processing pathways, known as the amyloidogenic and nonamyloidogenic processing pathways, as well as other minor APP processing pathways, to yield fragments, many of which have specific physiological functions.[22] The Aβ peptides generated by the amyloidogenic pathway are cytotoxic and have not been considered to have a physiological function, although recent studies have indicated possible antimicrobial role for the Aβ aggregates.[23]

The approximately 4-kDa Aβ peptide, derived from APP, is one of the hallmarks of AD. BACE1 is responsible for the initial step in Aβ production. The type-1 transmembrane aspartyl protease needs to be membrane bound and in close proximity with APP for cleavage, which highlights the importance of the spatial distribution of both APP and BACE1 within the membranes and intracellular compartments for processing.

Cleavage of APP by BACE1 results in $sAPP_{\beta}$, a soluble APP luminal domain that is subsequently secreted, and a membrane-bound C99-residue C-terminal fragment called C99 or CTF_{β}. This C99 fragment is cleaved by γ-secretase resulting in the release of hydrophobic Aβ peptides (Figure 1).

Various mutations of the APP gene are known to cause familial AD by increasing the extracellular Aβ load or shifting the ratio between Aβ1–40 and Aβ1–42 to the less favorable Aβ1–42.[24] The majority of these mutations affect the proteolytic processing by β- and α-secretases.[25] A reduction of amyloidogenic peptides is found in the Icelandic APP mutation (A673T), which confers protection against AD, as a result of reduced β-secretase cleavage of APP.[26]

In the alternate, nonamyloidogenic, pathway, α-secretase initially cleaves mature APP, resulting in the generation of a soluble luminal domain fragment, $sAPP_{\alpha}$, and a membrane-bound fragment C83 or CTF_{α}, in which the Aβ sequence is absent. Subsequent cleavage of the C83 fragment by γ-secretase yields a number of short peptides excluding Aβ.[27] A comprehensive review focusing on membrane trafficking and production of Aβ has been published recently by Tan and Gleeson.[14]

SECRETED Aβ

Aβ generated from the amyloidogenic pathway is secreted from the cell and subsequently aggregates to form amyloid plaques. The majority of secreted Aβ peptides are 40 amino acids (Aβ1–40), and 42 amino acids (Aβ1–42). The Aβ peptides have the propensity to nucleate and drive production of amyloid fibrils.[28] Aβ is usually removed from the brain by export into the cerebrospinal fluid, the blood vessels and local degradation by microglia. The different mechanisms by which Aβ can be

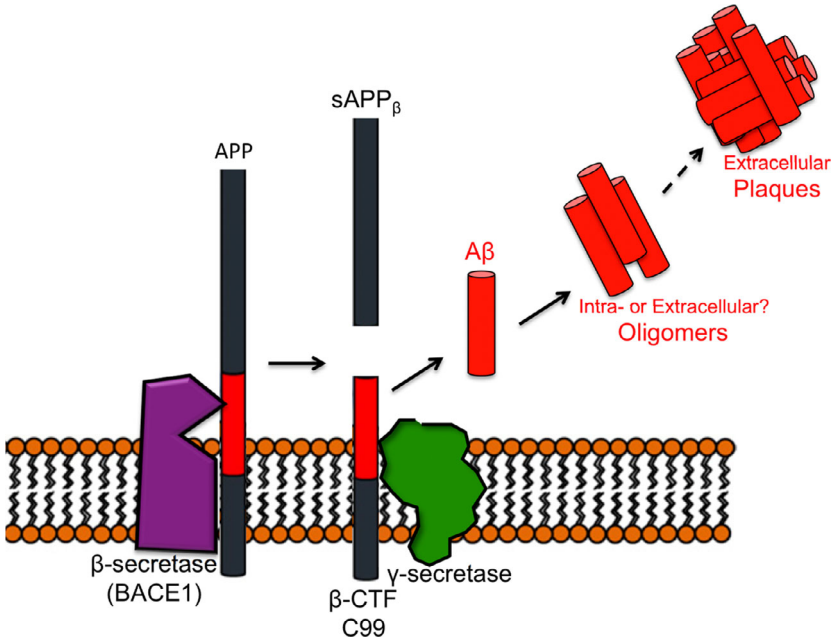

Figure 1. Amyloidogenic processing of amyloid-beta precursor protein (APP). The APP undergoes processing by BACE1 (beta-site APP cleaving enzyme 1), resulting in the $sAPP_{\beta}$ and the C99 fragment. This is further processed by γ-secretase, resulting in the Aβ monomer. The Aβ monomer can assemble into oligomers and fibrils and eventually form the characteristic Aβ plaques.

cleared or degraded has been reviewed by Tarasoff-Conway *et al.*[29] Microglia can clear Aβ by uptake and degradation or can degrade extracellular Aβ by the release of enzymes such as neprilysin. The rapid removal of Aβ released by neurons is imperative as Aβ is a hydrophobic peptide with a tendency to aggregate. Thus, there is a fine balance between pathways to clear Aβ and the generation of Aβ aggregates, which will accumulate. An increase in secreted Aβ concentration by neurons above a certain level results in formation of Aβ oligomers and plaques.[24]

NEUROFIBRILLARY TANGLES

Neurodegenerative tauopathies, characterized by abnormal tau, is another characteristic hallmark of AD. Tau is a microtubule-associated protein predominantly expressed in neurons and is required for microtubule stabilization as well as their assembly.[30] Pathological tau protein is hyperphosphorylated, and aggregated into insoluble neurofibrillary tangles. Accumulation of toxic intracellular aggregates, together with the loss of soluble tau to stabilize microtubules, may synergistically lead to compromised neuronal survival accounting for the strong correlation between neurofibrillary tangle burden and cognitive decline in AD.[31] Synaptic loss has been described with tangle accumulation. Studies of the rTg4510 tauopathy mouse model have further revealed that impaired synaptic plasticity also contributes to the neurodegenerative process in AD, and both Aβ and tau contribute to this degeneration.[32] Bussian *et al.* have recently shown that tau formation is enhanced by the presence of senescent microglia and astroglial cells.[33]

MICROGLIA

There has been considerable recent research to identify the pathways of neuroinflammation and the cell types involved. It has become clear that microglial cells are a major contributor to the inflammatory process in the brain, and are not just bystanders as originally thought. Microglia are now a major focus of neurodegenerative disease research and defining the physiological properties of microglia is crucial to understanding their potential role in neuronal loss and AD.

Microglia are resident innate immune cells of the brain and first described as migratory phagocytic cells of the CNS.[34] Given their properties, microglia are regarded as the resident macrophages of the brain. Microglia account for approximately 10% of cells in the CNS and are the most abundant mononuclear phagocytes in this tissue.[3,35] These resident myeloid cells of the CNS control the patterning and wiring of the brain in early development and contribute to homeostasis. Erythromyeloid progenitors in mice develop in the yolk sac from E8.5 onward and a subset of these cells become microglia progenitors which migrate to the brain from E9.5

onward. This original pool of microglia is the only source of myeloid cells in the healthy mouse brain. However, under pathological circumstances other myeloid cells such as bone marrow-derived monocytes may infiltrate the brain,[36] but this is unlikely to be a contributing factor in AD where the resident microglia, which are able to undergo local renewal, are considered the primary source of myeloid cells involved in the innate immune response.[37]

Microglia function in both prenatal development and postnatal development.[38] In postnatal development microglia influence learning and memory by regulating the strength of synapses, referred to as synaptic plasticity, the refinement or pruning of unwanted synaptic connections and the ability to clear dying neurons and cell debris, including misfolded proteins, via very active phagocytosis and macropinocytosis activity.[38,39] Collectively, these functions arise from the highly dynamic microglial cells which make intimate contact with dendrites and axons via their extensive cell processes constantly screening the local environment.

Microglia express chemokine receptors including CXCR4 and CX3CR1, as well as integrins such as CD11b, which is constitutively expressed, and CD11c, which is upregulated in activated microglia.[40,41] Chemokine receptors and integrins control the migration and the position of microglia within the CNS and enhance their ability to phagocytose and eliminate target cells. Proinflammatory and anti-inflammatory cytokines, such as interferon-γ, tumor necrosis factor α (TNFα), interleukin-1β (IL-1β), IL-10 and transforming growth factor β, tightly regulate the activity of microglia.[35] Furthermore, activated microglia are capable of releasing cytotoxicity mediators such as reactive oxygen and nitrogen species, arachidonic acid metabolites and histamine, among others.[42] Studies in rodents have shown that the precise profile of the neurotophic or cytotoxic factors released by microglia depends on the specific stimulus the microglia has been exposed to.[43,44] In particular, lipopolysaccharide (LPS) stimulation is known to result in the secretion of a number of proinflammatory cytokines in mice.[45]

Microglia, like macrophages, have previously been classified according to their M1 and M2 polarization. The M1/M2 paradigm has helped conceptualize the pathways of microglia activation *in vitro*, but is now considered inadequate for an *in vivo* understanding as microglia rarely display bias toward either phenotype.[46] A number of transcriptome studies have revealed that microglia activation is both varied and situation dependent.[47] Different mouse models of neurodegeneration have revealed that microglia express both neurotoxic and neurotrophic factors.[46] Microglia under resting or noninflammatory conditions have a small soma and numerous processes extending into the microenvironment. This allows microglia to penetrate throughout the parenchyma in the normal adult brain and survey the environment, one of their main functions. The term "resting" microglia, commonly used in the past, is therefore a misnomer,[48] as microglia triggering may occur intermittently. Additional roles of microglia in healthy conditions include the maintenance of homeostasis during neurogenesis, and shaping synaptic fields through synaptic pruning.[49]

Microglia in AD

The precise role of microglia in contributing to chronic disease is incomplete. We need to better understand the balance between their protective role and one where healthy tissue is destroyed. This scenario is akin to the role of macrophages in the development of chronic inflammatory autoimmune disease. For microglia, it can be argued that their role is mainly protective, to remove cell debris and/or infectious agents.[49]

Microglia are involved in Aβ clearance,[50] which is both beneficial, to inhibit Aβ buildup, and deleterious, when levels of Aβ are elevated, as prolonged inflammation will result. Aβ is usually removed from the brain by export into the cerebrospinal fluid, the blood vessels and local degradation by microglia.[29] Microglia can clear Aβ by uptake and degradation or can degrade extracellular Aβ by the release of enzymes such as neprilysin. The rapid removal of Aβ released by neurons is imperative as Aβ is a hydrophobic peptide with a tendency to aggregate. Thus, there is a fine balance between pathways to clear Aβ and the generation of Aβ aggregates, which will accumulate. An increase in the secretion of Aβ by neurons above a certain level results in the formation of Aβ oligomers and plaques.[24]

Microglia express a range of different receptors that can bind Aβ and trigger inflammation, such as different Toll-like receptors and NACHT-, LRR- and pyrin domains-containing protein 3 (NLRP3), and can be stimulated by danger-associated molecular patterns such as adenosine triphosphate.[51,52] Engagement of these receptors induces release of TNFα and IL-1β, which mediate neuroinflammation and neurotoxicity and cause sustained low-grade inflammation[51] (Figure 2). Deletion of receptors such as TLR4 reduces Aβ-induced cytokine production.[53] Stimulation of proinflammatory cascades can directly mediate neuronal damage by microglia via complement-mediated synapse loss,[54] and indirectly via astrocytes.[55]

The NLRP3 inflammasome is essential for the secretion of the proinflammatory cytokines such as IL-1β and IL-18.[56] A role for NLRP3 inflammasome has also been demonstrated in the pathogenesis of AD mouse models; NLRP3 deficiency in mice resulted in a decrease in Aβ deposition.[52] In addition, enhanced caspase 1 activity in patients with early onset AD[52] is consistent with inflammasome-mediated events associated with secretion of IL-1β. Caspase 1 is recruited and activated via interactions with the adaptor protein apoptosis-associated speck-like protein containing a CARD. Notably, associated speck-like protein containing a CARD specks have been detected in brain sections of patients with AD and mouse transgenic models of AD, located both within microglia and extracellularly, and which can bind to Aβ deposits and may thereby act to enhance the inflammation-driven pathology.[57] Moreover, associated speck-like protein containing a CARD specks were shown to promote Aβ deposition *in vivo*.[57] A relationship between systemic inflammation and neuroinflammation is strongly suggested by a recent report which demonstrated that LPS-mediated systemic inflammation reduces the clearance of Aβ by microglia in the mouse brain, a process shown to be dependent on the NLRP3 inflammasome.[58]

The importance of microglia in AD pathogenesis is also demonstrated by the concentration of activated microglia around amyloid plaques from AD patients and in AD animal models[59,60] and the genetic data identifying AD risk genes from GWAS analysis as microglia membrane proteins.

Microglia communicate and interact with other cells of the CNS, in particular astrocytes and neurons[5] and as indicated previously, astrocytes may also play a role in neurodegeneration. Astrocytes contribute to synapse formation and synaptic strength regulation[61] and, like microglia, are involved in monitoring neuronal activity. Astrocytes both sense and modulate synaptic output and may play a role in regulating in the overall computing function of the brain.[61] Although a role for astrocytes in neurodegenerative diseases has been largely ignored in the past, potential interactions between microglia and astrocytes are now attracting considerable attention. Astrocytes guide microglia to synapses that have been pruned via the complement pathway.[62] Hence, astrocytes can influence the interaction of microglia with neurons, and thereby indirectly affect the delivery of neurotoxic and neurotrophic factors to neurons released by microglia. A recent example demonstrating the intimate and functional relevance of microglia-astrocyte interactions involves the regulation of localized release of active transforming growth factor (TGF) β1; LRRC33 on the surface of microglia interacts with the pro-TGFβ1–αVβ8 integrin complex

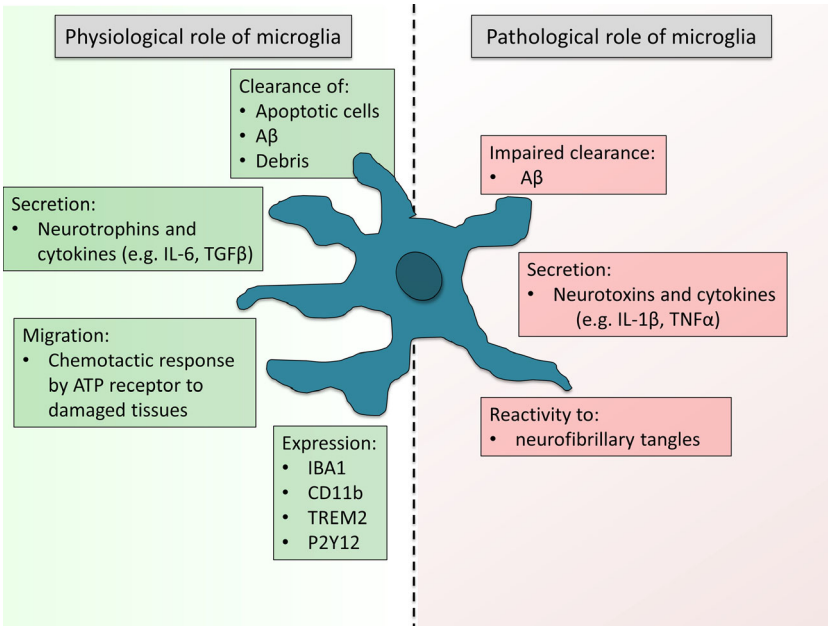

Figure 2. Differences in microglia function under physiological and pathological conditions. Microglia mediate immune responses by clearing apoptotic cells, debris and Aβ, and by the secretion of various neurotrophins and cytokine. Under pathological conditions, the clearance of Aβ is often impaired, leading to Aβ buildup and the secretion of neurotrophins, neurotoxins together with proinflammatory cytokines. ATP, adenosine triphosphate; IL, interleukin; TGF, transforming growth factor; TNF, tumor necrosis factor.

on the surface of astrocytes to promote release of active transforming growth factor β1.[63] For a detailed discussion of the role of astrocytes, see review articles by Kim *et al.*[64] and Frost and Li.[65]

Microglia and Other Neurodegenerative Diseases

Microglia involvement has also been associated with several other neurodegenerative diseases, in addition to AD, such as amyotrophic lateral sclerosis, where the release of proinflammatory cytokines by activated microglia leads to neuronal damage and neurotoxic activity.[66] They have further been implicated in Parkinson's disease as well as in Huntington's disease. In these examples, neuroinflammation is the major causative neurotoxic effector, demonstrating yet again the relevance of a better understanding of the link between microglia activation and its neurotoxic effects.[35]

NEUROINFLAMMATION

Evidence that inflammation has a causal role in the pathogenesis of AD comes both genetic and immunological analysis. Genes for various immune receptors such as triggering receptor expressed in myeloid cells 2 (TREM2) and CD33 are associated with AD and are expressed on the cell surface of microglia. In addition, microglia have been shown to be capable of binding to soluble Aβ oligomers and fibrils via cell surface receptors including CD36, CD14, CD47 and Toll-like receptors (TLR2, 4, 6 and 9). Moreover, Aβ binding to CD36, TLR4 or TLR6 results in activation of microglia, to produce proinflammatory cytokines and chemokines.[67] Proinflammatory cytokines secreted by activated microglia include TNFα and IL-1β; moreover, these cytokines are known to be upregulated in brains of AD patients and in transgenic mice with AD-like pathology, and in addition are secreted by primary microglia in culture.[68] TNFα secretion can either be harmful and promote neural damage or be beneficial and promote clearance of Aβ.[69] For example, transgenic expression of TNFα in the hippocampus of APP transgenic mice induced glial activation and did not appear to exacerbate Aβ pathology, rather it resulted in the reduction of Aβ plaques.[70] In addition, some studies indicate that TNFα may not always promote a proinflammatory response and the relative levels or balance of proinflammatory and anti-inflammatory cytokines is likely to be critical in defining the role of cytokines in actively promoting the disease.[71] Hence, the outcome of a proinflammatory TNFα response in the CNS may be dependent on the milieu of the microenvironment.

As for innate immune responses in the periphery, cytokines in the CNS are key regulators of neuroinflammation. The proinflammatory environment in the AD brain could directly and indirectly contribute to neuronal damage. For example, IL-1β and TNFα may directly impair neuronal function.[72] The rate of progression from mild cognitive impairment to the dementia stages of the disease is increased in patients who have elevated TNFα levels and decreased transforming growth factor β concentrations in the cerebrospinal fluid. Cytokines stimulate inducible nitric oxide synthase in microglia and astrocytes and inducible nitric oxide synthase is toxic to neurons at high concentrations. Notably, inducible nitric oxide synthase has been reported to be upregulated in the AD brain.[51] Consistent with this proposal, *in vitro* experiments have shown that binding of microglia to Aβ leads to the production of reactive oxygen species.[67]

Although Aβ deposition alone may be sufficient to induce an inflammatory reaction, risk factors such as systemic inflammation, obesity and traumatic brain injury might influence the development of AD through a sustained neuroinflammatory drive.[51] Inflammation preceding the development of AD may prime microglia, causing them to be highly responsive to further activation.[73] Subsequent stimulation by Aβ could then result in secretion of proinflammatory cytokines and chemokines, which could trigger neuronal hyperexcitability and synaptic dysfunction. Neurons had previously been considered to be a passive bystander in neuroinflammation; however, recent studies have demonstrated that neurons are also able to produce inflammatory mediators.[74] Activation of complement systems plays an important role in AD and neurons produce most of the components of the complement cascade. The complement system can be activated by pathogen-associated molecular patterns and/or danger-associated molecular patterns. C1q can directly bind to molecules such as Aβ, hence there is the potential for complement to be activated as a consequence of buildup of extracellular Aβ and/or release of components from dying neurons.[75] Moreover, neuronal production of complement components is increased in AD.[76] Neurons have also been postulated to be source of COX-2-derived prostanoids, a subclass of eicosanoids which are vasoactive lipid mediators, and several cytokines such as IL-1β, IL-6 and TNFα.[74]

A number of studies have shown that *in vivo* LPS treatment results in an increase level of Aβ1–42 and a decrease in the level of Aβ1–40.[77] This finding suggests a close connection between amyloidogenesis and neuroinflammation. However, the mechanisms responsible for LPS-induced amyloidogenesis are

unknown. Lee *et al.*[77] reported elevated β- and γ-secretase activity in cortical and hippocampal regions of ICR mice and Sprague-Dawley rats, as well as cell lysates upon LPS treatment, hence modified secretase activity could be a potential contributing factor. Furthermore, proinflammatory cytokines such as TNFα and IL-1β have been shown to increase levels of β-secretase messenger RNA, and BACE1 protein and enzymatic activity. Lee *et al.*[77] have proposed that LPS-induced inflammatory reactions influence APP processing through the enhancement of β- and γ-secretase activity and thereby affect amyloidogenesis.

Unfolded, misfolded and aggregated proteins are recognized by the DAMP receptors found on the cell surface of innate immune cells. An important finding in the neuroinflammation field is that aggregated Aβ acts as a DAMP, resulting in the activation of the innate immune system in the brain[78] with subsequent proinflammatory cytokine production. Hence, following an initial buildup of extracellular oligomeric Aβ, there is potential to stimulate an inflammatory response via microglia. As the proinflammatory response is directed to self-danger-associated molecular patterns, and as neurons are in turn impacted by the proinflammatory cytokines, a positive feedback loop is likely to be established, resulting in disease progression and establishing a chronic ongoing disease (Figure 3).

AD RISK GENES: INFLAMMATION AND MEMBRANE TRAFFICKING

For many years ApoE remained the only confirmed risk factor for LOAD. GWAS studies have now identified 29 risk genes.[79-81] The most recent GWAS study verified already known loci associated with AD and detected nine novel loci including *ADAMTS4* (secretase) and *CLNK* (a regulator of immunoreceptor signaling).[79] This recent study had a sample size eightfold larger than the previous GWAS and included genetic data of >600 000 individuals. Analysis of the identified single-nucleotide polymorphisms revealed that most of the variants are located in noncoding regions of the genome.[79]

The identified risk genes for LOAD can be broadly grouped into four categories: innate immune response, cholesterol metabolism, endosomal trafficking and APP catabolism/processing (summarized in Table 1).

Examples of innate immune responses include Clusterin, TREM2 and CD33. Clusterin, also known as apoJ, has several single-nucleotide polymorphisms that have been identified and shown to confer protection against LOAD. Clusterin is predicted to function in synapse turnover, Aβ aggregation, clearance and toxicity.[82] TREM2 is another example of a risk factor identified through GWAS studies which belongs to the innate immune response group. TREMs is a member of the immunoglobulin superfamily of receptors encoded by a gene cluster linked to the major histocompatibility complex.[83] TREM2 is a receptor expressed by myeloid cells, including microglia in the brain. TREM2 stimulates phagocytosis and plays a role in the reduction of inflammation by suppressing TLR-induced inflammatory cytokines and enhancing anti-inflammatory cytokine transcription.[83] TREM2 is also required for migration, lipid sensing and ApoE binding.[84] Overall, TREM2 as a surface receptor plays a fundamental role in the normal function of microglia in clearing Aβ deposition and controlling pathology in the brain.[85,86] A number of allelic variants of TREM2 confer an increased risk of AD.[87] The missense R47H variant of TREM2 is a major risk factor for AD and results in a partial loss of function of TREM2. The R47H variant of human TREM2 has been shown to have a detrimental effect on microglial function in a transgenic mouse model.[88] The risk associated with TREM2 mutations depends on the genetic background, as different populations show differences in their risk of developing AD for a given TREM2 mutation. For example, the TREM2 R47H variant confers an increased risk of developing AD similar to that of ApoE4 in Caucasians; however, there is no association between R47H status and risk of developing the disease in East Asian individuals.[87] For a detailed review on TREM2 and its link to microglia and AD, see Ulland and Colonna.[87]

CD33 has also been identified as a risk factor for LOAD. Two single-nucleotide polymorphisms in the CD33 gene have been associated with LOAD. Like TREM2, the CD33 variant associated with increased risk has been directly linked to impaired uptake of Aβ by microglia cells. CD33 is a member of the sialic acid-binding immunoglobulin-like lectins and mediates cell–cell interactions that inhibit or restrict immune responses.[89]

IMPACT OF NEUROINFLAMMATION ON TRAFFICKING OF APP AND BACE AND Aβ PRODUCTION

There is evidence that an increase of Aβ production arises as a direct result of neuroinflammation.[90] An increase in β- and γ-secretase activities may be one reason for the increased amyloidogenesis. In addition, proinflammatory cytokines in the brain (neuroinflammatory cytokines) have been reported to increase APP levels in neuronal cell models, suggesting that the expression of APP is upregulated.[90] The relationship between APP and inflammation is further discussed by Sastre *et al.*[91] In addition, another key issue is whether neuroinflammation has an effect on production of Aβ.

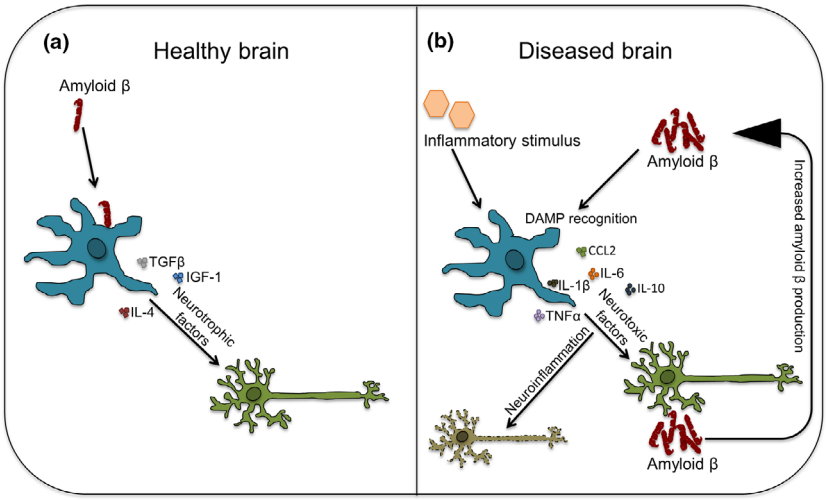

Figure 3. Potential positive feedback loop in the diseased brain between microglia and neurons. **(a)** In the healthy brain microglia cells clear amyloid β from the central nervous system microenvironment and secrete neurotrophic factors, maintaining a healthy neuron population. **(b)** A model illustrating the activation of microglia after danger-associated molecular pattern (DAMP) recognition of inflammatory stimuli or amyloid β stimulation. Microglia activation induces release of proinflammatory cytokines and mediates neuroinflammation and neurotoxicity. The resulting increase of amyloid β leads to further aggregation and the sustained low-grade inflammation found in Alzheimer's disease. IGF-1, insulin-like growth factor 1; IL, interleukin; TGF, transforming growth factor; TNF, tumor necrosis factor.

Neuronal BACE1 has been found to be induced in the proximity of activated glia cells,[91] hinting the possibility of an inflammation-dependent BACE1 upregulation. This suggestion was further supported by a study using neuronal cultures where exposure to proinflammatory cytokines and oxidative stress resulted in an increase in BACE1 protein.[91] As such, BACE1 has gained a lot of attention as a target for therapeutic inhibitors. While BACE inhibition has been shown to reduce Aβ levels, many clinical trials failed to rescue cognitive decline.[92] Selective targeting of BACE1 expression could present a more successful approach. An increase of matrix metalloproteinase 13 (MMP13) has been observed in both human AD brains and AD mouse models. The overexpression of MMP13 stimulates phosphatidylinositide kinase-3 signaling which in turn promotes the eukaryotic translation initiation factor 4B, which facilitates BACE1 messenger RNA translation. Selective targeting of MMP13 decreases eukaryotic translation initiation factor 4B phosphorylation and leads to a reduction of BACE1 synthesis. These findings highlight both MMP13 and phosphatidylinositide kinase-3/protein kinase B (Akt) signaling as therapeutic targets.[93]

To understand the direct connection between neuroinflammation and Aβ production, several studies have investigated the effect of LPS on Aβ production. *In vivo* animal experiments have shown that LPS injections induce memory loss and also the generation of Aβ1–42 in the cortex and hippocampus. How LPS induces amyloidogenesis remains unclear but it has been postulated to be related to changes in secretase activity, as LPS treatment was found to increase both β- and γ-secretase activities. This may be related to the activation of transcriptional upregulation of β-secretase messenger RNA.[77] Taken together, these findings indicate that LPS-augmented inflammatory reactions could influence APP processing through the enhancement of β- and γ-secretase activity and thereby affecting amyloidogenesis.[77]

Not only perturbations in the activity of the membrane-bound secretases, but also defects in membrane trafficking are linked to enhanced Aβ production. A number of trafficking machinery genes have been identified as LOAD risk genes.[94] The regulation of membrane trafficking plays an important role in APP processing and there is an intimate relationship between neuron activation and Aβ production. Increased neuronal activity results in increased Aβ production.[95] Aβ production resulting from increased neuronal activity has been linked to endosomal processing of APP and Aβ release from the cells.[96] Enhanced colocalization of exogenously expressed APP and BACE1 was observed following glycine-induced *N*-methyl-D-aspartate receptor activation or potassium activation,[97] findings suggesting that the trafficking of APP and BACE1 could be altered under different physiological conditions. Of relevance is that one of us has demonstrated that the phosphorylation of the BACE1 sorting motif, DISLL, is regulated by signaling in neurons[10] and that the

phosphorylation of the DISLL motif influences the endosomal trafficking of endogenous BACE1.[10] These studies highlight that external stimuli can induce changes to the trafficking itinerary of APP and BACE1 in primary neurons, which can also affect Aβ production. The question that arises from these findings is whether cytokines secreted from activated microglia can drive signaling events in neurons to influence trafficking and convergence of APP and BACE1. Of relevance is that TNFα has been shown to stimulate BACE1 expression and is also linked to enhanced Aβ production.[98] Collectively, these reports suggest that the inflammatory environment contributes to Aβ production.

Aβ-induced apoptosis is associated with cyclooxygenase-2 upregulation through activation of nuclear factor-κB signaling, and mediated by various kinases including extracellular signal-regulated kinase and p38 mitogen-activated protein kinase signaling. The increase of apoptotic neuronal cell death via elevation of Aβ1–42 could be an important mechanism in LPS-induced memory impairment.[99]

In summary, systemic inflammatory stimuli elevate amyloidogenesis through a number of likely mechanisms, including activation of β- and γ-secretases, inhibition of α-secretase and alterations in membrane trafficking of APP and BACE1, leading to elevated Aβ1–42 levels both *in vivo* and *in vitro*. The elevated inflammation and amyloidogenesis would then result in neuronal cell death and thus memory impairment.

RELATIONSHIP BETWEEN CHRONIC INFLAMMATION IN THE PERIPHERY AND AD

Given the importance of neuroinflammation in AD, it is important to consider the potential impact of peripheral chronic inflammation on the progression of AD. What do we know about conditions associated with chronic inflammation and susceptibility to AD? There is evidence of a correlation between systemic chronic inflammation, as determined by elevated C-reactive protein, and AD risk.[100] Inflammatory conditions are also increasingly linked to AD. Oral health and infection, such as periodontitis, are correlated with AD, reviewed recently by Teixeira *et al.*[101] The relationship between chronic inflammatory autoimmune diseases, such as rheumatoid arthritis (RA), which is characterized by both elevated C-reactive protein and TNFα, and AD has been studied. While one study found that AD was more prevalent among RA patients than those individuals not affected by RA,[102] Kao *et al.* claimed an inverse correlation between the two diseases.[103]

Is there any evidence of protection from AD by long-term anti-inflammatory medication? Several epidemiological studies have provided evidence for a reduced prevalence of AD among nonsteroidal anti-inflammatory drug users.[104,105] The effects appear to be strongly dependent on both the duration of treatment and the ApoE genotype. Individuals with at least one ApoE4 allele benefited significantly more from nonsteroidal anti-inflammatory drug use,[106] possibly because of their increased risk of AD. However, while some studies suggest beneficial effects of nonsteroidal anti-inflammatory drugs such as ibuprofen, other studies did not find a correlation. A more recent report, involving a very large cohort of 8.5 million participants, not only confirmed an increased AD risk among RA patients, but also indicated there could be an important connection between anti-TNFα therapy for RA and reduced risk of AD among RA patients. RA patients on anti-TNF therapy with etanercept have a lowered risk of AD.[102] This represents an exciting observation that needs further investigation.

CONCLUSION AND FUTURE DIRECTIONS

There is now substantial evidence that neuroinflammation contributes to the progression of AD and, as such, AD can now be considered as a chronic inflammatory disease. In addition, there is an increasing appreciation that the pathways of Aβ production and accumulation and of inflammation may converge and synergize the progression of this neurodegenerative disease. However, despite the recent advances, there remain considerable gaps in our knowledge on the interactions between the different cell types involved in AD and the molecular details of the pathways which link Aβ accumulation and on-going inflammation. Unravelling the underlying mechanisms of this system could help to identify new therapeutic targets and to provide a deeper understanding as to why current therapeutic strategies are often failing.

Given the relevance of inflammation to the progression and pathology of AD, current therapeutic treatments from other chronic inflammatory conditions could be exploited. The role of MMPs in AD was mentioned earlier. MMPs play a crucial role in the development of osteoarthritis. As for neuroinflammation, in osteoarthritis TNFα and IL-1β drive the inflammatory process.[107] Pharmacological inhibition of MMP13 is an effective strategy in mouse models of osteoarthritis.[108] Of relevance is that selective targeting of MMP13 decreases eukaryotic translation initiation factor 4B phosphorylation, causing a reduction of BACE1 synthesis, and that studies in a mouse model resulted in attenuated cerebral amyloid pathology, rescued learning and memory deficits in an AD mouse model.[109] Hence, these findings

provide support to investigate strategies for anti-inflammatory treatments in AD.

Another approach is to target the feedback loop between proinflammatory cytokine release by activated microglia and Aβ production and its dispersal. The molecular details of this feedback pathway need to be better defined both *in vitro* and *in vivo* as it is likely to provide crucial insights into understanding disease development and also to reveal novel therapeutic targets. More information is required on how activated microglia affect neurons. Coculturing primary neurons and microglial cells, or exposing one cell type to conditioned media of the other, would be worthwhile experiments to establish a system to define the effectors responsible for inflammation in AD. In addition, exploiting newly emerging three-dimensional models of brain tissue[110] containing activated microglia and neurons could also aid in defining the key events associated with Aβ secretion and turnover, and Aβ neurotoxicity under inflammatory conditions. Organoid cultures of neurons, microglia and astrocytes from induced pluripotent stem cells derived from cells from patients with familial mutations could provide a powerful approach to understand the impact of disease-causing mutations within a cellular environment that mimics the physiological tissue.

In addition to *in vitro* cell and tissue systems, existing mouse models used by immunologists and neurobiologists could be further exploited. For example, cytokine reporter mice,[111] used extensively in immunological studies of peripheral responses, could be used for the identification of cytokine expressing cells *in vivo*, and would be particularly useful for neuroinflammation studies. The application of sophisticated imaging technologies with AD mouse models would also be beneficial. These include combining fluorescent protein-labeled membrane proteins and secreted cytokines with cranial windows in AD mouse models to track the events *in situ*. Furthermore, laser capture microdissection-targeted mass spectrometry could also be used to identify inflammatory cytokines and other neurotoxic products in defined regions of the brain in AD mouse models. A recent study by MacDonald *et al.*[112] has successfully investigated this combined approach. Analyzing both human and monkey model postmortem they were able to quantify over 200 proteins in different cortical layers. This promising approach has the advantage of exceptional precision and throughput without losing sensitivity and has the potential to generate insights into proteomics of specific brain tissues.[112] This approach could potentially be exploited for cytokine profile analysis in human postmortem studies. Such approaches would provide a spatial map of the events associated with neuroinflammation and the impact on neurons.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Alzheimer A. Ueber einen eigenartigen schweren Erkrankungsprozess der Hirnrinde. *Neurol Central* 1906; **25**: 1134.
2. Probst A, Langui D, Ulrich J. Alzheimer's disease: a description of the structural lesions. *Brain Pathol*; **1**: 229–239.
3. Ginhoux F, Greter M, Leboeuf M, *et al.* Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science* 2010; **330**: 841–845.
4. Braak H, Braak E. Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol* 1991; **82**: 239–259.
5. Heneka MT, Kummer MP, Latz E. Innate immune activation in neurodegenerative disease. *Nat Rev Immunol* 2014; **14**: 463–477.
6. Hong S, Dissing-Olesen L, Stevens B. New insights on the role of microglia in synaptic pruning in health and disease. *Curr Opin Neurobiol* 2016; **36**: 128–134.
7. Block ML, Hong J-S. Chronic microglial activation and progressive dopaminergic neurotoxicity. *Biochem Soc Trans* 2007; **35**: 1127–1132.
8. Kang J, Lemaire H-G, Unterbeck A, *et al.* The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. *Nature* 1987; **325**: 733.
9. Vetrivel KS, Thinakaran G. Membrane rafts in Alzheimer's disease beta-amyloid production. *Biochim et Biophys Acta (BBA) - Mol Cell Biol Lipids* 2010; **1801**: 860–867.
10. Toh WH, Chia PZC, Hossain MI, Gleeson PA. GGA1 regulates signal-dependent sorting of BACE1 to recycling endosomes, which moderates Aβ production. *MBoC* 2018; **29**: 191–208.
11. Sisodia SS. Beta-amyloid precursor protein cleavage by a membrane-bound protease. *PNAS* 1992; **89**: 6075–6079.
12. Thinakaran G, Koo EH. Amyloid precursor protein trafficking, processing, and function. *J Biol Chem* 2008; **283**: 29615–29619.
13. Wang X, Zhou X, Li G, Zhang Y, Wu Y, Song W. Modifications and trafficking of APP in the pathogenesis of Alzheimer's disease. *Front Mol Neurosci* 2017; **10**: 294. https://doi.org/10.3389/fnmol.2017.00294.
14. Tan JZA, Gleeson PA. The role of membrane trafficking in the processing of amyloid precursor protein and production of amyloid peptides in Alzheimer's disease. *Biochim Biophys Acta (BBA) -Biomembr* 2019; **1861**: 697–712.
15. Rajendran L, Annaert W. Membrane trafficking pathways in Alzheimer's disease. *Traffic* 2012; **13**: 759–770.
16. Haass C. Take five—BACE and the γ-secretase quartet conduct Alzheimer's amyloid β-peptide generation. *EMBO J* 2004; **23**: 483–488.

17. Fukumoto H, Cheung BS, Hyman BT, Irizarry MC. β-secretase protein and activity are increased in the neocortex in Alzheimer disease. *Arch Neurol* 2002; **59**: 1381–1389.
18. Vassar R, Kovacs DM, Yan R, Wong PC. The β-secretase enzyme BACE in health and Alzheimer's disease: regulation, cell biology, function, and therapeutic potential. *J Neurosci* 2009; **29**: 12787–12794.
19. Yan R, Han P, Miao H, Greengard P, Xu H. The transmembrane domain of the Alzheimer's β-secretase (BACE1) determines its late Golgi localization and access to APP substrate. *J Biol Chem* 2009; **276**: 36788–36796.
20. Laird FM, Cai H, Savonenko AV, *et al.* BACE1, a major determinant of selective vulnerability of the brain to amyloid-beta amyloidogenesis, is essential for cognitive, emotional, and synaptic functions. *J Neurosci* 2005; **25**: 11693–11709.
21. Coimbra JRM, Marques DFF, Baptista SJ, *et al.* Highlights in BACE1 inhibitors for Alzheimer's disease treatment. *Front Chem* 2018; **6**: 178. https://doi.org/10.3389/fchem.2018.00178
22. Müller UC, Deller T, Korte M. Not just amyloid: physiological functions of the amyloid precursor protein family. *Nat Rev Neurosci* 2017; **18**: 281–298.
23. Gosztyla ML, Brothers HM, Robinson SR. Alzheimer's amyloid-β is an antimicrobial peptide: a review of the evidence. *J Alzheimer's Dis* 2018; **62**: 1495–1506.
24. Scheuner D, Eckman C, Jensen M, *et al.* Secreted amyloid β–protein similar to that in the senile plaques of Alzheimer's disease is increased *in vivo* by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. *Nat Med* 1996; **2**: 864.
25. Weggen S, Beher D. Molecular consequences of amyloid precursor protein and presenilin mutations causing autosomal-dominant Alzheimer's disease. *Alzheimer's Res Ther* 2012; **4**: 9.
26. Jonsson T, Atwal JK, Steinberg S, *et al.* A mutation in *APP* protects against Alzheimer's disease and age-related cognitive decline. *Nature* 2012; **488**: 96–99.
27. Vassar R, Bennett BD, Babu-Khan S, *et al.* Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. *Science* 1999; **286**: 735–741.
28. Jarrett JT, Berger EP, Lansbury PT. The C-terminus of the β protein is critical in amyloidogenesisa. *Ann N Y Acad Sci* 1993; **695**: 144–148.
29. Tarasoff-Conway JM, Carare RO, Osorio RS, *et al.* Clearance systems in the brain—implications for Alzheimer disease. *Nat Rev Neurol* 2015; **11**: 457–470.
30. Takemura R, Okabe S, Umeyama T, Kanai Y, Cowan NJ, Hirokawa N. Increased microtubule stability and alpha tubulin acetylation in cells transfected with microtubule-associated proteins MAP1B, MAP2 or tau. *J Cell Sci* 1992; **103**: 953–964.
31. Iba M, Guo JL, McBride JD, Zhang B, Trojanowski JQ, Lee VM-Y. Synthetic tau fibrils mediate transmission of neurofibrillary tangles in a transgenic mouse model of Alzheimer's-like tauopathy. *J Neurosci* 2013; **33**: 1024–1037.
32. Crimins JL, Pooler A, Polydoro M, Luebke JI, Spires-Jones TL. The intersection of amyloid β and tau in glutamatergic synaptic dysfunction and collapse in Alzheimer's disease. *Ageing Res Rev* 2013; **12**: 757–763.
33. Bussian TJ, Aziz A, Meyer CF, Swenson BL, van Deursen JM, Baker DJ. Clearance of senescent glial cells prevents tau-dependent pathology and cognitive decline. *Nature* 2018; **562**: 578–582.
34. del Rio-Hortega P. Microglia. *Cytol Cell Pathol Nerv Syst* 1932; **11**: 481–534.
35. Colonna M, Butovsky O. Microglia function in the central nervous system during health and neurodegeneration. *Annu Rev Immunol* 2017; **35**: 441–468.
36. Kierdorf K, Erny D, Goldmann T, *et al.* Microglia emerge from erythromyeloid precursors via Pu.1- and Irf8-dependent pathways. *Nat Neurosci* 2013; **16**: 273–280.
37. Prinz M, Priller J. Microglia and brain macrophages in the molecular age: from origin to neuropsychiatric disease. *Nat Rev Neurosci* 2014; **15**: 300–312.
38. Hammond TR, Robinton D, Stevens B. Microglia and the brain: complementary partners in development and disease. *Annu Rev Cell Dev Biol* 2018; **34**: 523–544.
39. Sarlus H, Heneka MT. Microglia in Alzheimer's disease. *J Clin Invest* 2017; **127**: 3240–3249.
40. Färber K, Kettenmann H. Physiology of microglial cells. *Brain Res Rev* 2005; **48**: 133–143.
41. Roy A, Fung YK, Liu X, Pahan K. Up-regulation of microglial CD11b expression by nitric oxide. *J Biol Chem* 2006; **281**: 14971–14980.
42. Nakajima K, Kohsaka S. Microglia: activation and their significance in the central nervous system. *J Biochem* 2001; **130**: 169–175.
43. Zhao H, Cheng L, Liu Y, *et al.* Mechanisms of anti-inflammatory property of conserved dopamine neurotrophic factor: inhibition of jnk signaling in lipopolysaccharide-induced microglia. *J Mol Neurosci* 2014; **52**: 186–192.
44. Nakajima K, Kikuchi Y, Ikoma E, *et al.* Neurotrophins regulate the function of cultured microglia. *Glia* 1998; **24**: 272–289.
45. Lee DC, Rizer J, Selenica M-LB, *et al.* LPS- induced inflammation exacerbates phospho-tau pathology in rTg4510 mice. *J Neuroinflamm* 2010; **7**: 56.
46. Ransohoff RM. A polarizing question: do M1 and M2 microglia exist? *Nat Neurosci* 2016; **19**: 987–991.
47. Hanisch U-K. Functional diversity of microglia – how heterogeneous are they to begin with? *Front Cell Neurosci* 2013; **7**: 65. https://doi.org/10.3389/fncel.2013.00065.
48. Nimmerjahn A, Kirchhoff F, Helmchen F. Resting microglial cells are highly dynamic surveillants of brain parenchyma *in vivo*. *Science* 2005; **308**: 1314–1318.
49. Chen Z, Trapp BD. Microglia and neuroprotection. *J Neurochem* 2016; **136**: 10–17.
50. Lee CYD, Landreth GE. The role of microglia in amyloid clearance from the AD brain. *J Neural Transm* 2010; **117**: 949–960.
51. Heneka MT, Carson MJ, El Khoury J, *et al.* Neuroinflammation in Alzheimer's disease. *Lancet Neurol* 2015; **14**: 388–405.

52. Heneka MT, Kummer MP, Stutz A, *et al.* NLRP3 is activated in Alzheimer's disease and contributes to pathology in APP/PS1 mice. *Nature* 2013; **493**: 674–678.
53. Fiebich BL, Batista CRA, Saliba SW, Yousif NM, de Oliveira ACP. Role of microglia TLRs in neurodegeneration. *Front Cell Neurosci* 2018; **12**: 329. https://doi.org/10.3389/fncel.2018.00329.
54. Fonseca MI, Chu S-H, Hernandez MX, *et al.* Cell-specific deletion of C1qa identifies microglia as the dominant source of C1q in mouse brain. *J Neuroinflammation* 2017; **14**: 48. https://doi.org/10.1186/s12974-017-0814-9.
55. Liddelow SA, Guttenplan KA, Clarke LE, *et al.* Neurotoxic reactive astrocytes are induced by activated microglia. *Nature* 2017; **541**: 481–487.
56. Walsh JG, Muruve DA, Power C. Inflammasomes in the CNS. *Nat Rev Neurosci* 2014; **15**: 84–97.
57. Venegas C, Kumar S, Franklin BS, *et al.* Microglia-derived ASC specks cross-seed amyloid-β in Alzheimer's disease. *Nature* 2017; **552**: 355–361.
58. Tejera D, Mercan D, Sanchez-Caro JM, *et al.* Systemic inflammation impairs microglial Aβ clearance through NLRP3 inflammasome. *EMBO J* 2019; **38**: e101064.
59. Frautschy SA, Yang F, Irrizarry M, *et al.* Microglial response to amyloid plaques in APPsw transgenic mice. *Am J Pathol* 1998; **152**: 307–317.
60. McGeer PL, Itagaki S, Tago H, McGeer EG. Reactive microglia in patients with senile dementia of the Alzheimer type are positive for the histocompatibility glycoprotein HLA-DR. *Neurosci Lett* 1987; **79**: 195–200.
61. Allen NJ. Astrocyte regulation of synaptic behavior. *Annu Rev Cell Dev Biol* 2014; **30**: 439–463.
62. Lian H, Yang L, Cole A, *et al.* NFκB-activated astroglial release of complement C3 compromises neuronal morphology and function associated with Alzheimer's disease. *Neuron* 2015; **85**: 101–115.
63. Qin Y, Garrison BS, Ma W, *et al.* A milieu molecule for TGF-β required for microglia function in the nervous system. *Cell* 2018; **174**: 156–171.e16
64. Kim YS, Jung HM, Yoon B-E. Exploring glia to better understand Alzheimer's disease. *Animal Cells Syst* 2018; **22**: 213–218.
65. Frost GR, Li Y-M. The role of astrocytes in amyloid production and Alzheimer's disease. *Open Biol* 2017; **7**: 170228. https://doi.org/10.1098/rsob.170228.
66. Boillée S, Yamanaka K, Lobsiger CS, *et al.* Onset and progression in inherited ALS determined by motor neurons and microglia. *Science* 2006; **312**: 1389–1392.
67. El Khoury JB, Moore KJ, Means TK, *et al.* CD36 mediates the innate host response to beta-amyloid. *J Exp Med* 2003; **197**: 1657–1666.
68. Grammas P, Ovase R. Inflammatory factors are elevated in brain microvessels in Alzheimer's disease. *Neurobiol Aging* 2001; **22**: 837–842.
69. Shohami E, Ginis I, Hallenbeck JM. Dual role of tumor necrosis factor alpha in brain injury. *Cytokine Growth Factor Rev* 1999; **10**: 119–130.
70. Chakrabarty P, Herring A, Ceballos-Diaz C, Das P, Golde TE. Hippocampal expression of murine TNFα results in attenuation of amyloid deposition *in vivo*. *Mol Neurodegener* 2011; **6**: 16.
71. Zheng C, Zhou X-W, Wang J-Z. The dual roles of cytokines in Alzheimer's disease: update on interleukins, TNF-α, TGF-β and IFN-γ. *Transl Neurodegener* 2016; **5**: 7. https://doi.org/10.1186/s40035-016-0054-4.
72. Ye L, Huang Y, Zhao L, *et al.* IL-1β and TNF-α induce neurotoxicity through glutamate production: a potential role for neuronal glutaminase. *J Neurochem* 2013; **125**: 897–908.
73. Colton CA, Mott RT, Sharpe H, Xu Q, Van Nostrand WE, Vitek MP. Expression profiles for macrophage alternative activation genes in AD and in mouse models of AD. *J Neuroinflammation* 2006; **3**: 27.
74. Heneka MT, O'Banion MK, Terwel D, Kummer MP. Neuroinflammatory processes in Alzheimer's disease. *J Neural Transm (Vienna)* 2010; **117**: 919–947.
75. Orsini F, De Blasio D, Zangari R, Zanier ER, De Simoni M-G. Versatility of the complement system in neuroinflammation, neurodegeneration and brain homeostasis. *Front Cell Neurosci* 2014; **8**: 380. https://doi.org/10.3389/fncel.2014.00380.
76. Eikelenboom P, Hack CE, Rozemuller JM, Stam FC. Complement activation in amyloid plaques in Alzheimer's dementia. *Virchows Arch B* 1989; **56**: 259–262.
77. Lee JW, Lee YK, Yuk DY, *et al.* Neuro-inflammation induced by lipopolysaccharide causes cognitive impairment through enhancement of beta-amyloid generation. *J Neuroinflammation* 2008; **5**: 37.
78. Heneka MT. Inflammasome activation and innate immunity in Alzheimer's disease. *Brain Pathol* 2017; **27**: 220–222.
79. Jansen IE, Savage JE, Watanabe K, *et al.* Genome-wide meta-analysis identifies new loci and functional pathways influencing Alzheimer's disease risk. *Nat Genet* 2019; **51**: 404.
80. Lambert J-C, Ibrahim-Verbaas CA, Harold DC, *et al.* Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. *Nat Genet* 2013; **45**: 1452–1458.
81. Lambert J-C, Heath S, Even G, *et al.* Genome-wide association study identifies variants at CLU and CR81 associated with Alzheimer's disease. *Nat Genet* 2009; **41**: 1094–1099.
82. Bettens K, Sleegers K, Van Broeckhoven C. Genetic insights in Alzheimer's disease. *Lancet Neurol* 2013; **12**: 92–104.
83. Jay TR, Miller CM, Cheng PJ, *et al.* TREM2 deficiency eliminates TREM2 + inflammatory macrophages and ameliorates pathology in Alzheimer's disease mouse models. *J Exp Med* 2015; **212**: 287–295.
84. Schlepckow K, Kleinberger G, An Fukumori A, *et al.* Alzheimer-associated TREM2 variant occurs at the ADAM cleavage site and affects shedding and phagocytic function. *EMBO Mol Med* 2017; **9**: 1356–1365. e201707672.

85. Lee CYD, Daggett A, Gu X, *et al.* Elevated TREM2 gene dosage reprograms microglia responsivity and ameliorates pathological phenotypes in Alzheimer's disease models. *Neuron* 2018; **97**: 1032–1048.e5.
86. Ulland TK, Song WM, Huang SC-C, *et al.* TREM2 maintains microglial metabolic fitness in Alzheimer's disease. *Cell* 2017; **170**: 649–663.e13.
87. Ulland TK, Colonna M. TREM2 — a key player in microglial biology and Alzheimer disease. *Nat Rev Neurol* 2018; **14**: 667–675.
88. Song WM, Joshita S, Zhou Y, Ulland TK, Gilfillan S, Colonna M. Humanized TREM2 mice reveal microglia-intrinsic and -extrinsic effects of R47H polymorphism. *J Exp Med* 2018; **215**: 745–760.
89. Griciuc A, Serrano-Pozo A, Parrado AR, *et al.* Alzheimer's disease risk gene CD33 inhibits microglial uptake of amyloid beta. *Neuron* 2013; **78**: 631–643.
90. Alasmari F, Alshammari MA, Alasmari AF, Alanazi WA, Alhazzani K. Neuroinflammatory cytokines induce amyloid beta neurotoxicity through modulating amyloid precursor protein levels/metabolism. *Biomed Res Int* 2018; **78**: 3087475. https://doi.org/10.1155/2018/3087475.
91. Sastre M, Walter J, Gentleman SM. Interactions between APP secretases and inflammatory mediators. *J Neuroinflammation* 2008; **5**: 25.
92. Zhu K, Peters F, Filser S, Herms J. Consequences of pharmacological BACE inhibition on synaptic structure and function. *Biol Psychiat* 2018; **84**: 478–487.
93. Zhu B-L, Long Y, Luo W, *et al.* MMP13 inhibition rescues cognitive decline in Alzheimer transgenic mice via BACE1 regulation. *Brain* 2019; **142**: 176–192.
94. Small SA, Simoes-Spassov S, Mayeux R, Petsko GA. Endosomal traffic jams represent a pathogenic hub and therapeutic target in Alzheimer's disease. *Trends Neurosci* 2017; **40**: 592–602.
95. Kamenetz F, Tomita T, Hsieh H, *et al.* APP processing and synaptic function. *Neuron* 2003; **37**: 925–937.
96. Cirrito JR, Kang J-E, Lee J, *et al.* Endocytosis is required for synaptic activity-dependent release of amyloid-β *in vivo*. *Neuron* 2008; **58**: 42–51.
97. Das U, Scott DA, Ganguly A, Koo EH, Tang Y, Roy S. Activity-induced convergence of APP and BACE-1 in acidic microdomains via an endocytosis-dependent pathway. *Neuron* 2013; **79**: 447–460.
98. Yamamoto M, Kiyota T, Horiba M, *et al.* Interferon-γ and tumor necrosis factor-α regulate amyloid-β plaque deposition and β-secretase expression in swedish mutant APP transgenic mice. *Am J Pathol* 2007; **170**: 680–692.
99. Jang J-H, Surh Y-J. β-Amyloid-induced apoptosis is associated with cyclooxygenase-2 up-regulation via the mitogen-activated protein kinase–NF-κB signaling pathway. *Free Radic Biol Med* 2005; **38**: 1604–1613.
100. Tao Q, Ang TFA, DeCarli C, *et al.* Association of chronic low-grade inflammation with risk of Alzheimer disease in ApoE4 carriers. *JAMA Netw Open* 2018; **1**: e183597.
101. Teixeira FB, Saito MT, Matheus FC, *et al.* Periodontitis and Alzheimer's disease: a possible comorbidity between oral chronic inflammatory condition and neuroinflammation. *Front Aging Neurosci* 2017; **9**: 327. https://doi.org/10.3389/fnagi.2017.00327.
102. Chou RC, Kane M, Ghimire S, Gautam S, Gui J. Treatment for rheumatoid arthritis and risk of Alzheimer's disease: a nested case-control analysis. *CNS Drugs* 2016; **30**: 1111–1120.
103. Kao L-T, Kang J-H, Lin H-C, Huang C-C, Lee H-C, Chung S-D. Rheumatoid arthritis was negatively associated with Alzheimer's disease: a population-based case-control study. *PLoS One* 2016; **11**: e0168106.
104. Gómez-Isla T, Blesa R, Boada M, *et al.* A randomized, double-blind, placebo controlled-trial of triflusal in mild cognitive impairment: the TRIMCI study. *Alzheimer Dis Assoc Disord* 2008; **22**: 21–29.
105. Rogers J, Kirby LC, Hempelman SR, *et al.* Clinical trial of indomethacin in Alzheimer's disease. *Neurology* 1993; **43**: 1609–1611.
106. Imbimbo BP, Solfrizzi V, Panza F. Are NSAIDs useful to treat Alzheimer's disease or mild cognitive impairment? *Front Aging Neurosci* 2010; **2**. https://doi.org/10.3389/fnagi.2010.00019.
107. Schlaak JF, Schwarting A, Knolle P, Meyer zum Büschenfelde KH, Mayet W. Effects of Th1 and Th2 cytokines on cytokine production and ICAM-1 expression on synovial fibroblasts. *Ann Rheum Dis* 1995; **54**: 560–565.
108. Wang M, Sampson ER, Jin H, *et al.* MMP13 is a critical target gene during the progression of osteoarthritis. *Arthritis Res Ther* 2013; **15**: R5.
109. Paumier J-M, Thinakaran G. Matrix metalloproteinase 13, a new target for therapy in Alzheimer's disease. *Genes Dis* 2019; **6**: 1–2.
110. Cho HJ, Verbridge SS, Davalos RV, Lee YW. Development of an *in vitro* 3D brain tissue model mimicking *in vivo*-like pro-inflammatory and pro-oxidative responses. *Ann Biomed Eng* 2018; **46**: 877–887.
111. Croxford AL, Buch T. Cytokine reporter mice in immunological research: perspectives and lessons learned. *Immunology* 2011; **132**: 1–8.
112. MacDonald ML, Favo D, Garver M, *et al.* Laser capture microdissection–targeted mass spectrometry: a method for multiplexed protein quantification within individual layers of the cerebral cortex. *Neuropsychopharmacology* 2019; **44**: 743.
113. Corder EH, Saunders AM, Strittmatter WJ, *et al.* Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* 1993; **261**: 921–923.