The role of astroglia in Alzheimer's disease: pathophysiology and clinical implications

Amaia M Arranz, Bart De Strooper

Lancet Neurol 2019; 18: 406–14

Published Online February 19, 2019 http://dx.doi.org/10.1016/S1474-4422(18)30490-3

VIB Center for Brain and Disease Research, Leuven, Belgium (A M Arranz PhD, B De Strooper MD); **Department of Neurosciences, Katholieke Universiteit Leuven, Leuven, Belgium** (A M Arranz, B De Strooper); **and Dementia Research Institute, University College London, London, UK** (B De Strooper)

Correspondence to: Dr Bart De Strooper, Dementia Research Institute, University College London, London WC1E 6BT, UK
b.destrooper@ucl.ac.uk

Summary

Background **Astrocytes, also called astroglia, maintain homoeostasis of the brain by providing trophic and metabolic support to neurons. They recycle neurotransmitters, stimulate synaptogenesis and synaptic neurotransmission, form part of the blood–brain barrier, and regulate regional blood flow. Although astrocytes have been known to display morphological alterations in Alzheimer's disease for more than a century, research has remained neurocentric. Emerging evidence suggests that these morphological changes reflect functional alterations that affect disease.**

Recent developments **Genetic studies indicate that most of the risk of developing late onset Alzheimer's disease, the most common form of the disease, affecting patients aged 65 years and older, is associated with genes (ie, *APOE*, *APOJ*, and *SORL*) that are mainly expressed by glial cells (ie, astrocytes, microglia, and oligodendrocytes). This insight has moved the focus of research away from neurons and towards glial cells and neuroinflammation. Molecular studies in rodent models suggest a direct contribution of astrocytes to neuroinflammatory and neurodegenerative processes causing Alzheimer's disease; however, these models might insufficiently mimic the human disease, because rodent astrocytes differ considerably in morphology, functionality, and gene expression. In-vivo studies using stem-cell derived human astrocytes are allowing exploration of the human disease and providing insights into the neurotoxic or protective contributions of these cells to the pathogenesis of disease. The first attempts to develop astrocytic biomarkers and targeted therapies are emerging.**

Where next? **Single-cell transcriptomics allows the fate of individual astrocytes to be followed in situ and provides the granularity needed to describe healthy and pathological cellular states at different stages of Alzheimer's disease. Given the differences between human and rodent astroglia, study of human cells in this way will be crucial. Although refined single-cell transcriptomic analyses of human post-mortem brains are important for documentation of pathology, they only provide snapshots of a dynamic reality. Thus, functional work studying human astrocytes generated from stem cells and exposed to pathological conditions in rodent brain or cell culture are needed to understand the role of these cells in the pathogenesis of Alzheimer's disease. These studies will lead to novel biomarkers and hopefully a series of new drug targets to tackle this disease.**

Introduction

Alzheimer's disease is characterised clinically by memory loss and pathologically by amyloid-β accumulation, neurofibrillary tangle formation, extensive neuroinflammation, synaptic toxicity, neurodegeneration, and brain dysfunction.[1] Development of drugs targeting amyloid-β has been unsuccessful and no cure for patients with Alzheimer's disease currently exists,[2] perhaps because the focus in the field has been too narrow. Novel concepts and ideas are, therefore, needed. One way to broaden the scope of research is to move away from the biochemical theory of the disease to a cellular theory.[1] It is becoming clear that not only neurons, but also glial cells of the brain react to amyloid and tau pathology.[1] This line of thought implies the possibility of novel therapies, diagnostics, and ways to define the disease.

Genetic data are driving this change in viewpoint. More than 40 genetic loci have been associated with the risk of developing late onset Alzheimer's disease, the most common form of the disease, affecting patients aged 65 years and older, and many of the linked genes are expressed in astrocytes, microglia, and oligodendrocytes (panel 1; table 1).[17] As a result, glial cells are becoming central to research into Alzheimer's disease. Despite complex signalling between astroglia and microglia, the focus in the field has been mainly on microglia. Microglia have been associated with neuroinflammation, whereas astroglia were considered as passive, supportive cells for a long time.[19,20] Therefore, although microglia have received much attention, reflected in many excellent reviews discussing their role in Alzheimer's disease,[19,20] new information[21] also supports a role for astroglia in Alzheimer's disease and other neurological disorders.

Microglia[22,23] and astroglia[21,24] adopt many different states in Alzheimer's disease, which might explain their disparate roles in the development and progression of pathology. Functional evidence provides insights into how astroglia and microglia converge in the disease process. When instigated by microglia, astrocytes become reactive and have major roles in the neuroinflammatory and neurodegenerative processes in Alzheimer's disease and other neurological disorders (eg, Parkinson's disease and multiple sclerosis).[21,25–27] Moreover, astroglia regulate the vascular unit and affect clearance of tau and amyloid-β.[28] In this Rapid Review, we summarise emerging insights into the role of astroglia early in the disease process and

explore how further study will yield novel biomarkers and therapeutics for Alzheimer's disease and neurodegenerative disorders in general.

Physiology and pathophysiology of astroglia

Astrocytes are specialised glial cells of neuroepithelial origin.[29] They regulate neurotransmitter and calcium homoeostasis, modulate synapse formation, maturation, and elimination, regulate blood–brain barrier function through neuron-glia-vascular units, control extracellular space volume and ion homoeostasis, and provide nutritional and trophic support to the brain.[29,30] The proportion of astrocytes in the brain is not well defined, and the ratio of glia to neurons varies between 3·7:1 in cortical regions, 11:1 in the brain stem, and 0·2:1 in the cerebellum.[29] Quantitative studies using unbiased stereology and isotropic fractionation estimate that glia comprise about 40% of the total human brain cell population and that the astrocyte proportion ranges from 20% to 40% of all glial cells.[29,31]

Methodological limitations have made the study of human astrocytes challenging; therefore, most of our knowledge on astrocyte physiology in health and disease is extrapolated from experiments in rodents. However, evidence exists of human-specific morphological, transcriptional, and functional features that we summarise in this section.

Morphological features

Human astrocytes are much larger and more complex than rodent astrocytes and they show brain region-dependent diversity.[32] Four morphologically distinct glial fibrillar acidic protein (GFAP)-expressing cells have been described in humans, whereas two have been described in rodents. Although protoplasmic and fibrous astrocytes are present in humans and rodents, interlaminar and varicose-projection astrocytes are unique to primates (panel 2; appendix).[32]

Transcriptional features

Techniques to purify astrocytes required the use of serum that induced unwanted reactive changes in these cells. However, in 2016, Zhang and colleagues[16] isolated both human and mouse astroglia by use of antibodies targeting different cell types to purify them in serum-free conditions (so-called immunopanning) and did the first transcriptomic analysis of human astrocytes. Their study showed that substantial overlap exists in astrocyte-specific genes between humans and mice (eg, *GFAP*, *ALDH1L1*, *AQP4*, *GLUL*, *SLC1A2*, and *SLC1A3*).[16] However, only 30% of the most highly expressed genes in human astrocytes are also highly expressed in mice astrocytes,[16] indicating high variability in transcriptomes between the two species. Among the genes enriched in human astrocytes, several encode proteins involved in calcium signalling (*RYR3*, *MRVI1*, and *RGN*) and metabolism (*APOC2* and *AMY2B*), suggesting that regulation of calcium homoeostasis and metabolism are particularly important in human astrocytes. The transcriptomes of isolated human and mouse neurons, oligodendrocytes, microglia, and endothelial cells were also analysed,[16] but the greatest interspecies differences were found in astroglial transcripts, suggesting that astrocytes are the most evolutionary plastic cells.[33]

Panel 1: **Glossary of terms related to astrocytes**

Astroglia
Heterogeneous class of neural cells of ectodermal, neuroepithelial origin that includes many specialised astrocytes, including protoplasmic astrocytes of the grey matter, fibrous astrocytes of the white matter, cerebellar Bergmann glia, Müller retinal glial cells, tanycytes, ependymal astrocytes, perivascular glia, marginal glia, and velate glia. Two additional types are unique to primates: interlaminar astrocytes and varicose-projection astrocytes.

Atrophic and reactive astrocytes
Morphological alterations in astrocytes have been reported in many CNS diseases (eg, Alzheimer's disease and amyotrophic lateral sclerosis). Atrophic astrocytes have reduced cell soma volume and reduced number or loss of processes and probably lose their homoeostatic capabilities. In contrast, reactive astrocytes show increased volume, thicker processes, and increased expression of glial fibrillar acidic protein.

A1 and A2 astrocytes
Two forms of reactive astrocytes, activated by different stimuli (neuroinflammation *vs* ischaemic insults) and characterised by different gene expression profiles. Neuroinflammation induces A1 astrocytes that are neurotoxic and upregulate expression of genes of the complement cascade, whereas ischaemia gives rise to A2 astrocytes that are protective and upregulate expression of neurotrophic genes.

Microglia
Resident immune cells of the CNS, of mesodermal, mesenchymal origin. They are constantly surveilling their environment and maintain homoeostasis. In pathological conditions, microglia become activated and adopt different gene expression profiles. They are involved in amyloid phagocytosis and neuroinflammation and likely direct the neurotoxic responses of astroglia.

Oligodendroglia
Type of neural cells of ectodermal, neuroepithelial origin whose main function is to form and maintain the myelin that surrounds and insulates CNS axons. Each oligodendrocyte sheathes multiple axons.

Single-cell transcriptomics
High-throughput technology that examines the gene expression of individual cells by simultaneously measuring the messenger RNA concentration of hundreds to thousands of genes.

See Online for appendix

	Entrez gene name	Glial cell type (*Homo sapiens*)	Pathway
ABCA7 [3]	ATP binding cassette subfamily A member 7	All glial cell types (low expression)	Lipid metabolism, immune response
AKAP9 [4]	A-kinase anchoring protein 9	Astrocytes, oligodendrocytes, microglia	Unknown
APOE [5]	Apolipoprotein E	Astrocytes, microglia	Lipid metabolism, immune response
BIN1 [3]	Bridging integrator 1	Microglia, oligodendrocytes	Endocytosis, synaptic transmission
CASS4 [6]	Cas scaffold protein family member 4	Microglia	Unknown
CD33 [7]	CD33 molecule	Microglia	Immune response, endocytosis
CELF1 [8]	CUGBP Elav-like family member 1	Astrocytes, oligodendrocytes, microglia	Unknown
CLU (*APOJ*) [3]	Clusterin or apolipoprotein J	Astrocytes	Lipid metabolism, immune response
FERMT2 [9]	Fermitin family member 2	Astrocytes	Unknown
HLA cluster [10]	Major histocompatibility complex, class II cluster	Microglia	Immune response
IL1RAP [11]	Interleukin 1 receptor accessory protein	Astrocytes, oligodendrocytes	Immune response
INPP5D [12]	Inositol polyphosphate-5-phosphatase D	Microglia	Immune response
MEF2C [13]	Myocyte enhancer factor 2C	Microglia	Immune response, endocytosis, synaptic transmission
MS4A cluster [3]	Membrane spanning 4-domains A	Microglia	Immune response
PICALM [14]	Phosphatidylinositol binding clathrin assembly protein	All glial cell types	Endocytosis, synaptic transmission
PTK2B [15]	Protein tyrosine kinase 2 beta	Microglia, astrocytes	Immune response, endocytosis, synaptic transmission
SLC24A4/RIN3 [6]	Solute carrier family 24 member 4/ and Ras and Rab interactor 3	All glial cell types	Lipid metabolism, endocytosis
SORL1 [14]	Sortilin related receptor 1	Microglia, astrocytes	Lipid metabolism, endocytosis
TREM2 [5]	Triggering receptor expressed on myeloid cells 2	Microglia	Immune response

Classification based on data and overviews from Lambert et al (2013),[6] Zhang et al (2016),[16] and Verheijen and Sleegers (2018).[17] Please note that the genome-wide association study data on which this list is based only provides information about loci associated with Alzheimer's disease. In many cases the locus contains several additional genes and further work is needed to establish whether the genes listed are indeed linked to Alzheimer's disease or not.[18]

Table 1: **Risk for developing Alzheimer's disease is associated with genes expressed by glial cells**

Functional roles

In vitro, human astrocytes promote neuronal survival and are involved in synapse formation, function, and elimination, similar to rodent astrocytes.[16] As in the rodent brain, human astrocytes also mediate rapid removal of neurotransmitters from the extracellular space, maintaining synaptic transmission, and avoiding excitotoxicity.[33] Both human and rodent astrocytes respond to ATP and glutamate through rises in intracellular calcium. A crucial advancement in the study of the function of human astrocytes was the generation of chimeric mice by engraftment of human glial progenitor cells into the forebrain of immunodeficient neonatal mice.[34] Remarkably, human grafted astrocytes displayed hominid features, such as larger size, complex morphologies, and faster calcium waves. They integrated into the mouse brain and improved synaptic transmission and long-term potentiation. Enhanced cognitive function was observed in these hybrid mice.[34]

Under pathological conditions, human astrocytes undergo several changes that can be classified into three broad, morphologically defined categories: astroglial atrophy or astrodegeneration, astroglial pathological remodelling, and reactive astrogliosis.[30,35,36] Atrophic astrocytes have reduced volume and decreased numbers of processes (panel 1).[37–39] They probably lose their homoeostatic capabilities (ie, the control of neurotransmission, glutamate uptake, and the neuron–glia vascular unit; figure 1), although few functional analyses are available in the literature that confirm this claim.[38,39] Astroglial atrophy occurs in many CNS disorders (eg, Alzheimer's disease, frontotemporal dementia, amyotrophic lateral sclerosis, epilepsy, and schizophrenia).[35] Astroglial pathological remodelling is a separate category characterised by specific cytoplasmic inclusions called Rosenthal fibres.[30] It is observed in leukodystrophies, for instance Alexander disease, a genetic disorder caused by mutant GFAP leading to severe leukomalacia.[35] Reactive astrogliosis is common in many CNS disorders, including Alzheimer's disease, and is characterised by astroglial hypertrophy (ie, increased volume, thicker processes, and increased expression of GFAP; figure 1).[30]

Astroglia in Alzheimer's disease

Genetic data show that most of the total risk for developing Alzheimer's disease is associated with genes mainly expressed in glial cells (table 1). Among these, Clusterin (ApoJ), Sortilin-related receptor 1, Fermitin family member 2 and the major risk factor for Alzheimer's disease, ApoE, are mainly expressed by astrocytes, suggesting

a crucial role of astroglia in the pathogenesis of Alzheimer's disease. In fact, astrocytes undergo several morphological, molecular, and functional changes in Alzheimer's disease.[21,38–40]

Atrophic and reactive astrocytes are found in post-mortem tissue of patients with Alzheimer's disease[37] and various Alzheimer's disease mouse models, such as the 3xTg-AD animals (expressing mutant genes for APPSwe, PS1M146V, and tauP301L) and PDAPP-J20 mice (expressing the mutant APPSwe).[38] In these mouse models, such morphological alterations in astrocytes are present even before the appearance of amyloid plaques.[38,39] iPSC-derived astrocytes from patients with Alzheimer's disease have atrophic phenotypes and less complex morphology in vitro compared with control cells.[41]

Hypertrophic, reactive astrocytes are found close to amyloid plaques.[38] They maintain their normal territory and do not overlap with neighbouring astrocytes, but produce spontaneous calcium oscillations and abnormal intercellular calcium waves (figure 1).[42] Reactive astrocytes contribute to the neuroinflammatory processes in Alzheimer's disease.[40] Transcriptional analysis of isolated astrocytes and microglia from an Alzheimer's disease mouse model (the APPswe/PS1dE9 double transgenic mouse)[40] showed that transcripts of several inflammatory genes (eg, *CST7*, *CCL4*, *IL1B*, *CLEC7A*, and *TYROBP*) displayed a greater fold increase in number in astrocytes, although steady state transcription of these genes might be greater in microglia. This study analysed the expression of genes in pooled cells, which yields population averages. Differences in gene expression that might exist in subsets, called cell states, will be missed when sequencing all cells in bulk.[43] Such cell states have been elegantly demonstrated for microglia, in which homoeostatic and disease associated cells could be discerned.[22,23] This concept of cell states changes the line of thought in the field; cells adopt dynamically different expression profiles in response to amyloid pathology or ageing.[22,23] Although in-depth single-cell data for astroglia in Alzheimer's disease are not yet available, such an approach will be instrumental in understanding the complexity of the different astroglial responses.

A major question in Alzheimer's disease research is whether astroglia are innocent bystanders or pivotally involved in the neurodegeneration process. Data from a mouse study[21] suggest that astrocytes are promoters of neuronal death in Alzheimer's disease after instigation by microglia (figure 2). Activated microglia secrete interleukin-1α (IL-1α), tumour necrosis factor α (TNFα), and complement component 1q (C1q), which together induce the A1 neurotoxic phenotype.[21] Mouse A1 reactive astrocytes upregulate expression of genes of the complement cascade, including complement component 3 (C3) and release an unidentified neurotoxin that induces the death of neurons and oligodendrocytes.[44–46] Moreover, mouse A1 astrocytes show decreased ability to promote synapse formation and function, to phagocytose synapses and myelin debris, and to promote neuronal survival and growth.[21] About 60% of the astrocytes in the prefrontal cortex of post-mortem brains of patients with Alzheimer's disease are expressing C3;[21] therefore, they might repesent human A1 neurotoxic astrocytes, although further analysis is needed, for instance by single-cell transcriptomics. C3-expressing reactive astrocytes are also reported in post-mortem brain tissue of patients

***Panel 2:* Four types of human glial fibrillar acidic protein expressing astrocytes[32]**

Protoplasmic astrocytes
The most abundant astroglia type in humans. They are located in layers II to VI of the cortex and organised in precise territorial domains with no overlapping processes. Their cell body is relatively small, similar to that of rodents (about 10 μm diameter). However, their processes are longer (about 100 μm *vs* 40 μm in rodents) and larger in number (about 40 *vs* four in rodents), so the human cells have a volume 27 times greater than that of rodent cells.

Interlaminar astrocytes
Primate-specific cells that reside in layer I of the cortex. Unlike protoplasmic astrocytes, they overlap and do not respect the domain boundaries of their neighbours. Their cell body is about 10 μm diameter and they extend two types of long, unbranched processes: tangential fibres travelling radially near the pial surface, and very long (≤1 mm), tortuous, vertical projections that terminate in layers III or IV of the cortex. Although their functions remain unknown, they might have a role in long-distance intracortical communication.

Varicose-projection astrocytes
Primate-specific cells located in cortex layers V to VI. They are not organised in territorial domains and their processes travel through other protoplasmic astrocyte domains. They are relatively sparse and, although they strongly express GFAP, their appearance is neuronal. They extend between one and five very long processes (≤1 mm) that are frequently straight, unbranched, and possess numerous varicosities distributed about 10 μm apart. Their shorter processes are also straight and slightly branched. Their functions are unknown but, similar to interlaminar astrocytes, they might have a role in long-distance communication across cortical layers.

Human fibrous astrocytes
Cells that reside in the white matter, which are much larger than their rodent counterparts (about 185 μm *vs* 85 μm diameter). They are simpler than protoplasmic astrocytes, with fewer glial fibrillar acidic protein expressing processes that are straight and less branched. These fibres intermingle and overlap, but their somas do not overlap and are roughly equidistant from one another. As white matter tracts are devoid of synapses and neuronal cell bodies, they probably do not modulate neuronal activity and their functions might be limited to metabolic support.

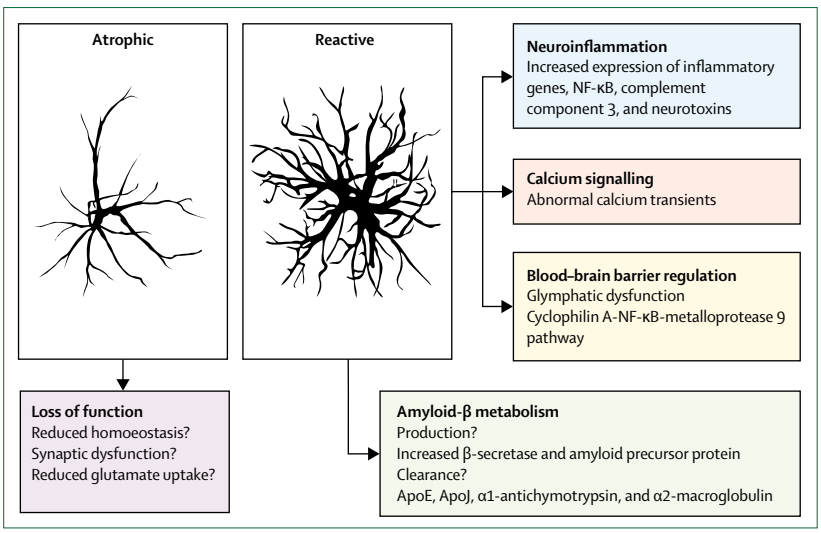

Figure 1: **Atrophic and reactive astrocytes in rodent models of Alzheimer's disease**
Atrophic astrocytes show reduced volume and decreased numbers or loss of processes that might lead to loss of their basic functions.[38,39] Conversely, reactive astrocytes display increased volume and thicker processes and have essential roles in neuroinflammation, calcium signalling, amyloid-β metabolism, and the regulation of the blood–brain barrier.[38,39] NF-κB=nuclear factor κ-light-chain-enhancer of activated B cells. Adapted from Rodríguez-Arellano et al.[39]

with Huntington's disease, Parkinson's disease, amyotrophic lateral sclerosis, and multiple sclerosis.[21] The A1 neurotoxic phenotype, therefore, might represent part of a generic pathway in neurodegeneration.

Direct links between astrocytes and one of the hallmark pathologies of Alzheimer's disease, the amyloid plaques, have been reported.[47,48] Theoretically, reactive astrocytes could be involved in amyloid-β generation in the diseased brain because they appear to upregulate the amyloid precursor protein and β-secretase 1 (figure 1).[47] However, astrocytes more likely participate in amyloid-β clearance, not in amyloid-β production, by secreting ApoE, ApoJ, α1-antichymotrypsin, and α2-macroglobulin that promote amyloid-β transport over the blood–brain barrier through low-density-lipoprotein-receptor-related-protein-1 and very-low-density-lipoprotein receptors (figure 1).[48] Astrocytes express many amyloid-β degrading enzymes as well. However, no quantitative studies show a major contribution of astroglia to the overall amyloid-β burden in the brain.

Astrocytes are the main cells that express ApoE in the brain in physiological conditions.[49] A study in iPSC-derived human glia and neurons demonstrated that ApoE4 astrocytes show impaired amyloid-β uptake and cholesterol accumulation compared with ApoE3 astrocytes.[50] Reduced amyloid-β uptake in ApoE4 astrocytes was related to impaired autophagy and excessive endosomal acidification.[51,52] Astrocytic ApoE is also involved in initial seeding of amyloid deposits, with ApoE4 driving seed formation more potently than ApoE3.[53,54] Once amyloid plaques are nucleated, ApoE does not have much effect on total amyloid load but instead affects plaque size and neuritic dystrophy.[53,54] Astrocytic ApoE4 activates the cyclophylin A-NF-κB-metalloproteinase 9 pathway in pericytes, increasing the permeability of the blood–brain barrier.[55] Increases in cyclophilin A and metalloproteinase 9 concentrations in the CSF and in brain samples in patients with homozygous ApoE4 Alzheimer's disease correlate with pericyte degeneration and blood–brain barrier breakdown.[55] Blood–brain barrier breakdown contributes to proinflammatory responses and neurodegeneration in Alzheimer's disease.[56]

In mouse models of Alzheimer's disease, amyloid-β itself can activate the NFκB pathway in astroglia, resulting in the release of C3 into the extracellular space (figure 2).[24] Astrocytes activated by amyloid-β probably display an A1-phenotype,[24,57] but transcriptomic analysis has not been performed. C3 binding to neurons through the C3aR receptor disrupts dendritic morphology and network function, and C3 binding to microglia alters amyloid-β phagocytosis.[57] Both might contribute to Alzheimer's disease pathogenesis.[57] NF-κB and C3 are activated in patients with Alzheimer's disease and in Alzheimer's disease mouse models.[21,24] Increased concentrations of complement factors, including C3 and C1q, have been reported in astrocyte-derived exosomes from patients with Alzheimer's disease compared with those from healthy individuals,[58] which supports the idea that A1 astrocytes contribute to Alzheimer's disease through complement proteins.

Neuroinflammation induces neurotoxic A1 astrocytes, whereas ischaemia gives rise to protective A2 astrocytes, characterised by upregulation of neurotrophic genes such as cardiotrophin-like cytokine factor 1, transglutaminase 1, pentraxin 3, S100 calcium-binding protein A10, or sphingosine kinase 1.[44] A2 astroglia secrete neurotrophic factors promoting survival and growth, and thrombospondins involved in synapse repair.[44] Protective astrocytes might also be present in Alzheimer's disease. In fact, amyloid-β-activated astrocytes and microglia secrete the neurotrophic factor transforming growth factor β,[57] which enhances microglial uptake of amyloid-β and protects neurons from amyloid-β toxicity.[57,59] However, transforming growth factor β 1 mediated neuronal signalling also promotes amyloid precursor protein transcription and amyloid-β production.[57] Further evidence for a beneficial role of reactive astroglia in Alzheimer's disease comes from morphological analyses showing that reactive astrocytes surrounding amyloid-β plaques have phagocytic activity and engulf neuritic dystrophies in both patients with Alzheimer's disease and mouse models.[60]

Current studies tend to stress the dual character of astrocytes—ie, reactive versus atrophic or A1-phenotype versus A2-phenotype[21,44]—which is an oversimplification of the different cellular states that astroglia probably adopt during the slowly evolving process of Alzheimer's disease. Description of the different pathological cell states of astrocytes by use of single-cell transcriptomics and other

single-cell approaches, such as single-cell proteomics and fluorescence in-situ hybridisation, will become a major research direction in the coming years.

Implications for diagnosis and therapeutic development

The knowledge that astrocytes adopt different reactive states has important implications for the development of new therapies. In fact, development of therapies that block the formation of neurotoxic (ie, A1) reactive astrocytes are of interest not only for Alzheimer's disease but for other neurodegenerative disorders (eg, Parkinson's disease, multiple sclerosis, and amyotrophic lateral sclerosis).[21,25–27] Some emerging attempts, based upon the neurotoxic-protective or A1–A2 concept (figure 2)[44] are summarised below, starting with those for which some preliminary evidence in humans has already been provided. The most promising approaches involve blocking microglia activation or targeting specific neuroinflammatory factors secreted by these cells, such as TNFα, IL1α, and C1q.

TNFα antagonism to block the conversion of astrocytes to the neurotoxic A1 phenotype is a possible therapy. TNFα therapy might attenuate inflammation in Alzheimer's disease, and elevated serum TNFα concentrations have been found in patients with Alzheimer's disease compared with controls.[61] TNFα has a crucial role in rheumatoid arthritis. Therapy with etanercept, a TNFα inhibitor, appeared to reduce the relative risk for developing Alzheimer's disease in patients with rheumatoid arthritis compared with healthy untreated controls in a large nested case-control study.[62] However, a phase 2 trial in patients with Alzheimer's disease with subcutaneous administration of etanercept was not conclusive.[63] The use of etanercept in Alzheimer's disease has become controversial because other studies announced spectacular results in single case studies without appropriate controls.[61] This controversy has clearly affected the development of further clinical trials.[63,64]

Other drugs used for treatment of rheumatoid arthritis and sepsis[65] have been considered for treatment in Alzheimer's disease,[44] such as the IL1α recombinant antagonist (anakinra) and an antibody inhibiting C1q. The anti-C1q antibody has been reported to be safe in a toxicity study.[66] Although testing such compounds in Alzheimer's disease should be relatively easy, no clinical trials are ongoing.

Targeting C3, secreted by NF-κB-activated astrocytes (figure 2),[24] or blocking its receptor, C3aR, might also be considered for intervention in Alzheimer's disease.[67] C3aR is a G protein-coupled receptor and, therefore, a pharmacologically attractive target. Data are limited to cell culture and rodent studies, but a C3aR small molecule antagonist or genetic deletion of C3aR restores cognitive deficits in APPswe transgenic mice.[24,57]

NLY01, a long-acting glucagon-like peptide-1 receptor (GLP1R) agonist, blocks microglia activation and inhibits the conversion of astroglia to the neurotoxic A1 phenotype in mice.[25] NLY01 protects against loss of dopaminergic neurons in various mouse models of Parkinson's disease.[25] Other studies in mouse models show that exendin-4, another GLP1R agonist, prevents microglial activation in amyotrophic lateral sclerosis, ischaemia, and multiple sclerosis.[68–70] Thus, GLP1R agonists might have broad neuroprotective properties in several neurodegenerative disorders.

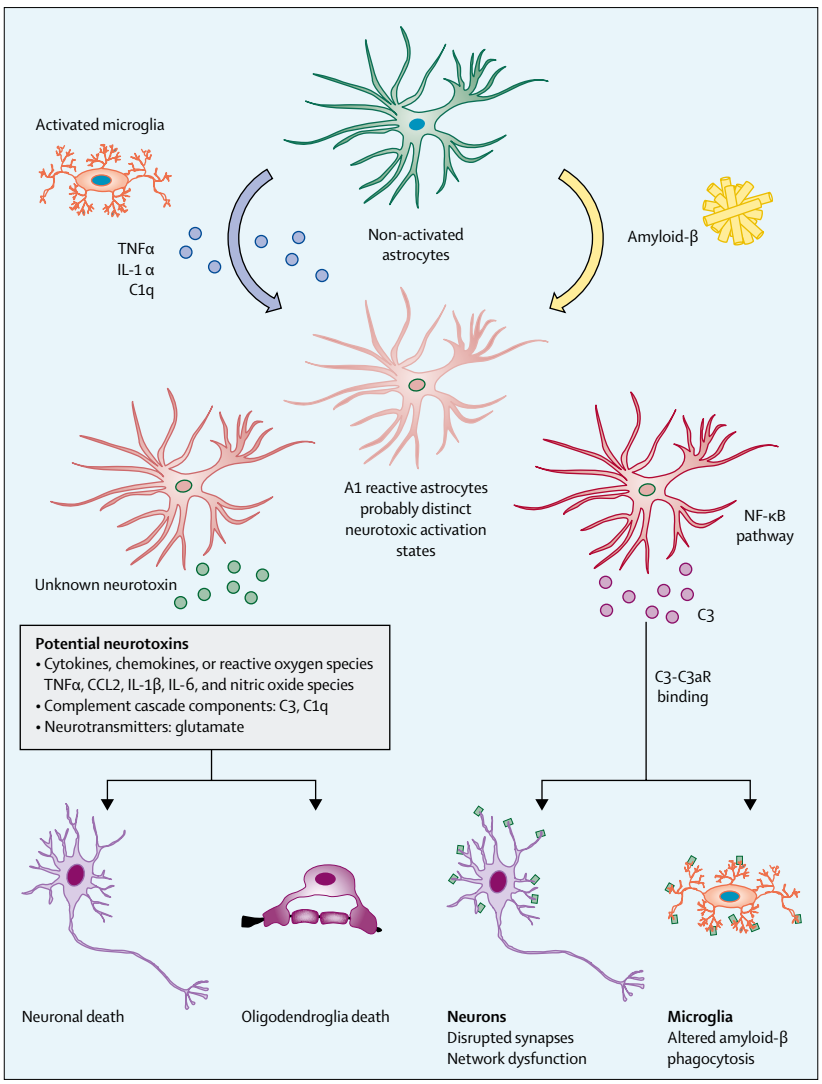

Figure 2: **Model of astroglial activation states in Alzheimer's disease**
Activated microglia secreting IL-1α, TNFα, and C1q,[21] amyloid-β activating the NF-κB pathway,[24] or potentially other factors (eg, apoptotic neurons or other aggregating proteins or viruses) could induce A1 reactive astrocytes and related neurotoxic reactive cell states of astrocytes. The idea of distinct cell states for astrocytes is speculative and requires further work, but the concept of different inflammatory cellular states is accepted for microglia.[22,23] Neurotoxic A1 reactive astrocytes release an unknown neurotoxin (in the figure some potential neurotoxins are indicated) that induces the death of neurons and oligodendrocytes.[21] When astrocytes are activated by the NF-κB pathway, they release the complement protein C3 to the extracellular space.[24] C3 binding to the neuronal C3aR receptor disrupts dendritic morphology and network function and C3 binding to the microglial C3aR alters amyloid-β phagocytosis.[24] C1q=complement component 1q. C3=complement component 3. C3aR=complement component 3a receptor. IL-1α=interleukin-1α. IL-6=interleukin-6. NF-κB=nuclear factor κ-light-chain-enhancer of activated B cells. TNFα=tumour necrosis factor α.

Search strategy and selection criteria

We searched PubMed and ScienceDirect for papers published in English from Jan 1, 2016, to Nov 10, 2018, using combinations of the terms "astrocytes", "Alzheimer's disease", "human astroglia", "microglia", "neuron", "neuroinflammation", and "transcriptome". We identified additional relevant papers by searching the reference lists of selected papers. The final reference list was generated on the basis of relevance to the topic of this Rapid Review.

Reactive astrocytes near amyloid-β plaques strongly express the purinergic receptor P2Y1. One study[71] reported that astrocyte hyperactivity in a mouse model of Alzheimer's disease can be inhibited using antagonists of P2Y1. This treatment normalises neuronal-astroglial network activity, restores structural and functional synaptic integrity, reduces neuritic dystrophy, and attenuates cognitive decline. These beneficial effects were associated with increased morphological complexity of astrocytes around amyloid-β plaques, indicating that functional and morphological alterations are linked.

Additionally, specific biomarkers and neuroimaging to analyse presymptomatic pathological processes in astroglia would be of great help. For example, monoamine oxidase B activity in astrocytes can be followed by PET. Monoamine oxidase B-activated astrocytes are found at early stages of Alzheimer's disease with the largest signals seen in prodromal Alzheimer's disease.[72] YKL-40, also known as chitinase 3-like 1 protein, has been described as a general marker of glial inflammation.[73] Increased concentrations of YKL-40 in CSF were reported in patients with amnestic mild cognitive impairment and correlated with cognitive decline compared with healthy controls.[73] Biomarkers of astrogliosis need further development as they have great potential to become a diagnostic tool in Alzheimer's disease.

Conclusions and future directions

Genetic insights strongly suggest that glial cells have a major role in Alzheimer's disease (table 1). Astrocytes are essential for maintenance of brain homoeostasis and protection of neurons. However, under diverse pathological conditions, including Alzheimer's disease, they become reactive and cause neuroinflammation and neurodegeneration.[21,25–27] The discovery of different types of reactive astroglia[21,44] shows that we need an improved understanding of how astroglia evolve during pathology. This effort needs to take brain region, state of biochemical pathology (plaques and tangles), white versus grey matter, and gender into account, as emerging studies with microglia indicate that these all influence cell state and probably cellular behaviour.[22,23] A complete description of such cellular states involves analysing morphology, metabolomics, transcriptomics, and proteomics. Single-cell transcriptomics is the most advanced method for description of cell states and will provide deep insights into how astrocytes evolve. In the coming years, we will be able to relate different states of astrocyte reactivity to different stages of Alzheimer's disease.

One major challenge that remains is the difference between mouse and human astroglia. Human iPSC-derived astrocytes in vitro or transplanted in vivo to generate chimeric mice provide a powerful approach to overcome this challenge.[34] Single-cell transcriptomics is possible for nuclei and maybe for cells isolated from brain at autopsy or even from frozen brain samples. This approach will allow for more comprehensive study of the human cell biology of Alzheimer's disease, which will lead to novel biomarkers, and hopefully new drug targets to tackle it.

Contributors

Both authors contributed equally to conception of the Review, literature search, and writing. Both authors agree with the content of this manuscript.

Declaration of interests

BDS has received grants from the Geneeskundige Stichting Koningin Elisabeth, the Bax-VanLuffelen foundation, the Alzheimer's Association, Methusalem (Flemish government and Katholieke Universiteit Leuven), and the Fonds Wetenschappelijk Onderzoek and consultancy fees from Janssen pharmaceutics, Biogen, and Eisai. AMA has received grants from the Alzheimer's Association and the Fonds Wetenschappelijk Onderzoek.

Acknowledgments

We thank Carlos Matute, Elena Alberdi, Matthew Holt, Renzo Mancuso, Pranav Preman, and Wei-Ting Chen for reading the manuscript and critical feedback.

References

1 De Strooper B, Karran E. The cellular phase of Alzheimer's disease. *Cell* 2016; **164:** 603–15.
2 Weller J, Budson A. Current understanding of Alzheimer's disease diagnosis and treatment *F1000Res* 2018; **7:** 1161.
3 Almeida JFF, dos Santos LR, Trancozo M, de Paula F. Updated meta-analysis of BIN1, CR1, MS4A6A, CLU, and ABCA7 variants in Alzheimer's disease. *J Mol Neurosci* 2018; **64:** 471–77.
4 Ikezu T, Chen C, DeLeo AM, et al. Tau phosphorylation is impacted by rare AKAP9 mutations associated with Alzheimer disease in African Americans. *J Neuroimmune Pharmacol* 2018; **13:** 254–64.
5 Shi Y, Holtzman DM. Interplay between innate immunity and Alzheimer disease: APOE and TREM2 in the spotlight. *Nat Rev Immunol* 2018; **18:** 759–72.
6 Lambert JC, Ibrahim-Verbaas CA, Harold D, et al. Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. *Nat Genet* 2013; **45:** 1452–58.
7 Liu G, Jiang Q. Alzheimer's disease CD33 rs3865444 variant does not contribute to cognitive performance. *Proc Natl Acad Sci USA* 2016; **113:** E1589–90.
8 Karch CM, Ezerskiy LA, Bertelsen S, et al. Alzheimer's disease risk polymorphisms regulate gene expression in the *ZCWPW1* and the *CELF1* loci. *PLoS One* 2016; **11:** e0148717.
9 Chapuis J, Flaig A, Grenier-Boley B, et al. Genome-wide, high-content siRNA screening identifies the Alzheimer's genetic risk factor FERMT2 as a major modulator of APP metabolism. *Acta Neuropathol.* 2017; **133:** 955–66.
10 Wang ZX, Wang HF, Tan L, et al. Effects of HLA-DRB1/DQB1 genetic variants on neuroimaging in healthy, mild cognitive impairment, and Alzheimer's disease cohorts. *Mol Neurobiol* 2017; **54:** 3181–88.
11 Wes PD, Sayed FA, Bard F, Gan L. Targeting microglia for the treatment of Alzheimer's disease. *Glia*. 2016; **64:** 1710–32.
12 Jing H, Zhu J-X, Wang H-F, et al. INPP5D rs35349669 polymorphism with late-onset Alzheimer's disease: a replication study and meta-analysis. *Oncotarget* 2016; **7:** 69225–30.

13 Tang S-S, Wang H-F, Zhang W, et al. MEF2C rs190982 polymorphism with late-onset Alzheimer's disease in Han Chinese: a replication study and meta-analyses. *Oncotarget* 2016; **7:** 39136–42.

14 Wang Z, Lei H, Zheng M, Li Y, Cui Y, Hao F. Meta-analysis of the Association between Alzheimer Disease and Variants in GAB2, PICALM, and SORL1. *Mol Neurobiol* 2016; **53:** 6501–10.

15 Li YQ, Tan MS, Wang HF, et al. Common variant in PTK2B is associated with late-onset Alzheimer's disease: a replication study and meta-analyses. *Neurosci Lett* 2016; **621:** 83–87.

16 Zhang Y, Sloan SA, Clarke LE, et al. Purification and characterization of progenitor and mature human astrocytes reveals transcriptional and functional differences with mouse. *Neuron* 2016; **89:** 37–53.

17 Verheijen J, Sleegers K. Understanding Alzheimer disease at the interface between genetics and transcriptomics. *Trends Genet* 2018; **34:** 434–47.

18 Efthymiou AG, Goate AM. Late onset Alzheimer's disease genetics implicates microglial pathways in disease risk. *Mol Neurodegener* 2017; **12:** 43.

19 Hansen D V, Hanson JE, Sheng M. Microglia in Alzheimer's disease. *J Cell Biol* 2017; **217:** 459–72.

20 Salter MW, Stevens B. Microglia emerge as central players in brain disease. *Nat Med* 2017; **23:** 1018–27.

21 Liddelow SA, Guttenplan KA, Clarke LE, et al. Neurotoxic reactive astrocytes are induced by activated microglia. *Nature* 2017; **541:** 481–87.

22 Krasemann S, Madore C, Cialic R, et al. The TREM2-APOE pathway drives the transcriptional phenotype of dysfunctional microglia in neurodegenerative diseases. *Immunity* 2017; **47:** 566–81.

23 Keren-Shaul H, Spinrad A, Weiner A, et al. A unique microglia type associated with restricting development of Alzheimer's disease. *Cell* 2017; **169:** 1276–90.

24 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:** 101–16.

25 Yun SP, Kam T, Panicker N, et al. Block of A1 astrocyte conversion by microglia is neuroprotective in models of Parkinson's disease. *Nat Med* 2018; **24:** 931–38.

26 Rothhammer V, Borucki DM, Tjon EC, et al. Microglial control of astrocytes in response to microbial metabolites. *Nature* 2018; **557:** 724–28.

27 Ouali Alami N, Schurr C, Olde Heuvel F, et al. NF–κB activation in astrocytes drives a stage-specific beneficial neuroimmunological response in ALS. *EMBO J* 2018; **37:** e98697.

28 Rasmussen MK, Mestre H, Nedergaard M. The glymphatic pathway in neurological disorders. *Lancet Neurol* 2018; **17:** 1016–24.

29 Verkhratsky A, Nedergaard M. Physiology of astroglia. *Physiol Rev* 2018; **98:** 239–389.

30 Ferrer I. Diversity of astroglial responses across human neurodegenerative disorders and brain aging. *Brain Pathol* 2017; **27:** 645–74.

31 von Bartheld CS, Bahney J, Herculano-Houzel S. The search for true numbers of neurons and glial cells in the human brain: a review of 150 years of cell counting. *J Comp Neurol* 2016; **524:** 3865–95.

32 Oberheim NA, Takano T, Han X, et al. Uniquely hominid features of adult human astrocytes. *J Neurosci* 2009; **29:** 3276–87.

33 Vasile F, Dossi E, Rouach N. Human astrocytes: structure and functions in the healthy brain. *Brain Struct Funct* 2017; **222:** 2017–29.

34 Han X, Chen M, Wang F, et al. Forebrain engraftment by human glial progenitor cells enhances synaptic plasticity and learning in adult mice. *Cell Stem Cell* 2013; **12:** 342–53.

35 Pekny M, Pekna M, Messing A, et al. Astrocytes: a central element in neurological diseases. *Acta Neuropathologica* 2016; **131:** 323–45.

36 Verkhratsky A, Zorec R, Parpura V. Stratification of astrocytes in healthy and diseased brain. *Brain Pathol* 2017; **27:** 629–44.

37 Hsu ET, Gangolli M, Su S, et al. Astrocytic degeneration in chronic traumatic encephalopathy. *Acta Neuropathol* 2018; **136:** 955–72.

38 Verkhratsky A, Zorec R, Rodríguez JJ, Parpura V. Astroglia dynamics in ageing and Alzheimer's disease. *Curr Opin Pharmacol* 2016; **26:** 74–79.

39 Rodríguez-Arellano JJ, Parpura V, Zorec R, Verkhratsky A. Astrocytes in physiological aging and Alzheimer's disease. *Neuroscience* 2016; **323:** 170–82.

40 Orre M, Kamphuis W, Osborn LM, et al. Isolation of glia from Alzheimer's mice reveals inflammation and dysfunction. *Neurobiol Aging* 2014; **35:** 2746–60.

41 Jones VC, Atkinson-Dell R, Verkhratsky A, Mohamet L. Aberrant iPSC-derived human astrocytes in Alzheimer's disease. *Cell Death Dis*. 2017; **8:** e2696.

42 Zorec R, Parpura V, Vardjan N, Verkhratsky A. Astrocytic face of Alzheimer's disease. *Behav Brain Res* 2017; **322:** 250–57.

43 Mathys H, Adaikkan C, Gao F, et al. Temporal tracking of microglia activation in neurodegeneration at single-cell resolution. *Cell Rep* 2017; **21:** 366–80.

44 Liddelow SA, Barres BA. Reactive atrocytes: production, function, and therapeutic potential. *Immunity* 2017; **46:** 957–67.

45 Sekar A, Bialas AR, De Rivera H, et al. Schizophrenia risk from complex variation of complement component 4. *Nature* 2016; **530:** 177–83.

46 Hong S, Beja-Glasser VF, Nfonoyim BM, et al. Complement and microglia mediate early synapse loss in Alzheimer mouse models. *Science* 2016; **352:** 712–16.

47 Frost GR, Li Y-M. The role of astrocytes in amyloid production and Alzheimer's disease. *Open Biol* 2017; **7:** 170228.

48 Ries M, Sastre M. Mechanisms of Aβ clearance and degradation by glial cells. *Front Aging Neurosci*. 2016; **8:** 160.

49 Zhao J, Davis MD, Martens YA, et al. APOE ε4/ε4 diminishes neurotrophic function of human iPSC-derived astrocytes. *Hum Mol Genet* 2017; **26:** 2690–700.

50 Lin YT, Seo J, Gao F, et al. APOE4 causes widespread molecular and cellular alterations associated with Alzheimer's disease phenotypes in human iPSC-derived brain cell types. *Neuron* 2018; **98:** 1141–54.

51 Simonovitch S, Schmukler E, Bespalko A, et al. Impaired autophagy in APOE4 astrocytes. *J Alzheimers Dis* 2016; **51:** 915–27.

52 Prasad H, Rao R. Amyloid clearance defect in ApoE4 astrocytes is reversed by epigenetic correction of endosomal pH. *Proc Natl Acad Sci USA* 2018; **115:** E6640–49.

53 Huynh TP V., Liao F, Francis CM, et al. Age-dependent effects of apoE reduction using antisense oligonucleotides in a model of β-amyloidosis. *Neuron* 2017; **96:** 1013–23.

54 Liu CC, Zhao N, Fu Y, et al. ApoE4 accelerates early seeding of amyloid pathology. *Neuron* 2017; **96:** 1024–32.

55 Sweeney MD, Ayyadurai S, Zlokovic B V. Pericytes of the neurovascular unit: key functions and signaling pathways. *Nat Neurosci* 2016; **19:** 771–83.

56 Sweeney MD, Sagare AP, Zlokovic B V. Blood–brain barrier breakdown in Alzheimer disease and other neurodegenerative disorders. *Nat Rev Neurol* 2018; **14:** 133–50.

57 Lian H, Zheng H. Signaling pathways regulating neuron-glia interaction and their implications in Alzheimer's disease. *J Neurochem* 2016; **136:** 475–91.

58 Goetzl EJ, Schwartz JB, Abner EL, Jicha GA, Kapogiannis D. High complement levels in astrocyte-derived exosomes of Alzheimer disease. *Ann Neurol* 2018; **83:** 544–52.

59 Diniz LP, Tortelli V, Matias XI, et al. Astrocyte transforming growth factor beta 1 protects synapses against Aβ oligomers in Alzheimer's disease model. *J Neurosci* 2017; **37:** 6797–809.

60 Gomez-Arboledas A, Davila JC, Sanchez-Mejias E, et al. Phagocytic clearance of presynaptic dystrophies by reactive astrocytes in Alzheimer's disease. *Glia* 2018; **66:** 637–53.

61 Ekert JO, Gould RL, Reynolds G, Howard RJ. TNF alpha inhibitors in Alzheimer's disease: a systematic review. *Int J Geriatr Psychiatry* 2018; **33:** 688–94.

62 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:** 1111–20.

63 Decourt B, Lahiri DK, Sabbagh MN. Targeting tumor necrosis factor alpha for Alzheimer's disease. *Curr Alzheimer Res* 2017; **14:** 412–25.

64 Chang R, Knox J, Chang J, et al. Blood–brain barrier penetrating biologic TNF-α inhibitor for Alzheimer's disease. *Mol Pharm* 2017; **14:** 2340–49.

65 Goldbach-Mansky R. Blocking interleukin-1 in rheumatic diseases. *Ann N Y Acad Sci* 2009; **1182:** 111–23.

66 Lansita JA, Mease KM, Qiu H, Yednock T, Sankaranarayanan S, Kramer S. Nonclinical development of ANX005: a humanized anti-C1q antibody for treatment of autoimmune and neurodegenerative diseases. *Int J Toxicol* 2017; **36:** 449–62.

67 Lian H, Litvinchuk A, Chiang AC-A, Aithmitti N, Jankowsky JL, Zheng H. Astrocyte-microglia cross talk through complement activation modulates amyloid pathology in mouse models of Alzheimer's disease. *J Neurosci*. 2016; **36:** 577–89.

68 Gonçalves A, Lin CM, Muthusamy A, et al. Protective effect of a GLP-1 analog on ischemia-reperfusion induced blood–retinal barrier breakdown and inflammation. *Investig Ophthalmol Vis Sci* 2016; **57:** 2584–92.

69 Gullo F, Ceriani M, D'Aloia A, et al. Plant polyphenols and exendin-4 prevent hyperactivity and TNF-α release in LPS-treated in vitro neuron/astrocyte/microglial networks. *Front Neurosci* 2017; **11:** 500.

70 Lee CH, Jeon SJ, Cho KS, et al. Activation of glucagon-like peptide-1 receptor promotes neuroprotection in experimental autoimmune encephalomyelitis by reducing neuroinflammatory responses. *Mol Neurobiol* 2018; **55:** 3007–20.

71 Reichenbach N, Delekate A, Breithausen B, et al. P2Y1 receptor blockade normalizes network dysfunction and cognition in an Alzheimer's disease model. *J Exp Med* 2018; **215:** 1649–63.

72 Rodriguez-Vieitez E, Saint-Aubert L, Carter SF, et al. Diverging longitudinal changes in astrocytosis and amyloid PET in autosomal dominant Alzheimer's disease. *Brain* 2016; **139:** 922–36.

73 Alcolea D, Vilaplana E, Pegueroles J, et al. Relationship between cortical thickness and cerebrospinal fluid YKL-40 in predementia stages of Alzheimer's disease. *Neurobiol Aging* 2015; **36:** 2018–23.