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

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SUMMARY

Background. Astrocytes, also called astroglia, maintain homeostasis of the brain by providing trophic and metabolic support to neurons. They recycle neurotransmitters, stimulate synaptogenesis and synaptic neurotransmission, are part of the blood-brain barrier and regulate regional blood flow. Research into Alzheimer's disease (AD) has been neurocentric for a very long time, although more than a century ago it was already known that astrocytes display remarkable morphological alterations during disease progression. Emerging evidence suggests that these morphological changes reflect functional alterations with major impact on disease.

Recent developments. Novel studies indicate that most of the risk in late onset AD is associated with genes that are mainly expressed by glial cells, i.e. astrocytes, microglia, and oligodendrocytes. This insight has moved the focus of research in the field away from neurons towards microglia and neuroinflammation. Surprisingly, although APOE, the major risk factor for AD, and APOJ/Clusterin and SORL, two other important genetic risk factors for AD, are mainly expressed by astrocytes, these cells remain underinvestigated in the field. Recent molecular studies of these cells in rodent models point indeed to a direct contribution of astrocytes to neuroinflammatory and neurodegenerative processes causing AD. However, rodent models for AD might insufficiently mimic the human situation with regard to the contribution of astrocytes. Rodent astroglia differ morphologically considerably from human astroglia, and this is reflected in important differences in gene expression. Exciting novel studies using stem-cell derived human astrocytes in vivo allow to explore human specific aspects of disease and provide already novel insights. The first attempts to develop astrocytic biomarkers and even astrocytic targetted therapies are emerging.

Where next? Single cell transcriptomic analyses allow following the fate of individual astrocytes in situ and provide the granularity needed to describe healthy and pathological cellular states that astroglia adopt over different stages of progressing AD. Given the differences with rodent astroglia, it will be crucial to focus this type of studies on human cells. While refined single cell transcriptome analysis of human postmortem brain are important to document the pathological scenery, this only provides 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 AD. This will lead to novel biomarkers and hopefully a series of new drug targets to tackle this devastating disease.

1. INTRODUCTION

Alzheimer disease (AD) is characterized clinically by memory loss and pathologically by amyloid-β (Aβ) accumulation, neurofibrillary tangle formation, extensive neuroinflammation, synaptic toxicity, neurodegeneration and brain dysfunction (1). Drug development, mainly targeting Aβ, has been disappointingly unsuccessful (2), perhaps because the focus in the field has been too narrow. Novel concepts and ideas are thus needed. One way to broaden this scope is to move away from the biochemical to a cellular theory of the disease (1). It is becoming clear that not only neurons but also glial cells of the brain react to amyloid and tau pathology. This novel way of thinking implies novel therapies, diagnostics, and ways to define the disease.

Recent genetic data are driving this process. More than forty genetic loci have been associated with late onset AD, and many of the linked genes are expressed in astrocytes, microglia and oligodendrocytes (3) (panel 1). As a result, glial cells are taking central stage in AD. Despite the complex back and forward signaling between astroglia and microglia, the focus in the field has been mainly on the latter. Microglia are associated with neuroinflammation. Astroglia, in contrast, were considered for long time as passive supportive cells. While microglia have thus received already "VIP" status, reflected in many excellent reviews discussing their role in AD (4,5), new information supports now also the emerging role of astroglia.

Microglia (6,7) and astroglia (8,9) adopt many different states in AD, which might explain their disparate roles in the development and progression of pathology. Functional evidence provides now insights in how astroglia and microglia converge in the disease process. When instigated by microglia, astrocytes become reactive and have major roles in the neuroinflammation and neurodegeneration processes in AD but also other neurological disorders (9–12). Moreover, astroglia regulate the vascular unit and affect clearance mechanisms of Tau and Aβ (13). In this Rapid Review, we summarize emerging insights in the role of these cells early in the disease process and explore how further study will yield novel biomarkers and therapeutics for AD and neurodegenerative disorders in general.

2. PHYSIOLOGY AND PATHOPHYSIOLOGY OF ASTROGLIA

Astrocytes are specialized glial cells of neuroepithelial origin (14). They regulate neurotransmitter and calcium homeostasis; modulate synapse formation, maturation and elimination; regulate blood-brain barrier (BBB) function through the neuron-glia-vascular units; control extracellular space volume and ion homeostasis; and provide nutritional-trophic support to the brain (14,15). The proportion of astrocytes in the brain is not well defined and varies in different brain regions. Quantitative studies using unbiased stereology and isotropic fractionation estimate that all glia comprise ~40% of the total human brain cell population, and that the astrocyte proportion ranges from 20% to 40% of all glia cells (14,16).

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

Morphological features
Human astrocytes are much larger and more complex than rodent astrocytes and they show brain region-dependent diversity (17). Four morphologically distinct glial fibrillar acidic protein (GFAP) expressing cells are described in humans, only two in rodents (panel 2). While protoplasmic and fibrous astrocytes are present in humans and rodents, interlaminar and varicose-projection astrocytes are unique to humans (17) (panel 2, appendix).

Transcriptional features
Until 2 years ago, techniques to purify astrocytes required the use of serum that induces unwanted reactive changes in these cells. In a new study, both human and mouse astroglia were isolated by immunopanning and the first transcriptomic analysis of astrocytes was performed in serum-free conditions (18). This study shows substantial overlap in astrocyte-specific genes in human and mice (e.g., GFAP, ALDH1L1, AQP4, GLUL, SLC1A2, and SLC1A3). However, only 30% of the most highly expressed genes in human astrocytes are also highly expressed in mice astrocytes (18) indicating high transcriptome variability between the two species. Among the human astrocyte-enriched genes, several encode proteins involved in calcium signaling (RYR3, MRVI1 and RGN) and metabolism (APOC2, AMY2B) suggesting that regulation of calcium homeostasis and metabolism are of particular importance in human astrocytes. In this study, transcriptome analysis was also performed on isolated human neurons, oligodendrocytes, microglia and endothelial cells (18). When comparing the human versus mouse transcriptome in all these different cell types, greatest differences were found in astroglial transcripts, suggesting that astrocytes are evolutionary most plastic (19).

Functional roles
Human astrocytes *in vitro* promote neuronal survival and are involved in synapse formation, function, and elimination, similar to rodent astrocytes (18). As in the rodent brain, human astrocytes also mediate rapid removal of neurotransmitters from the extracellular space, maintaining synaptic transmission and avoiding excitotoxicity (19). Both human and rodent astrocytes respond to ATP and glutamate through rises in intracellular calcium. A crucial step forward in studying the function of human astrocytes involved the generation of chimeric mice by engraftment of human glial progenitor cells into the forebrain of immunodeficient neonatal mice. Remarkably, human grafted astrocytes displayed hominid features such as larger size and complex morphologies as well as faster calcium waves. They integrated into the mouse brain and improved synaptic transmission and long-term potentiation. There was even indication of enhanced cognitive function in these hybrid mice (20).

Under pathological conditions, human astrocytes undergo a variety of changes that can be classified into three broad morphologically defined categories: (1) astroglial atrophy or astrodegeneration, (2) astroglial pathological remodeling, and (3) reactive astrogliosis (15,21,22). Atrophic astrocytes display reduced volume and decreased processes. They most probably lose their homeostatic capabilities, i.e. the control of neurotransmission, glutamate uptake and the neuron-glia-vascular unit (figure 1), although little functional analyses are available in the literature that confirm these claims. Astroglial atrophy occurs in many CNS disorders, eg, AD, frontotemporal dementia, amyotrophic lateral sclerosis, epilepsy, and schizophrenia (21). Astroglial pathological remodeling is a separate category characterized by specific cytoplasmic inclusions called Rosenthal fibers (15). It is observed in leukodystrophies, for instance Alexander disease, a genetic disorder caused by mutant GFAP leading to severe leukomalacia (21). Reactive astrogliosis is also common in many CNS disorders including AD and is characterized by astroglial hypertrophy, i.e. increased volume, thicker processes and increased expression of GFAP (15) (figure 1).

3. ASTROGLIA IN ALZHEIMER'S DISEASE

Recent genetic data shows that most of the total risk for AD is associated with genes mainly expressed in glial cells (Table 1). Among these, Clusterin/Apolipoprotein J (CLU/ApoJ), Sortilin-related receptor (SORL1), Fermitin family member 2 (FERMT2) and the major risk factor for AD, Apolipoprotein E (ApoE), are mainly expressed by astrocytes (Table 1) pointing to a crucial role of astroglia in the pathogenesis of AD. In fact, astrocytes undergo several morphological, molecular and functional changes during the course of AD.

Atrophic and reactive astrocytes are found in post mortem tissue of AD patients (23) and various AD mouse models (24). In mouse models these are observed even before the appearance of amyloid plaques (24,25). Interestingly, iPSC-derived astrocytes from AD patients show atrophic phenotypes and less complex morphology *in vitro* compared to control cells (26).

Hypertrophic, reactive astrocytes are found close to amyloid plaques (24). They maintain their normal territory and do not overlap with neighbouring astrocytes but produce spontaneous Ca2+ oscillations and abnormal intercellular Ca2+ waves (27) (figure 1). Reactive astrocytes contribute to the neuroinflammatory processes in AD. Transcriptional analysis of isolated astrocytes from an AD mouse model revealed a strong, tenfold induction of inflammatory genes like Cst7, Ccl4, Il1b, Clec7a and Tyrobp, which is larger than the induction seen in simultaneously isolated microglia, although steady state levels of the genes might be higher in the latter (28). This study analyzed 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 middled out and the response of these cell subsets will go unnoticed when sequencing all cells in bulk (29). Such cell subsets or 'cell states' are important as demonstrated elegantly for microglia recently where different cell states such as homeostatic or disease associated microglia could be

discerned (6,7). This novel concept of cell states changes the way of thinking in the field: it is clear that cells adopt dynamically different expression profiles while reacting on amyloid pathology or when aging. While in depth single cell data for astroglia in AD are not yet available, such approach will be instrumental to understand the complexity of the different astroglia responses.

A major question in AD research is whether astroglia are innocent bystanders or pivotally involved in the neurodegeneration process. Data from mouse studies suggest that astrocytes are indeed promoters of neuronal death in AD. They do so after instigation by microglia (9) (figure 2). Activated microglia secrete interleukin-1 alpha (IL-1α), tumor necrosis factor alpha (TNFα), and complement component 1q (C1q) which together induce the A1 neurotoxic phenotype (9). Mouse A1 reactive astrocytes upregulate genes of the complement cascade including complement component 3 (C3) and release a not yet identified neurotoxin that induces the death of neurons and oligodendrocytes (30–32). 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 (9). Remarkably, approximately 60% of the astrocytes in the prefrontal cortex of post-mortem AD brain are expressing C3 (9) and therefore might represent human A1 neurotoxic astrocytes although this needs further analysis, for instance by single cell transcriptome investigations as discussed above. C3-expressing reactive astrocytes are also reported in post-mortem tissue of patients with Huntington's disease, Parkinson's disease, Amyotrophic Lateral Sclerosis and Multiple Sclerosis (9) and the A1-phenotype therefore might represent part of a generic pathway in neurodegeneration.

Direct links between astrocytes and one of the hallmark pathologies of AD , i.e. the amyloid plaques, have been made repeatedly in the literature. Theoretically, reactive astrocytes could be involved in Aβ generation in the diseased brain as they appear to upregulate the amyloid precursor protein (APP) and beta secretase 1 (BACE1) (figure 1) (33). Astrocytes however more likely participate in Aβ clearance, not in Aβ production, by secreting apolipoprotein E (ApoE), apolipoprotein J (ApoJ), α1-antichymotrypsin (ACT) and α2-macroglobulin (α2-M) that promote Aβ transport over the BBB via low-density-lipoprotein-receptor-related-protein-1 (LRP1) and very-low-density-lipoprotein (VLDL) receptors (figure 1) (34). Astrocytes express many Aβ degrading enzymes as well. There is however a lack of quantitative studies demonstrating a major contribution of astroglia to the overall Aβ burden in the brain.

Astrocytes are the main cells expressing ApoE in the brain in physiological conditions (35). A study in iPSC-derived human glia and neurons demonstrated that APOE4 astrocytes display impaired Aβ uptake and cholesterol accumulation compared to APOE3 cells (36). Reduced Aβ uptake in APOE4 astrocytes was related to impaired autophagy and excessive endosomal acidification (37,38). Astrocytic ApoE is also involved in initial seeding of amyloid deposits, with APOE4 driving seed formation more potently than

APOE3 (39,40). Once amyloid plaques are nucleated, ApoE does not have much influence on total amyloid load but instead affects plaque size and neuritic dystrophy (39,40). Astrocytic APOE4 activates the cyclophylin A-NFkB-Metalloproteinase 9 pathway in pericytes, affecting the integrity of the BBB (41). Increases in cyclophilin A and metalloproteinase 9 levels in the CSF and in brain samples in homozygous APOE4 AD patients correlate with pericyte degeneration and BBB breakdown (41). BBB breakdown contributes to proinflammatory responses and neurodegeneration in AD (42).

In AD mouse models, Aβ itself can also activate the NFkB pathway in astroglia, resulting in the release of C3 to the extracellular space (8) (figure 2). It is likely that astrocytes activated by Aβ display an A1 phenotype, but transcriptome analysis was not performed. C3 binding to neurons via the C3aR receptor disrupts dendritic morphology and network function, and C3 binding to microglia alters Aβ phagocytosis (43). Both might contribute to AD pathogenesis (43). NFkB and C3 are activated in human AD brain and in AD mouse models (8,9). Interestingly, high levels of complement factors, including C3 and C1q, have been reported in astrocyte-derived exosomes from AD patients compared to healthy individuals (44), in support of the idea that type A1 astrocytes contribute to AD via complement proteins.

While neuroinflammation induces neurotoxic A1 astrocytes, ischaemia gives rise to protective A2 astrocytes, characterized by upregulation of neurotrophic genes such as cardiotrophin-like cytokine factor 1 (CLCF1), transglutaminase 1 (TGM1), pentraxin 3 (PTX3), S100 calcium-binding protein A10 (S100A10) or sphingosine kinase 1 (SPHK1) (30). A2 astroglia secrete neurotrophic factors promoting survival and growth, and thrombospondins involved in synapse repair (30). Protective astrocytes might also be present in AD. In fact, Aβ-activated astrocytes and microglia secrete the neurotrophic factor transforming growth factor beta (TGFβ) (43) that enhances microglial uptake of Aβ and protects neurons from Aβ toxicity (43,45). However, transforming growth factor beta 1 (TGFβ1) mediated neuronal signaling also promotes APP transcription and Aβ production (43). Further evidence for a beneficial role of reactive astroglia in AD comes from morphological analyses showing that reactive astrocytes surrounding Aβ plaques have phagocytic activity and engulf neuritic dystrophies in both AD patients and mouse models (46).

The available studies tend to stress the dual character of astrocytes, i.e. reactive versus atrophic or A1- versus A2-phenotypes (9,30). This is an oversimplification of the different cellular states that astroglia likely adopt over the course of the slowly evolving AD process. Single cell transcriptomics and other single cell approaches such as single cell proteomics and fluorescence in situ hybridization (FISH) are instrumental to describe the landscape of different pathological cell states of astrocytes and the study of this will become a major research direction in the coming years.

4. IMPLICATIONS FOR DIAGNOSIS AND THERAPEUTIC DEVELOPMENT

The understanding that astrocytes adopt different reactive states has important implications for the development of new therapies. In fact, developing therapies that block the formation of neurotoxic (A1) reactive astrocytes are of interest not only for AD but for other neurodegenerative disorders (9–12). Some emerging attempts, evolving around the neurotoxic-protective or A1-A2 concept (30) (figure 2) are summarized below. The most promising involve blocking microglia activation or targeting specific neuroinflammatory factors secreted by these cells such as TNFα, IL1α and C1q. We discuss here the few emerging ideas around astroglia directed therapies starting with those for which some preliminary evidence in human has already been provided.

TNFα antagonism to block the conversion of astrocytes to the neurotoxic A1-phenotype is a possible therapeutic road. TNFα therapy might attenuate inflammation in AD, and elevated serum-TNFα has been linked to worse evolution in AD (47). TNFα plays a critical role in rheumatoid artritis (RA). Anti-TNFα therapy with etanercept, a TNFα inhibitor, to treat these patients appeared to lower also their relative risk for AD in a large nested case-control study (48). Unfortunately, a phase II trial in patients with AD with subcutaneous administration of etanercept was not conclusive (49). The use of etanercept in AD has become controversial now because other studies announced spectacular results in single case studies without appropriate controls (47). It is clear that this has affected the development of further clinical trials (49,50).

Other drugs used for treatment of RA and sepsis (51) are considered for treatment in AD (30) such as the IL1α recombinant antagonist (anakinra) and an antibody inhibiting C1q. Toxicity studies for the latter are reported to be safe (52). Although it should be relatively easy to test such compounds now in AD, no clinical trials are going on at this moment.

Targeting C3, secreted by NFkB-activated astrocytes (8) (figure 2) or blocking its receptor C3aR might be considered for intervention in AD (53) as well. 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 an AD mouse model (8,43).

NLY01, a long-acting glucagon-like peptide-1 receptor (GLP1R) agonist, blocks microglia activation and inhibits in that way the conversion of astroglia to the neurotoxic A1-phenotype in mice (10). NLY01 protects against loss of dopaminergic neurons in various mouse models of Parkinson's disease (10). Other studies in mouse models show that exendin-4, another GLP1R agonist, prevents microglial activation in ALS, ischemia and multiple sclerosis (54–56). Thus, GLP1R agonists might have broad neuroprotective properties in a variety of neurodegenerative disorders.

Reactive astrocytes near Aβ plaques strongly express the purinergic receptor P2Y1. A study reports that astrocyte hyperactivity in a mouse model of AD can be inhibited using antagonists of P2Y1 (57). This treatment normalizes neuronal-astroglial network activity, restores structural and functional synaptic integrity, reduces neuritic dystrophy and attenuates cognitive decline. Interestingly, these beneficial effects were associated with

increased morphological complexity of astrocytes around Aβ plaques, indicating that functional and morphological alterations are linked.

Additionally, specific biomarkers, neuroimaging and other diagnostics to analyze presymptomatic pathological processes in astroglia would be of great help. For example, monoamine oxidase B (MaoB) activity in astrocytes can be followed by PET. Indeed, MaoB-activated astrocytes are found at early stages of AD with the largest signals seen in prodromal AD (58) compared to later stages of the disease. YKL-40, also known as chitinase 3-like 1 protein, has been described as a general marker of glial inflammation (59). Increased levels of YKL-40 in CSF were observed in patients with amnestic mild cognitive impairment and correlated with cognitive decline compared with healthy controls (59). Biomarkers of astrogliosis need further development as they have great potential to become a diagnostic tool in AD.

5. CONCLUSIONS AND FUTURE DIRECTIONS

Novel genetic insights strongly point towards glial cells as major players in AD (Table 1). Astrocytes are essential to maintain brain homeostasis and protect neurons. However, under diverse pathological conditions including AD, they become reactive and cause neuroinflammation and neurodegeneration (9–12). The discovery of different types of reactive astroglia (9,30) illustrates that we need a better understanding of how astroglia evolve over the landscape of pathological cellular states. This effort needs to take into account, for example, brain region, state of biochemical pathology (plaques, tangles); white versus grey matter, gender, as emerging studies with microglia indicate that these all influence cell state and likely cellular behavior (6,7). A complete description of such cellular states involves analyzing morphology, metabolomics, transcriptomics, and proteomics. Transcriptome analysis at single cell level is the most advanced method to describe cell states and will provide deep insights in how astrocytes evolve. Soon we will be able to relate different states of astrocyte reactivity to different stages of AD.

One major challenge remains 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 this end (20). Single cell transcriptome sequencing is possible on nuclei and maybe on cells isolated from brain at autopsy or even from frozen brain samples. This will provide a complete novel look into human cell biology of AD which will undoubtedly lead to novel biomarkers, and hopefully new drug targets to tackle AD.

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 based on relevance to the topic of this Rapid Review.

Author contribution

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

Declaration of interests

Acknowledgements

Figure legends

Figure 1. Atrophic and reactive astrocytes in Alzheimer's disease. Atrophic astrocytes show reduced volume and decrease or loss of processes that might lead to loss of their basic functions (24,25). Conversely, reactive astrocytes display increased volume and thicker processes and have essential roles in neuroinflammation, Ca2+ signaling, Aβ metabolism and the regulation of the blood-brain-barrier (24,25). Data obtained in rodent models of AD. NFkB: nuclear factor kappa-light-chain-enhancer of activated B cells, C3: complement component 3, Ca2+: calcium, BBB: blood-brain-barrier, Cyp A: cyclophilin A, MMP9: metalloprotease 9, Aβ: amyloid-beta, BACE: beta-secretase 1, APP: amyloid precursor protein, ApoE: apolipoprotein E, ApoJ: apolipoprotein J, ACT: α1-antichymotrypsin, α2-M: α2-macroglobulin. Astrocyte illustrations taken from (25).

Figure 2. Model of astroglial activation states in Alzheimer's disease. Activated microglia secreting IL-1α, TNFα, and C1q (9), or Aβ which, in turn, activates the NFkB pathway (8), or potentially other factors (apoptotic neurons, other aggregating proteins eg, alpha-synuclein, virusses) could potentially induce A1 reactive astrocytes and related neurotoxic reactive cell states of astrocytes. The idea of distinct cell states for astrocytes is speculative at this moment and requires further work but the concept of different inflammatory cellular states is accepted for microglia (6,7). Neurotoxic reactive astrocytes release an unknown neurotoxin (in the figure some possible factors are indicated) that induces the death of neurons and oligodendrocytes (9). When astrocytes are actived by the NFkB pathway, they release the complement protein C3 to the extracellular space (8). C3 binding to the neuronal C3aR receptor disrupts dendritic morphology and network function and C3 binding to the microglial C3aR receptor alters Aβ phagocytosis (8). Further work is needed to elucidate the mechanisms that contribute to

neurodegeneration in AD. IL-1α: interleukin 1 alpha, TNFα: tumor necrosis factor alpha, C1q: complement component 1q, NFkB: nuclear factor kappa-light-chain-enhancer of activated B cells, C3: complement component 3, C3aR: complement component 3a receptor.

Panel 1: Glossary of terms related to astrocytes

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

Atrophic and reactive astrocytes. Morphological alterations observed in astrocytes in many CNS diseases. Atrophic astrocytes display reduced volume and decreased or loss of processes and most probably lose their homeostatic capabilities. In contrast, reactive astrocytes show increased volume, thicker processes and increased expression of GFAP.

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

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

Neuroinflammation. Activation of astroglia and microglia and subsequent expression of proinflammatory cytokines and chemokines initiated in response to a variety of cues, including infection, traumatic brain injury, toxic metabolites, or autoimmunity.

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. Novel high throughput technology that examines the gene expression level of individual cells by simultaneously measuring the messenger RNA concentration of hundreds to thousands of genes.

Panel 2. Four types of human glial fibrillar acidic protein (GFAP) expressing astrocytes (17).

Protoplasmic astrocytes are the most abundant astroglia type in humans. They are located in layers II to VI of the cortex and organized in precise territorial domains with no overlapping processes. Their cell body is relatively small, similar to that of rodents (≈ 10 μm diameter). However, their processes are longer (≈ 100 μm vs 40 μm in rodents) and more in number (≈40 vs 4 in rodents) so the human cells display a 27-fold greater volume compared to rodents.

Interlaminar astrocytes are primate-specific and reside in layer I of the cortex. Unlike protoplasmic astrocytes, they overlap and do not respect the domain boundaries of their neighbors. Their cell body is ≈ 10 μm diameter and they extend two types of long, unbranched processes: tangential fibers traveling radially near the pial surface, and very long (up to 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 intra-cortical communication.

Varicose-projection astrocytes are primate specific as well and located in cortex layers V-VI. They are not organized 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 (up to 1 mm) that are frequently straight, unbranched and possess numerous varicosities distributed ≈ 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 reside in the white matter and are much larger than their rodent counterparts (≈185 vs 85 μm diameter). They are simpler than protoplasmic astrocytes: they have fewer GFAP expressing processes that are straight and less branched. These fibers 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.

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