Unravelling the glial response in the pathogenesis of Alzheimer's Disease

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ABSTRACT: Alzheimer's disease is a progressive, incurable neurodegenerative disease targeting specific neuronal populations within the brain while neighboring neurons appear unaffected. The focus for defining mechanisms has therefore been on the pathogenesis in affected neuronal populations and developing intervention strategies to prevent their cell death. However, there is growing recognition of the importance of glial cells in the development of pathology. Determining exactly how glial cells are involved in the disease process and the susceptibility of the aging brain provides unprecedented challenges. The present review examines recent studies attempting to unravel the glial response during the course of disease and how this action may dictate the outcome of neurodegeneration. The importance of regional heterogeneity of glial cells within the CNS during healthy aging and disease is examined to understand how the glial cells may contribute to neuronal susceptibility or resilience during the neurodegenerative process.—Alibhai, J. D., Diack, A. B., Manson, J. C. Unravelling the glial response in the pathogenesis of Alzheimer's disease. FASEB J. 32, 5766–5777 (2018). www.fasebj.org

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In 2017, Alzheimer's disease (AD) was estimated to have affected 50 million people worldwide, with the number likely to double every 20 yr (1). The pathology of AD is characterized by the accumulation of aggregates of 2 misfolded proteins, amyloid-β (Aβ) and tau, in the brain; this accumulation occurs over many decades, well before clinical signs of disease become apparent (2, 3). The mechanisms leading to the cerebral deposits and the relationship between the misfolded proteins and clinical disease have been the focus of decades of scientific investigation. These studies have provided potential targets for therapeutic intervention, almost all of which have been failures (4). Thus, we still lack sufficient knowledge of the process of neurodegeneration and its association with the misfolded proteins to successfully interrupt or significantly attenuate its course.

Over the last decade, some insights into the understanding of AD have come from studies of the prion diseases in which cell-to-cell transmission is achieved by templated misfolding of the normal protein substrate by the conformationally variant prion. AD is believed to exist in a number of subtypes, with experimental data indicating that different conformations of Aβ and tau misfolded protein might underlie distinct pathologic phenotypes, a phenomenon possibly analogous to the multiple strains of the prion diseases (5–8). In addition, the misfolded proteins in AD act as seeds for "prion-like" conversion of normally folded protein to abnormal conformations (9, 10). Similar to prions, the misfolded proteins Aβ and tau are able to induce the pathologic conformational changes in their respective naive proteins and through this mechanism spread within the CNS and occasionally from the periphery to the brain (11). This effect was first noted in the brains of patients with Parkinson's disease (PD) who received implants of embryonic neurons that subsequently displayed the characteristic Lewy body inclusions seen in PD dopaminergic neurons (12); this effect has also been shown in animal models of AD, PD, and amyotrophic lateral sclerosis (ALS) (13). This finding has also been reported in subjects who underwent dura mater grafts or treatment with human growth hormone extracted from cadaveric human pituitaries in which foci of misfolded tau and Aβ proteins were found (in addition to the prion protein) in their brains (14–19). However, these subjects did not develop any clinical symptoms of AD, suggesting that

ABBREVIATIONS: Aβ, amyloid-β; AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; GFAP, glial fibrillary acidic protein; GLT-1, glutamate transporter 1; PD, Parkinson's disease; PRR, pattern recognition receptor

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the misfolded tau and Aβ proteins were not sufficient for the development of clinical disease.

In common with prion diseases, misfolded protein accumulation, spread, and distribution in AD has been believed to predict the regions of neurodegeneration. Thus, the misfolded proteins, their predisposition to form amyloid (20), and their relationship to neurodegeneration have been the major focus for defining AD and prion disease mechanisms. Studies of the prion diseases have also generated new tools and robust animal models for studying misfolded proteins. The *in vitro* conversion assays, in particular the protein misfolding cyclic amplification assay first developed to study prion diseases, have now been adapted for the study of other protein misfolding diseases, including AD, and have greatly enhanced our ability to study the misfolded protein in the brain (21, 22). Unlike AD models, the prion models show all the elements of an infectious chronic neurodegenerative process, from the long preclinical phase through to clinical disease, neurodegeneration, and death. Although we recognize that there are many differences between AD and prion diseases, there are also striking similarities. The accumulation and clearance of the misfolded protein, glial responses, and the patterns of neurodegeneration strongly imply that common mechanisms may operate (13, 23, 24). Thus, the study of these diseases in parallel has the potential to more rapidly advance knowledge.

Studies in recent years have dramatically altered our perception of the neurodegenerative process by examining the brain not as a single entity but in a regional and temporal manner. Specific cell types have been examined as well as, importantly, interactions between different cell types. Neuronal cells and glial cell interactions within the CNS seem to be important contributors to the process of neurodegeneration but differ between brain regions and vary with age (25). Understanding how these interactions between and among different cell types in the complex environment of the aging brain contribute to neurodegeneration is key to developing effective intervention strategies for these diseases.

The common mechanisms in protein misfolding diseases offer a wealth of human and animal data that provide insights into the process of neurodegeneration; thus, intervention strategies developed for any one of these disorders may prove to be effective across a range of neurodegenerative conditions. Determining exactly how the different cells of the brain orchestrate the neurodegenerative process in the complex arena of the aging brain provides interesting challenges. The present article discusses the role of glial cells during aging and disease and the importance of understanding regional and cell-specific changes.

THE COMPLEXITY OF THE BRAIN

Aging is the major risk factor for many neurodegenerative diseases. Although the brain is one of the organs most resilient to aging, recent studies have highlighted numerous aging-associated changes in all the cells of the brain. Understanding the interactions among the different cell types during aging and in response to disease is key to defining the effects of how and why aging is the principal risk factor in many chronic neurodegenerative diseases such as AD. Recent studies critical to this understanding have examined the brain not as a single compartment at one time point but in a region-, time-, and cell-specific manner (**Fig. 1**).

For several decades, the neuron has been subject of the majority of research into protein misfolding diseases. It is now apparent that glial cells are also important players in the neurodegenerative process. Many of the protein misfolding diseases (including AD, PD, ALS, and prion diseases) demonstrate activation of glial cells concomitant with the accumulation of the misfolded protein, but their precise role in the neurodegenerative disease process is unknown. Much evidence now exists to suggest that misfolded protein alone may not be sufficient to induce neurodegeneration. We have shown that misfolded proteins accumulate in both degenerating and non-degenerating regions of the brain (26). Thus, one important question is, "what leads to resilience and susceptibility in different brain regions?"

The involvement of glial cells in the process of neurodegeneration was noted as early as 1910 (27). However, it is only relatively recently that a major investigative focus has returned toward understanding the role of the glial cells. Although many of the early studies concentrated on the clinical stages of disease, current efforts to understand the events leading to neurodegeneration have revealed the prominence of glial cells as an early feature of the process. The present article focuses on 2 glial cell types, microglia and astrocytes, because these have been the subject of a number of recent region-specific studies. Although the disruption of oligodendrocyte and NG2 glia function has been implicated in AD, considerably more research is required to clarify the roles of these cells in a regional and temporal manner.

Microglia heterogeneity and AD

Microglia are the resident macrophage cells in the CNS, representing 10% of the cells in the healthy brain. They form a 3-dimensional lattice in which each cell inhabits a unique nonoverlapping domain. In the normal state, they are considered a heterogeneous population with density differences across brain regions, with the hippocampus, basal ganglia, substantia nigra, and olfactory telencephalon more densely populated than the cerebellum and brain stem (28). They display region-dependent functional signatures, which are enhanced further by age (29). In the normal or resting state, they are highly arborized cells with multiple fine processes that are constantly in motion surveying the parenchymal environment (30, 31) and providing trophic support to neurons (32). The processes make contact with neurons, astrocytes, and blood vessels, and variation in morphology exists depending on location. Although the importance of variability in morphology and density between different brain regions remains unclear, a number of distinct microglial subsets have been identified. For example, distinct subsets of microglia in specific brain regions in murine and human tissues express differing levels of inflammatory cytokines (33). Furthermore, microglial subpopulations have been shown to be

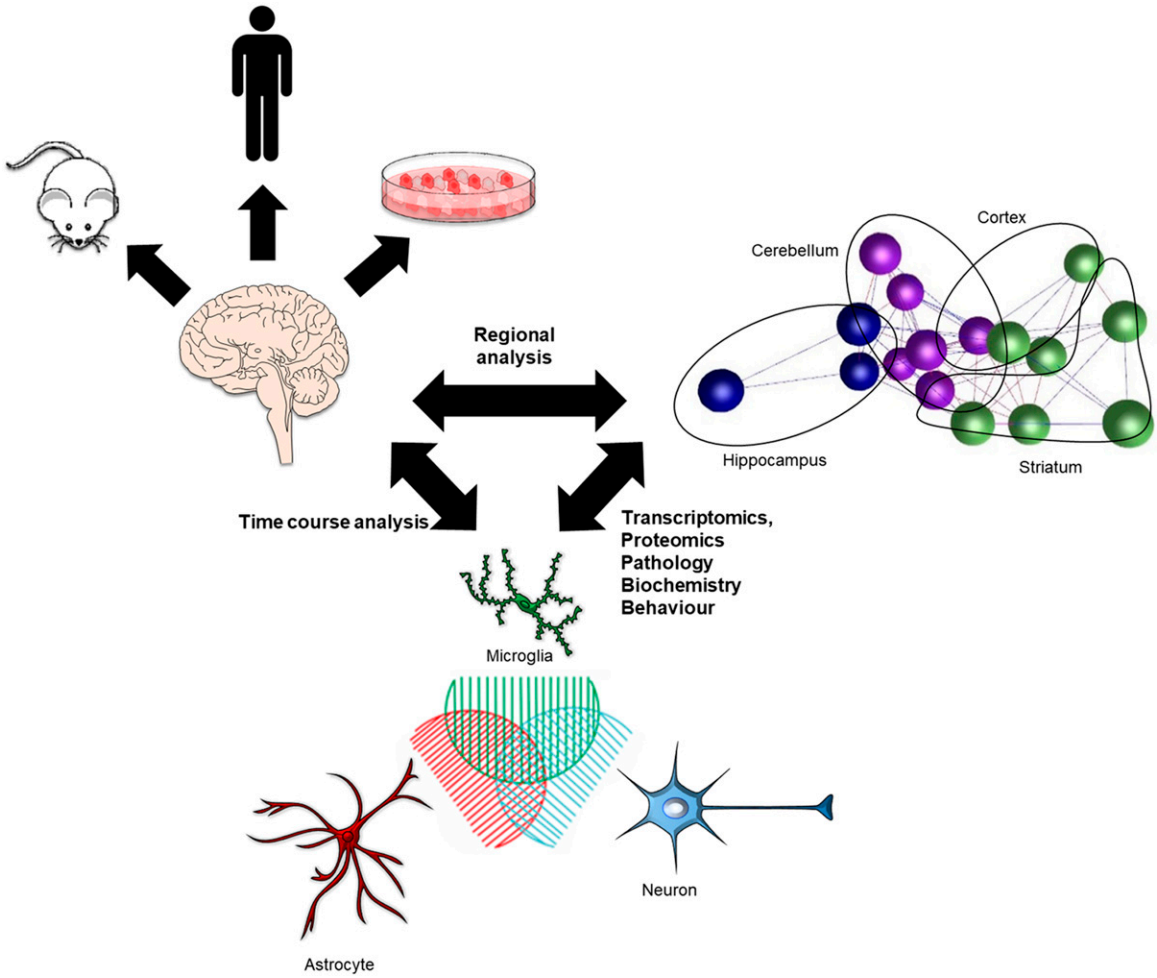

Figure 1. Unraveling disease mechanisms. To fully understand the mechanisms of disease, a combination of approaches must be used. The most powerful approach is to utilize the different models available to us and employ a range of techniques in time-course, regional, and cell-specific manners. Only in this way will we be able to decipher the processes of disease and identify therapeutic targets.

differentially responsive to neurotransmitters (34), indicating that microglial subtypes coordinate their functions with particular neuronal populations. A more comprehensive characterization of microglial subtypes was performed on hippocampal slices, suggesting that microglial diversity occurs in response to the differing neuronal-architecture changes throughout the hippocampus (35). The heterogeneity observed in distribution, morphology, and function has been suggested to be determined by environmental cues in the brain (28). A recent study in which microglia were depleted either genetically or chemically and allowed to repopulate has provided compelling evidence that local cues are responsible for the heterogeneity (36).

Microglial cells can be easily distinguished from other cells in the CNS by using markers such as Iba1, a protein associated with calcium homeostasis, and CD11b, a complement receptor. The expression of these markers increases with microglial activation, at which point major histocompatibility complex class II structures can also be used as markers (31).

Microgliosis can be triggered by a pathologic insult or a disturbance to homeostasis. The microglia adapt their phenotype from "resting" to "activated" by modifying both their morphology and biologic function (30, 37, 38). Their morphology changes from that with a small cell body and long fine processes to an amoeboid shape in which the cell bodies enlarge and processes shorten and cover a more limited area. Microglia activation is often defined as M1, a proinflammatory and neurotoxic state, or M2, an anti-inflammatory and healing state. However, it is now recognized that they are much more diverse than these previously defined M1 and M2 phenotypes (39–42).

Microglia have been shown to express pattern recognition receptors (PRRs) that respond to pathogen- or danger-associated molecular patterns, termed PAMP or DAMP, respectively [reviewed elsewhere (43, 44)]. With the binding of these patterns to PRRs, an enhanced proinflammatory response is activated, which in AD experimental models has been shown to compromise microglial phagocytosis (45). It has also been shown that Aβ species can activate microglia *via* PRRs, such as TLRs (46) or receptors for advanced glycation end-products (47). Aβ derived from human AD cortex has the potential to activate microglia when injected into mice intracerebrally (48). It has also been proposed that microglia can switch from one phenotype to another (49–51) and are sensitive to signals from the peripheral immune system (52–54).

Microglia in different brain regions have been shown to respond variably to a peripheral inflammatory stimulus (55), raising the issue of whether viral or bacterial infections that occur during the progression of AD could lead to region-specific activity and functional changes in microglia. In addition, microglia have recently been shown to become epigenetically modified in response to peripheral inflammatory stimuli, thus affecting their reaction to additional insults for up to 6 mo (56). It is also clear that a complicated interconnected network of CNS cells contributes to the activated profile adopted by microglia, with signaling from both astrocytes and neurons having significant impact (57–60). It is possible that neurodegeneration occurring in different brain regions can result in variable microglial activation states.

Reactive gliosis is a hallmark of AD, with numbers of microglia reported to increase and correlate with disease severity in both human AD brains and mouse models of disease (61, 62). Microglia can be observed surrounding amyloid plaques. Their regional distribution follows that of the pathologic changes in AD. Recent studies indicate that the age of the individual also plays a role in microglial pathogenesis (63). The presence of microglia can be both detrimental and beneficial to AD pathogenesis. It has been suggested that during the early phases of experimental animal models of AD, microglial activation is potentially beneficial to the modulation of plaque composition and the reduction in Aβ42 affinity; however, the beneficial effect is lost with aging (64). In human studies, microglial activation is also observed at the earliest stages of clinical disease and is associated with slower progression of clinical symptoms (65). In addition, a recent study has shown evidence for a novel disease-specific microglial subtype in AD, which expresses a number of genes that have been associated with genetic forms of AD, perhaps hinting at a protective role of this subset of microglia (66).

As the disease progresses, chronic activation results in substantial microglial proliferation, which can occur to a differing extent in specific brain regions (67). There is also an increase in the number and degree of expression of inflammatory genes (68). However, a potential contribution from infiltrating peripheral cells cannot be excluded because the blood–brain barrier is known to be compromised during disease (69–72). The progressive change in microglial phenotype is believed to have detrimental effects, such as synapse loss (73, 74), a potential role in the spread of tau pathology (75), and damage to neurons through release of proinflammatory factors (76, 77). A distinct microglial subtype, termed "dark microglia," has also been characterized in a murine model of AD. These cells closely surround synaptic clefts in amyloid plaques (78) and have been proposed to play a role in the pathologic synaptic stripping in AD (78, 79).

Microglia pathways have been implicated in a number of studies relating to both AD pathogenesis and risk. A significant proportion of genes identified in genome-wide association studies as being associated with risk for sporadic AD are involved in microglial functions [reviewed elsewhere (79)]. A rare variant of a microglial-expressed gene, TREM2, is associated with a significantly increased risk for developing AD (80, 81). Studies have shown that the ablation of TREM2 from microglia impairs their abilities to phagocytose and increases proinflammatory cytokine production. It has been hypothesized that TREM2 mutations contribute to AD pathogenesis and thus could be a therapeutic target. However, results of studies in various mouse models and at different stages of disease have been inconsistent, with some suggesting a beneficial role for TREM2 (82, 83) and others reporting a negative effect (84). Other genes expressed by microglia that are associated with AD susceptibility include CD33 (85, 86), clusterin, and complement receptor 1 (86).

With the increasing identification of multiple disease-specific subtypes of microglia, understanding how different microglial subtypes function and affect disease progression will prove critically important.

Astrocyte heterogeneity and AD

Astrocytes are the most abundant glial cell in the CNS and are distributed in a highly organized fashion with no overlap apart from the distal tips. Astrocytes exist in great heterogeneity, with at least 11 different astrocyte classes having been defined to date (87, 88). Their cellular density and morphology are believed to play a role in their contribution to local functional and metabolic demands as well as region-specific cyto-architecture. A number of studies have examined the structure and morphology of astrocytes at differing levels of resolution. Astrocytic processes penetrate deep into neuropil, forming tripartite synapses, interacting with neuronal and neighboring glial cells, and encompassing microvessels.

Because of their diversity, astrocytes are difficult to study. For instance, there are currently no defined molecular or functional features that distinguish them. They are most commonly defined by the expression of the molecular marker, glial fibrillary acidic protein (GFAP). Healthy mature astrocytes express GFAP but generally at levels below the limit of detection of immunohistochemistry, with some exceptions (*e.g.*, those compartmentalized to the hippocampus). During neuronal injury, levels of GFAP rise exponentially at the site of injury, although GFAP is only expressed in a fraction of the total cell mass (89). It is not expressed by all astrocyte populations nor is it exclusively expressed by astrocytes within the CNS. Recent studies have begun to more precisely characterize the variability in GFAP expression patterns among astrocyte populations during AD. For instance, a frame-shift variant of GFAP has been shown to be generally increased in AD, whereas in the hippocampus, a different GFAP isoform was shown to be expressed exclusively in the dentate gyrus and CA4 subregions, with the only exceptions being in astrocytes surrounding plaques (90). In total, 8 different isoforms of GFAP are expressed in AD (91).

In the normal state, astrocytes exhibit a steady low resting potential due to their high density of voltage-gated potassium channels (92); however, they are not electrically excitable (93). More recent studies have shown differences in the types and expression levels of potassium channels that could account for the differences in physiologic function between various brain regions (94–96). The role of

such ion channel diversity within astrocyte populations in neurodegeneration is as yet unclear, as are the functional consequences of astrogliosis on the distribution and proportions of ion channels. Previous studies have shown that the levels of astrocytic potassium channels are decreased during AD progression, indicating a gross loss of neuromodulation of astrocytes (97). Astrocytes form a lattice of gap junctions and communicate by using a chemically excitable system. For instance, astrocytes can take up glutamate and propagate a calcium wave to neighboring astrocytes (98). Glutamate uptake by astrocytes is known to prevent excitotoxicity and support excitatory neurotransmission of neurons; however, other studies have shown that inflammation can exacerbate excitotoxicity (99). Astrocytes achieve glutamate uptake primarily through expression of glutamate-aspartate transporter and glutamate transporter 1 (GLT-1). Previous studies have shown that glutamate-aspartate transporter is primarily expressed in immature and developing astrocytes but that GLT-1 exhibits regional expression differences in the adult CNS. For instance, GLT-1 is expressed at significantly higher levels in the brain compared with the spinal cord (100), and differences in GLT-1 transcripts exist among astrocyte populations within the adult brain (101), which may lead to variation in glutamate uptake levels in different brain regions.

During AD progression, levels of GLT-1 are significantly reduced in the hippocampus and cerebral cortex (102, 103) but do not change in the prefrontal cortex (104). Although GLT-1 expression is largely decreased during AD, a series of alternate splice variants of GLT-1 mRNA have been identified as being up-regulated in temporal, frontal, and parietal cortices in human AD tissue, which were functionally compromised for glutamate transport (105). The significance of this observation is unclear.

The combination of reduced or compromised astrocyte ion physiology and glutamate uptake in AD indicates a loss of neuromodulation of astrocytes. Incidentally, astrocytes have been shown to respond differently to various combinations of neurotransmitters in the healthy CNS; for example, in the hippocampus, astrocytes respond to glutamate, ATP, GABA, acetylcholine, endocannabinoids, and prostaglandins, whereas in the cerebral cortex, astrocytes respond only to glutamate and norepinephrine (106–112). These findings suggest functional differences in the interaction between astrocytes and neurons in various brain regions, which may be differentially disrupted based on the functional deficits occurring during AD. One particular example would be the loss of norepinephrine as a result of the degeneration of locus coeruleus neurons [reviewed elsewhere (113)], which are the major producers of norepinephrine for the entire CNS. This action which would affect neuron–astrocyte communication in the cerebral cortex but not in the hippocampus.

Astrocytes are also involved in the development and support of synapses and synaptic function. Astrocytes come into direct contact with synapses and neuronal dendrites and can influence synapse strength and dendrite maturation (114, 115). As a result, astrocytes are understood to be vital for modulating neuronal activity. It is well established that synaptic degeneration and loss is an early pathology and a functional correlate of cognitive decline (116, 117). More recent evidence has shown astrocyte atrophy at early stages of AD progression leading to disruption of synapse connectivity, synaptic dysfunction, and eventual loss (118–120).

In addition, astrocytes provide trophic support to neurons. In particular, astrocytes have been shown to secrete lactate in response to glutamate release by neurons (121). Although the prominence of lactate as a main or secondary source of energy for neurons is under intense debate, it is widely acknowledged that lactate plays an important role in neuronal trophic support, at least in brain regions of high metabolic activity. Furthermore, glucose, lipids, and other important neuronal metabolites are transferred from the periphery to the CNS through the blood–brain barrier. The role of the astrocyte in the regulation and maintenance of the blood–brain barrier has been shown to be compromised during early stages of AD pathology (70–72, 122).

Our current understanding of astrocytes as neuroprotective, neuromodulatory, and neurodevelopmentally important suggests that loss of function of these cells could play a critical role in neurodegeneration. The majority of literature examining the astrocyte in animal models of disease and in human tissue has reported compromised astrocytic function, which is likely to have a role in defining the severity of neurodegeneration in the compromised areas. However, as discussed, astrocytes are a heterogeneous population of cells and thus will almost certainly exhibit differences in the type and intensity of the disease-associated functional changes between brain regions. To date, most studies examining astrocyte function have failed to take into account the heterogeneity of astrocyte populations. It is certainly possible, therefore, that astrocytes in some brain regions may act protectively, whereas in other areas, astrocytes become functionally compromised and contribute to neurodegeneration. As such, our understanding of the exact role of the astrocyte in AD and how this class of cells could be targeted for therapeutic intervention seems a long way off.

Oligodendrocytes

Oligodendrocytes are the myelinating cells of the CNS and are the end-product of a cell lineage that includes NG2-glia. The function of the mature oligodendrocytes is to produce myelin, which insulates the axons (123). The myelin sheath maintains the action potentials and ion currents in the axons, thus reducing energy consumption and increasing conduction velocity, whereby a myelinated axon is more efficient at transducing a signal than an unmyelinated one (124). The disruption of oligodendrocyte function has been implicated in a number of disorders, including AD, PD, multiple sclerosis, and cerebral palsy (reviewed elsewhere (125–128)]. Any event that targets oligodendrocytes will invariably result in demyelination.

As individuals age, myelin starts to break down, a process that accelerates as aging progresses (129) and may contribute to cognitive decline and dementia. Alterations in myelination and oligodendrocyte status have been

observed before Aβ and tau deposition in *in vivo* models of AD; however, these findings can vary according to the mouse model examined (130–132).

The presence of Aβ may in itself have toxic effects on oligodendrocytes and myelin. This theory has been reviewed in more detail by Cai *et al.* (124). It is clear that myelin disruption correlates with temporal and spatial progression of cognitive impairment. A number of studies have observed a loss of oligodendrocyte lineage cells in the gray matter associated with amyloid plaques (133, 134). Changes in myelin damage and oligodendrocytes in the white matter have also been observed and have been reviewed in detail by Nasrabady *et al.* (125). These alterations may be caused by oxidative stress, apoptosis, or neuroinflammation or a combination thereof (124, 125). As yet, the mechanisms involved in oligodendrocyte dysfunction in AD have not been deciphered and remain a potential clinical target.

Expression profiles of glial cells reveal that regional specificity is altered with age

Age is the primary risk factor for AD, suggesting an overlap between aging and the consequential drive toward neurodegeneration in an unfortunately high proportion of individuals (25). The brain is unparalleled in its structural, cellular, and functional complexity, which makes molecular studies of glial cells and the role they may play in both healthy aging and during neurodegeneration extremely challenging. Although such focused molecular studies have been useful in elucidating how and why glial cells could be critically involved during neurodegeneration, many are limited by studying only a specific glial function, in a specific brain region in a particular animal model of AD. Of course, it is well known that glial cells do not exist in a single, one-cell-does-all capacity but are highly heterogeneous across the CNS. Thus, attempting to tie together the validity of studies examining glial cell responses in the context of the pros and cons of different animal models of disease is nearly impossible. Recently, however, a number of studies have overcome such problems by examining the transcriptome of glial cells sampled across the brain and during aging in both human tissue and in animal models.

The elucidation of microglial regional identity first during adulthood (135) and then during aging (136) represented landmark studies into understanding the complexity of glial cells across the CNS. Grabert *et al.* (136) showed that microglia exist in a regionally diverse expression pattern in wild-type mice. However, as mice age, microglial specificity diminishes. For example, in the hippocampus, the unique youthful expression signature becomes more like that of other brain regions. This progression hints that a slow loss of specialization might contribute to deficits during the aging process. This theory was further corroborated by a study examining the expression of microglial-specific genes from the transcriptome of brain regions extracted from human aging samples (137). Other studies have examined microglia isolated from the cerebral cortex (138) or from biopsy material (139) and shown a similarity between the murine and human microglial signatures, with some exceptions between immune-related genes, which exhibit a higher expression level in human tissue. A notable exception was the difference in the aging profile from human cortex compared with mouse, indicating that microglia in adult brain regions are broadly similar in expression across species, but human microglia age differently (138). While recognizing that the differences in life span and time delays in postmortem between species could result in different profiles, there are clear commonalities being revealed between human and animal studies.

The expression profile of astrocytes is highly heterogeneous in the adult brain (140), consistent with the earlier identification of multiple classes of astrocytes (87, 88). During aging, astrocytes have been shown to change their gene expression profile in cell bodies within the murine cortex, with the predominant change appearing to be an increase in an inflammatory phenotype (141). Astrocytes isolated from multiple brain regions in adult and aged mice showed region-specific, age-associated changes in the astrocyte transcriptome with many genes associated with an inflammatory and reactive phenotype (142). The observation is consistent with an earlier report that suggested astrocyte brain region–specific alterations during aging in humans (137). Furthermore, aged human astrocytes increase expression of genes typically involved in synapse elimination during development, indicating that the astrocyte may be critically involved in aging-associated cognitive decline (142). Such findings have greatly increased our understanding of glial cells and their varied expression profiles throughout the brain and during aging. It is known, however, that only a relatively small percentage of expressed transcripts will be translated to proteins; thus, future research will also require advancing our understanding of the proteome heterogeneity. Indeed, one such study has examined the proteome of microglia from the brains of young and aged mice but without analyzing the varied profiles from different brain regions (143).

EXPRESSION PROFILE OF GLIAL CELLS DURING NEURODEGENERATIVE DISEASE

Understanding the complex glial responses in disease ideally will utilize animal models of a progressive neurodegenerative disease, assessing the temporal sequence of multiple brain region changes. Prion diseases have offered a significant advantage in understanding glial cell responses in the context of a neurodegenerative disease. Mice experimentally infected with prions undergo a predictable progressive neurodegenerative disease and display the complete repertoire of pathologies also found in human prion diseases. This outcome suggests that studies of murine prion disease may be more translatable to human disease than animal models of many other neurodegenerative diseases. Indeed, a recent study isolating microglia from prion-infected mice over multiple time points provides a useful resource by characterizing the

disease-specific inflammatory response of microglia in a progressive neurodegenerative disease (144). In animal models of AD, a number of genes have been shown to be differentially expressed in isolated microglia (67, 141, 145–147), many of which overlap with those identified in prion disease (144). Among the expression of inflammatory or anti-inflammatory cytokines, genes associated with other functions, such as phagocytosis, lipid metabolism and lysosomal genes are also shown to be highly activated in microglia during disease.

Transcriptomic analysis of the astrocyte in AD is remarkably understudied. One landmark study performed laser microdissection of astrocytes from brain regions at different stages of AD as judged according to the Braak neurofibrillary stages (148). This study showed increasingly dissimilar, differentially expressed genes between brain regions at early Braak stages compared with other brain regions at late stages. Among the gene expression changes observed were many associated with astrocyte cytoskeletal proteins consistent with the known reactive astrogliosis that progressively occurs during AD. Moreover, at earlier stages of AD progression, genes involved in apoptosis and ubiquitin-mediated proteolysis were shown to be differentially expressed, whereas at later stages, genes involved in tight and adherent junctions as well as many intracellular signaling pathways (*e.g.*, insulin) were shown to differ. Many of the differentially expressed genes have also been shown to occur in the same or other brain regions of patients with AD at late stages of disease (149–152). However, these data are difficult to interpret because they represented whole tissue transcriptomes rather than being cell type specific.

It is notable that many of these studies examine glia either at a whole brain level or within a specific brain region. Given the current understanding of the variability in glial gene expression among brain regions, a greater understanding of the glial response in different brain regions during the progression of disease could reveal novel aspects and improve our understanding regarding the potential of glial targeting for therapeutic intervention. Indeed, a study in our laboratory showed that glial cell responses are altered during disease progression in brain regions that are either susceptible or show resilience to neurodegeneration, even when the misfolded protein is detected in all brain regions tested (26). The expression of a number of microglial genes is differentially altered in brain regions undergoing neurodegeneration overlapping with those identified in other studies of prion disease and AD (66, 141, 144–147). Importantly, a unique disease-specific response was also observed associated with microglia in brain regions that show no apparent neurodegeneration (26). This heterogeneity in microglial response has also been highlighted in a recent meta-analysis in AD, whereby distinct classes of microglia were identified in neurodegeneration-affected brain regions, including an interferon-related phenotype (153). The role that variable glial activation states has upon progression of neurodegeneration is unclear but could indicate an important role of glia in defining the severity of neurodegeneration (26).

GLIAL CELL INTERACTIONS IN NEURODEGENERATION

During disease, microglia respond early in pathogenesis by expressing a range of innate immune activation genes (144). We have discussed the potential role such differing activation states of microglia could have on the neurodegenerative process. Although astrocytes exhibit a number of functional deficits associated with disease progression, for many years, our understanding of the impact of astrogliosis and astrocytic dysfunction has upon neuronal health has been limited. Certainly, astrocytes are known to express a number of immune-related genes and thus are likely to play a coordinated immune-activated response to neurodegeneration. Studies in prion diseases have indicated a mechanism by which this glial communication could occur (**Fig. 2**). An early study showed that a prion-infected neuron/astrocyte culture inoculated into mice had chemotactic properties not present in similar preparations from control cultures (154). This finding indicates that either, or both, the neurons or astrocytes were responsible for microglial recruitment to the site of injection. Another study showed that the proinflammatory cytokine IL-1β, which could be up-regulated by many factors [including NLRP3 inflammasome pathway activation (155)], is important in astrogliosis activation (156), and IL-1β synthesis is highly robust in microglial populations during disease (157). This finding suggests that microglial activation may be integral for astrogliosis through the secretion of cytokines such as IL-1β, which in turn produces a chemokine response to attract additional populations of microglia, thus acting as a feedback communication pathway between glial cells during disease. A recent study showed that a class of activated microglia can induce an astrogliosis response through the secretion of a combination of IL-1β, TNF-α, and C1q (76), demonstrating overlapping glial interaction mechanisms in AD and other neurodegenerative diseases such as prion disease. Furthermore, such activated astrocytes are functionally impaired and can also lead to neuronal death. This class of astrogliosis has been termed A1, as the activation appears functionally analogous to a "classic" activation state of microglia. Importantly, such A1 astrocytes have been shown to be present in a range of neurodegenerative diseases, including AD. These data suggest that resolution of glial cell activation or disruption of glial cell interaction during disease might be an important therapeutic intervention strategy. A recent study showed that microglia can induce a neuroprotective astrocytic phenotype through down-regulation of the $P2Y_1$ receptor (158), thereby providing a tantalizing prospect of glial-targeted therapeutic intervention.

CONCLUSIONS

Our understanding of the complex process of neurodegeneration has, in recent years, been transformed by the manner in which we are examining these diseases. However, we are still hampered to some extent by the limitations of the animal models of AD and our ability to compare data among model systems, as AD models display only some

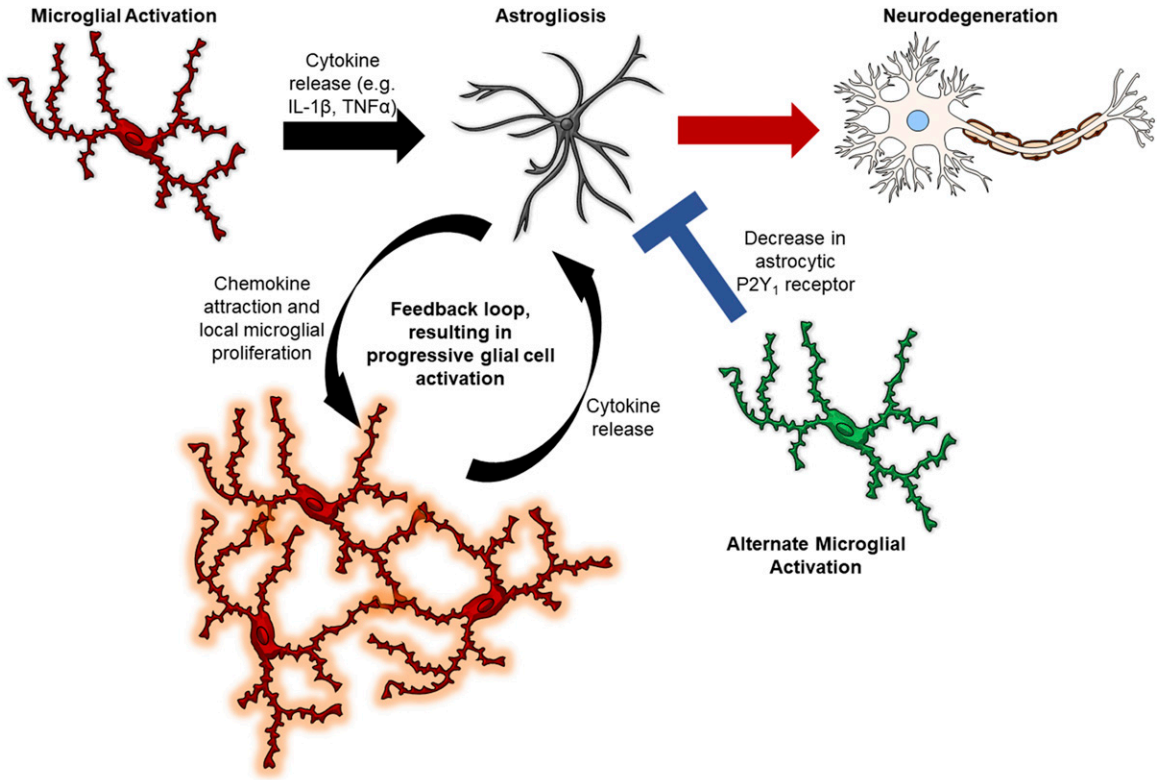

Figure 2. Mechanism of glial cell interaction during disease. At early stages of disease, microglial activation results in secretion of a number of cytokines that induce astrogliosis. In turn, astrocytes increase expression of chemokines, which in combination with local microglial proliferation, result in increased numbers and reactive activity of microglia. Reactive microglia then secrete increased levels of cytokines into the local environment, leading to progressive astrogliosis. During disease, there appears to be no resolution of the glia activation states over time; instead, glial activation progressively increases over time, indicating that this feedback loop is a glial-specific perpetuating cycle. Recent studies indicate that the astrogliosis state activated by cytokines such as IL-1β results in neurodegeneration in AD. However, other studies have found evidence for microglial-mediated resolution of astrogliosis, highlighting the therapeutic potential for targeting this glial cell interaction in AD.

aspects of the disease process. The understanding of commonalities between the different protein misfolding diseases has accelerated our understanding of these chronic neurodegenerative disease processes.

A realistic focus is on prevention of these diseases reaching the devastating clinical manifestations of symptoms such as dementia, and to achieve this goal, it is important to unravel the early events of which the glial cells are clearly a key feature. Both microglia and astrocytes seem to have a dual role in the disease process by, on the one hand, attempting to resolve the damage in the CNS and, on the other hand, perhaps through loss of function leading to the damaging inflammatory reactions that ultimately result in neurodegeneration. It is therefore important to tease apart these different roles with a view to preserving and enhancing the protective functions. It is also clear that only certain regions of the brain are vulnerable to the neurodegenerative process; therefore, by examining the differences between susceptible and resistant regions within the brain, we are likely to define new intervening targets to preserve the integrity of the brain.

We are now on the precipice of an explosion of data aimed at understanding the complex and heterogeneous roles of glial cell responses during aging and neurodegeneration by studying the brain in both a regional and temporal manner. Large-scale genomic, proteomic, and metabolomic data are being generated from both human studies and from animal models. The next decade will, with the tools now available, unravel the complexity of the pathways of neurodegenerative processes and provide solutions to the therapeutic intervention into these devastating diseases. FJ

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AUTHOR CONTRIBUTIONS

J. D. Alibhai, A. B. Diack, and J. C. Manson conceived and wrote the paper.

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