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Interplay between innate immunity and Alzheimer's disease: APOE and TREM2 in the spotlight

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Abstract

Alzheimer's disease is more than a pure proteopathy. Chronic neuroinflammation stands out during the pathogenesis of the disease and in turn modulates disease progression. The central nervous system (CNS) is separated from the blood circulation by the blood-brain barrier. In Alzheimer's disease, neuroinflammation heavily relies on innate immune responses that are primarily mediated by CNS-resident microglia. Apolipoprotein E (ApoE) is the strongest genetic risk factor for Alzheimer's disease and was recently shown to affect the disease in part through its immunomodulatory function. This function of ApoE is likely linked to triggering receptor expressed on myeloid cells 2 (TREM2), which is expressed by microglia in the CNS. Here, we review the rapidly growing literature on the role of disease-associated microglia, TREM2 and ApoE in the pathogenesis of Alzheimer's disease, and present an integrated view of innate immune function in Alzheimer's disease.

Introduction

Alzheimer's disease is the most common cause of dementia. It was first reported by Alois Alzheimer in 1906 in a description of a 51-year-old woman who presented with cognitive deficits and behavioral abnormalities¹. Her autopsy showed brain atrophy with 'miliary foci' and characteristic neurofibrillary changes, which represent two distinct pathological hallmarks: extracellular deposition of amyloid plaques consisting predominantly of amyloid- β [G] peptides, and intraneuronal accumulation of neurofibrillary tangles comprising aggregated, hyperphosphorylated tau [G] protein. Alzheimer's disease pathology begins decades prior to the onset of clinical symptoms. Extensive biomarker and imaging studies indicate that amyloid- β aggregation is the earliest pathological event in Alzheimer's disease, followed at a later stage by pathological changes of tau aggregation extending beyond the

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Author contributions

Y.S. wrote the draft of the manuscript. D.M.H. reviewed and edited the manuscript.

Competing interests statement

D.M.H. co-founded and is on the scientific advisory board of C2N Diagnostics. D.M.H. is on the scientific advisory board of Denali, Genentech, and Proclara. D.M.H. consults for AbbVie and Eli Lilly.

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limbic system to the neocortex, which correlates with brain atrophy and cognitive decline^{2,3}. The amyloid hypothesis poses that amyloid- β accumulation initiates a cascade of downstream pathological events and drives Alzheimer's disease progression⁴. Although Alzheimer's disease was initially considered a cell autonomous neurodegenerative disorder, marked neuroinflammation is observed in the brains of patients with Alzheimer's disease, alongside amyloid- β and tau pathology. Accumulating genetic and functional evidence strongly indicate an active role of brain innate immunity in Alzheimer's disease pathogenesis and disease progression. In this Review, we summarize the exciting research progress in understanding the roles of innate immunity in Alzheimer's disease. We first highlight the significance of the innate immune system in Alzheimer's disease by describing their genetic linkage. We then discuss the current understanding of how brain innate immunity affects Alzheimer's disease pathogenesis, with a particular focus on the microglia-apolipoprotein E (ApoE)-triggering receptor expressed on myeloid cells 2 (TREM2) axis.

Linking the innate immune system to Alzheimer's disease

Genetic evidence.

Alzheimer's disease is divided into two main categories. A small percentage (<1%) of Alzheimer's disease is inherited within families in an autosomal-dominant fashion that manifests with early dementia onset, typically between age 30 and 60. This form of familial Alzheimer's disease (FAD) is caused by mutations in genes affecting amyloid- β production, A β 42/A β 40 ratio [G], or amyloid- β structure, such as amyloid precursor protein (*APP*), presenilin-1 (*PSEN1*) and presenilin-2 (*PSEN2*)⁵. Over 99% of Alzheimer's disease cases occur later in life, typically in people over 65 years old, and are referred to as 'late-onset' Alzheimer's disease (LOAD). While there is no identified 'cause' for LOAD, the two most important risk factors are age and genetics. To date, more than 30 Alzheimer's disease-risk loci have been identified via a combination of genetic linkage, candidate gene, genome-wide association studies (GWAS), and whole genome/exome sequencing (WGS/WES) studies⁶. Of these risk loci, over 50% of gene variants validated by functional genomics are implicated in microglial and innate immune cell function, including the top two risk genes, *APOE* and *TREM2*. In addition, epigenomic analysis has shown that Alzheimer's disease GWAS loci are preferentially enriched in enhancer sequences implicated in innate immune processes⁷. These findings indicate an essential role of the innate immune response in Alzheimer's disease pathogenesis. Major Alzheimer's disease genetic risk factors and their linkage to innate immune functions are shown in Box 1–Box 3.

Why does innate immunity matter in Alzheimer's disease?

Chronic glial activation is a prominent feature accompanying pathological protein accumulation in the Alzheimer's disease brain. Prolonged neuroinflammation may induce neuronal injury and death via multiple ways. First, activated glia produce toxic substances such as reactive oxygen species (ROS) and nitric oxide (NO) that can directly damage or kill neurons; second, inflammatory stress and sub-lethal pathological insults can induce stressed-but-viable neurons to expose reversible 'eat-me' signals such as phosphatidylserine or calreticulin, causing the neurons to be phagocytosed and killed by activated microglia via a process called phagoptosis⁸; third, activation of the complement system promotes membrane

attack complexes (MAC) formation, creating pores on cell membranes to induce cell lysis; fourth, activated microglia can crosstalk with astrocytes, inducing reactive astrocytes to rapidly kill neurons via secreted neurotoxic factors and loss of neurotrophic function⁹, and fifth, neuronal NLRP1 (NACHT, LRR and PYD domains-containing protein 1) inflammasome and caspase-1 activation in Alzheimer's disease can induce neuronal injury and death^{10,11}. In addition to a direct effect on neuronal viability, innate immunity also affects amyloid- β and tau pathology buildup, which in turn can influence neurodegeneration. Therefore, innate immunity is an indispensable component in Alzheimer's disease pathogenesis and may constitute a driving force for disease progression.

As the principle immune cell type in the brain, microglia play a key role in mediating brain innate immunity. Microglial cell function in the pathogenesis in Alzheimer's disease will be discussed in the following sections.

Microglia biology

Origin and functions in the brain.

Microglia are the macrophages of the brain and play central roles in neuroinflammation. They are derived from embryonic yolk sac (YS) precursors and maintain their CNS population via local self-renewal, with little contribution from bone marrow-derived monocytes^{12,13}. They retain many features of macrophages, but also adapt themselves to meet specific requirements of the neural tissue. During development, microglia prune complement-tagged immature synapses^{14,15}, eliminate redundant neural precursor cells¹⁶, and promote developmental neuronal apoptosis along with removal of cell corpses^{17,18}. In the adult brain, highly dynamic microglia processes constantly survey the local environment^{19,20} in a speed estimated to cover the entire parenchyma within a few hours²⁰. Comprehensive signaling components, including major classes of neurotransmitter receptors, pattern recognition receptors, cytokine and chemokine receptors, as well as other receptor systems, converge on microglia²¹, making them a superior sensor and reactor to environmental cues. This grants microglia the capacity to monitor neuronal activity and modulate synaptic plasticity²². During stresses, such as brain injury, infection, or neuropathological diseases, microglia are activated in an effort to combat the insult and control damage. However, in this instance, both protective and detrimental effects can occur.

Disease-associated microglia: an updated understanding.

The signal transduction pathways for microglial cell activation have been well documented (Box 4). Initial characterization of microglial cell polarization into pro-inflammatory 'M1' and reparative 'M2' phenotypes was based on the M1/M2 macrophage classification.²³ Macrophages acquire an M1 phenotype in response to interferon γ (IFN γ) and synthesize toxic substances, such as ROS, NO, and lysosomal enzymes to kill microbes. By contrast, M2 macrophages are induced by interleukin-4 (IL-4) and express enzymes for collagen synthesis and fibrosis to promote tissue repair. Likewise, activated microglia are divided into an M1 phenotype, characterized by upregulation of inflammatory genes such as *TNF* and *IL1 β* , and an M2 phenotype, characterized by the expression of pro-repair genes such as arginase 1 (*Arg1*) and chitinase-like 3 (*Chi3l3*, also known as *Ym1*)²⁴. However, this nomenclature is

now deemed inaccurate as accumulating evidence has revealed a broad diversity of macrophage activation statuses and shown that macrophages show remarkable plasticity to change phenotypes in response to environmental cues^{25,26}.

In neurodegenerative diseases, the precise gene signatures of microglia that mediate pathology-and neurodegeneration-associated sterile inflammation have not been clearly elucidated. Recent single-cell RNA-seq data identified a two-stage microglial cell activation process that occurs during disease progression in an amyloid- β -depositing mouse model, where the transition from the first to the second stage is determined by TREM2²⁷. The end-stage activated microglia appear to display a conserved core-expression profile harbouring certain degrees of diversity across individual disease phenotypes^{27,28}. The core expression profile is likely induced by neuronal cell death or damage, whereas distinct pathologies may contribute to the diversity. The common disease-associated microglial cell signature was defined by different groups as the ‘microglial neurodegenerative’ (MGnD) phenotype²⁸ or the ‘disease-associated microglia’ (DAM) phenotype²⁷. This signature is characterized by a suppression of microglial homeostatic genes, and an induction of pro-inflammatory genes as well as genes involved in microglial phagocytotic function targeting degenerated/injured neurons and amyloid plaques^{27,28}. The MGnD phenotype can largely be recapitulated by injecting apoptotic neurons into mouse brain parenchyma, where the subset of microglia that are activated to phagocytose apoptotic neurons show a similar molecular phenotype to the MGnD phenotype²⁸. The MGnD microglia can also be activated via intracerebral administration of kainic acid [G] that induces neuronal cell death²⁸, supporting the idea that neuronal death and damage determines the central microglial cell gene signature in neurodegenerative diseases. Importantly, although the microglia that phagocytose apoptotic neurons share many molecular signatures with lipopolysaccharide (LPS) and IFN γ -stimulated M1 microglia, they also exhibit significant differences, such as contrasting gene expression patterns of secreted phosphoprotein 1 (*Ssp1*), CC-chemokine ligand 3 (*Ccl3*), and early growth response 1 (*Egr1*). But the most distinguishing difference is that the expression of *ApoE* — the hallmark gene of the MGnD phenotype that has been found to be upregulated in microglia in all currently-assessed neurodegenerative diseases — is suppressed in M1 microglia. In addition, certain classical M2 microglial cell markers, such as *Arg1* and *Ym1*, are also induced in microglia that phagocytose apoptotic neurons, indicating a unique microglial activation profile in neurodegenerative diseases that is distinct from the M1/M2 classification²⁸ (Table 1).

Microglia and amyloid- β pathology

Global immune modulation in the brain: effects on amyloid- β pathology.

Numerous studies have examined the effect of neuroinflammation on amyloid- β pathogenesis by targeting cytokines and other inflammatory molecules to create either a pro-inflammatory or anti-inflammatory environment in the brain. The results of these studies have been inconsistent. Several studies have found that activating pro-inflammatory pathways — for example, by overexpressing TNF²⁹, IFN γ ³⁰ or IL-6³¹, or by inhibiting anti-inflammatory cytokines such as IL-10³² — reduces plaque deposition, and vice versa^{33,34}. These effects have been proposed to mainly result from enhanced phagocytotic activity of

microglia induced by potent inflammation, leading to greater amyloid- β clearance, whereas APP synthesis or processing is not affected. However, other studies have suggested that inhibition of inflammation attenuates amyloid- β pathology^{35–37}. Although these studies in which brain inflammation is manipulated featuring conventional M1 or M2 microglial cell phenotypes provide important insights, it is likely that they do not truly reflect the disease-associated inflammatory signatures found in neurodegenerative diseases.

Microglia function prior to pathology onset.

Microglia have long been hypothesized to regulate amyloid- β pathogenesis via phagocytosis. However, depleting microglia after amyloid- β pathology onset via pharmacological inhibition of colony-stimulating factor 1 receptor (CSF1R) signalling³⁸ or by using the CD11b-HSVTK (TK) system³⁹ for 4 weeks, does not affect total plaque burden. In contrast, a recent study shows that a three-month depletion of microglia via CSF1R inhibition starting prior to amyloid- β pathology onset drastically reduces plaque deposition in 5XFAD mice [G]⁴⁰. This indicates that microglia may be involved in the initial seeding stage of plaque formation. Interestingly, activation of the NLRP3 inflammasome in cultured microglia leads to the release of ASC specks that rapidly bind to and cross-seed amyloid- β to promote amyloid- β aggregation⁴¹. In the brains of patients with Alzheimer's disease and amyloid- β depositing mice, the NLRP3 inflammasome is activated and ASC specks are found to present in the core of amyloid plaques⁴¹, indicating an active role of ASC specks in plaque seeding and initiation. ASC-deficient APP_{Swe}PSEN1_{ΔE9} mice [G] have significantly reduced cerebral plaque loads, and show little seeding and spreading of amyloid- β pathology when receiving cerebral injection of brain homogenates containing amyloid- β seeds⁴¹. Consistent with this, two-photon live imaging of GFP-labelled microglia in 5XFAD mice show that microglia activated by laser-induced cerebral injury migrate to the damaged sites and induce de novo plaque formation in the injured site within two weeks⁴². This emerging evidence suggests that activated microglia may facilitate amyloid- β plaque formation by promoting amyloid- β seeding in the initiation stage. However, more research is needed to verify this effect and to exclude potential artefacts caused by specific animal models or off-target drug properties.

TREM2 and amyloid- β pathology.

Despite the fact that no notable changes in global plaque loads have been observed when microglia are deleted after pathology onset, genetic evidence from TREM2 studies indicate a role of microglia in regulating amyloid- β pathology post plaque formation. Microglia have long been observed to cluster around amyloid- β plaques in postmortem Alzheimer's disease brains and in mouse models of amyloid- β deposition. Unlike non-plaque-associated microglia, which retain a more homeostatic phenotype^{27,43}, plaque-associated microglia show major alterations in their molecular signatures and constitute the population of DAM in amyloid- β -depositing mouse models²⁷. TREM2 was recently demonstrated to be essential for promoting microglial cell association with plaques^{44–49}. TREM2 haploinsufficiency, ablation, or the TREM2^{R47H} mutation that causes a missense variant of TREM2, leads to a significant reduction of plaque-associated microglia compared to their TREM2-sufficient counterparts^{44–49}. Corresponding to loss of microglial enclosure around plaques, TREM2-deficient amyloid- β depositing mice develop significantly enlarged and less compact plaques

containing longer and more diffuse star-shaped amyloid fibrils that project from loosely packed cores^{48,49}. Such morphological changes in plaques are frequently associated with a greater degree of neuritic dystrophy with enlarged, swollen axons and dendrites in close vicinity^{48,49}, indicating that plaque-associated microglia perform plaque trimming and compacting functions that help to restrict plaque growth, and that these cells serve as a barrier to limit plaque-induced neuronal toxicity. Patients with Alzheimer's disease who carry the *TREM2*^{R47H} mutation show patterns of plaque morphology and neurite dystrophy that resemble the plaques observed in *TREM2*-deficient amyloid- β depositing mice⁴⁹, indicating that this mutation causes a loss of function.

In terms of the mechanistic basis of these observations, *TREM2* was recently identified as an amyloid- β receptor with a higher affinity for amyloid- β oligomers than monomers⁵⁰. Notably, *TREM2* expression is specifically upregulated in plaque-associated microglia in both mice and humans^{27,45}. Keren-Shaul *et al.* showed that *TREM2* expression switches on the late-stage DAM signature featuring an enhanced phagocytotic activity⁵¹, suggesting that plaque-associated microglia may be able to phagocytose plaques. Supporting this idea, plaque-associated microglia have been shown to contain plaque-derived materials^{27,49}, indicating their capability to phagocytose plaque components. This plaque-phagocytotic capacity is impaired with *TREM2* deficiency^{48,49}, indicating that plaque-associated microglia trim and compress plaques via *TREM2*-dependent plaque phagocytosis.

Initial studies on *TREM2* reported contradicting results regarding the effect of *TREM2*-deficiency on plaque loads^{45,46}, which was subsequently identified to be a disease progression-dependent effect⁴⁷. Overall, *TREM2* deficiency leads to a mild reduction of plaque deposition in early-stage disease, but causes enhanced amyloid- β pathology in advanced stages of the disease^{45–47}. This disease-stage-dependent effect was initially confusing. However, new insights into microglial cell functions suggest a possible explanation. Given the role of activated microglia in promoting amyloid- β seeding, reduced microglial association with the initial plaque seeds due to *TREM2* deficiency may impede the seeding efficiency, leading to a prolonged lag phase and therefore reducing pathology in the early stage. In addition, *TREM2*^{-/-} amyloid- β depositing mice consistently show downregulation of inflammatory genes, such as *Tnf* and *Il1b*^{45–47}, and also of key genes associated with the MGnD phenotype, such as c-type lectin domain family 7 member A (*Clec7a*) and integrin subunit alpha X (*Itgax*)^{27,28}, indicating immature polarization of microglia into the microglial neurodegenerative phenotype and insufficient microglial activation. Such incompletely activated microglia may fail to produce sufficient inflammatory factors⁴³, such as ASCs, to effectively initiate plaque seeding.

By contrast, during the advanced disease stage plaques are already formed, hence seeding efficiency is no longer a determining factor. Instead, microglial plaque-trimming function becomes a dominating factor influencing plaque growth. Lack of *TREM2* results in impaired plaque trimming, which leads to enlarged plaques with reduced compaction^{48,49}. The increased plaque size may be the primary cause of the enhanced amyloid- β pathology observed in late-stage disease in *TREM2*^{-/-} amyloid- β depositing mice revealed by amyloid- β antibody staining. Supporting this notion, fibrillar plaque staining indicated no

difference in the total amount of pathology between TREM2^{+/+} and TREM2^{-/-} amyloid- β depositing mice at the late stage of disease⁴⁷.

Interestingly, a recent study showed that expressing human TREM2 under the endogenous human regulatory elements on top of murine TREM2 in 5XFAD mice leads to reduced fibrillar amyloid- β deposition with denser cores, accompanied by ameliorated neuritic dystrophy⁵². Surprisingly, plaque-associated microglia drop in number with TREM2 overexpression and exhibit elongated and ramified processes with a sharp reduction of ionized calcium binding adaptor molecule 1 (IBA1) [G] signal, indicating an altered activation status, likely reflecting less activation⁵². These effects are interpreted to derive from enhanced microglial phagocytotic capacity induced by TREM2 overexpression which requires less microglial activation to trim plaques. Transcription analysis from crude cortical lysates reveals mixed microglial activation patterns with suppression of certain DAM genes, but further activation of other DAM genes that appear to favour phagocytosis⁵². These gene signatures need to be verified in isolated microglia in future studies and the human TREM2 overexpression effect would need to be confirmed in a murine TREM2^{-/-} background to avoid potential confounding effects.

Microglia and tau pathology

In the brain, tau is primarily an intracellular neuronal protein, but is normally secreted into the interstitial fluid (ISF)⁵³ and is believed to transmit tau pathology in a prion-like manner⁵⁴. High-molecular-weight phosphorylated tau is found in the ISF, and is proposed to be the species mediating tau propagation⁵⁵. Insoluble tau isolated from postmortem Alzheimer's disease brain is shown to be taken up by microglia in vitro and in vivo⁵⁶. In a tau-spreading mouse model where human P301L tau was focally expressed in medial entorhinal cortex (MEC) layer II/III neurons, microglial cell depletion markedly suppressed tau propagation from the entorhinal cortex to the dentate gyrus by inhibiting microglial secretion of exosomes that contain tau oligomers for seeding⁵⁷, indicating microglia may actively participate in tau spreading by taking in and subsequently releasing some form of tau.

Others assessed the effect of microglial activation on tau pathogenesis. Microglial activation is shown to precede tau pathology in a tauopathy mouse model⁵⁸, and administering an immunosuppressant drug FK506 from an early age drastically reduces tau pathology, neurodegeneration and mortality⁵⁸. In addition, chronic intraperitoneal administration of LPS to 3xTg-AD mice[G] for 6-weeks is shown to induce marked microglial activation and promote site-specific tau hyperphosphorylation⁵⁹. IL-1 receptor signalling is found to play a key role in neuroinflammation-induced tau phosphorylation by enhancing the activity of tau kinases including cyclin-dependent-like kinase 5/p25 (CDK5/p25), glycogen synthase kinase 3 β (GSK3 β), and p38 mitogen-activated protein kinase (p38-MAPK)^{60,61}. Importantly, deleting the microglial cell-expressed homeostatic gene CX3C chemokine receptor 1 (*Cx3cr1*) in htau mice [G] leads to amplified microglial activation and enhanced tau hyperphosphorylation/aggregation^{62,63}; whereas overexpressing a soluble form of CX₃CL1, the ligand for CX₃CR1, reduces microglial cell activation and tau pathology in rTg4510 mice [G]⁶⁴. Interestingly, there also appears to be a disease-stage-dependent

microglial effect on tau pathology. CX₃CR1-induced differences in tau phosphorylation are only observed in early to middle disease stages^{62–64}, but is largely absent in late-disease stage, when an overall reduction of phosphorylated-tau (p-tau) signal occurs^{58,63}, likely due to loss of neurons from neurodegeneration. Similarly, TREM2 deficiency is reported to enhance tau hyperphosphorylation at an early disease stage in htau mice⁶⁵ and a mid-disease stage in P301S mice [G]⁶⁶, but does not affect tau pathology at a late-disease stage in P301S mice⁶⁷, whereas overexpressing murine TREM2 in microglia is found to reduce tau hyperphosphorylation in the mid-disease stage in P301S mice⁶⁸.

Microglia and neurodegeneration

Microglia directly regulate neurodegeneration.

Apart from regulating Alzheimer's disease pathologies, microglia likely play a more crucial role in modulating neurodegeneration. This capacity is partially attributed to their pathology-modulating functions, as greater pathology may cause more neuronal injury and death. However, end-stage MGnD microglia that harbour high phagocytotic and inflammatory capacity may directly kill stressed neurons, such as tau-loaded neurons, via phagoptosis; or damage and kill healthy neurons by secreting neurotoxic substances, independent of their effects on Alzheimer's disease pathology. Interestingly, microglia are shown to intimately associate with tau-loaded neurons in human brain tissue⁵⁶. End-stage TREM2^{-/-} P301S mice that exhibit significantly reduced microgliosis and downregulated MGnD genes such as *ApoE* and cystatin 7 (*Cst7*), show significantly attenuated brain atrophy compared to TREM2^{+/+} P301S mice despite no change of tau pathology⁶⁷. Similarly, overexpression of soluble CX₃CL1 reduces microglial activation and neuronal loss in rTg4510 mice⁶⁴. The same principle also applies to other neurodegenerative diseases. In mouse models of Parkinson's disease and amyotrophic lateral sclerosis (ALS), CX₃CR1 ablation leads to marked microglial activation and loss of dopaminergic or motor neurons compared to CX₃CR1^{+/+} mice. Hence, activated microglia actively modulate neurodegeneration.

Microglia-astrocyte crosstalk.

In addition to showing a direct impact, microglia also indirectly regulate neurodegeneration by communicating with astrocytes, another major CNS cell type constituting approximately 30% of CNS cells. Unlike microglia that are derived from yolk sac precursors, astrocytes resemble neurons in that they originate from ectodermal precursors and are not conventional immune cells. However, astrocytes are immune-competent. They respond to a wide range of brain insults and are activated in a wide range of neurodegenerative diseases including Alzheimer's disease^{9,69–71}. In physiological conditions, astrocytes provide essential nutritional and growth support to neurons to maintain their survival and function. They regulate extracellular ion and neurotransmitter concentrations and likely modulate synaptic transmission and synaptic plasticity⁷². In addition, astrocytes actively regulate synaptic pruning during development^{73,74} and in adulthood^{74,75} to maintain synaptic homeostasis and neuronal circuit function. They also form the glia limitans that serves as a critical barrier separating neural and non-neural tissue along perivascular spaces, meninges, and tissue lesions in the CNS to maintain brain homeostasis⁷⁶.

Astrocytes are highly heterogeneous populations that differ in a variety of dimensions including morphology, function, developmental origin, and activation profiles⁷². A recent study identified two distinct populations of reactive astrocytes induced by inflammation or ischemia, termed as A1 astrocytes and A2 astrocytes, respectively⁷⁷. A1 astrocytes strongly upregulate genes involved in classical complement activation which are shown to be destructive to synapses, therefore are considered harmful; whereas A2 astrocytes upregulate neurotrophic genes that promote neuronal survival and growth, and are deemed protective⁷⁷. Interestingly, LPS-induced inflammatory microglia are found to activate A1 astrocytes by producing a collection of molecules: TNF, IL-1 α and complement 1q (C1Q)⁹. A1 astrocytes demonstrate strong neurotoxicity by secreting toxic factors that can rapidly induce neuronal death, and by losing normal functions to support neuronal survival, growth or functionality and to maintain environmental homeostasis⁹. Complement 3 (C3), a secreted marker for A1 astrocytes, is found to signal through the C3a receptor (C3aR) expressed on neurons to disrupt neuronal morphology and function⁷⁸. In APP-transgenic mice, blocking A1 astrocyte activation rescues cognitive impairment⁷⁸. A1 astrocytes are found in post-mortem brain tissues from a broad panel of neurodegenerative diseases including Alzheimer's disease, Huntington's disease, Parkinson's disease, ALS, and multiple sclerosis⁹, indicating a common mechanism through which these reactive astrocytes affect neurodegeneration.

Interestingly, activated A1 astrocytes are shown to signal back to microglia by binding to microglial C3aR to regulate microglial function⁷⁹. In vitro, acute C3/C3a activation promotes, whereas chronic C3/C3a treatment reduces microglial phagocytosis. In vivo, C3 elevation via NF- κ B hyperactivation significantly exacerbates amyloid- β pathology and neuroinflammation in APP-transgenic mice, whereas treatment with a C3aR antagonist ameliorates plaque load and microgliosis⁷⁹. Microglia-astrocyte crosstalk is essential for the immune regulation of Alzheimer's disease pathogenesis and neurodegeneration, and should be further explored in future studies.

Other innate immune cells in Alzheimer's disease

Three additional CNS macrophage cell types other than classical microglia, namely perivascular, meningeal, and choroid plexus macrophages, are present at the interface between the parenchyma and the circulation. Perivascular and meningeal macrophages are generated from yolk sac precursors and are maintained by self-renewal, whereas choroid plexus macrophages have dual embryonic and adult hematopoietic origins⁸⁰. These macrophages express high levels of CD45 and MHCII as opposed to low expression of these markers in parenchymal microglia⁸⁰, and are speculated to play a role in CNS surveillance and parenchyma-peripheral immune connection, but their functions are still not clear. The role of potential-infiltrating monocytes in affecting amyloid- β pathology is assessed in a microglia-repopulation model, where depleting microglia using the CD11b-HSVTK (TK) system is followed by a rapid repopulation by peripherally derived myeloid cells in the absence of additional stimuli such as irradiation⁸¹. Monocyte repopulation for up to 6 months does not modify amyloid load^{82,83}, indicating peripheral monocytes may have no additional effects on parenchymal amyloid- β pathology. However, patrolling monocytes are reported to be actively involved in vascular amyloid- β clearance⁸⁴. Others report that neutrophils infiltrate brain parenchyma of amyloid- β depositing mouse models and human

Alzheimer's disease brain, and migrate towards amyloid- β plaques^{85,86}. Neutrophil infiltration is shown to exacerbate tau phosphorylation, microgliosis, and behavioral deficits in 3xTg-AD mice that develop both amyloid- β and tau pathology⁸⁶.

Overview of neuroinflammation in Alzheimer's disease

Neuroinflammation in Alzheimer's disease can be beneficial or detrimental depending on disease stages and targets of activated microglia. There appear to be three major disease stages during the course of Alzheimer's disease (FIG. 1). The early stage (FIG. 1a) features plaque initiation, when microglial activation appears to facilitate plaque formation via secreting inflammatory factors, therefore inflammation at this stage may be harmful. It's unclear why microglia may have evolved to promote amyloid- β seeding during the plaque initiation stage. While amyloid- β deposition is likely an incidental event as a price paid for aging beyond active evolutionary selection, one perspective holds that amyloid- β peptides act as antimicrobial molecules that bind to and trap invading pathogens via oligomerization to inhibit their binding to host cells⁸⁷. Hence, enhanced seeding may expedite pathogen insulation. Although intriguing, this theory needs more evidence to support. The mid-stage (FIG. 1b) is characterized by plaque accumulation, during which time activated microglia target growing plaques to restrict their expansion and toxicity to neighboring neurons, hence playing a protective role. The late stage (FIG. 1c) is represented by accumulating tau pathology with plateaued amyloid- β pathology. During this time, cell death increases and MGNd microglia are primarily involved in removal of dead neurons and targeting stressed-but-viable neurons injured by tau pathology for phagoptosis. Activated microglia may also exacerbate tau pathology by activating tau kinases and enhancing tau spreading. Meanwhile, they may generate ROS and NO and induce A1 astrocytes to aggravate neuronal loss. The massive neuronal death may further exacerbate neuroinflammation to kill more neurons, forming a vicious circle. This stage may be similar in all neurodegenerative diseases, and detrimental neuroinflammation during this stage may constitute a major mechanism for neurodegeneration across different diseases.

Where does ApoE fit in the picture?

ApoE biology and current view of ApoE as an Alzheimer's disease risk factor.

ApoE as an apolipoprotein forms lipoprotein particles with lipids and cholesterol to mediate their transport. It delivers lipids and cholesterol to cells via receptor-mediated endocytosis of lipoproteins and also mediates cellular cholesterol efflux in a process called reverse cholesterol transport, where cellular cholesterol is transferred to the lipoprotein via ATP-binding cassette transporter (ABCA1)^{88,89}. ApoE is most highly expressed in the liver and the CNS. In the brain, ApoE is the most abundantly produced apolipoprotein, and is synthesized primarily by astrocytes and to a lesser extent by microglia^{90,91}.

APOE is by far the strongest genetic risk factor for late-onset Alzheimer's disease. The $\epsilon 4$ allele is significantly enriched in patients with Alzheimer's disease, and $\epsilon 4$ -carrying patients show earlier disease onset⁹², heavier amyloid- β plaque load in the brain⁹³, greater brain atrophy⁹⁴, and accelerated disease progression⁶⁹ compared to patients who do not carry this allele. The prominent effect of ApoE on Alzheimer's disease triggered broad enthusiasm in

understanding how ApoE contributes to Alzheimer's disease. As ApoE was initially identified as an amyloid- β -binding protein⁹⁵, extensive follow-up studies have focused on its relationship with amyloid- β pathology. To date, substantial evidence has demonstrated ApoE affects amyloid- β deposition in a dosage and isoform-specific fashion (ApoE4>ApoE3>ApoE2)^{96–100}, and we recently reported that ApoE4 exacerbated tau pathogenesis and tau-mediated neurodegeneration relative to other ApoE isoforms independent of amyloid- β ⁶⁹. Mechanistically, ApoE isoform-dependent effects on ApoE-amyloid- β interaction, amyloid- β aggregation, and amyloid- β clearance have been comprehensively investigated¹⁰¹, and ApoE isoform-dependent interaction with tau is also reported^{102,103}. Interestingly, ApoE effects do not appear to be restricted to Alzheimer's disease. *APOE4* has also been reported to be a risk factor for other neurodegenerative diseases, such as frontotemporal dementia¹⁰⁴, pure synucleinopathies including pure dementia with Lewy bodies and Parkinson disease dementia¹⁰⁵, although the data in these instances is less clear. Notably, genetic ablation of ApoE in various neurodegenerative mouse models — including tauopathy⁶⁹, synucleinopathy¹⁰⁶, and experimental autoimmune encephalomyelitis (EAE)¹⁰⁷ — shows a similar protective effect against neurodegeneration and disease severity, indicating a prevalent function of ApoE in neurodegenerative conditions based on a potential common mechanism in addition to its specific effects in individual pathologies. We think this shared mechanism is likely linked to ApoE's immunomodulatory function, as we discuss below.

Immunomodulatory function of ApoE: insights from LPS studies.

In fact, the capacity of ApoE to modulate inflammation has long been observed. In healthy humans challenged with intravenous LPS infusion, ϵ 4-carriers demonstrate significantly higher elevation of body temperature and plasma TNF levels compared to ϵ 4 non-carriers¹⁰⁸. Similarly, when whole blood isolates from human subjects were stimulated *ex vivo* with TLR ligands, enhanced production of a wide panel of cytokines and chemokines was observed in blood from ϵ 4⁺ donors compared with ϵ 4⁻ donors¹⁰⁸. A greater immune response associated with the ϵ 4 allele is also observed in human ApoE-targeted replacement (APOE-TR) mice [G] and in cultured microglia/macrophages upon LPS stimulation^{108–110}.

How ApoE achieves this is not well understood. Evidence shows that lipid rafts play an essential role in immune activation by serving as platforms for signalling complex assembly; hence minor intervention on membrane lipids such as cholesterol loading or efflux is sufficient to induce membrane remodelling and subsequent signal transduction¹¹¹. Higher retention of cholesterol in lipid rafts due to lack of the cholesterol efflux transporters ABCA1 or ABCG1, enhances TLR signalling in macrophages¹¹¹ and induces leukocytosis under hypercholesterolemia conditions¹¹², whereas myeloid cell surface-bound ApoE promotes cholesterol efflux in a cell-autonomous fashion to inhibit myelopoiesis¹¹³. ApoE4 is reported to be less effective than ApoE3 in inducing cholesterol efflux from macrophages¹¹⁴, which causes cholesterol accumulation on cell membranes^{108,114}. This mechanism has been proposed to explain the higher immune reactivity associated with ApoE4. Supporting this theory, ApoE-deficient mice behave similarly to ApoE4-targeted replacement (ApoE4-TR) mice (mice with the human *APOE4* coding sequence replacing the murine *apoe* coding sequence) in response to intracerebroventricular LPS stimulation with

enhanced gliosis and cytokine production compared with ApoE2-TR and ApoE3-TR mice¹¹⁰. However, this study is based on LPS stimulation, which has key differences from neurodegeneration-associated sterile neuroinflammation. Above all differences, the lack of ApoE induction may determine the distinct nature of the response.

Immune functions of ApoE in neurodegeneration

Contrary to LPS treatment where ApoE deletion amplifies the immune response, under neurodegenerative or neuroinflammatory conditions, ApoE deficiency attenuates inflammation^{28,69,97,107} and disease severity^{69,106,107}. In a tauopathy mouse model, genetic ablation of ApoE markedly reduces activation of microglia and astrocytes activation and ameliorates brain atrophy⁶⁹. In a mouse model of Parkinson's disease featuring synucleinopathy, ApoE deletion significantly attenuates the loss of spinal cord motor neurons, delays disease onset, and improves survival¹⁰⁶. ApoE ablation in EAE mice also reduces inflammation and disease severity¹⁰⁷.

How can one explain this apparent contradiction? Interestingly, in EAE, the disease-exacerbating effect of ApoE only occurs in the effector phase¹⁰⁷ when recruited effector immune cells, primarily macrophages, are activated by helper T cells and start to attack and ingest myelin. In the initiation stage, however, T cell priming by myelin antigens is enhanced in ApoE-deficient mice compared to wild-type mice¹⁰⁷. These results indicate multi-faceted immunomodulatory functions of ApoE. ApoE may reduce the excitability of immune cells by modulating lipid raft content on cell membranes, but may also be required for intact functionality of MGnD microglia. ApoE deletion prevents full acquisition of the microglial neurodegenerative phenotype with suppression of key MGnD genes²⁸, and therefore may prohibit disease-associated microglia from being fully developed to carry out their pro-inflammatory and phagocytotic functions. In addition, as neurons express a number of ApoE receptors, such as low-density lipoprotein receptor-related protein 1 (LRP1), heparan sulfate proteoglycans (HSPGs), and low-density lipoprotein receptor (LDLR) on cell membranes, we propose that ApoE may accumulate on the surfaces of damaged and dead neurons as a result of impaired neuronal uptake function, and serve as an opsonin to promote neuronal phagocytosis by microglia. The opsonization effect may be achieved via ApoE interacting with TREM2 on microglia, as TREM2 has been demonstrated to be an ApoE receptor^{116–119}. Supporting this hypothesis, ApoE binding to cell surface was shown to be significantly enhanced in apoptotic *N2a* cells, a murine neuroblastoma cell line, compared to healthy cells, and cultured microglia phagocytose significantly more apoptotic *N2a* cells with increased levels of ApoE in the medium¹¹⁶. TREM2^{-/-} microglia, in contrast, show impaired phagocytosis of apoptotic *N2a* cells, supporting an ApoE-TREM2 axis in mediating microglial phagocytotic function.

Hence, despite higher excitability of microglia in the absence of ApoE, their impaired neurodegenerative functionality may hinder their capacity to kill and damage neurons via phagocytosis or inflammatory mechanisms, which may underlie the protective effect of ApoE deficiency in various neurodegenerative settings. Supporting this view, global or conditional deletion of ApoE in microglia reduces neuronal loss in the facial motor nucleus after facial nerve axotomy²⁸. Interestingly, TREM2 shares the same pathways with ApoE in regulating

the microglial neurodegenerative phenotype. TREM2 deletion in APPPS1–21 [G] or superoxide dismutase 1 (SOD1) mice [G] results in ApoE downregulation and a similar suppression of core inflammatory MGnD genes and restoration of homeostatic genes²⁸. Targeting TREM2 signaling in the facial nerve axotomy model ameliorates neuronal loss²⁸, and ablation of TREM2 in tauopathy mice attenuates neuroinflammation and neurodegeneration⁶⁷, similar to the effect of ApoE deletion. Whether ApoE isoform-specifically regulates MGnD microglia activation or potential opsonization for neuronal phagocytosis are intriguing questions to be further investigated in future studies.

ApoE and Alzheimer's disease pathologies

ApoE and amyloid- β pathology.

ApoE has long been observed to affect amyloid- β pathogenesis. Compared with other ApoE isoforms, ApoE4 exacerbates the deposition of amyloid plaques in the brain^{98–100}. Furthermore, regardless of ApoE isoforms, ablating one allele or both alleles of ApoE all lead to reduction of fibrillar amyloid- β deposits^{96,97,120}. These effects were attributed to ApoE affecting amyloid- β fibrillization and clearance¹⁰¹. However, recent data suggest new mechanisms of ApoE function. ApoE was found to be essential for plaque formation during the initial seeding stage^{121,122}. Targeting ApoE4 with antisense oligonucleotides (ASOs) from birth reduces amyloid- β plaque deposition, whereas ASO treatment after pathology onset in adult mice does not affect fibrillar plaque load¹²¹. Vice versa, induction of ApoE4 expression during the initial seeding stage, but not during the plaque growing phase, increases amyloid- β pathology¹²². However, although no change in fibrillar plaques was observed in adult ASO-treated mice when X-34 staining (which stains for bona fide fibrillary beta-sheet structures) was used, staining by amyloid- β antibodies revealed significantly increased plaque sizes¹²¹. Interestingly, ApoE ablation in APP_{Swe}PSEN1_{dE9} [G] or APPPS1–21 [G] mice also results in enlarged plaques with wispy fibre-like structures projecting from loosely packed cores¹²⁰, similar to the plaques observed in TREM2-deficient amyloid- β -depositing mice^{48,49}. Corresponding to loose and enlarged plaques, ApoE deletion leads to impaired microglial cell response to plaques¹²⁰, the same phenotype observed with TREM2 deficiency^{44,46}. These findings indicate that the ApoE-TREM2 axis implicated in neurodegeneration may share similar mechanisms in regulating microglial cell responses to amyloid- β pathology.

Microglia-mediated compaction and insulation of amyloid- β plaques may require phagocytotic MGnD microglia activation that is dependent on ApoE, and the abundance of ApoE in amyloid plaques^{123,124} may also play an important role in opsonizing plaques for targeting by activated microglia. In addition, microglia are found to present in the core of ApoE-labelled senile plaques in areas of ApoE-like immunoreactivity⁹¹, suggesting microglial cell targeting amyloid- β seeds to promote plaque seeding during the plaque initiation stage may also involve ApoE participation as an opsonin to enhance microglia-plaque interaction. The ApoE-TREM2-microglia connection is strongly supported by the observation that the presence of ApoE, TREM2, or microglia similarly enhances amyloidogenesis during the early amyloid-depositing stage, and that ApoE and TREM2 deficiency share the same plaque phenotype mediated by impaired microglial cell function at

the late amyloid-depositing stage. Of note, ApoE4 is associated with higher microglial cell reactivity around amyloid- β plaques than ApoE3 in deep cortical layers¹²⁵. The effect of ApoE isoforms on plaque deposition may be related to their different capacities in mediating microglial cell activation and opsonization, in addition to direct isoform-dependent effects on amyloid- β aggregation or clearance.

ApoE and tau pathology.

Early studies showed that ApoE3, but not ApoE4, avidly binds to human tau in vitro^{102,103}, indicating a potential link between ApoE and tau. ApoE is normally produced by glia and is secreted into the extracellular space, but rare ApoE immunoreactivity is also observed in neuronal cell bodies in brain tissues of Alzheimer's disease and tauopathy patients^{123,126,127}. Specific overexpression of human ApoE4 in neurons, but not astrocytes, increases tau phosphorylation in mouse brains compared to ApoE3 overexpression^{128,129}. In addition, amyloid- β pathology is reported to induce higher levels of endogenous murine tau phosphorylation in ApoE4 amyloid- β depositing mice compared to their ApoE3 counterparts¹³⁰. We recently found that human ApoE4 expressed under the endogenous murine ApoE promoter exacerbates tau pathology compared to other ApoE isoforms in a tauopathy mouse model independent of amyloid- β pathology⁶⁹. However, the key point is that in addition to affecting tau pathology, ApoE also potently modulates neurodegeneration in the setting of tauopathy. ApoE4 significantly exacerbates neurodegeneration whereas ApoE ablation strongly attenuates the damage compared to the presence of ApoE regardless of ApoE isoforms⁶⁹. While ApoE may directly regulate tau pathogenesis and affect neurodegeneration via tau-induced cell-autonomous neuronal toxicity, we think the effects of ApoE on both tau pathology and neurodegeneration at least partially fit in the broader picture of the immunomodulatory function of ApoE. The specific lipid composition of ApoE4 lipoprotein may affect lipid raft structures on microglial cell membranes and induce a stronger MGnD phenotype that exacerbates tau pathogenesis and neurodegeneration. It may also make ApoE4 a more competent opsonin to potentiate neuronal loss via enhanced phagoptosis. In contrast, failure to acquire a complete MGnD phenotype and loss of ApoE opsonization in the absence of ApoE may underlie its protective effect in tauopathy.

An integrated view of ApoE function in Alzheimer's disease is summarized in FIG. 2.

Concluding remarks and future directions

Substantial genetic and functional evidence point to a central role of innate immunity in neurodegenerative diseases. In Alzheimer's disease, neuroinflammation plays different roles depending on disease stages. Activated microglia appear to accelerate amyloid plaque formation during the plaque initiation stage, but serve to restrain plaque growth and plaque-associated neuronal damage during the plaque deposition phase. In the late-disease stage when pathological tau accumulates and neuronal death increases, activated microglia exacerbate tau pathology and may serve as a major driving force for neurodegeneration. Targeting neuroinflammation may be an effective therapeutic approach to intervene with disease progression, but therapeutic designs should take into account the disease stage to target the appropriate pathophysiology.

ApoE affects Alzheimer's disease risk in large part due to its effects on amyloid- β seeding and clearance; it is also a core immune regulator that may influence Alzheimer's disease through its immunomodulatory functions. The shared features between ApoE and TREM2 suggest an ApoE-TREM2 axis linking ApoE to microglial function. Deeper characterization of disease-associated microglia signatures and functions will be key to understanding the role of microglia-mediated neuroinflammation in disease. Further investigation connecting ApoE-dependent lipid metabolism with microglia profiling will provide crucial insights into mechanisms underlying ApoE immunomodulatory functions.

Despite all the exciting findings deciphering roles of neuroinflammation in disease pathogenesis and progression with mechanistic and therapeutic implications, the fundamental problem is not solved. Alzheimer's disease, like many other neurodegenerative diseases, begins with disruption of proteostasis. This process, once initiated, cannot currently be self-resolved and will inevitably lead to pathological protein accumulation that eventually progresses to neurodegeneration. The failure to effectively maintain brain proteostasis is likely due to dampened innate immunity and lack of adaptive immunity in brain parenchyma, which jeopardize the brain's clearance machinery compared to peripheral tissues. Boosting immune activity in the brain to enhance waste clearance under homeostatic or pathological status without damaging neurons may be an alternative strategy. Specific reprogramming of microglia to enhance the clearance of amyloid- β seeds and plaques without amplifying inflammatory stress or targeting neurons; or connecting adaptive immunity with brain innate immunity, such as pathology-directed antibody treatment or T cell-based strategies, are promising fields that should be worked out with caution to avoid potential side effects. For intracellular tau pathology that may be difficult to target by immune cells without damaging neurons, activating intrinsic or applying extrinsic neuronal protein degradation programmes could be considered.

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Glossary terms:

Amyloid- β

Small peptides that are major components of amyloid deposits in the brain and cerebrovasculature. They are generated from cleavage of the amyloid precursor protein (APP). Depending on the C-terminus cleavage site, amyloid- β of varying lengths (36–43 amino acids, denoted as A β 36–A β 43) can be generated, among which A β 40 and A β 42 are the most prevalent species.

Tau

A microtubule-binding protein synthesized primarily in neurons. Under normal conditions, tau is most abundantly located in axons and serves to stabilize microtubules. In Alzheimer's disease, tau becomes hyperphosphorylated, dissociates from microtubules, translocates from axons to neuronal cell bodies and dendrites, and self-aggregates to form neurofibrillary tangles.

A β 42/A β 40 ratio

A β 42 is more fibrillogenic than A β 40. The level of A β 42 produced relative to A β 40 determines the propensity of amyloid plaque formation.

Kainic acid

A potent neuroexcitatory amino acid that serves as an agonist for kainate-class ionotropic glutamate receptors. High doses of kainic acid induce neuronal death by overexcitation of neurons.

APP_{Swe}PSEN1_{dE9} mice

An amyloid- β depositing mouse model that expresses a chimeric mouse/human *APP* transgene carrying the Swedish mutation (K670N, M671L) and a mutant *PSEN1* transgene lacking exon 9 (dE9) under the prion promoter. These mice begin to develop amyloid- β pathology around six months of age.

5XFAD mice

An amyloid- β depositing mouse model that overexpresses mutant human APP carrying the Swedish (K670N, M671L), Florida (I716V), and London (V717I) FAD mutations along with human PS1 carrying the M146L and L286V mutations. Both transgenes are controlled under the Thy1 promoter. These mice accumulate high levels of intraneuronal A β 42 around six weeks of age, followed by plaque deposition around two months of age.

APPPS1–21 mice

An amyloid- β depositing mouse model that harbours mutant human transgenes of *APP* (K670N, M671L) and *PSEN1* (L166P), both under the control of the Thy1 promoter. These mice begin to develop amyloid plaques in the neocortex at approximately six weeks of age, followed by deposits in the hippocampus at three to four months of age.

Ionized calcium binding adaptor molecule 1 (IBA1)

A microglia marker protein in the CNS that binds calcium and actin. It is involved in RacGTPase-dependent membrane ruffling and phagocytosis during microglial cell activation.

3xTg-AD mice

This mouse model harbours three mutant human genes (*APP*^{K670N M671L}, *pS1*^{M146V}, and *MAPT*^{P301L}), and sequentially develop amyloid- β pathology and tau pathology starting at six months of age.

htau mice

This mouse model expresses all six human tau isoforms, including 3R and 4R tau, under the endogenous human *MAPT* promoter, and expresses no murine tau. These mice start to develop tau pathology around nine months of age.

rTg4510 mice

A tauopathy mouse model overexpressing the 0N4R human tau isoform carrying the P301L mutation linked to familial frontotemporal dementia. Tau transgene expression is largely restricted to the forebrain by the *Camk2a* promoter, and is regulatable by doxycycline. These

mice develop tau pathology around three to four months of age and show significant neuronal loss by six months of age.

P301S mice

A tauopathy mouse model overexpressing the 1N4R human tau isoform carrying the P301S mutation found in frontotemporal dementia patients. These mice begin to accumulate tau pathology at four to five months of age and develop severe brain atrophy at 9 months of age.

ApoE-targeted replacement (APOE-TR) mice

These mice express human APOE in place of the murine ApoE under the endogenous murine *ApoE* promoter. Therefore, the expression level and pattern of human APOE is maintained in a physiological form.

superoxide dismutase 1 (SOD1) mice

A mouse model for ALS that overexpresses human Cu-Zn superoxide dismutase (SOD1) carrying the G93A mutation. These mice recapitulate phenotypes in human ALS patients showing motor neurons loss in the spinal cord and paralysis in one or more limbs.

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Box 1:**APOE as a risk factor for Alzheimer's disease**

APOE is the first identified Alzheimer's disease risk gene reported in 1993⁹⁵. It remains the strongest genetic risk factor for late-onset Alzheimer's disease. Three common *APOE* alleles ($\epsilon 2$, $\epsilon 3$, and $\epsilon 4$) are present in the human population, with $\epsilon 4$ being a risk factor, and $\epsilon 2$ being protective relative to the $\epsilon 3$ allele. One copy of $\epsilon 4$ increases Alzheimer's disease risk by ~3-fold and two copies by ~12-fold⁵. The initial discovery of ApoE as an Alzheimer's disease risk factor was unexpected. As a lipid-carrier regulating lipid homeostasis, ApoE was considered to be primarily involved in influencing atherosclerosis. In the early 1990s, ApoE was found to be a constituent of amyloid plaques^{123,124}, yet its significance was not clear until *APOE* was identified as an Alzheimer's disease risk factor shortly afterwards. The discovery was inspired by two converging lines of evidence, the first showing an Alzheimer's disease linkage at chromosome 19q13¹³¹ where *APOE* is located, and the second indicating a physical interaction of ApoE with amyloid- β ⁹⁵. The close link between ApoE and amyloid- β in the initial discovery studies has largely driven ApoE research in the Alzheimer's disease field along an amyloid- β track. Important roles of ApoE in regulating amyloid- β pathogenesis have been identified¹⁰¹, which likely drive a large part of its linkage to Alzheimer's disease risk. However, how ApoE's function as a lipid-carrier is in itself related mechanistically to Alzheimer's disease, either in relation to amyloid- β or other factors, is not entirely clear. Lipids serve more than pure nutritional purposes; they also play essential roles in immune regulation. As a major component of cell membranes, lipids regulate immune cell signalling and function by affecting membrane fluidity¹¹¹. Lipids and certain lipoproteins also serve as ligands for various immune receptors to mediate immune cell activation and phagocytosis^{132,133}. Other lipid-mediated mechanisms such as regulation of gene transcription and eicosanoid production have also been proposed to affect immune function¹³⁴. Being a major protein regulating lipid homeostasis, it is not surprising that ApoE plays an immunomodulatory role. Therefore, a previously overlooked immunomodulatory function of ApoE may serve as an important mechanism in Alzheimer's disease pathogenesis.

Box 2:**TREM2 as a risk factor for Alzheimer's disease**

TREM2 is second only to *APOE* in terms of the magnitude of its effects as a late-onset Alzheimer's disease risk factor. The most common *TREM2* variant R47H increases Alzheimer's disease risk by 2- to 3-fold^{135,136}. *TREM2* is a cell surface receptor of the immunoglobulin superfamily that is expressed by tissue macrophages, such as bone osteoclasts, CNS microglia, alveolar macrophages, peritoneal macrophages and intestinal macrophages¹³⁷. Upon ligand binding, *TREM2* associates with an adaptor protein DNAX-activation protein 12 (DAP12), which contains a cytosolic immunoreceptor tyrosine-based activation motif (ITAM) that upon phosphorylation, recruits the spleen tyrosine kinase (SYK)¹³⁷. SYK activation initiates a cascade of signalling that promotes cell anabolic metabolism¹³⁸, survival^{138,139}, proliferation¹⁴⁰ and phagocytosis^{140,141}. Therefore, *TREM2* activation is essential for macrophage viability and immune function. Homozygous *TREM2* mutations that cause a complete loss of function of *TREM2* are associated with Nasu-Hakola disease (NHD), an autosomal recessive disorder featured by a presenile form of frontal-lobe dementia and bone cysts with fractures¹⁴². Heterozygous *TREM2* variants leading to partial *TREM2* loss of function are found to confer higher Alzheimer's disease risk¹⁴². Mutations that cause NHD or increase Alzheimer's disease risk lead to premature protein truncation (Q33X), impaired receptor trafficking and surface expression (T66M, Y38C), defective ligand binding (R47H, R62H, T66M, Y38C) and signalling (R47H, R62H), and reduced phagocytosis (R47H, R62H, T66M, Y38C)¹⁴³. Conversely, a missense variant S144G in *TREML2*, a *TREM*-like gene located within the same *TREM* gene cluster that encodes a structurally similar protein showing opposite effects on microglial cell proliferation and inflammatory responses as *TREM2*¹⁴⁴, was reported to be protective against Alzheimer's disease¹⁴⁵. These genetic findings suggest that immune functions mediated by *TREM2*-expressing immune cells critically regulate Alzheimer's disease pathogenesis.

Box3 |**Other major genetic risk factors for Alzheimer's disease**

Adenosine triphosphate-binding cassette transporter (*ABCA7*) and complement receptor 1 (*CR1*) are two additional highly replicated Alzheimer's disease risk factors⁶. *ABCA7* is abundantly expressed in myeloid cells and microglia, and is found to promote macrophage phagocytosis¹⁴⁶. *ABCA7* ablation exacerbates amyloid- β pathology in amyloid- β depositing mice^{147,148}. *CR1* is a receptor for the complement fragments C3b and C4b, and is implicated in regulating the complement cascade via interacting with C3b¹⁴⁹. Other common Alzheimer's disease risk genes such as clusterin (*CLU*, lipid metabolism, phagocytosis¹⁵⁰), transcription factor PU.1 (*SPI1*, phagocytosis¹⁵¹, *CELF1* in the same locus), and cluster of differentiation 33 (*CD33*, phagocytosis that influences amyloid- β deposition^{152,153}), share a common immune function, indicating an immune-linkage with Alzheimer's disease.

Box 4 |**Signal transduction pathways for microglial activation**

Microglia express pattern recognition receptors (PRRs) that recognize two categories of molecular structures for activation: pathogen-associated molecular patterns (PAMPs) that are products of microbial pathogens, and damage-associated molecular patterns (DAMPs) that are derived from damaged cells, such as misfolded/aggregated proteins, or mislocalized nucleic acids. PRRs consist of toll-like receptors (TLRs) located on the cell surface and intracellular membranes, and cytosolic NOD-like receptors (NLRs).

Conventional LPS-induced microglial activation involves TLR4 activation, which recruits adaptor proteins MYD88 (myeloid differentiation primary response 88) or TRIF (TIR-domain-containing adapter-inducing interferon- β) that activates NF- κ B (nuclear factor kappa-light-chain-enhancer of activated B cells) and IRFs (interferon regulatory factors), respectively. NF- κ B stimulates the expression of pro-inflammatory genes, such as tumor necrosis factor (*Tnf*), interleukin-1(*Il1*) and chemokine C-C motif ligand 2 (*Ccl2*), whereas IRFs promote production of type 1 interferons that are important for anti-viral responses.

In Alzheimer's disease, the NLRP3 inflammasome is activated in microglia¹⁵⁴. Upon ligand binding, multiple NLRP3 proteins interact to form an oligomer, where each NLRP3 molecule binds an adaptor protein called apoptosis-associated speck-like protein containing a carboxy-terminal CARD (ASC). The adaptors then bind to pro-caspase 1, an inactive precursor of caspase 1. The recruitment of pro-caspase 1 to the inflammasome induces auto-proteolytic conversion of the pro-enzyme into active caspase 1, which cleaves precursors of IL-1 β and IL-18 into active forms for subsequent release. Canonical NLRP3 inflammasome activation requires two signals: the first signal is induced by microbial or endogenous molecules, typically TLR ligands such as LPS, to prime microglia for NLRP3 and pro-IL-1 β expression; whereas the second signal is triggered by a variety of stimuli such as ATP, pore-forming toxins, and crystals that induce a common cellular signal (K⁺ efflux or others) to initiate inflammasome assembly¹⁵⁵. In Alzheimer's disease, protein pathologies and injured neurons may provide the two signals respectively. Cleaved IL-1 β and IL-18 signal through their respective receptors, IL-1R1 and IL-18R, triggering NF- κ B activation. IL-1 β is a key initiator of inflammation that potently induces microglia cellular activation and cytokine production, whereas IL-18 regulates interferon- γ responses¹⁵⁶.

Figure1 | Roles of neuroinflammation in different stages of Alzheimer's disease

a | At an early stage disease prior to or just following the initiation of plaque deposition, NLRP3 inflammasome activation by pathological changes results in release of ASC specks from microglia that promote amyloid- β seeding. Activated microglia may capture amyloid- β seeds via TREM2-amyloid- β interaction and boost plaque formation by generating local high concentrations of ASC specks around amyloid- β seeds to facilitate amyloid- β aggregation for plaque core initiation. **b** | In the mid-stage disease when plaques accumulate, microglia cluster around plaques in a TREM2-dependent fashion and trim amyloid- β fibrils on the edge to compact the plaque. Plaque-associated microglia also serve as a barrier between the plaque and surrounding tissues to reduce amyloid- β -induced toxicity to neurites. TREM2 deficiency hinders full activation of DAM/MGnD microglia and significantly reduces the number of plaque-associated microglia. This results in enlarged amyloid- β plaques with wispy fibre-like structures projecting from loosely packed cores and greater neuritic dystrophy in close vicinity. **c** | In the late-stage disease, tau pathology accumulates. Microglia may accelerate tau pathology spreading by taking in extracellular tau and subsequently releasing certain tau species. Activated microglia also secrete IL-1 β that signals on neurons and likely indirectly through astrocytes as well to enhance neuronal tau phosphorylation by activating tau kinases. **d** | Along with tau pathology buildup, neurodegeneration occurs. DAM/MGnD microglia appear to exacerbate neuronal loss. Activated microglia induce A1 astrocytes, which kill/damage neurons via secreting neurotoxic factors and loss of neurotrophic functions. The large amounts of ROS/NO generated during microglia activation can also directly injure/kill neurons. With increased

neuronal death, DAM/MGnD microglia are engaged in removing neuron corpses. However, they can also target stressed-but-viable neurons that present 'eat-me' signals for phagoptosis, causing more neuronal loss.

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Figure 2: Model of immunomodulatory functions of ApoE in Alzheimer's disease pathogenesis and neurodegeneration along disease progression

a | ApoE exacerbates amyloid- β pathology during the initial amyloid- β seeding stage. This is likely partially due to a direct pro-aggregating effect of ApoE on amyloid- β through ApoE-amyloid- β interactions. In addition, ApoE may serve as an opsonin bridging microglia with amyloid- β seeds by binding to TREM2 on microglia. This may result in recruitment of more activated microglia to amyloid- β seeds that promote plaque formation via secreting ASC specks or other mechanisms. **b** | During the plaque accumulation phase, DAM/MGnD microglia clustering around amyloid- β plaques for plaque trimming and insulation is strictly regulated by ApoE. ApoE is required for full DAM/MGnD microglial activation that allows them to associate with plaques. In addition, ApoE is abundantly present in amyloid plaques and may promote DAM/MGnD microglia to target plaques via ApoE-TREM2 interaction. **c** | In the late-stage disease when intracellular pathological tau accumulation and other factors result in more neuronal injury and death, ApoE may accumulate on the cell surface of jeopardized neurons likely due to their impaired cellular uptake function, and may opsonize these neurons for phagoptosis by DAM/MGnD microglia via interaction with TREM2, thus

exacerbating neurodegeneration. In addition, ApoE is required for full DAM/MGnD microglial polarization. Fully activated DAM/MGnD microglia in the presence of ApoE may further aggravate neuronal death by elevating oxidative stress, enhancing A1 astrocytic activation and exacerbating tau pathology.

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Table 1:

Comparison of different microglial cell phenotypes [ED:?]

Cell phenotype	‘M1’ microglial cell	‘DAM’ or ‘MGnD’ microglial cell	‘M2’ microglial cell (alternative activation)	‘M2’ microglial cell (acquired deactivation)
Stimulus	IFN γ , TNF, LPS	Disease-associated pathologies, neuronal cell death, tissue damage	IL-4 and IL-13	TGF β and IL-10
Marker genes	<ul style="list-style-type: none"> IL1b, Tnf, Il6, Il23 iNOS and ROS-producing genes <i>MHCII</i> (?) * 	<i>Apoe, Trem2, Clec7a, Lpl, Itgax, Axl, Csf1, Lilrb4, Spp1, Ccl2, Cst7</i>	<i>Arg1, Ym1, Mrc1, Fizz1, Clec7a</i>	<i>Il10, Tgfb</i>
Functions	<ul style="list-style-type: none"> Oxidative stress Kill microbes or host cells Phagocytosis Antigen presentation (?) * 	<ul style="list-style-type: none"> Phagocytose dead and injured neurons Trim amyloid p plaques Inflammasome/ASC formation, promote plaque seeding Inflammatory stress and damage 	<ul style="list-style-type: none"> Extracellular matrix production and remodeling for tissue repair and wound healing Phagocytosis Immunosuppression 	
Genes regulated in the same direction	<i>IL1b</i> $\uparrow$, <i>Cybb</i> $\uparrow$, <i>Lilrb4</i> $\uparrow$, <i>Ccl2</i> $\uparrow$, <i>Axl</i> $\uparrow$, <i>Itgax</i> $\uparrow$, <i>Tspo</i> $\uparrow$, <i>P2ry12</i> $\downarrow$, <i>Tmem119</i> $\downarrow$, <i>Olfr13</i> $\downarrow$, <i>Tgfb</i> $\downarrow$, <i>Csf1r</i> $\downarrow$, <i>Sall1</i> $\downarrow$, <i>Hexb</i> $\downarrow$, <i>Rhob</i> $\downarrow$...		<i>Arg1</i> $\uparrow$, <i>Ym1</i> $\uparrow$, <i>Clec7a</i> $\uparrow$...	
Genes regulated in opposite directions	<i>Apoe, Trem2, Erg1, Spp1</i> ...		<i>Many genes...</i>	

* Inconclusive