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Review: Astrocytes in Alzheimer's disease and other age-associated dementias; a supporting player with a central role

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Abstract

Astrocytes have essential roles in the central nervous system and are also implicated in the pathogenesis of neurodegenerative disease. Forming non-overlapping domains, astrocytes are highly complex cells. Immunohistochemistry to a variety of proteins can be used to study astrocytes in tissue, labelling different cellular components and subpopulations, including GFAP, ALDH1L1, CD44, NDRG2 and amino acid transporters, but none of these label the entire astrocyte population. Increasing heterogeneity is recognised in the astrocyte population, a complexity that is relevant both to their normal function and pathogenic roles. They are involved in neuronal support, as active components of the tripartite synapse and in cell interactions within the neurovascular unit, where they are essential for blood brain barrier maintenance and neurovascular coupling. Astrocytes change with

age, and their responses may modulate the cellular effects of neurodegenerative pathologies, which alone do not explain all of the variance in statistical models of neurodegenerative dementias. Astrocytes respond to both the neurofibrillary tangles and plaques of Alzheimer's disease, to hyperphosphorylated tau and Aβ, eliciting an effect which may be neuroprotective or deleterious. Astrocyte hypertrophy, in the form of gliosis, occurs, but also astrocyte injury and atrophy. Loss of normal astrocyte functions may contribute to reduced support for neurons and dysfunction of the neurovascular unit. Understanding how astrocytes contribute to dementia requires an understanding of the underlying heterogeneity of astrocyte populations, and the complexity of their responses to pathology. Enhancing the supportive and neuroprotective components of the astrocyte response has potential translational applications in therapeutic approaches to dementia.

Abbreviations: Aβ Beta-amyloid; AD Alzheimer's disease; ALDH1L1 aldehyde dehydrogenase 1 family member L1; ARTAG aging related tau astrogliopathy; BBB blood brain barrier; CAA cerebral amyloid angiopathy; CBF cerebral blood flow; CFAS Cognitive Function and Ageing Study; CNS central nervous system; CLU clusterin; CR1 complement receptor 1; EAAT excitatory amino acid transporter; EGFP – enhanced green fluorescent protein; ET-1 Endothelin-1; FTD frontotemporal dementia; GFAP glial fibrillary acidic protein; GLAST glutamate/aspartate transporter; GLT-1 glutamate transporter-1; GWAS genome-wide association studies; MND Motor Neurone Disease; NDRG-2 N-myc downregulated gene 2; NFT neurofibrillary tangle; NVU Neurovascular unit; SASP senescence associated secretory phenotype; SOD-1 superoxide dismutase-1; TREM2 triggering receptor expressed on myeloid cells 2

Astrocytes in the Context of Dementia Research

The neuron is the target for many of the cellular pathologies of dementia, such as neurofibrillary tangles (NFTs) and Lewy bodies, whilst extracellular beta-amyloid (Aβ) may also target neurons and their processes. These classical pathologies remain central to theories of dementia pathogenesis and to neuropathological diagnosis, and much of the focus in dementia research has been on the neuron. However, although important correlates of dementia, other factors may also be important. Population based studies such as the UK Medical Research Council Cognitive Function and Ageing Study (CFAS), and other large autopsy series, have shown that classical neuropathological lesions alone do not account for all of dementia in statistical models, particularly at the oldest ages (1-8). This is also recognised in clinical trials with the identification of individuals with Alzheimer's Disease (AD)-like clinical presentations but with little pathology on amyloid imaging or on neuropathology, so-called AD with insufficient neuropathology (9). Different molecular forms of amyloid, tau and other protein pathologies are important on-going areas of therapeutic targets, but other factors may contribute to

age-related cognitive decline either independently or by modulating the effect of the classical cellular and molecular pathologies on neuronal fate and function. This includes other cell types and the interactions between them.

Neurons function within the context of other cell types, and recent theories have emphasised the importance of dysfunction of the neurovascular unit (NVU) in ageing and in development of neurodegeneration and dementia (10, 11). The NVU includes the neuron, the microvasculature composed of endothelial cells and pericytes, astrocytes and the blood brain barrier (BBB). Oligodendrocytes and microglia are additional key components. A neurovascular hypothesis of dementia would then seek to integrate changes in the neuron with alterations of other cell types and their interaction within the NVU.

Long thought of as "just" a supporting cell, or brain glue, the astrocyte has important physiological roles in relation to the neuron, to synaptic function, and to blood brain barrier and neurovascular coupling. Astrocyte dysfunction may therefore be an important contributor to dysfunction of the NVU, with effects on neurons and microvasculature. This review is focused on the role of the astrocyte in the brain and how perturbation of its function may contribute to dementia pathogenesis, particularly focusing on AD.

Astrocyte Biology

Classification

Astrocytes were first described by Virchow in 1846 and were originally thought to be a homogenous population of cells which function to support neurons. The term 'astrocyte' was first used in 1893 by Michael von Lenhossek and refers to the stellate morphology of the cells (12). There has been much debate around what constitutes an astrocyte given the diversity of this cell type and the commonalities with other cell types of the central nervous system (CNS). A review by Kimelberg et al., sets out the following criteria for defining this cell type : 1) Nonexcitability; 2) A very negative membrane potential which is determined by the K^+ gradient; 3) Glutamate and GABA uptake by astrocyte-specific transporters; 4) the presence of intermediate filament bundles; 5) the presence of glycogen granules; 6) cell processes surrounding blood vessels and synapses; 7) the presence of gap junctions formed from connexins (13).

Astrocytes have been classified into two major morphological groups on the basis on their morphology and anatomical location; the fibrous astrocytes of the white matter and the protoplasmic astrocytes of the grey matter. They have distinct morphologies, initially identified through the use of Golgi staining, with protoplasmic astrocytes having a more complex structure, with many finely branching processes (12). Although both Golgi staining and immunolabelling of protoplasmic astrocytes have provided insights into the structure of these cells it is estimated that they reveal only a small proportion of total astrocyte volume. Immunohistochemistry for glial fibrillary acidic protein (GFAP), a structural protein found only in the main branches of the astrocyte, has been estimated to identify around 15% of the cell. Die injection studies enable a larger proportion of the total astrocyte volume to be revealed, and have identified that protoplasmic astrocytes organise themselves into distinct domains with no overlap apparent (14). The significance of these domains is not well understood but they are functional territories with autonomous features that are thought to be central to the astrocytes ability to coordinate synaptic activity and blood flow (12, 15). Fibrous astrocytes send out long processes which are less elaborate than their grey matter counterparts. These processes contact axons at nodes of Ranvier as well as establishing perivascular end-feet. Fibrous astrocytes are organised along white matter tracts and unlike protoplasmic astrocytes do not organise themselves into domains.

This binary classification, however, is an oversimplification. Several morphological types can be recognised in human cortex and are more complex than those found in other animals, such as rodents, that are often used for disease modelling. For example, the interlaminar astrocyte, which is found in superficial cortex with long processes extending into cortex, is unique to higher primates (16, 17). Much of the complexity of human brain is thought to arise from this phylogenetic evolution of astrocytes. Functional complexity is also being recognised but, as yet, has not been accounted for in neurodegeneration research.

Developmental origin

There are three distinct pools of glial progenitors in the cerebral cortex, radial cells of the ventricular zone, postnatal glial progenitor cells of the subventricular zone (SVZ) and glial restricted precursors. The protoplasmic astrocytes of the grey matter are mostly generated by embryonic radial glia whereas the fibrous astrocytes of the white matter are derived from neonatal SVZ progenitors (18). It is generally accepted that astrocytes develop after neurons in the developing central nervous system although in spite of this they are thought to play a role in guiding axon migration and synaptic pruning (19) Further, astrocyte-like cells residing in the adult subventricular zone can function as multi-potent stem cells *in vivo* and are the major source of adult neurogenesis in the olfactory bulb and hippocampus (20, 21). However, it is important to consider that the developmental origins of

astrocytes are fundamentally different across species, suggesting that the contributions of astrocytes to the functioning CNS also differ across species (22).

Glial Fibrillary Acidic Protein

Since their initial classification numerous techniques have been used to further investigate astrocyte morphology and phenotype including immunohistochemical techniques. GFAP is the prototypical marker for identifying astrocytes immunohistochemically. GFAP is a class III intermediate filament protein, encoded by a gene on chromosome 17q21. There are several isoforms, including GFAP-α, which is the main isoform in the CNS, and GFAP-δ, which is particularly expressed by neurogenic subventricular astrocytes. A single nucleotide frameshift produces GFAP+1 variants that are expressed in a subset of astrocytes (23). Mutation in GFAP produces Alexander disease, a primary astrogliopathy characterised by Rosenthal fibre accumulation (24).

GFAP expression increases with age in animal models, in which oxidative stress may play a role (25). It is also upregulated non-specifically under pathological conditions in the hypertrophic response of (astro)gliosis. This is a complex phenomenon, reflecting a spectrum of changes, from up-regulation of GFAP, increasing GFAP with proliferation and loss of domain structure through to compact glial scar formation with disruption of tissue architecture (26). The term isomorphic gliosis has been used where gliosis does not disrupt the underlying cytoarchicture of the brain, so that remodelling of neuronal networks is permitted, whereas the term anisomorphic gliosis has been used where a permanent glial scar disrupts tissue architecture, preventing remodelling (27).

GFAP up-regulation, detectable by immunohistochemistry, is a reliable marker for identifying reactive astrocytes, but astrogliosis also involves up-regulation of other intermediate filament proteins, namely vimentin, nestin and synemin (28, 29). Although astrogliosis appears to be a stereotypic reaction histologically, transcriptomic analysis shows that the pattern of gene expression within reactive astrocytes varies according to the stimulus, which is relevant to the functional role that astrocytes are likely to play in different pathological states (30).

Astrocytes in the neurovascular unit

The main areas of astrocyte function are summarised in Figure 2. Protoplasmic astrocytes form extensive contacts with blood vessels and the microvasculature and play a central role in regulating blood flow in the brain. They release prostaglandins, nitric oxide and arachidonic acid in order to cause vasoconstriction or vasodilation of blood vessels, often in response to changes in synaptic activity. They have a role in neurovascular coupling, by which local cerebral blood flow is matched to neuronal activity and energy requirements (31). In addition to effects on vasculature from neurons

themselves, astrocytes appear to be able to mediate both vasoconstriction and vasodilation. Changes in astrocyte intracellular calcium with activity lead to formation of arachidonic acid and release of vasoactive substances. Although incompletely understood, astrocyte responses may contribute to changes in functional MRI signals and these responses may be altered in ageing and CNS disease states.

Close apposition of astrocytic end-feet with the vasculature is important in the induction and maintenance of the BBB (32). *In vitro* studies have demonstrated that astrocytes can induce barrier properties in cerebral endothelial cells and it has been shown *in vivo* that loss of GFAP-positive astrocytes from the inferior colliculus of rat brain is accompanied by widespread loss of the tight junctional proteins claudin-5, occludin and ZO-1 (33, 34). Perivascular astrocytes are also vital for ionic and water homeostasis. Their end-feet are enriched in potassium channels, important for the spatial buffering of K^+ in the extracellular space, whilst water movement through aquaporin-4 channels is essential to osmotic balance (35).

The tripartite synapse and astrocyte signalling

Astrocytes have been shown to regulate the stability, dynamics, and maturation of dendritic spines (36, 37) and take part in the regulation of synaptic plasticity and synaptic transmission (38). The tripartite synapse was a phrase coined 16 years ago (39) to define the close proximity of astrocyte processes to pre- and post-synaptic neuronal elements (40) (Figure 2). This strategic positioning of astrocytes enables an interactive dialogue to occur between these two cell types which is central to the local and widespread communication of information across the brain.

The exchange begins with the release of neurotransmitters from the pre-synaptic cleft, which triggers activation of metabotropic or ionotropic receptors on the astrocyte cell surface. Neuronally released transmitters such as glutamate, noradrenaline, histamine and acetylcholine are all capable of binding to receptors on the surface of astrocytes, which leads to a transient increase in calcium levels within the cell, the extent of which is dependent on the intensity of synaptic activity. In rat brain a single hippocampal astrocyte is estimated to be associated with approximately140000 hippocampal synapses (41) meaning that the processes of one astrocyte are in close proximity to a number of stimuli of differing intensities. Astrocytes integrate external synaptic activity and generate a calcium wave of corresponding magnitude that can exist in the small microdomain of a process or propagate throughout the cell. More recently it has been shown that calcium signalling in astrocytic processes can occur independently of signalling in the cell body, and this can compartmentalise neuronal activity to a specific region of the astrocyte (42). Calcium waves are not restricted to one astrocyte and can propagate via gap junctions to neighbouring astrocytes. Importantly, calcium oscillations in astrocytes are not just a chemical conversion of synaptic activity but can occur spontaneously and

excite neighbouring neurons (43).

In response to neuronal activity astrocytes produce gliotransmitters to facilitate communication between neurons. Gliotransmitter release occurs via a number of different mechanisms; calcium dependent exocytosis (44), non-exocytotic release from cytosolic pools; and calcium-independent channel mediated release. The molecular mechanisms that regulate the release of these neuroactive molecules (e.g glutamate, adenosine, GABA, ATP and D-serine) is not fully understood but is thought to be predominantly controlled by calcium (45). Astrocytic release of gliotransmitters offers multiple ways of controlling synaptic activity as astrocytes are able to selectively release a range of different gliotransmitters to individual synaptic inputs, each one having different mechanisms of action at the pre or post-synaptic neuronal elements (46). In addition gliotransmitters interact with other cell types in the vicinity such as microglia and vascular cells.

Astrocytes can further regulate signalling in the brain via the efficient removal of synaptically released inhibitory and excitatory neurotransmitters respectively known as ϒ-aminobutyrate (GABA) and glutamate, which are taken up through transporters on perisynaptic astrocytic processes (47). Astrocytes can directly convert glutamate to glutamine (48) which upon release from the astrocyte is used by neurons to replenish intracellular stores of glutamate and GABA. Uptake of glutamate is also important for astrocytic amino acid metabolism. The speed astrocytes clear neurotransmitters from the synaptic cleft affects the level of post-synaptic activation (49). Thus activity at the synapse can be regulated by both the release and uptake of neuroactive molecules, and activity at a single synapse can quickly lead to ripples of bidirectional chemical crosstalk between a network of astrocytes and neurons.

Finally, astrocytes are also capable of simultaneously integrating information beyond the synapse, such as from vascular cells and microglia, which allows them to finely tune the complex neuronal circuitry within a dynamic microenvironment. Astrocytes form networks, connected via gap junctions, which include connexins, which add a further layer to the integration of astrocytic and neuronal function (50).

Astrocyte metabolism and neuronal support

Astrocyte support in the form of neurotransmitter recycling, provision of energy substrates and cholesterol are important mechanisms by which astrocytes support the viability and function of neurons (Figure 2). Such astrocyte metabolic activities are important in astrocyte involvement in memory and learning (51) and the positioning of these cells means they are able to provide glucose and other molecules for neuronal metabolism, and to sense changes in neural activity in order to facilitate this. Astrocytes are known to avidly take up glucose and typically present a high

glycolytic rate (52) and the metabolic needs of active neurons are at least partly met by non-oxidative glucose metabolism. The astrocyte-neuron lactate shuttle hypothesis proposes that astrocytes metabolise glucose to lactate which is then taken up by neurons and used as a fuel source (53). In addition to this proposed function, astrocytes also store glucose in the form of glycogen which can act as a short-term energy buffer during periods of high neuronal activity, for example during learning and memory when astrocytic glycogen can be used to liberate lactate as an energy substrate for neurons (54, 55). Astrocyte cholesterol metabolism is a further important area. Cholesterol is essential for normal brain function, including synaptic and receptor function. Astrocytes are the main source for neuronal cholesterol, via the APOE lipoproteins (56, 57).

Cytopathology of Astrocytes in Ageing and Neurodegeneration

Identifying astrocytes in tissue-based studies

Many neuropathological studies of astrocytes have relied on the use of GFAP to detect these cells. However, many healthy astrocytes do not express detectable levels of GFAP, and expression of GFAP can depend upon the anatomical location of astrocytes as well as the species in which GFAP expression is being examined (26). This can skew studies into the role of astrocytes in disease as only the population with up-regulated GFAP, reflecting the more reactive cells, is detected. This limitation of current detection methods should be kept in mind when interpreting the results of pathological studies. Thus, although GFAP might still be considered the 'gold standard' astrocyte marker there is a need for additional tissue markers to better capture the full spectrum of astrocytic cells. A number of other potential markers are available and can be used on human post mortem tissue (Figure 1).

1. S100β marks many astrocytes, but is non-specific, labelling many other neuroglial cells (58-60).
2. The excitatory amino acid transporters (EAATs), involved in glutamate uptake from the synaptic cleft. There are 5 types of EAAT in human brain with EAAT1 and EAAT-2 primarily expressed by astrocytes (61). The rodent analogues of these are designated GLAST (glutamate/aspartate transporter) and GLT-1 (glutamate transporter-1) respectively. Double labelling shows that EAAT-2 and GFAP do not entirely overlap, so that there are EAAT2+/GFAP+, EAAT2+/GFAP- and EAAT2-/GFAP+ populations (62).
3. Glutamine synthetase is expressed in cell bodies and processes of astrocytes throughout the cortex (63).
4. CD44 is a cell surface glycoprotein that is a receptor for hyaluronic acid and may act as an adhesion molecule for astrocytes. This marker appears to be distributed more to processes in astrocytes and to identify particular subsets (64).

5. ALDH1L1 (aldehyde dehydrogenase 1 family, member L1, an enzyme involved in folate metabolism) is a marker identified from transcriptomic analysis of astrocytes. ALDH1L1 immunohistochemistry labels astrocyte cell bodies and processes and appears to label more astrocytes than GFAP (65). In our hands, ALDH1L1 immunohistochemistry also labels oligodendrocytes. In mice, it is expressed in cortical and spinal astrocytes and studies in the G93A mutant SOD1 (superoxide dismutase 1) motor neuron disease model suggest that it is upregulated in chronic neurodegeneration. These authors also showed that the gene expression profiles of ALDH1L1+ and GLT-1+ cells are similar, suggesting that they share astroglial identity (66).
6. NDRG2 (N-myc downregulated gene 2) is expressed in the cell bodies and processes of astrocytes in humans and rodents. It appears to be involved in responses to stress but is downregulated in reactive astrocytes near a cortical glial scar. It has been suggested that NDRG2 may be a marker for mature non-reactive astrocytes (67, 68).

In summary, there are a number of astrocytic markers in addition to GFAP, which show varying labelling patterns. To date, ALDHL1 and NDRG2 hold out the best promise for general markers that pick up the largest proportion of the astrocyte population. However, some of these markers require further validation in human tissue and whilst they provide options for the analysis of astrocytes, cannot be considered a universal marker; considerations of astrocyte heterogeneity and subpopulations will still be required.

Astrocytes in ageing

Ageing is the leading risk factor for the common dementias, including AD, dementia with Lewy bodies and vascular dementia.. Whilst age may operate partly via development of the classical neurodegenerative and vascular pathologies, age-related mechanisms may impair cells of the brain via a number of mechanisms (69) which could damage NVU function to act independently or in concert with classical neuropathologies. Astrocytes are affected by ageing, potentially impairing their complex functions or biasing their response to pathology to a less advantageous pattern.

GFAP mRNA and protein levels appears to increase with ageing in rodents and humans, accompanied (in some studies) by S100β (25, 70-72). Astrocytes in the ageing brain show features of senescence and expression of a senescence associated secretory phenotype (SASP). Transcriptomic studies also suggest a shift to a more proinflammatory phenotype in astrocytes with ageing (73-75) . These age-related astrocytic changes might contribute to declining brain function via loss of function and neuroinflammation, and contribute to the background in which neurodegenerative disease develops.

Astrocytic inclusions

AD is generally not associated with astroglial inclusions. However, tau positive thorny astrocytes are a feature of brain ageing (76, 77) . These are found particularly in subpial, subependymal and perivascular locations, particularly in mesial temporal structures and around brainstem. The inclusions may be Gallyas positive, suggesting that some of the tau is fibrillar, and immunohistochemical studies suggest that the inclusions are of 4-repeat tau. The significance and origin of these is currently uncertain, but they do not appear to be associated with dementia and studies have found either no, or a weak association with AD neuropathology. Those in a subpial location, however, are associated with subpial tau positive neuritic processes (78). Whether the astrocytes derive the tau from neuronally produce phospho-tau is currently unknown. Recently a consensus approach has been developed for assessment of the age related form of tau, which also includes "astrocytes with finely granular (tau) immunoreactivity in processes", under an umbrella term of Aging Related Tau Astrogliopathy (ARTAG) (79).

Astrocytes in Alzheimer's disease

The initial observation that astrocytes appeared 'activated' in AD brain was thought to be a secondary and non-specific response to the disease process (80), and a neurocentric view has predominated in AD and neurodegeneration research generally. However, astrocytes are central to pathogenic mechanisms in neurodegeneration. This may include toxic gains-of-function, such as production of cytokines and chemokines, or loss of their complex physiological functions, such as neuronal support (e.g. neurotransmitter recycling, energy substrate provision) and spatial buffering (e.g. K^+, H^+) (26). Essential to synaptic maintenance, disruption of the normal glio-neuronal interaction can lead to synaptic dysfunction and contribute to cognitive impairment (81). Studies in Motor Neurone Disease (MND) have shown that astrocytes affect disease progression (82-84), giving rise to a non-cell-autonomous theory of MND, and establishing the principle that astrocytes have a deterministic role in pathogenic progression of a neurodegenerative disease.

The astrocytic response to beta-amyloid

The first clear evidence that astrocytes play an active role in the AD process was provided by Wyss-Corey et al., (2003) who showed that astrocytes were able to uptake and degrade Aβ using an *in vitro* system of cultured mouse astrocytes (85). However, this was not the first evidence for the uptake of Aβ by astrocytes since earlier studies had shown the presence of Aβ-containing astrocytes (86, 87). Astrocytes respond to plaques, along with microglia and their activation is important in pathogenesis. Since they can take up Aβ they are involved in plaque progression, with death of Aβ loaded astrocytes giving rise to secondary plaques (88).

The mechanisms governing the receptor-mediated uptake of Aβ are not fully understood, particularly whether the uptake of Aβ induces a change in astrocyte phenotype which alters their usual neuro-supportive function. One receptor identified as being involved in uptake and clearance of Aβ is the Low density lipoprotein receptor-related protein 1 (LRP1) (89, 90). LRP1 is also a receptor for the uptake of apoE and complexes of apoE-Aβ and highlights the importance of this receptor, as well as apoE, in astrocytic clearance of Aβ (91). However, it is possible that a number of as yet unidentified receptors are also involved in Aβ uptake.

Whether the response of astrocytes to Aβ is a protective mechanism or results in further damage is not clear with conflicting evidence which likely reflects differences in experimental approach, although it is worth considering that there may be an impact of age on the ability of astrocytes to uptake and degrade Aβ effectively. Evidence from cell culture models of AD suggest that the astrocyte response to Aβ actively contributes to the disease process; Garwood et al. (2011) have shown that the toxic properties of Aβ are enhanced in cultures of primary neurons with small contaminating numbers of astrocytes and that changes in tau phosphorylation and cleavage are only observed in the presence of astrocytes (92). This toxicity is associated with a distinct astrocytic inflammatory profile which might, amongst other things, increase the amyloidogenic processing of APP in astrocytes (93). This same paper has evidence showing $A\beta_{1-42}$ itself can further increase astrocytic BACE1, APP and β-secretase processing resulting in further increases in oligomeric and fibrillary Aβ, providing evidence astrocytes contribute to the production, as well as the degradation, of Aβ.

Astrocytes and neuroinflammation in AD

Neuroinflammation is a prominent and early feature of AD which plays a key role in modulating the progression of disease via a range of inflammatory mediators and neurotoxic compounds. Genome-wide association studies (GWAS) have identified several immune-associated genes that are associated with an increased risk of developing AD, including CLU (clusterin), CR1 (complement receptor 1) and TREM2 (triggering receptor expressed on myeloid cells 2) (94-97). While the role of microglia in the neuroinflammatory response in AD is well established [reviewed in (98)], several studies indicate that astrocyte-mediated inflammatory processes also contribute to neurodegeneration in AD through increased astrocytic expression of pro-inflammatory cytokines and chemokines, activation of the complement cascade, as well as reactive oxygen and nitrogen species [reviewed in ((99-101)]. Transcriptional analysis of $GFAP^+$ astrocytes isolated from cortex of Alzheimer's mice (APPswe/PS1dE9) reveals a proinflammatory phenotype compared to their wildtype littermates, with the number of genes induced and the fold-change in expression more pronounced in astrocytes than in microglia, suggesting that astrocytes contribute to cytokine production and play a central role in the

disease process. These findings were further supported by comparisons with a human AD astrocyte transcriptomic data set, which showed a similar alteration in inflammatory changes (102). Astrocytes can also suppress innate immunity through αβ-crystallin, suggesting that they have a modulatory effect on neuroinflammation. Loss of this function (through knockout of the dopamine D2 receptor) enhances immune responses (103). Anti-inflammatory therapies targeting astrocytes in animal models of AD suggest that the astrocyte contribution to neuroinflammation is a potential and relevant therapeutic target (104).

Astrocyte hypertrophy and atrophy in Alzheimer's disease

Astrocyte hypertrophy has previously been documented in Alzheimer's disease brain (105, 106). The hypertrophic response, as described earlier, is characterised typically by increased GFAP expression and loss of domain structure. The CFAS neuropathology cohort is a population-based ageing brain (>65yrs) cohort that spans the spectrum of ageing brain pathologies. CFAS, and other population cohorts, allows assessment of relationships between pathology, risk factors and dementia without the biases inherent in pre-selection into clinical diagnostic groups [reviewed in (3, 107, 108)], providing complementary information to the usual case-control design. Studies in CFAS showed that the astrocyte hypertrophic response, characterised by GFAP expression, increases early in relation to the development of AD neuropathology as defined by Braak NFT stage. GFAP showed a closer relationship to compact than diffuse plaques. The trend for GFAP expression was the opposite of that for EAAT2 expression, suggesting loss of astrocyte function with AD progression. (62).

Although there is some differential expression of GFAP isoforms in terms of astrocyte sub-populations, transcript levels of most isoforms appear to increase in concert in AD and mouse AD-models, without evidence for differential AD-related isoform changes (109, 110). However, isoform GFAP-ε (also designated GFAP-δ) appears able to bind presenilin-1 (111), so it remains to be determined whether isoforms play selective functional roles in the disorder.

Astrogliosis, may have functional pathological effects. Gliosis contributes to neuronal hyperexcitability through alterations to glutamate, GABA and chloride channels (112). Neuronal hyperexcitability occurs in AD, whilst in APPmt-transgenic AD models hyperexcitability is worsened by Aβ immunization (113). Whether astrocytes contribute to this as a further mechanism by which astrocytes contribute to neuronal dysfunction is unclear at present.

Whilst much of the work in human tissue has focused on hypertrophy, which is easily visualised using the currently available markers, there is also evidence for astrocyte atrophy. Careful morphological studies in transgenic models have suggested that, in addition to gliosis, astrocytes undergo atrophy

particularly at early disease stages, supporting the importance of reduced function and atrophy in addition to hypertrophic and gain of function responses (114, 115). The fragmentation of calpain-positive glial processes (116) and the loss of EAAT2 (62) in human tissue may be reflections of this process. Loss of astrocyte processes are also seen in age related white matter lesions associated with serum plasma protein uptake and BBB dysfunction, producing clasmatodendritic astrocytes (117, 118). Astrocyte atrophy may reflect loss of function, so confining study to hypertrophic astrocytes identified by GFAP up-regulation may miss important changes.

Oxidative stress and the DNA damage response in AD

In addition to the hypertrophic response, there is evidence of cellular damage to astrocytes. Oxidative DNA damage can be found in astrocytes in the ageing brain, and is early in relation to AD stage, and the DNA damage response in astrocytes appears to parallel that in neurons and endothelium (119, 120). Unrepaired DNA damage may induce senescence or apoptosis. Glia express apoptosis-related markers in AD, such as p53 and CD95 (121), and β-galactosidase expression, a marker for senescence, can be found in astrocytes, although the quantitative relationship of this to AD is unclear (120).

Astrocyte injury may contribute to loss of their function or to a proinflammatory state via the SASP, and a number of factors may drive this injury. Oxidative stress is likely to be important and an early feature of AD (122-124). Oxidative stress is also a feature of ageing in the brain (125), and indeed the presence of oxidative DNA damage at the earliest AD stages suggests that oxidative DNA damage in astrocytes may be partly related to ageing mechanisms, a further point of interaction between ageing and neurodegeneration. AD-related molecular pathology may also contribute to astrocyte injury; aggregated Aβ can induce DNA damage and apoptosis (126, 127) and astrocytes around amyloid deposits in both cored and non-cored plaques express apoptosis related molecules caspase 3 and CD95 (128).

Whilst it is clear that multiple factors may drive astrocyte pathology, related to both ageing and neurodegeneration, the relative partitioning of injury *in vivo* is uncertain. Further, different pathological drivers may produce different pathological molecular responses in astrocytes. So, for example, DNA damage but not AD pathology correlates with nuclear retention of the signalling molecule FOXO3a, suggesting differential effects of stressors on signalling in astrocytes (63).

Alterations in astrocytic signalling pathways.

Astrocytes host a complex network of signaling pathways which are altered with the development of disease pathology. Indeed signalling pathways involved in orchestrating glial activation which include STAT3, NFκB, and MAP kinases, have previously been investigated as potential therapeutic targets (129).

Gene expression analysis is one approach to dissecting out molecular responses in tissue and, combined with laser capture microdissection, can be applied to specific cell types (130). It should be noted that the laser capture method will produce a sample that, whist highly enriched for the cell-type targeted, is not a pure sample. Our group have previously used microarray-based gene expression analysis to define changes in astrocyte gene expression associated with AD progression. Analysis showed down-regulation of key cellular pathways, particularly in relation to signalling, including insulin, IGF-1, calcium and MAP-kinase signalling (131). The protein components of the insulin and IGF-1 pathways are present and functional in human cultured astrocytes and can be modulated by insulin-fructose treatment and IGF-1R monoclonal antibody treatment respectively (132). Insulin regulates metabolic processes such as glycogen synthesis and proliferation in astrocytes (133). IGF-1 signalling is important for astrocyte protection of neurons from oxidative stress (134) and interaction of IGF-1 and calcineurin in astrocytes has been implicated in pathogenesis in AD models (135). Insulin resistance is described in neurons in AD, and is a potential therapeutic target (136-139). Derangement of metabolic pathways regulated by insulin and IGF-1 in astrocytes may therefore also contribute to impairment of astrocyte function and their support for neurons as AD progresses.

Astrocyte subtypes in relation to AD pathology

In addition to physiological subtypes, there may also be pathological heterogeneity of astrocytes related to their spatial relationships to specific pathological lesions. Increased gliosis with AD progression correlates with NFTs, but the relationship to plaques is more complex in that gliosis diverges from plaque burden, but glia increase in the vicinity of plaques (140). The association of peri-plaque astrocytes with better cognitive preservation may suggest that the response of astrocytes to this lesion is neuroprotective (141). Calpain upregulation with AD progression is observed in the long processes of interlaminar astrocytes (116), and there are alterations in astrocytes in white matter in AD (142, 143). Astrocytes cannot be considered as a homogeneous population and the differential effects of astrocytes in these various pathological compartments is a significant question.

The influence of *APOE* on astrocyte responses.

The mechanisms by which possession of the ε4 allele contributes to a higher risk of late-onset AD remain to be fully defined, but influence on astrocyte responses may be a contributory factor. Astrocytes respond to Aβ in an APOE-dependent manner (144) and possession of the ε4 allele is associated with a lower peri-plaque astrocyte response (141). Possession of ε4 is associated with higher degree of GFAP expression in demented individuals (145). There may also be interaction with therapy. Use of non-steroidal anti-inflammatory drugs in AD is associated with lower astrocytic counts that is more marked in association with ε4 (146). In a gene expression analysis of astrocytes, transcriptomic changes associated with AD progression were seen at earlier Braak NFT stage in association with ε4 allele possession, although this was a subsidiary analysis of the study based on few cases (131). Overall, however, these studies suggest a picture whereby the astrocyte response is more detrimental in individuals with the ε4 polymorphism.

Evidence for the role of astrocytes from other dementias

The involvement of astrocytes in dementia is not restricted to Alzheimer's disease. Vascular dementia is associated with dysregulation of cerebral blood flow, blood-brain barrier breakdown and increased inflammation and oxidative stress (147). There is considerable overlap between vascular dementia and AD, with evidence of cerebral amyloid angiopathy (CAA) frequently found in AD brains. CAA is the vascular accumulation of Aβ. Interactions of astrocytic endfeet with the vasculature, a critical component of the NVU, is thought to be disrupted by Aβ, with impairment of vascular responses. Hence the presence of CAA is likely to disrupt the NVU and could contribute to reduce blood flow to the brain in AD (148). In the ArcAβ mouse model, which exhibits severe vascular pathology, there are changes in astrocytes early in the disease process. These changes include retraction and swelling of astrocyte endfeet surrounding vascular Aβ deposits and reduced expression of the glucose transporter GLUT1 and monocarboxylate transporter 1 (MCT1) and were accompanied by evidence of neurovascular uncoupling and disruption of the BBB (149).

There is also evidence that changes in astrocytic endothelin-1 are involved in dementia associated with stroke. Endothelin-1 (ET-1), a potent constrictor, is synthesized by both astrocytes and endothelial cells in stroke and in AD brain where it plays an important role in neurovascular reactions. In a study investigating the role of astrocytic ET-1 in post-stroke cognitive deficit it was found that astrocytic ET-1 overexpression contributed to neurodegeneration (in the GET-1 mouse model) (150).

In frontotemporal dementia (FTD), there is evidence that the severity of the disease is directly related to the degeneration of astrocytes. This is evidenced by an increase in astrocytes with beaded processes as well as apoptotic features which correlated with the degree of neuronal loss and the

stage of the disease (151) and there is a suggestion that as the disease progresses unreactive, quiescent astrocytes become involved, further exacerbating the situation. An earlier study investigating astrocytic degeneration in sporadic FTD found that degenerating astrocytes correlated with decreased cerebral blood flow (CBF) and that areas of significant astrogliosis corresponded to areas of hypoperfusion, suggesting a potential causal role for astrocytes in disturbed CBF in FTD (152). It should be noted that these studies preceded current molecular subclassification of the frontotemporal lobar degenerations (the pathological pattern that underlies FTD).

Finally, astrocytic star-like inclusions, which resemble tufted astrocytic fibrillary tangles, are present in the brains of those with Dementia with Lewy Bodies and may contain α-synuclein, suggesting that in DLB a primary degenerative process takes place in both glial cells and neurons (153, 154). Astrocytes may be directly affected by inclusion formation, particularly with the deposition of phosphorylated tau, in specific neurodegenerative disorders. These include tufted astrocytes in progressive supranuclear palsy, astrocytic plaques in corticobasal degeneration and globular astroglial inclusions in globular glial tauopathy (155).

CONCLUSION

So what is the involvement of astrocytes in dementia? In MND there is evidence for a central role of astrocytes in the disease process; the "non-cell autonomous" theory (156, 157). Whether this applies to AD and other dementias is unknown, but the evidence for their cellular pathology and perturbation of function certainly suggests an important role. Whilst the potential dichotomy between loss of normal function and gain of a toxic function is well recognised, the complexity of this cell, including intrinsic heterogeneity and different responses in relation to different molecular and cell pathologies suggest that a more detailed and nuanced understanding of the variations in astrocyte responses is required. Given the central role of this cell in microvascular regulation and neuronal and synaptic support, enhancing the potential neurosupportive and neuroprotective functions of astrocytes, whilst inhibiting their proinflammatory injurious effects offers an additional therapeutic avenue to support brain function in dementia.

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AUTHOR CONTRIBUTIONS

CJG and SBW wrote the paper with input from LER, JES, PRH and PGI.

There are no conflicts of interest to disclose.

FIGURE LEGENDS

Figure 1: Immunohistochemistry illustrating variation in immunostaining patterns for astrocyte markers in human cerebral cortex and white matter which showed gliosis. Sections from an (anonymised) case (Braak NFT stage I) from the CFAS ageing brain cohort. The left column shows low power views of cortex, the centre column high power view of cortex, right column high power view of white matter. Variation in staining patterns with antibodies to: GFAP (A, B, C); EAAT2 (D, E, F); glutamine synthetase (G, H, I); ALDH1L1 (J,K,L). Note variation in relative staining of cortical vs white matter astrocytes.

Figure 2: **The many roles of the astrocyte**: (A) Astrocytes provide energy substrates for neurons, primarily in the form of lactate which is converted from glucose as well as providing cholesterol to support neuronal function. (B) The intimate association of astrocytic processes with neuronal synapses enables the finely-tuned regulation of synaptic transmission. Neurotransmitters are released from the pre-synaptic terminal such as glutamate, noradrenaline, histamine or ACh that are capable of binding to post-synaptic receptors or to receptors present on the surface of astrocytic processes. This binding can trigger a calcium response in the astrocyte that results in the release of gliotransmitters such as GABA, ATP or D-serine that will regulate synaptic activity. Glutamate can also be recycled back to the precursor glutamine, which is released into the extracellular space and taken up by neurons to be resynthesised into glutamate. (C) Astrocytes couple together synaptic activity with local blood flow to ensure neurons have a sufficient energy supply. Prostaglandin (PG), nitric oxide (NO) and arachidonic acid (AA) are all released in response to changes in intracellular calcium levels, which causes vasoconstriction or dilation. Aquaporin 4 and K^+ channels are also present to maintain osmotic balance and for spatial K+ buffering, respectively. (D). Gap junctions such as connexin 43 enable dialogue between neighbouring astrocytes, which is mediated by intracellular changes in calcium levels.

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