Astrocyte in prion disease: a double-edged sword

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Waqas Tahir[1, 2, 3], Simrika Thapa[1, 2, 3], Hermann M. Schatzl[1, 2, 3, *]

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Abstract

Prion diseases are infectious protein misfolding disorders of the central nervous system that result from misfolding of the cellular prion protein (PrP^C) into the pathologic isoform PrP^{Sc}. Pathologic hallmarks of prion disease are depositions of pathological prion protein PrP^{Sc}, neuronal loss, spongiform degeneration and astrogliosis in the brain. Prion diseases affect human and animals, there is no effective therapy, and they invariably remain fatal. For a long time, neuronal loss was considered the sole reason for neurodegeneration in prion pathogenesis, and the contribution of non-neuronal cells like microglia and astrocytes was considered less important. Recent evidence suggests that neurodegeneration during prion pathogenesis is a consequence of a complex interplay between neuronal and non-neuronal cells in the brain, but the exact role of these non-neuronal cells during prion pathology is still elusive. Astrocytes are non-neuronal cells that regulate brain homeostasis under physiological conditions. However, astrocytes can deposit PrP^{Sc} aggregates and propagate prions in prion-infected brains. Additionally, sub-populations of reactive astrocytes that include neurotrophic and neurotoxic species have been identified, differentially expressed in the brain during prion infection. Revealing the exact role of astrocytes in prion disease is hampered by the lack of *in vitro* models of prion-infected astrocytes. Recently, we established a murine astrocyte cell line persistently infected with mouse-adapted prions, and showed how such astrocytes differentially process various prion strains. Considering the complexity of the role of astrocytes in prion pathogenesis, we need more *in vitro* and *in vivo* models for exploring the contribution of sub-populations of reactive astrocytes, their differential regulation of signaling cascades, and the interaction with neurons and microglia during prion pathogenesis. This will help to establish novel *in vivo* models and define new therapeutic targets against prion diseases. In this review, we will discuss the complex role of astrocytes in prion disease, the existing experimental resources, the challenges to analyze the contribution of astrocytes in prion disease pathogenesis, and future strategies to improve the understanding of their role in prion disease.

Key Words: Alzheimer's disease; astrocytes; central nervous system; Creutzfeldt-Jakob disease; glial cells; neurodegeneration; prion; prion disease; prion protein; scrapie

Introduction

Prion diseases are fatal infectious neurodegenerative disorders and prototypic protein misfolding diseases. Prion diseases affect both humans and animals. Human prion diseases can occur either as sporadic (80–95%), genetic/ familial (10–15%) or acquired (< 1%) forms. Sporadic Creutzfeldt Jakob's disease (sCJD) is an example of sporadic form, whereas Gerstmann-Sträussler-Scheinker syndrome and fatal familial insomnia are the genetic forms and, and Kuru and variant CJD (vCJD) are the acquired forms of human prion diseases. sCJD is a human prion disease characterized mainly by rapidly progressive dementia along with impaired sensory, motor and behavioural functions. These neurological symptoms get worse rapidly and fatal within a few months, hence making this group of diseases extremely deadly (reviewed in Geschwind, 2015). Examples of animal prion diseases include scrapie in sheep and goats, bovine spongiform encephalopathy (BSE, mad cow disease) in cattle, and chronic wasting disease in deer, elk, moose and reindeer (reviewed by Orge et al., 2021). The central event in prion pathogenesis is the accumulation of pathological prion protein PrP^{Sc}, the pathologic and misfolded isoform of the cellular prion protein (PrP^C: encoded by *PRNP* gene), accompanied by neuronal death, spongiform degeneration, and gliosis in the central nervous system (CNS) (reviewed by Prusiner, 1998; Orge et al., 2021). Neuronal death in the CNS is considered the main underlying mechanism driving prion pathogenesis and clinical disease development. However, after decades of research, questions such as, what induces neurodegeneration and what is the role of non-neuronal cells in this process, still remain elusive. There are studies suggesting a crucial involvement of glial cells, such as astrocytes and microglia, in prion pathogenesis (Diedrich et al., 1991; Ye et al., 1998; Baker et al., 2002). Moreover, astrocytosis is one of the pathologic hallmarks in prion disease, however, it is considered a reactive event, and the exact role of astrocytes in prion propagation and pathogenesis is yet to be established. Astrocytes are non-neuronal cells in the CNS, which express PrP^C (Moser et al., 1995) and play a vital role in brain homeostasis. Accumulation of PrP^{Sc} in astrocytes and prevalence of reactive astrocytes (also termed astrogliosis) predominantly in white matter and variably in grey matter have been consistently reported in multiple forms of human prion disease, including sporadic, familial, iatrogenic and variant CJD and Gerstmann-Sträussler-Scheinker syndrome. Reactive astrocytes were found mainly in thalamus, hypothalamus and cerebellum in the human patients (Fraser et al., 2003; Frau-Méndez et al., 2017). Moreover, animal prion diseases, including scrapie and BSE exhibit similar features of intra-astrocytic

[1]Department of Comparative Biology & Experimental Medicine, Faculty of Veterinary Medicine, University of Calgary, Calgary, AB, Canada; [2]Calgary Prion Research Unit, University of Calgary, Calgary, AB, Canada; [3]Hotchkiss Brain Institute, University of Calgary, Calgary, AB, Canada

*__Correspondence to:__ Hermann M. Schatzl, MD, hschaetz@ucalgary.ca.

https://orcid.org/0000-0003-4972-8740 (Hermann M. Schatzl)

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PrP^{Sc} deposition, predominantly in white matter and variably in grey matter (Bons et al., 1999; González et al., 2002; Hedman et al., 2016). Interestingly, the widespread presence of reactive astrocytes during prion pathology (Kushwaha et al., 2021), deposition of PrP^{Sc} in astrocytes during prion infection (Diedrich et al., 1991; Kovács et al., 2005), and prion propagation in PrP-null mice (Büeler et al., 1993) expressing a PrP^{C} transgene under the glial fibrillary acidic protein (GFAP) promoter (Raeber et al., 1997; Jeffrey et al., 2004) indicate an important role of astrocytes in prion disease pathogenesis. However, the complex interplay of astrocytes with other cells in the CNS (e.g., neurons and microglia) complicates the understanding of their role in prion pathology. Additionally, the identification of sub-populations of reactive astrocytes, neurotrophic and neurotoxic, and their differential expression and downstream signaling in the prion disease add an additional layer of complexity. In this review, we discuss the controversial role of astrocytes during prion pathogenesis and future perspectives for better understanding of their involvement in prion disease.

During drafting this review article, we used PubMed (https://pubmed.ncbi.nlm.nih.gov/) to search the most relevant literature published from 1990 to 2021. The majority of cited literature in this article corresponds to 2010–2021. Key words used in the search included: prion diseases, functions of astrocytes in relation to PrP^{C}, astrocytes in human and animal prion diseases, propagation and replication of prions in astrocytes, sub-populations of reactive astrocytes in prion disease, role of astrocytes in neurodegeneration in prion disease, modulation of signaling pathways in astrocytes during prion infection, and role of astrocytes in therapeutic strategies against prion diseases.

Functional Role of Astrocytes Linked to Expression of PrP^{C}

During physiological conditions, astrocytes are involved in the regulation of brain development, synaptic function, neuronal survival, formation of blood brain barrier, and neural plasticity via interaction with neurons (Goss et al., 1998; Christopherson et al., 2005; Cavaccini et al., 2020; Huang et al., 2020; Heithoff et al., 2021) (**Figure 1**). Astrocytes highly express PrP^{C} (Moser et al., 1995), which is associated with their neuroprotective roles. Studies using neuron-astrocyte co-cultures from PrP knockout mice have suggested that astrocytic PrP is critical for neuritogenesis (Lima et al., 2007). Moreover, astrocyte-restricted PrP^{C} expression was sufficient to impede neurodegeneration induced by truncated prion protein in a transgenic mouse model (Race et al., 2009), by rescuing 50% of mice from disease and prolonging survival up to 200 days in the remaining 50%. These results indicate that astrocytic PrP exerts a neuroprotective role just like the neuronal PrP. Similarly, astrocytic PrP was found to regulate the secretion of soluble factors needed for neuronal survival. Laminin and stress-inducible protein-1 were identified as the factors secreted by astrocytes that supported the PrP-mediated neuronal survival and differentiation (Lima et al., 2007), and enhanced cellular protection from apoptosis (Lopes et al., 2005). Over-expression of PrP^{C} and its binding to stress-inducible protein-1 promote development, maturation and survival of astrocytes (Hartmann et al., 2013). Furthermore, astrocytic PrP^{C} has been reported to be crucial for neuronal protection from oxidative stress, and hypoxic and ischemic conditions. Firstly, astrocytic PrP^{C} acts as a sensor of oxidative stress followed by scavenging of reactive oxygen species. Secondly, astrocytes release exosomes with elevated PrP^{C} levels under stress conditions that further regulate laminin receptor, apolipoprotein E and the ribosomal proteins S3 and P0, and enhance neuroprotection under hypoxia and ischemia (Bertuchi et al., 2012; Guitart et al., 2016). Interestingly, PrP on astrocytes is also vital for providing metabolic support to neurons under hypoxic conditions (Kleene et al., 2007). Neuronal survival was shown to be associated with glutamate-dependent lactate release by astrocytes, which is regulated by astrocytic PrP (Kleene et al., 2007). Moreover, lactate is essential for neurotrophic support, particularly in metabolically active brain regions where glutamate from excitatory neurons induces lactate release from astrocytes (Pellerin and Magistretti, 1994; Pellerin et al., 2005; Kleene et al., 2007). Cerebrospinal fluid of CJD patients and PrP-knockout mice contained elevated levels of lactate, which might indicate loss-of-function of PrP^{C} in astrocytes, resulting in dysregulation of lactate transport and leading to neuronal damage (Awerbuch et al., 1988; Kleene et al., 2007). Another role assigned to PrP^{C} in astrocytes is the regulation of copper uptake by astrocytes from the extracellular space to provide protection to neurons against copper toxicity (Brown, 2004).

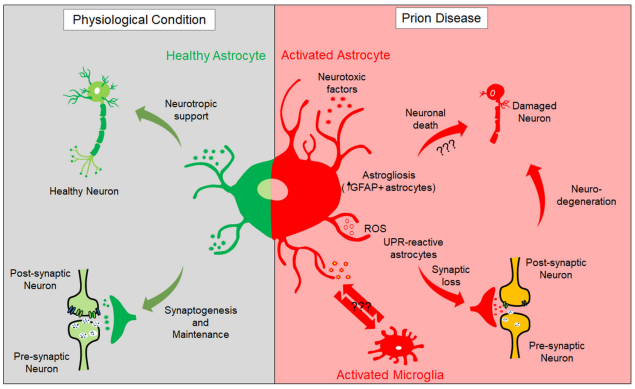

Figure 1 | Schematic presentation of functional role of astrocytes in central nervous system under physiological condition (left) and prion disease condition (right).
Resident astrocytes contribute in synaptogenesis and maintaining neuronal activity by providing neurotrophic support under physiological conditions. During prion disease, astrocytes become activated (a phenomenon called astrogliosis), resulting in their hypertrophy and an increase in glial fibrillary acidic protein. Activation of astrocytes may lead to disruption of their homeostatic role, release of neurotoxic factors and activated unfolded protein response, resulting in synaptic loss and neuronal damage, and consequently neurodegeneration. The exact mechanism of induction of neuronal death by reactive astrocytes during prion infection is not clear. Interaction between reactive astrocytes and activated microglia and subsequent downstream effects of their interaction in prion disease are also a matter of debate. Unveiling the downstream effects and inter-cellular interactions of activated astrocytes during prion disease pathogenesis may help to better understand the mechanism of neurodegeneration. GFAP: Glial fibrillary acidic protein; ROS: reactive oxygen species; UPR: unfolded protein response.

Transformation of astrocytes into their reactive state is an important feature of prion pathogenesis (Carroll et al., 2016). Activated astrocytes exist either as pro-inflammatory or toxic forms, referred as A1 astrocytes, or neuroprotective form, referred as A2 astrocytes (Ferrer, 2017; reviewed by Liddelow and Barres, 2017). A significant increase in the mRNA levels of A2 sub-population-specific markers in thalamus and hippocampus, and A1 sub-population-specific markers in hippocampus and cortex of 22L prion-infected mouse brains (Makarava et al., 2019) indicates a complex and heterogeneous response of reactive astrocytes in different brain regions during prion disease. Given the differential expression of sub-populations of reactive astrocytes in various brain regions, analyzing astrocytes as a single entity may not fully explore the neuroprotective and neurotoxic aspects of reactive astrocytes in prion disease. Studying the roles of specific astrocyte sub-populations and exploring the functional role of PrP^{C} in each of those subpopulations, for example by utilizing single cell RNASeq analysis, might help to identify new therapeutic avenues. Recently, Boisvert and colleagues have shown that astrocytes can lose a homeostatic and neurotransmission regulating profile (Boisvert et al., 2018) and acquire characteristics of neuro-inflammatory reactive A1-astrocytes (Clarke et al., 2018) during aging. These findings can further explain the role of PrP^{C} levels in the transformation from physiological to toxic functions in these astrocytes.

Role of Astrocytes in Prion Propagation

Astrogliosis is one of the hallmarks of prion disease pathology (**Figure 2**) and reactive astrocytes are mostly considered synaptotoxic in prion disease pathogenesis (Kushwaha et al., 2021). PrP^{C} expression confers astrocytes with the ability to successfully propagate prions both *in vivo* and *in vitro* (Raeber et al., 1997; Tahir et al., 2020). Deposition of PrP^{Sc} in astrocytes during prion disease has been widely reported (Diedrich et al., 1991; Ye et al., 1998; Kovács et al., 2005). The accumulation of PrP^{Sc} in the PrP^{C}-expressing astrocytes was sufficient to induce significant neuronal damage to the adjacent neurons devoid of PrP^{C} *in vivo* (Jeffrey et al., 2004). Moreover, PrP-knockout mice supported 263K hamster prion replication after hamster PrP was expressed under GFAP promoter (Raeber et al., 1997). In addition, transgenic mice with astrocyte-restricted hamster PrP expression were susceptible to intraocular prion infection leading to brain neuropathology, although the incubation period was longer in such mice compared to those expressing neuron-restricted PrP (Kercher et al., 2004).

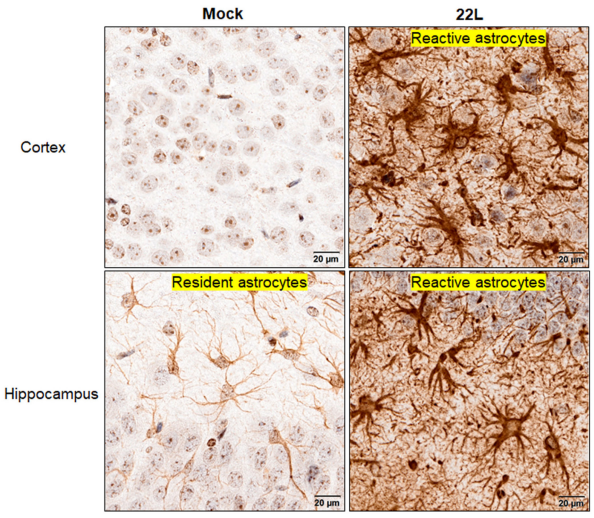

Figure 2 | **Detection of astrogliosis with immunohistochemistry using anti-glial fibrillary acidic protein antibody at terminal stage of prion infection.** Activated astrocytes are visible by their hypertrophy and an increase in the intensity of glial fibrillary acidic protein staining in cortex and hippocampus of a terminally sick mouse infected with 22L prions, compared to an age-matched mock-infected mouse. Resident astrocytes are seen in the hippocampus of the mock-inoculated mouse brain. Scale bars: 20 μm. Unpublished data.

However, later it was found that in these PrP-KO mice expressing PrP^{C} under GFAP promoter, the Cre-lox recombination approach resulted in the ectopic expression of a component of GFAP promoter construct in certain neural stem cells (Zhuo et al., 2001; Casper and McCarthy, 2006), thus raising a question if prion propagation observed in these transgenic mice was really an outcome related to PrP-expressing astrocytes alone. Recently, Lakkaraju and his colleagues used a CAG-CAT expression system to address the issue of ectopic expression of GFAP promoter construct in neurons and to induce astrocyte-specific expression of PrP^{C}. This study also confirmed that astrocytes are indeed able to replicate prions at their own. However, these transgenic mice expressing astrocyte-specific PrP^{C} neither developed prion disease nor showed any pathological features except deposition of PrP^{Sc} in astrocytes (Lakkaraju et al., 2021). On the contrary, depletion of neuronal PrP^{C} in adult prion-infected mice reversed neuronal loss, neuropathology and clinical disease, despite accumulation of PrP^{Sc} in astrocytes, suggesting neuronal PrP^{C} as fundamental for inducing neurotoxicity as well as a non-essential involvement of astrocytes in prion pathogenesis (Mallucci et al., 2003). This study used a conditional knock-out mouse model infected with a single prion strain (Mallucci et al., 2003). Findings of Mallucci et al. (2003) are also validated by Lakkaraju and his colleagues who showed that neuronal PrP^{C} is indispensable for initiating neurodegeneration (Lakkaraju et al., 2021). Here, it is also important to mention the discrepancies in the results from the studies of Raeber et al. (1997) and Mallucci et al. (2003). In comparison to scrapie (RML) infected mouse used by Mallucci et al. (2003), which upon neuronal PrP^{C} depletion remained asymptomatic, mouse expressing hamster PrP^{C} in astrocytes used by Raeber et al. (1997) succumbed to infection with hamster scrapie strain 263K. Interestingly, these two models are very different in regards to their genetic backgrounds, the species of PrP^{C} expressed, the promoters used, as well as the scrapie strains inoculated for infection. Additionally, mouse expressing astrocytic hamster PrP^{C} overexpressed PrP^{C} in astrocytes because of exponential induction of the GFAP promoter during prion infection, whereas scrapie-infected mouse upon neuronal PrP^{C} depletion expressed astrocytic mouse PrP^{C} at endogenous level under its own promoter. Such subtle differences might be responsible for the discrepancy in the outcomes of two above mentioned studies.

Recently, our lab showed differential propagation of various mouse-adapted prion strains in a murine astrocyte cell line, indicating a prion strain-specific role of astrocytes in prion conversion (Tahir et al., 2020). Additionally, *in vivo*, a brain region-specific response of activated astrocytes to PrP^{Sc} accumulation was observed, unlike microglia, following infection with 22L prions. This suggests that the region-specific microenvironment could play a role in differential distribution of astrocyte subtypes and functional states in response to prion infection (Makarava et al., 2019). However, it is unclear whether such heterogeneity of reactive astrocytes in different brain regions might influence prion disease pathogenesis, leading to neuroprotection in some regions while modulating neurotoxicity in others. Furthermore, different prion strains were associated with unique patterns of heterogeneous activated astrocytes within a brain region, indicating a critical role of astrocytes in strain-specific neuropathogenesis (Bradford et al., 2019). Until now, the detection of reactive astrocytes in prion disease is mainly based on morphological changes and glial fibrillary acidic protein (GFAP) upregulation in brain histology (Monzón et al., 2018). Of note, GFAP-knockout mice showed no effect on prion disease pathogenesis (Gomi et al., 1995; Tatzelt et al., 1996). Additionally, astrocytes have also been classified on the basis of their morphology and distribution in the brain: fibrous astrocytes with many glial filaments composed of GFAP and located mainly in white matter; and protoplasmic astrocytes with few glial filaments and located mainly in grey matter (Miller and Raff, 1984). In another study, Caverzasi et al. (2014) reported distinct variations in reactive astrocytes in both white and grey matter of sCJD patients, irrespective of the amount of PrP^{Sc} deposition. The grey matter showed less patchy reactive astrocytes, but mostly co-localizing with PrP^{Sc}, while white matter showed much stronger reactive astrocytes, not localizing with PrP^{Sc} deposits. These results indicate that protoplasmic astrocytes (which are mainly located in grey matter) are not efficiently activated despite the presence of intense PrP^{Sc} deposits in grey matter, whereas fibrous astrocytes (which are mainly in white matter) are capable of becoming reactive astrocytes irrespective of PrP^{Sc} deposits in the white matter (Caverzasi et al., 2014). Altogether, these studies indicate the importance of analyzing the subsets of activated astrocytes in different brain regions, rather than relying on GFAP expression to delineate their functional role in prion pathology.

Activation of Astrocytes during Prion Pathogenesis

An important question is whether astrocyte activation is triggered by accumulation of PrP^{Sc} (in astrocyte specifically or anywhere) in the brain directly or is caused indirectly by neurodegenerative changes in the prion-infected brain, including microglia activation. Answering this question is relevant to understand the role of astrocytes in neuronal degeneration, which could be causal or more of reactive nature (bystander effect). A recent study found that genes associated with microglial proliferation and astrocytosis are upregulated after a substantial quantity of PrP^{Sc} is detectable in the brain, but well before any signs of disease or neuronal damage are evident (Hwang et al., 2009). These findings indicate that brain inflammation might be an early and direct response to PrP^{Sc}. A similar inference was made by neuropathological time-course studies which showed that astrocyte and microglia activation occur many weeks before neuronal loss and coincide with the earliest changes in neuronal morphology in the mouse models of prion disease (Williams et al., 1997a, b; Jeffrey et al., 2000). Clustering analysis of regulatory genes associated with 22L and ME7 prion infection in mice revealed that astrocyte- related functional pathways responded to prion infection prior to microglia, neuron and neurotransmission associated pathways (Makarava et al., 2020). Recently, Guijarro and colleagues showed an increased reactive glial response to administration of an anti-inflammatory drug at the pre-clinical stage of scrapie infected sheep, along with reduced vacuolation and PrP^{Sc} deposition. These data also hint to glial phenotypes as neuroprotective responders at early clinical stages compared to advanced stages of prion infection where more neurotoxic phenotypes prevail (Guijarro et al., 2020a, b). Additionally, astrocyte and microglia-enriched genes from mouse models of BSE and RML infection represented an inflammatory profile comprised of inflammatory cytokines, genes related to phagocytosis and proteolysis (Majer et al., 2019). In addition to activated astrocytes, the activation of microglia is also seen profoundly in prion disease pathology. This could be neuroprotective, however there are reports demonstrating their detrimental effect (Zhu et al., 2016; Muth et al., 2017; Carroll et al., 2018; Krbot et al., 2018). The phagocytic function of microglia cannot clear PrP^{Sc} in late prion disease stages, rather their pro-inflammatory response seems to aid in neuronal damage (Hughes et al., 2010). A link has been reported between activated microglia and reactive astrocytes, where tumor necrosis factor alpha (TNF-α), interleukin 1 alpha (IL-1α) and C1qa cytokines secreted by activated microglia could induce astrocytes into reactive forms with neurotoxic property (A1 phenotype) (Liddelow et al., 2017). Moreover,

microglia-induced A1 astrocytes were found to be neurotoxic in Parkinson's disease mouse models (Yun et al., 2018). Genetic or immunological ablation of these cytokines (IL-1α, TNFα, and C1q) individually significantly promoted neuronal survival by decreasing A1 astrocyte formation (Liddelow et al., 2017). Of note, microglia activation and upregulation of pro-inflammatory cytokines, such as TNF-α, IL-1α and C1qa, has been reported in mouse models of prion disease and in human CJD patients (Hwang et al., 2009; reviewed by Aguzzi and Zhu, 2017; Carroll et al., 2018). On the contrary, increased expression of A1 and A2 reactive astrocyte related genes in RML infected mice in the absence of microglia has been recently reported (Carroll et al., 2020). Similarly, microglia induced pro-inflammatory cytokines (IL-1β and TNFα) were also shown to induce astrocytic YKL-40, a neuroprotective protein (Llorens et al., 2017). Furthermore, the phenotype of A1 reactive astrocytes is also stratified by the type of PrP^{Sc} present during prion infection (Ugalde et al., 2020). Overall, these data highlight the existence of multiple subtypes of reactive astrocytes in the context of prion disease.

Recently, complement 3-positive (C3+) reactive A1 astrocytes were described in prion-infected mice and in sCJD-affected human brain samples (Hartmann et al., 2019). However, triple knockout mice, devoid of microglia-induced TNF-α, IL-1α and C1qa cytokines, upon inoculation with scrapie (RML) prions unexpectedly showed a marked acceleration of prion disease despite the reduction of C3+ reactive astrocytes (Hartmann et al., 2019). Interestingly, distinct astrocyte subpopulation, termed as C3+ PrP^{Sc}-reactive astrocytes, were found activated in the thalamus region of these mice following prion infection, which had activation profile different from that of A1 astrocytes. This finding highlights a complex interaction between activated microglia and reactive astrocytes in prion disease, and the effects of activated microglia on neuroprotection or neurodegeneration in the context of astrocyte reactivity are not yet fully understood. Therefore, more work is necessary to understand how prion infection leads astrocytes towards reactive forms, either neurotoxic or neuroprotective, and what role microglia plays in this context. This is in line with the reports that chemokines released by reactive astrocytes during prion infection are involved in the recruitment of microglia to the site of infection (Marella and Chabry, 2004), and that activated microglia is neuroprotective to some extent (Zhu et al., 2016). Moreover, astrocyte activation as measured by differential expression of the cell adhesion molecule CD44 is affected by prion strain and host *PRNP* genotype in prion infection, suggesting a role of reactive astrocytes in prion strain-specific pathology (Bradford et al., 2019). Indeed, CD44 was shown to be highly expressed by A1 neurotoxic astrocytes (Bradford et al., 2019). Further investigation of the astrocyte-microglia homeostasis and the crosstalk between astrocytes, microglia and neurons in the context of prion strains is important to understand the strain-specific prion disease pathophysiology and neurodegeneration.

Role of Astrocytes in Neurodegenerative Cascades during Prion Pathogenesis

The cellular signaling in astrocytes has been linked to neurodegeneration in prion disease. Recently, the involvement of prion infection-mediated unfolded protein response in astrocytes in a non-cell-autonomous manner was suggested in neurodegeneration (Smith et al., 2020). Phosphorylated protein kinase R-like endoplasmic reticulum (ER) kinase (p-PERK) signaling led to the formation of reactive astrocytes with an altered secretome, resulting in neuronal damage in *in vitro* and *in vivo* models of prion infection (Smith et al., 2020). Interestingly, blocking the p-PERK pathway genetically in astrocytes alone reduced neuropathology and extended survival in prion-infected mice (Smith et al., 2020). Of note, prion infection can result in cellular ER stress that supports prion conversion and, *vice versa*, unfolded protein response helps cells to cope with ER stress (Torres et al., 2010; Nunziante et al., 2011; Thapa et al., 2018). However, excessive unfolded protein response can be detrimental and facilitate neurodegeneration (Moreno et al., 2012). Moreover, elevated expression of aquaporin 1 and 4 that are involved in maintaining cerebrospinal fluid production and contact with blood vessels was described in astrocytes in brains of patients with sCJD, Gerstmann-Sträussler-Scheinker syndrome and fatal familial insomnia (Iwasaki et al., 2007; Sadashima et al., 2020). This indicates a role of astrocytes in maintaining cellular homeostatic processes in the brain during prion pathogenesis. Of note, aquaporins are water channel proteins responsible for water homeostasis, cell migration and cell adhesion in the CNS (Moftakhar et al., 2010). In addition, reactive astrocytes specifically activate distinct set of cytokines, which are capable of inducing inflammatory responses in the CNS. Tribouillard-Tanvier and colleagues showed that primary cultures of astrocytes and microglia exposed to 22L prions activate astrocyte-specific CCL2, CCL3, CCL5, CXCL1, G-CSF, IL-1β, IL-6, IL-12p70, and IL-13 cytokines (Tribouillard-Tanvier et al., 2009), indicating a crucial role of astrocytes in triggering neuro-inflammatory processes in the CNS during prion pathogenesis.

Cellular Models of Astrocytes for Prion Propagation

Cell culture models are used to study the molecular and cellular mechanisms of prion infection and pathogenesis as well as for screening compounds with therapeutic potential. Experiments using animal models of prion infection on the other side are time-consuming and hardly can be used for screening approaches. Cell culture models are also ideal for studying the mechanisms of prion replication and cellular pathways involved, but very few cell lines recapitulate prion neuropathology, in contrast to primary cells (Schätzl et al., 1997; Hannaoui et al., 2014). There are several neuronal and non-neuronal cell lines that support stable replication of rodent-adapted scrapie (strains RML, 22L, 139A and Me7) and human (e.g., Fukuoka-1) prions, however, single cell-cloning is often required to obtain highly susceptible and persistently infected cell clones from heterogeneous cell populations (Schätzl et al., 1997; Mahal et al., 2007; Tahir et al., 2020). Moreover, genetically engineered neuronal and non-neuronal cell lines were developed to study a variety of prions (Bourkas et al., 2019; Walia et al., 2019), as prion transmission depends on the homology in PrP primary structure between host and incoming prion strain (Dickinson and Meikle, 1971; Wadsworth et al., 2004). Importantly, not all cells that express PrP^{C} are permissive to prion replication, and not all naturally occurring prion strains can be propagated in cellular models (Mahal et al., 2007; Marbiah et al., 2014).

Although both neuronal loss and astrogliosis are observed in prion pathology, the role of glia in prion disease has not been fully investigated. Of note, astrogliosis occurs in early stages of prion pathogenesis, long before neuronal loss can be observed (Sandberg et al., 2014). Studying the involvement of glial cells has now gained momentum in prion research, given their inter-cellular communication and brain region-specific impact on neurodegeneration (Marella and Chabry, 2004; Monzón et al., 2018; Makarava et al., 2019; Smith et al., 2020). The few cell culture models of astrocytes, mainly primary cell cultures, shown to be susceptible to prion uptake and/or replication are listed in **Table 1**. Primary astrocyte cultures from cerebellum extracted from transgenic Tg338 mice overexpressing ovine PrP^{C} were found to propagate 127S scrapie prions *in vitro* (Cronier et al., 2004). The human astrocyte cell line SVG was shown to uptake and degrade human sCJD prions, comprising both PK-sensitive and PK-resistant PrP^{Sc} forms. In spite of their biochemical differences, both forms were taken up and degraded in a similar fashion by these cells. However, prion infection in these cells was only followed for 48 hours and persistent infection is unlikely (Choi et al., 2014). Primary astrocytes were reported to internalize 263K hamster prions more efficiently than neurons and astrocytic processes were found in contact with neurons, which could help the spread of PrP^{Sc} from astrocytes to neurons (Hollister et al., 2015). Another study suggested the permissiveness of cerebellar astrocytes to propagate 22L prions and their role in spreading PrP^{Sc} to neurons via direct cell-to-cell contact and tunneling nanotubes (Victoria et al., 2016). Recently, our lab described an immortalized murine astrocyte cell line, C8D1A, which replicates different mouse-adapted scrapie strains. Interestingly, these cells propagate prion strains 22L and RML; thought to be similar, very differently with stable propagation maintained by only 22L prions when using immunoblotting for proteinase K resistant PrP^{Sc} as read-out. Although negative in immunoblot, RML prions were detected in these cells in large amounts by the prion conversion assay RT-QuIC and also by bioassay in mice, suggesting effective propagation of PK-sensitive RML prions by C8D1A cells (Tahir et al., 2020, and unpublished data). This study indicates that astrocytes can differentially process prion strains and thus could act as a tool for prion strain analysis. This aligns with aforementioned data that prion strains lead to different patterns of astrocyte activation in the brain (Bradford et al., 2019). An astrocyte cell model derived from induced pluripotent human stem cells has been reported to replicate human sCJD and vCJD prions (Krejciova et al., 2017), which also confirmed the association of the

Table 1 | **Existing models of astrocytes used to propagate prions *in vitro* and *in vivo***

Models	Host	Prion strain	Propagation/ Uptake	References
***In vitro* models**				
Astrocyte cell type				
Cerebellar astrocytes (primary culture)	tg338 mice overexpressing ovine PrPC	127S	Propagation	Cronier et al., 2004
Cerebellar astrocytes	Mouse	22L	Propagation	Victoria et al., 2016
Astrocyte cell-line, SVG p12 from ATCC	Human	sCJD	Prion uptake without propagation	Choi et al., 2014
Astrocyte cell-line, C8D1A	Mouse	22L	Propagation	Tahir et al., 2020
Astrocyte cell-line, C8D1A	Mouse	Me7	Prion uptake without propagation	Tahir et al., 2020
Astrocyte cell-line, C8D1A	Mouse	RML	Propagation	Tahir et al., 2020
Astrocytes derived from induced pluripotent stem cells (iPSCs)	Human	vCJD	Propagation	Krejciova et al., 2017
Astrocytes derived from iPSCs	Human	sCJD	Propagation	Krejciova et al., 2017
***In vivo* models**				
Animal type				
PrP KO mice expressing Hampster PrP transgene under GFAP promoter	C57BL/10	263K	Propagation	Raeber et al., 1997
GFAP KO	C57BL	RML	Propagation	Tatzelt et al., 1996
GFAP KO	C57BL/6	Obihiro scrapie strain	Propagation	Gomi et al., 1995
Triple-KO-mice (knockout of TNF-α, IL-1α and C1qa)	C57BL/6	RML	Propagation	Hartmann et al., 2019

GFAP: Glial fibrillary acidic protein; IL-1α: interleukin 1 alpha; iPSCs: induced pluripotent stem cells; KO: knock out; TNF-α: tumor necrosis factor alpha.

codon 129 polymorphism (Methionine or Valine) in the human *PRNP* gene with the susceptibility to vCJD (Asante et al., 2002; Wadsworth et al., 2004). vCJD resulted from the interspecies transmission of BSE to humans with the majority of patients expressing methionine at codon 129 at both alleles, except for two heterozygote cases (Bruce et al., 1997; Mok et al., 2017). Taken together, astrocyte-based cell models can be used to study cellular mechanisms of prion propagation and determinants of prion strain characteristics, and might open new avenues for delineating therapeutic targets.

Animal Models to Explore the Role of Astrocytes in Prion Propagation and Pathogenesis

Mouse models with ablated functions of complete astrocytes or sub-populations thereof were used to address the role of activated astrocytes in prion pathogenesis (**Table 1**). PrP-knockout mice reconstituted with a hamster PrP transgene under a GFAP promoter successfully propagated 263K hamster prions (Raeber et al., 1997), providing convincing experimental evidence that astrocytes are capable of replicating prions. As GFAP expression, widely used as a marker for activated astrocytes, is up-regulated in all prion diseases, it was obvious to knock-out GFAP in mice and then to infect them with prions. Surprisingly, GFAP-knockout mice showed no effect on prion disease pathogenesis indicating that manipulating only GFAP is not enough to address the entire spectrum of roles played by astrocyte subsets (Gomi et al., 1995; Tatzelt et al., 1996). Recently, Hartmann et al. (2019) tested the role of $C3^+$ neurotoxic astrocyte subset in prion disease by infecting A1 astrocyte-negative triple knock-out (TNF-α, IL-1α, and C1qa) mice with RML prions and observed the acceleration of disease in such mice, with unchanged levels of PrP^{Sc} deposition and neuropathological changes. These studies suggest a complex role of astrocytes in prion pathogenesis, and leave room for speculating that subsets of reactive astrocytes might be triggered in prion infection having various beneficial or detrimental effects.

Future Perspectives

Reactive astrocytes are consistently found in prion-infected animals and humans, and astrocytosis is considered a hallmark of prion disease. However, whether astrocytosis is a reactive event in prion pathogenesis or is causally involved in it is still a matter of debate. Similarly, the molecular mechanisms underlying the postulated roles of reactive astrocytes in prion disease are still elusive. One possible explanation is a lack of appropriate *in vitro* and *in vivo* models to study the role of reactive astrocytes in prion disease, alone or in concert with neurons and microglia. Since prion strains are associated with differences in prion pathology and reactive astrocyte profiles in the brain, versatile *in vitro* models of astrocytes that propagate different animal and human prion strains will help to better understand which roles astrocytes have in prion pathogenesis and strain characteristics. Gene editing of existing astrocytic cell models (Krejciova et al., 2017; Tahir et al., 2020) using CRISPR/Cas9 technology can also be used to expand the repertoire of astrocytic cell models by expressing prion proteins of other species and making them permissive to prion infection from these species (Bourkas et al., 2019; Walia et al., 2019).

Given the unclear role of astrocytes in prion pathogenesis, whether it is protective or deleterious, it is currently difficult to outline therapeutic strategies targeting astrocytes. Fine characterization of the potential roles of the various sub-populations of reactive astrocytes is another vital requirement before designing therapeutic strategies involving astrocytes. Approaches to identify and analyze sub-population specific reactive astrocyte responses are needed, making use of their specific markers. Establishing sub-population specific cellular models of reactive astrocytes could help to differentiate and understand neuroprotective versus neurotoxic signaling pathways in these sub-populations. Single cell-based transcriptomics and proteomics studies in these models when infected with various prion strains could generate new important insights and open the door for new therapeutic strategies. Modulation of neurotoxic functions or augmentation of neuroprotective functions of reactive astrocytes could be achieved by application of small molecules or genetic manipulations. Importantly, reactive astrocytes and astrocytosis is a common characteristic of many neurodegenerative disorders, not only prion diseases. It is therefore likely that studying the role of astrocytes in prion diseases will also unveil important insights into the role of astrocytes and astrocytosis in other neurodegenerative disorders.

Conclusion

The molecular mechanisms underlying prion pathogenesis are not fully known and the complex interplay of neuronal and non-neuronal cells in the CNS and the differential responses of these cell populations to various prion strains further complicate the situation. The potential role of reactive astrocytes in prion disease is not well characterized, also because of lack of appropriate *in vivo* and *in vitro* models. The existence of neuroprotective and neurotoxic sub-populations of reactive astrocytes requires a detailed analysis of their differential impact on prion pathogenesis and prion strain manifestation. Development of prion infection models that well recapitulate sub-populations of reactive astrocytes and their modulation using pharmacologic or genetic tools will be important to understand the role of astrocytes in prion pathogenesis. Ultimately, characterization of neurotoxic and neuroprotective signaling cascades in these astrocytes will provide new therapeutic strategies for prion diseases.

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