Master Thesis:

Astrocytes in Alzheimer's Disease

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Foreword

This master thesis is the product of the two year Master program Neuroscience and Cognition at the Utrecht University. It is the result of an intensive literature investigation concerning the effects of astrocytes on Alzheimer's disease patients and with minor content on the blood-brain barrier of Alzheimer's disease patients. After I approached Dr. Albertus de Boer, Professor of Pharmacology and Head of the Blood-Brain Barrier research group at the Leiden University, we discussed the subject of this project and I was immediately interested and eager to start with this project.

The modulating effects of astrocytes have gained increased attention from many research groups as it becomes more evident that these cells are highly important for the integrity of the blood-brain barrier and for the progression of Alzheimer's disease. It is clear that they play great roles in Alzheimer's disease and most likely also in the blood-brain barrier impairments during Alzheimer's disease. Much research has been done on astrocytes in Alzheimer's disease patients without regard to the blood-brain barrier. In this thesis I will mostly focus on the altered activity of astrocytes in Alzheimer's disease and I will attempt to extrapolate these results to the impaired blood-brain barrier in Alzheimer's disease patients. However, regard to the blood-brain barrier will be limited due to minimal data on this subject. Therefore, more investigation of astrocytes on the blood-brain barrier in Alzheimer's disease is needed and could provide evidence how the underlying mechanisms of astrocytes work in this condition.

I would like to thank my supervisor Dr. Albertus de Boer for providing me the liberty to write this thesis to my own perception and for his continuous support. I would also like to thank Dr. Rou-Afza Gunput for suggesting me to write about this subject under the supervision of Dr. Albertus de Boer.

Amila Zuko,
Rotterdam, December

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Summary

The blood-brain barrier (BBB) is essential for the normal function of the central nervous system since it restricts influx of most compounds from blood to brain. During several neurologic conditions the integrity of the BBB is impaired and progressively loses its function resulting in BBB breakdown and damage to neurons and other brain cells, such as during Alzheimer's disease (AD). Astrocytes play a major part in the formation and maintenance of the BBB and are implicated in the progression of Alzheimer's disease.

In AD, the β-amyloid peptide (Aβ) accumulates in plaques which results in initiation of a cascade of events leading to neurodegeneration and dementia. This peptide activates astrocytes which are found to surround Aβ plaques and to intracellularly contain Aβ. Several data indicate that astrocytes in close proximity to Aβ plaques are capable to intracellularly accumulate substantial amounts of Aβ. Aβ-overburdened astrocytes have been shown to produce astrocyte-derived plaques. In contrast, the amyloid precursor protein and the β-site APP-cleaving enzyme have been localized in activated astrocytes which are able to produce Aβ. Thus, it remains elucidated whether activated astrocytes produce Aβ or whether they phagocytose and degenerate Aβ.

Astrocytes are also implicated in chronic inflammation in AD. They have been found to produce pro- and anti-inflammatory factors and facilitate complex interactions with several inflammatory cells.

Astrocytes express several Aβ receptors which are responsible for Aβ phagocytosis, mainly the low-density lipoprotein receptor (LDLR) and the LDLR-related receptor (LRP-1). ApoE is implicated in the clearance of Aβ by forming an ApoE-Aβ complex and binding the LRP-1 receptor on astrocytes. The frequency of certain isoforms of ApoE in late-onset familial AD patients is almost four times higher than in the general population.

In addition, mechanisms which as well add to the progression of AD are the aquaporin channels which facilitate the osmotic driven bidirectional water transport across cell membranes. Several studies suggest there is a possible association of astrocyte APQ1 with Aβ deposition in AD brains. The prostaglandin transporter mediates the secretion of several pro-inflammatory molecules which were postulated to be upregulated in patients with AD. However, the levels of the prostaglandin transporter is found to be decreased in astrocytes in AD patients.

In patients with AD, inefficient clearance of Aβ seems to be the fundamental event leading to accumulation of Aβ in the brain. To clear Aβ from the brain and prevent neurotoxicity, Aβ has to be transported across the BBB. Accumulation of Aβ in the vessel walls is the primary pathogenic event in AD and results in disruption of the BBB. The methods by which astrocytes are implicated in AD remain to be elucidated. However, the altered activity of astrocytes seem to play an important role in the disruption of the BBB in AD patients.

1. General introductions

1.1. The blood-brain barrier

The blood-brain barrier (BBB) is a metabolic diffusion barrier which impedes influx of most compounds from blood to brain, essential for the normal function of the central nervous system. The BBB consists of endothelial cells which differ from endothelial cells in the rest of the body by absence of fenestrations, more extensive tight junctions (TJ), and sparse pinocytic vesicular transport (Brightman and Reese 1969). The main function of the BBB is to restrict the paracellular passage of hydrophilic molecules across the BBB by the endothelial TJs. Small lipophilic substances diffuse freely across plasma membranes along their concentration gradient (Grieb, Forster et al. 1985). Nutrients including glucose and amino acids are able to pass the BBB and enter the brain via transporters, whereas receptor-mediated endocytosis mediates the uptake of larger molecules such as insulin, leptin, and iron transferrin (Pardridge, Eisenberg et al. 1985; Zhang and Pardridge 2001).

In addition to endothelial cells, the BBB is composed of pericytes with smooth muscle-like properties that reside adjacent to capillaries, and astroglial processes that ensheath more than 95% of the abluminal blood vessel surface (Kandel, Schwartz et al. 2000). However, in several neurologic conditions the integrity of the BBB is altered which can trigger signal transduction cascades leading to loss of TJ molecules and BBB breakdown.

1.1.1. Astrocytes in the blood-brain barrier

A number of studies have suggested that the ability of endothelial cells in the CNS to form a BBB is not intrinsic to these cells, but CNS environment induces the formation of the barrier into the blood vessels (Stewart and Wiley 1981). Astrocytes implanted into areas with normal leaky vessels in the BBB have induced tightening of the endothelium (Janzer and Raff 1987; Bauer and Bauer 2000). Direct contact between endothelial cells and astrocytes is necessary to generate an optimal BBB (Rubin, Barbu et al. 1991). Neuhaus et al. has reintroduced high transendothelial resistance in human and bovine endothelial monolayer cells by culturing these cells in astrocyte-conditioned media, suggesting that induction of BBB characteristics in endothelial cells is due to an astrocyte-derived soluble factor (Neuhaus, Risau et al. 1991). In addition, anatomical examination of the brain microvasculature provides strong support for the involvement of perivascular astrocytic glia in metabolic coupling. Astrocyte endfeet are in proximity to the outer surface of the endothelium and this allows direct metabolic changes between the astrocytes and endothelium, while not forming a physical barrier, so preserving free diffusion between the endothelium and brain parenchyma (Kacem, Lacombe et al. 1998). The endfeet of perivascular astrocytes play an essential role in maintaining the integrity and permeability of the BBB (Abbott, Ronnback et al. 2006). The neural microenvironment including astrocytes, play a key role in inducing BBB function in capillary endothelial cells during the gradual formation of the BBB shortly after intraneural neuvascularization (Bauer and Bauer 2000).

In Alzheimer's disease, astrocytes are activated by the β-amyloid protein and related oligopeptides, leading to a cascade of events producing toxic molecules, neuronal damage, and synaptic dysfunction (Giulian, Haverkamp et al. 1995). However, the mechanism underlying how astrocytes are implicated in AD remains to be elucidated. This review will cover implications of astrocytes in Alzheimer's disease patients and to a minor extent attempt to extrapolate these astrocyte implications to BBB impairments during Alzheimer's disease.

1.2. Alzheimer's disease

Dementia is a syndrome characterized by failure of recent memory and other intellectual functions that is usually insidious in onset but steadily progresses. Alzheimer's disease (AD) is the most common dementia, accounting for 60-70% of cases in the elderly (Hebert, Scherr et al. 2003). AD affects 20 to 30 million individuals worldwide (Selkoe 2005). The prevalence of AD increases with age, affecting approximately 1% to 3% of the population round 60 years of life, 3% to 12% of the population between 70 and 80 years, and up to 25% to 35% of the population older than 85 years (Walsh and Selkoe 2004). Life expectancy has been constantly increasing in industrialized countries, predicting that the incidence of AD will increase three fold over the next 50 years.

The earliest sign of AD is typically an impairment of recent memory function and attention followed by failure of language skills, abstract thinking and judgment and visual-spatial orientation. AD gradually progresses to severe dementia and stupor. These defects are accompanied by alterations of personality (Nestor, Scheltens et al. 2004). The earliest symptoms appear as subtle, sporadic deficits in remembering minor events of everyday life, including forgetfulness and difficulties recalling new names or recent conversations, referred to as loss of episodic memory. At a later stage, a profound dementia develops affecting multiple cognitive and behavioral abilities. The patient is unaware of time and place and cannot even identify close family members. These symptoms are frequently accompanied by additional neurological symptoms such as extrapyramidal motor signs, slowed movements and hampered motor coordination. Death occurs, on average, 9 years after the initial clinical diagnosis, usually caused by respiratory complications such as aspiration of pneumonia.

At autopsy, the AD brain shows a macroscopically visible severe cerebral atrophy involving brain regions associated with learning and memory processes, including the temporal, parietal and frontal cortex as well as the hippocampus and amygdala. A reduction in brain weight of usually more than 35% is visible in AD brains. The histopathology in the brain of AD patients consist of several principal features such as, neurofibrillary tangles, amyloid plaques and a diffuse loss of neurons. These changes are most apparent in the neocortex, limbic structures and selected brainstem nuclei (Selkoe and Schenk 2003).

1.2.1. Amyloid precursor protein-processing pathways

The leading theory of progression of AD is the amyloid hypothesis, which poses that an imbalance in the production or clearance of the β-amyloid peptide results in accumulation of β-amyloid and initiation of a cascade of events leading to neurodegeneration and dementia (Gandy 2005). A growing amount of evidence supports that altered processing of amyloid precursor protein (APP) is one of the early events in the pathogenesis of AD (Rossner, Ueberham et al. 1998; Selkoe and Schenk 2003). APP is a member of the glycosylated transmembrane protein family which are ubiquitously expressed, but are most abundant in the brain. The APP gene is mapped on chromosome 21 in humans and constitutes a family of different isoforms, which are derived by alternative splicing of APP mRNA and named accordingly to their length in amino acids (Kang, Lemaire et al. 1987). APP can be processed by both amyloidogenic or non-amyloidogenic pathways. The non-amyloidogenic secretory pathway includes cleavage of APP by α-secretase within the β-amyloid sequence, generating a secreted water soluble protein with the membrane-anchored C-terminal

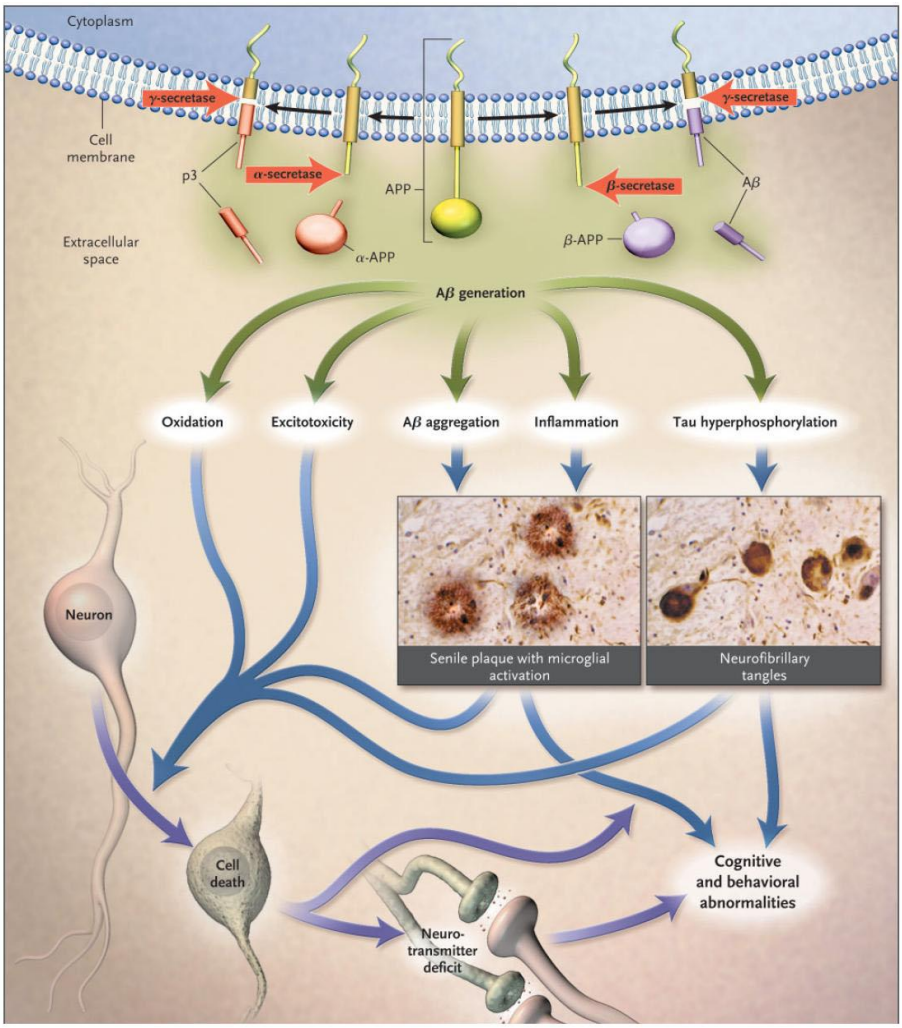

Figure 1. Proteolycal APP-processing pathways. In the β-seretase pathway (right), APP is initially cleaved by the β-seretase BACE1. After additional cleavage of the remaining APP fragment of γ-secretase, the β-amyloid peptide is released. Alternatively, in the α-secretase pathway (left), APP is cleaved by α-secretase. The γ-secretase cleaves the remaining APP fragment, which releases the p3 fragment. Adapted from Cummings et al. 2004

fragment of APP remaining in the cell (Haass, Koo et al. 1992; Haass, Schlossmacher et al. 1992). The α-APP formed is released into the extracellular space and the membrane-bound P3-CT fragment undergoes second proteolytic cleavage by γ-secretase after internalization (Francis, McGrath et al. 2002). The amyloidogenic pathway is initiated by β-secretase cleavage of APP within the lumen of the Golgi apparatus or endosomes to expose the N-terminus of the β-amyloid peptide (Seubert, Oltersdorf et al. 1993). The endoprotease catalyzing this APP cleavage at the N-terminus of the β-amyloid peptide has been identified as β-site APP-cleaving enzyme (BACE1) (Vassar, Bennett et al. 1999). After additional cleavage of the remaining APP fragment by γ-secretase, the β-amyloid peptide (Aβ) is released (Francis, McGrath et al. 2002) (Figure 1.).

Several studies suggest that Aβ is involved in the neuro-degenerative cascade of AD. The subsequent cleavage of γ-secretase generates the $A\beta_{38\text{-}42}$ peptides, of which $A\beta_{42}$ is the most neurotoxic. Mutations in the APP, presenilin-1, and presenilin-2 genes, which are required for γ-secretase activity, as well as other less well-characterized hereditary and environmental influences increase $A\beta_{42}$ production (Francis, McGrath et al. 2002). Accumulation and oligomerization of $A\beta_{42}$ or reduced clearance from the brain results in the formation of

amyloid plaques and initiates a cascade of events associated with neuronal and synaptic dysfunction, inflammatory responses, neuronal death, dementia and ultimately death (Cummings 2004).

2. Implications of astrocytes in Alzheimer's disease

Besides maintaining the BBB, astrocytes organize the structural architecture of the brain, communication throughout the brain and plasticity. Astrocytes are the most prominent microglia in the CNS. They make extensive contacts with adjacent neurons, encase synaptic terminals, ensure normal neuronal excitability by maintaining extracellular ion homeostasis, clear glutamate and potassium from the region of synapses, stabilize synapses and may participate in synaptic plasticity. Neurons co-cultured with astrocytes develop seven-fold more synapses and have an increased synapse activity (Pfrieger and Barres 1997). Recent studies have demonstrated that reciprocal paracrine interactions between astrocyte, endothelial cells and ependymal cells can regulate neurogenesis and gliogenesis from resident precursor cells (Song, Stevens et al. 2002). These reactivated astrocytes are identified by their elevated expression of the glial fibrillary acidic protein (GFAP) (Eng and Ghirnikar 1994). Astrocytes become activated with ensuing hypertrophy and proliferation under pathophysiological conditions such as an infection, injury, demyelinating and neurodegenerative disorders. Reactive astroglia can form a glial scar in response to injury, leading to an isolation of injured tissue and accompanying inflammatory processes. Several studies have reported that astrocytes are involved in the processing of Aβ, chronic inflammation, and several other processes which will be described below.

2.1. Astrocytes in Aβ processing

Astrocytes play significant roles during AD. Senile plaques found in the brain of patients with AD are surrounded by clusters with active astrocytes. Although the trigger that causes activation of astrocytes under these circumstances is not clear, studies have shown that both aggregated Aβ and the intact cores of Aβ plaques isolated from human AD brain tissue can stimulate activation of astrocytes in vitro (DeWitt, Perry et al. 1998).

2.1.1. Aβ accumulation in astrocytes

In AD brains, activated astrocytes can be found in the cortical molecular layer as well as in the pyramidal cell layers. While microglia infiltrate Aβ plaques in AD, astrocytes aggregate around the periphery, walling off the plaque. This positions three key players, Aβ, microglia and astrocytes in close proximity (Schwab and McGeer 2008) (Figure 2). From this location, astrocytes project thick processes that envelop the Aβ plaque and thinner branches that infiltrate deep into the plaque interior, suggesting that they participate in eventual glial scarring an possibly mediate other changes within the confines of plaques that may contribute to the observed variety of plaque morphologies.

The origin of astrocytic $A\beta_{42}$ and the mechanism by which it accumulates selectively in activated astrocytes and not in

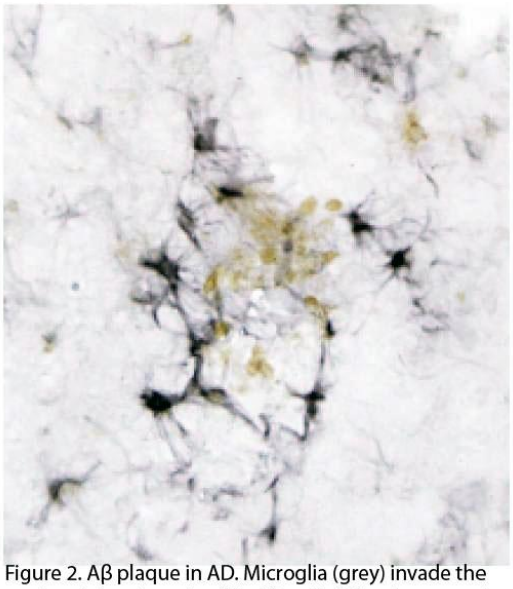

Figure 2. Aβ plaque in AD. Microglia (grey) invade the center while astrocytes (black) wall of the plaque and are found in the periphery.
Adapted from Schwab et al. 2008

their more quiescent counterparts is unknown. $A\beta_{42}$ found in astrocytes could be either internally produced or extracellulary taken up. Since expression of the $A\beta_{42}$ precursor protein (APP) in astrocytes is known to be extremely low, internal production is unlikely to be a major source of accumulated $A\beta_{42}$. Thus, internalization of extracellular $A\beta_{42}$ is more apparent and could occur via receptor-mediated endocytosis and/or phagocytosis. Aldskogius et al. demonstrated the phagocytic capability of activated astrocytes which includes elimination of synapses (Aldskogius, Liu et al. 1999). Activated astrocytes in close proximity with $A\beta_{42}$ plaques can intracellularly accumulate substantial amounts of $A\beta_{42}$ often to the extent that this material dominates the cytoplasmic volume of astrocytes (Kurt, Davies et al. 1999; Nagele, D'Andrea et al. 2003). Pihlaja et al. has applied astrocytes to brain sections containing human plaque and shown that like microglial cells, astrocytes are able to phagocytose $A\beta_{42}$, but they can do this spontaneously and without stimulation (Pihlaja, Koistinaho et al. 2008). Nagele et al. has provided strong evidence that $A\beta_{42}$-immunopositive material within astrocytes is localized to prominent granules in the perinuclear cytoplasm, and $A\beta_{42}$ accumulation within these cells is not dependent on the local presence of plaques, since activated astrocytes exhibiting prominent intracellular $A\beta_{42}$ deposits often populate the cortical molecular layer, even when Aβ plaques are completely absent (Nagele, D'Andrea et al. 2003).

Nagele et al. has shown that accumulated $A\beta_{42}$ in activated astrocytes residing in the cortical molecular layer is of neuronal origin and is derived from internalization of degenerating synapses and dendrites belonging to neurons in the underlying pyramidal cell layers. Additional evidence for the uptake of $A\beta_{42}$ by astrocytes comes from the fact that $A\beta_{42}$ in activated astrocytes is co-localized with other neuron-specific proteins, including choline acetyltransferase (ChAT) and the alpha7 nicotinic acetylcholine receptor (α7nAChR) (Nagele, D'Andrea et al. 2003). The selective accumulation of these neuronal proteins in activated astrocytes is an expected consequence of their debris-clearing activity in response to AD-related degeneration of dendrites and synapses. As result, ChAT and α7nAChR-immunopositive material is most prominent in astrocytes, reflecting the particularly high density of fine dendritic branches and associated synapses (Nagele, D'Andrea et al. 2003).

These data indicate that astrocytes are capable of accumulating $A\beta_{42}$ intracellularly. The reason that the astrocytic cytoplasm is accumulated with $A\beta_{42}$ and neuronal debris lies in the fact that $A\beta_{42}$ binds with exceptionally high affinity to α7nAChRs on neuronal surfaces. The cortical molecular layer is densely packed with fine α7nAChR-rich dendrite branches emanating from underlying neurons. The $A\beta_{42}$-α7nAChR complex is present on degenerating dendrites and synaptic surfaces, suggesting that astrocyte-mediated clearing of this debris via phagocytosis and endocytosis and the targeting of this material to the lysosomal system explains both the source of astrocytic $A\beta_{42}$ as well as its co-localization in astrocytes with other neuron-specific proteins, such as α7nAChR and ChAT (Nagele, D'Andrea et al. 2003). This mechanism also explains why $A\beta_{42}$, rather than $A\beta_{40}$ (which has much less binding affinity for α7nAChR), is the dominant Aβ peptide species in astrocytic intracellular deposits and in amyloid plaques throughout AD brain (Nagele, D'Andrea et al. 2002). In conclusion, these findings suggest that activated astrocytes are capable of internalizing neuron-derived materials,

including $A\beta_{42}$, presumably through their endocytic and phagocytic activity. The enhanced levels of cathepsin D, a lysosome specific enzyme, in these astrocytes in AD brains confirms the increased activity of this cellular compartment (Nagele, D'Andrea et al. 2003).

In addition to the clearing ability of astrocytes in AD, astrocytes are also able to act as a neurotoxin suggesting a consequence of their phagocytotic abilities. Giulian et al. showed that isolated human astrocytes placed in contact with plaques in vitro are activated to release a certain neurotoxin. This neurotoxin is found in AD brain tissue and the highest concentrations are found in brain areas with the most astrocytes, suggesting that plaque-activated astrocytes contribute to neuronal damage and impaired cognition in patients with AD (Giulian, Haverkamp et al. 1995).

2.1.2. Amyloidogenic APP processing

There is increasing evidence for a role of astrocytes in Aβ generation. The expression of the BACE1 enzyme has been localized, in particular in neurons, indicating that neurons are the major source of Aβ in the brain. A BACE1 gene was identified in a human astrocyte cDNA library, indicating that astrocytes can express BACE1 (Yan, Bienkowski et al. 1999). However, there are reports of BACE1 expression being restricted to neurons in the rodent brain (Rossner, Apelt et al. 2001).

Rossner et al. have observed BACE1 expression using cultured rat primary astrocyte cultures and animal models of amyloid plaque formation and astrogliosis. Astrocytic BACE1 expression was demonstrated in proximity to Aβ plaques in brains of APP transgenic Tg2576 mice with Aβ pathology (Rossner, Apelt et al. 2001). This observation raised the question whether astrocytic BACE1 expression in the Tg2576 brains is an event, which is specifically triggered by Aβ plaque formation or whether induction of BACE1 expression is a general feature of astrocytic activation. If the latter is correct, repeated chronic inflammatory processes in the brain accompanied by gliosis would increase the generation of Aβ resulting in the formation of Aβ plaques. To address this question experimentally, a number of lesion paradigms characterized by chronic astrocyte reactivation were studied regarding to BACE1 expression (Hartlage-Rubsamen, Zeitschel et al. 2003). They found no detectable expression of BACE1 by astrocytes after acute intoxification of the brain. However, BACE1 immunoreactive astrocytes were observed in the affected brain tissue after middle cerebral artery occlusion, after degeneration of cholinergic septohippocampal afferents by cholinergic immunolesion, in brain stem after induction of experimental autoimmune encephalomyelitis and in the hippocampus after cerebral infection with Borna disease virus (Hartlage-Rubsamen, Zeitschel et al. 2003). In all these experiments, BACE1 expression was detectable as early as the morphological features of reactive astrocytes were evident, peaked at the time point of maximum astrocyte activation, and declined in parallel with the loss of morphological features of reactive astrocytes. The astrocytic expression of BACE1 after induction of chronic gliosis was not only limited to experimental animals but also included astrocytes in close proximity to Aβ plaques in brains of AD patients (Hartlage-Rubsamen, Zeitschel et al. 2003). Repetitive mild traumatic brain injury accelerates Aβ deposition in APP-transgenic mice and induces astrocytic BACE1 expression in rats (Uryu, Laurer et al. 2002; Blasko, Stampfer-Kountchev et al. 2004). The increased generation of Aβ and accelerated formation of Aβ plaques had been reported for several animal models of chronic gliosis (Uryu, Laurer

et al. 2002). In addition, several *in vitro* studies demonstrate significant astrocytic BACE1 promoter activity and immunoreactivity by cultured rat primary astrocytes (Hartlage-Rubsamen, Zeitschel et al. 2003; Lange-Dohna, Zeitschel et al. 2003). In conclusion, astrocytes are able to express significant amounts of BACE1 both *in vitro* and *in vivo.*

These results suggest that increased BACE1 expression by reactive astrocytes may locally increase the generation of amyloidogenic APP fragments and potentially contribute to Aβ plaque formation. Furthermore, astrocytic BACE1 expression appears to be stimulus-dependent with the most increased BACE1 expression during chronic rather than acute gliosis. Microglial cells never expressed BACE1 immunoreactivity in any of the lesion paradigms studied, but it is likely that activated microglia release pro-inflammatory cytokines which activate astrocytic BACE1 expression. For example, interleukin-1β (IL-1β) plays a role in amyloidogenic APP processing in human astrocytes and transforming growth factor-β (TGF-β) increases APP mRNA expression in vitro and accelerates the deposition of Aβ in vivo (Gray and Patel 1993; Wyss-Coray, Masliah et al. 1997; Blasko, Veerhuis et al. 2000). The hypothesis that chronic inflammation triggers Aβ plaque formation via the release of pro-inflammatory cytokines by microglial cells and subsequent induction of astrocytic BACE1 expression is supported by the observation of diminished Aβ plaque formation in Tg2576 mice after suppression of microglial activation (Lim, Chu et al. 2001).

It is important to note that astrocytic BACE1 expression is only relevant for the development of AD if the astrocytes also express the BACE1 substrate and APP. There is ample evidence indicating that this is indeed the case. For example, primary astrocytes do express APP and generate significant amounts of Aβ (Gray and Patel 1993; Amara, Junaid et al. 1999; Blasko, Veerhuis et al. 2000). Additionally, APP is also expressed by reactive astrocytes in experimental models of chronic gliosis (Martins, Taddei et al. 2001) and this induced astrocytic APP expression results in the increased generation of Aβ and BACE1 cleavage-derived A4-CT fragments (Lesne, Docagne et al. 2003).

2.1.3. Plaque formation by astrocytes

As AD progresses continued synaptic loss in the cortical molecular layer can be observed and subsequently the intracellular load of $A\beta_{42}$-immunopositive material in local activated astrocytes gradually increases. Nagele et al. has demonstrated that a new population of amyloid plaques are formed, considerably smaller than those in the underlying pyramidal cell layers. They found that this new population of plaques is derived from the death and lysis of $A\beta_{42}$-overburdened astrocytes (Nagele, D'Andrea et al. 2003). The scattering of cytoplasmic material from ruptured astrocytes, including accumulated $A\beta_{42}$, may be facilitated by the activity of coincidentally released lysosomal enzymes, creating small, spherical, astrocyte-derived plaques. These typical plaques are observed only in regions where nearby astrocytes contain large intracellular deposits of $A\beta_{42}$-positive material (Nagele, D'Andrea et al. 2003). This mechanism of astrocytic plaque formation is nearly identical to the described larger neuron-derived plaques, many formed after the lysis of $A\beta_{42}$-overburdened neurons (Nagele, D'Andrea et al. 2002). Although the neuron-derived and the astrocyte-derived plaques are $A\beta_{42}$-immunopositive, astrocyte-derived plaques can be distinguished by their smaller size and particularly intense GFAP-immunoreactivity. The formation of these plaques (Figure 3), consistent with their spherical shape and size and the cells from which they are derived, argue strongly against

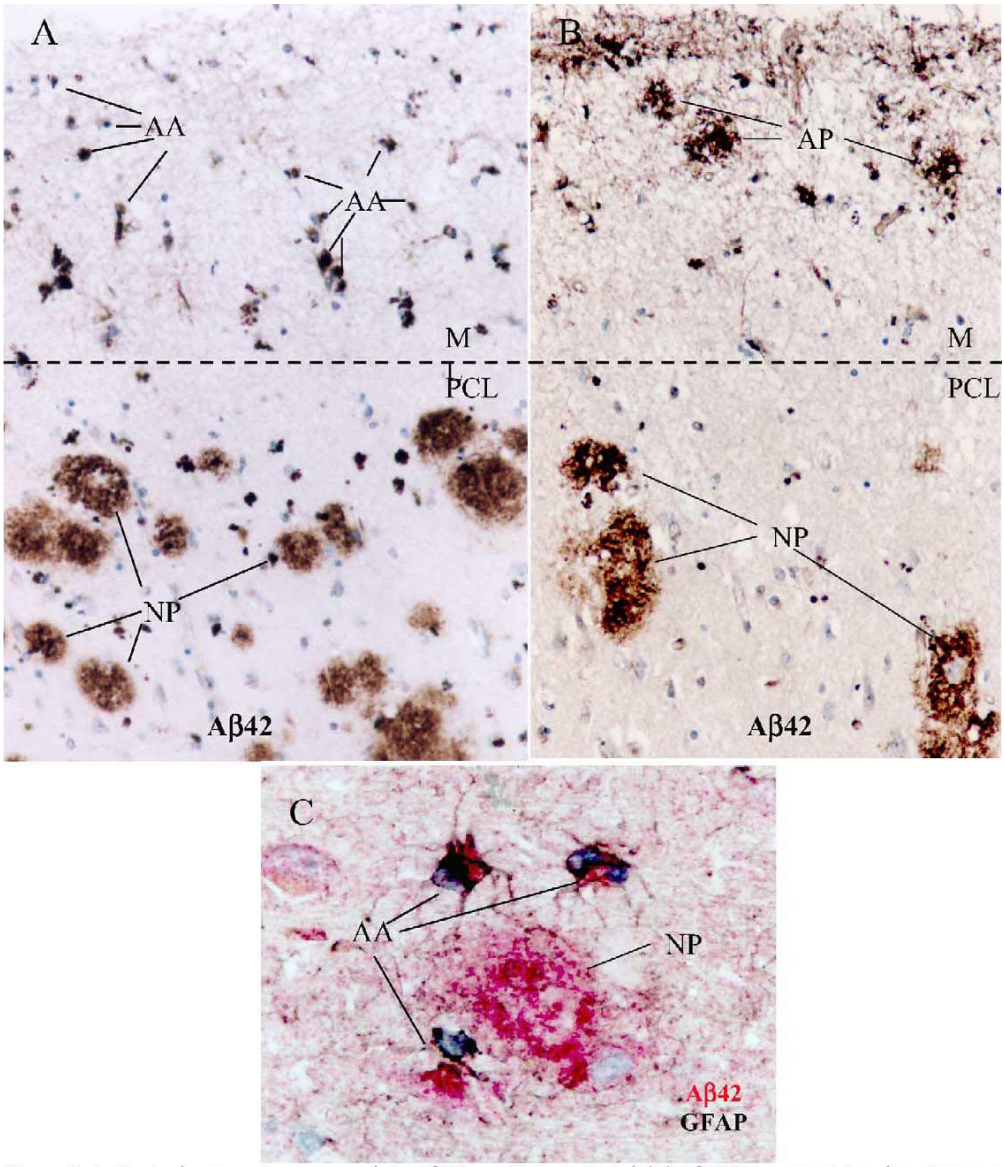

Figure 3. Activated astrocytes accumulate Aβ42-positive material. (A) Aβ42 immunostained section through the cortical molecular layer (M) shows acivated astrocytes (AA) burdened with Aβ42-positive material. In the underlying pyramidal cell layers, large neuron-derived plaques (NP) are visible. (B) Immunostained section of an AD brain showing small, Aβ42-positive, astrocyte-derived plaques (AP) in the molecular layer. (C) Double immunolabeling with GFAP (black) and Aβ42 (red) specific antibodies confirms the presence of accumulated Aβ42 in activated astrocytes. Adapted from Nagele et al. 2003

a mechanism which depends upon a gradual growth of plaques from a seeding site.

Accumulation of $A\beta_{42}$, α7nAChR and ChAT in activated astrocytes in the cortical molecular layer and the growth of $A\beta_{42}$-positive deposits in neurons of subadjacent pyramidal cell layers is a consequence of degeneration of the more distal dendrites and synapses in $A\beta_{42}$ accumulation in neurons (Nagele, D'Andrea et al. 2003). Oddo et al. have demonstrated that the location of significant synaptic degeneration was associated with the development of intraneuronal $A\beta_{42}$ accumulations, both occurring prior to the appearance of amyloid plaques (Oddo, Caccamo et al. 2003). This suggests that the activated astrocytes in those areas are likely to contribute to astrocytic plaque formation, after accumulation of $A\beta_{42}$ and neuronal debris.

The temporal aspect for neurons to accumulate $A\beta_{42}$, undergo lysis leading to

accumulation of $A\beta_{42}$ and debris in activated astrocytes and subsequently forming astrocytic plaques remains to be elucidated. This process could take months to years, which would suggest why memory and cognitive deficits in AD patients are occasionally detected prior to the appearance of a significant number of plaques in the brain.

2.1.4. Amount of Aβ is associated with AD progression

Accumulation of $A\beta_{42}$ in activated astrocytes varies throughout the cerebral cortex of AD brains, but appears to be both spatially and temporally correlated with AD progression (Nagele, D'Andrea et al. 2003). In the pyramidal cell layers, $A\beta_{42}$ accumulation within individual astrocytes is proportional to the relative amount of intracellular $A\beta_{42}$ contained within local neurons as well as the presence and local density of plaques. However, in the cortical molecular layer, astrocytes contain abundant $A\beta_{42}$, despite that this layer generally lacks $A\beta_{42}$-burdened neurons and plaques, especially in early stages of AD pathogenesis. Interestingly, the amount of $A\beta_{42}$ in these molecular layer astrocytes correlates closely with the severity of pathology exhibited in the cortical laminae directly subadjacent to these cells.

Nagele et al. demonstrated that in early AD brains and in age-matched control brains regions displaying AD pathology, hold cortical astrocytes which overlie pyramidal cell layers populated with $A\beta_{42}$-burdened neurons, invariably contained substantial intracellular deposits of $A\beta_{42}$. In addition, astrocytes overlying pyramidal cell layers lacking $A\beta_{42}$-burdened neurons and plaques, even in the same brain sections, were generally devoid of $A\beta_{42}$-positive material (Nagele, D'Andrea et al. 2003). These observations summarize the temporal and spatial link between $A\beta_{42}$ accumulation in neurons and the appearance of similar intracellular deposits in overlying astrocytes.

2.1.5. Neuroprotective action by astrocytes

There is evidence that activated astrocytes are implicated in Aβ clearance and degradation. Electron microscopy of AD brain tissues revealed Aβ in astrocyte processes (Kurt, Davies et al. 1999). This suggests that astrocytes are involved either with the synthesis or phagocytosis of Aβ. Yamaguchi et al. observed a new type of diffuse plaques associated with astrocytes, consisting of Aβ granules, suggesting that astrocytes might be involved in the phagocytosis of Aβ rather than the production of Aβ (Yamaguchi, Sugihara et al. 1998). They propose that plaque density reaches a plateau when plaque formation and destruction are equally balanced. Astrocytes can contain Aβ fragments and once activated, these astrocytes can bind and degrade $A\beta_{42}$, suggesting a direct role for these cells in Aβ degradation and clearance in AD brains (DeWitt, Perry et al. 1998). Wegiel et al. shows that in human and mouse brains, astrocytic processes infiltrate Aβ plaques and amyloid hemistars in capillaries (Wegiel, Imaki et al. 2003).

In addition, astrocytes have been shown to degrade Aβ deposits *in vivo* and an *in vitro* system of cultured astrocytes plated onto spot deposits of Aβ or onto mouse brain sections of APP-transgenic mice (Wyss-Coray, Loike et al. 2003) (Figure 4). This suggest that accumulation of astrocytes around Aβ plaques indicate active phagocytosis of Aβ and that possibly deficits in the clearance of Aβ by astrocytes is part of the pathology of AD. Aβ deposits can be degraded by the action of metalloproteases including neprilysin and insulysin (Kurochkin and Goto 1994). The expression of the Aβ degrading enzyme neprilysin is induced in reactive astrocytes

surrounding Aβ plaques in brains of APP transgenic mice (Apelt, Ach et al. 2003). It is not clear whether the mechanisms of astrocytic Aβ clearance described are functional in the AD brain (Wyss-Coray, Loike et al. 2003). Using three-dimentional reconstruction of Aβ plaques in different stages of development, it was shown that while microglial cells are the most important factor behind plaque formation, astrocytes are the major factor in plaque degradation (Wegiel, Wang et al. 2000).

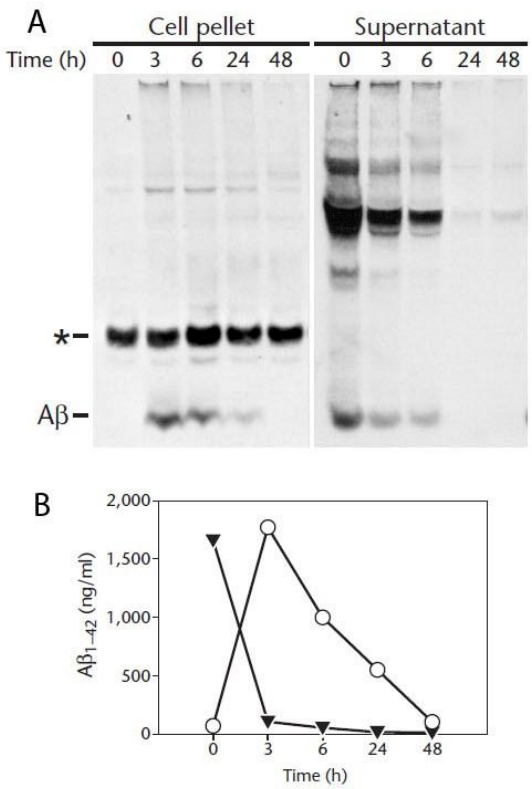

Figure 4. Degradation of Aβ1–42 by astrocytes, measured by western blot and ELISA. (A-B) Astrocytes were incubated with Aβ 1–42, and supernatant or adherent cells (pellet) were collected after the indicated time points. Aβ is detected in the cell pellet (A, left panel; B, ○) after 3 h and disappears at 48 h in both pellet and supernatant (A, right panel; B, ▼). * indicates, non-specific band. Adapted from Wyss-Coray et al. 2003

2.2. The role of astrocytes in chronic inflammation

In the last decade considerable attention has been paid to understand the role of astrocytes in the immune response in AD. Inflammation is a key component of the innate immune response. Innate immunity is a highly conserved system that protects the host from infections and injury in a relatively non-specific manner. Chronic inflammation has been implicated not only in diseases of the periphery, but also in the central nervous system in neurodegenerative disorders such as AD.

It is widely recognized that age is the most important risk factor for AD and that the innate immune system plays a role in the development of neurodegeneration. Very little information is available on how aging affects the innate immune system. However, there are clear indications that the development of AD is due to age-related changes that modulate innate immunity. It is interesting that Aβ and other proteins found in the senile plaques of AD patients are potent activators of the innate immune response because chronic stimulation of the innate immune system may lead to alterations of astrocytes. When the brain is injured, astrocytes are believed to react by putting down glial scar tissue as part of the healing process. Recently, it has been shown that astrocytes themselves actively contribute to the inflammatory response (Farina, Aloisi et al. 2007).

It has been shown that the neurotransmitter glutamate is released in neuroinflammatory conditions and to some degree under normal circumstances, which on the long term is proved to be toxic to neurons. The neuroprotective action of astrocytes has also been attributed to their capacity to take up the neurotransmitter glutamate, convert it to glutamine, and recycle it to neurons (Vesce, Rossi et al. 2007).

2.2.1. Astrocytes secrete pro-inflammatory factors

Several studies have shown that chronically activated astrocytes are able to secrete several neurotrophic molecules and cytokines *in vitro* and *in vivo* in AD patients. In response to chronic inflammation, cultured astrocytes express a wide range of molecules with neurotrophic properties including various cytokines, the nervous growth factor (NGF), the glial-derived growth factor (GDGF), and ciliary neurotrophic factor (CNTF) and the class I and II MHC antigens (Kraus, Schneider-Schaulies et al. 1992; Merrill and Jonakait 1995).

In AD brains, the release of pro-inflammatory factors is suggested to be further enhanced. As previously described, astrocytes can be activated by Aβ, and IL-1β and IL-6 positive astrocytes can be found in close proximity of both fibrillary and diffuse Aβ deposits (Benzing, Wujek et al. 1999). In AD transgenic mice, reactive astrocytes are able to produce TGF-β1, TGF-β3 and IL-10 (Apelt, Ach et al. 2003). In addition, Smits et al. have showed that reactive astrocytes can secrete pro-inflammatory mediators such as monocyte chemoattractant protein-1 (MCP-1), CCL5 (RANTES), tumor necrosis factor-α (TNF-α) and IL-1 in response to stimulation with $A\beta_{42}$ (Smits, Rijsmus et al. 2002). This might indicate that a vicious circle of pro-inflammatory factors could down-regulate anti-inflammatory responses and neutralize protective mechanisms.

Reactive astrocytes are able to react to cytokines and can produce additional pro-inflammatory factors. Exposure of human astrocytes to microbial components such as lipopolysaccharide (LPS) and IL-1β stimulates inflammatory mediators. These include TNF-α, IL-6 and IL-12 (Bsibsi, Bajramovic et al. 2007). Furthermore, an astrocytic response to IL-1β

and TNF-α results in an active secretion of colony stimulating factor 1 (CSF-1), granulocyte-macrophage CSF (GM-CSF), granulocyte CSF (G-CSF) (Aloisi, Care et al. 1992), MCP-1 and MIP-1α (Savarin-Vuaillat and Ransohoff 2007), which can lead to the recruitment of microglial cells to Aβ plaques (Hurwitz, Lyman et al. 1995). In addition, the CXCL10 ligands, IL-10 and Mig, which bind the CXCR3 receptor are present in subpopulations of astrocytes associated with senile plaques in AD brains compared to controls (Xia, Bacskai et al. 2000).

2.2.2. Astrocytes interact with macrophages

While previous studies suggest that astrocytes may play a role in Aβ processing, their main function is thought to be associated with the release of pro-inflammatory products. Various factors are involved in the overall immune response such as cytokines, the complement system, acute phase reactants and various cellular elements, which in concert compose a powerful reaction. Cytokines associated with AD include several interleukins, TNF-α and TGF-β along with several others. Their production is increased in inflammatory states and they function by regulating the intensity and duration of the immune response (Tuppo and Arias 2005).

The cytokine class of inflammatory mediators is secreted by astrocytes surrounding Aβ plaques. Interactions between macrophages and astrocytes have been observed in AD, which result in chronically activated astrocytes killing adjacent neurons by the release of highly toxic products such as IL-1β, TNF-α, TGF-β, complement proteins and reactive oxygen species (Klegeris, Walker et al. 1994; Giulian, Haverkamp et al. 1995; Klegeris and McGeer 1997; Klegeris, Walker et al. 1997). Cytokines such as TNF-α and IL-1β can be directly toxic, when chronically produced at high concentrations (Jeohn, Kong et al. 1998). These cytokines can also stimulate the synthesis of APP or may in combinations with other cytokines stimulate the production of Aβ (Goldgaber, Harris et al. 1989; Blasko, Schmitt et al. 1997). TNF-α and IL-1β can induce the synthesis of Aβ-binding proteins in astrocytes (Lieb, Fiebich et al. 1996). Thus, the chronic release of pro-inflammatory cytokines in the brain is likely to maintain the chronic protein secretion of activated astrocytes inducing Aβ formation.

The review of Minagar et al. postulates a response cascade in which microphages, astrocytes and neurons are involved. Microphage activation by Aβ results in the production of superoxide anions, TNF-α, and IL-1β which are neurotoxic and activate astrocytes which produce IL-1β and NO. Aβ is also capable to directly activate astrocytes (Minagar, Shapshak et al. 2002), which subsequently produce chemokines, cytokines and reactive oxygen species that may cause neuronal cell damage (Smits, Rijsmus et al.

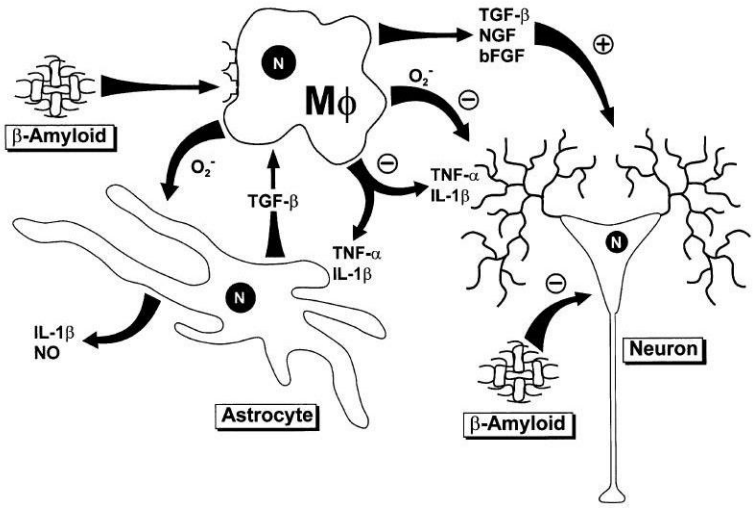

Figure 5. Scematic overview of astrocytes interacting with macrophages. Production of NO, IL-1β and TGF-β as a result of astrocyte activation by macrophages and Aβ. Adapted from Minagar et al. 2002

2002). The production of reactive oxygen species induces neurotoxicity in vitro (Meda, Cassatella et al. 1995) (Figure 5).

In addition, the astrocytes activated by macrophages produce four glial proteins interacting with $A\beta_{1-42}$ and subsequently activate surrounding astrocytes. These proteins concern alpha-1-anti-chymotrypsin (ACT), IL-1β, S100β and butyrylcholinesterase (BChE). Under the influence of IL-1β, astrocytes were found to upregulate the cytokine S100β in neuritic plaques (Mrak and Griffinbc 2001). IL-1β and IL-6 were able to induce human astrocytes to upregulate the gene for the acute phase protein α_1-antichymotrypsin (ACT) (Nilsson, Das et al. 2001). ACT mRNA was also shown to be expressed at a higher level by astrocytes in the gray matter of AD brains compared to controls and the ACT was shown to be tightly associated with Aβ plaques in AD brain (Pasternack, Abraham et al. 1989; Abraham 2001). ACT, IL-1β and S100β activate rat cortical astrocyte cultures and enhance the ability of Aβ to further activate the astrocytes, characterized by reactive morphology, upregulation of IL-1β and production of inducible NOS and NO. These proteins participate in the chronic activation of astrocytes in AD through their ability to stimulate these cells directly and to modulate Aβ-induced activation (Hu and Van Eldik 1999). In astrocytes, IL-1β induces IL-6 production, stimulates iNOS activity and induces the production of M-CSF (Aloisi, Care et al. 1992; Rossi and Bianchini 1996). In addition, IL-1β enhances neuronal acetylcholinesterase activity, microglial activation and additional IL-1β production, with consequent astrocyte activation and expression of the cytokine S100β by astrocytes thereby establishing a self propagating cycle (Mrak and Griffinbc 2001).

AD brains were found to have high levels of NOS positive astrocytes as compared to controls, suggesting increased production of NO in the AD brain (Simic, Lucassen et al. 2000). Aβ has been shown to activate cultured astrocytes to produce IL-1α, NOS-mRNA and NO (Hu, Akama et al. 1998). Under the stimulation of IL-1α and IL-1β, astrocytes have been shown to produce NO that leads to neuronal damage (Chao, Hu et al. 1996). Chemokines released by the astrocytes attract microglia, which further express pro-inflammatory products contributing to additional neuronal cell damage.

The presence of astrocytes suppresses the activity of iNOS produced by macrophages, probably due to TGF-β. Induction of iNOS by Aβ is associated with selective loss of cholinergic neurons (Vincent, Tilders et al. 1997; Weldon, Maggio et al. 1997). Thus, astrocytes may have protective effects in the neuropathogenesis of AD by inhibiting macrophage iNOS.

2.2.3. Astrocytes interact with microglia

Astrocytes are able to secrete immune modulators including CCL2, CXCL10, IL-6, IL-1β, IFN-γ, and TGF-β (Apelt and Schliebs 2001; Farina, Aloisi et al. 2007). Several of these molecules are involved in pathways for astrocytic interactions with microglia. The pro-inflammatory cytokines IFN-γ and TNF-α, produced by astrocytes, stimulate pro-inflammatory microglial activation, while in contrast, TGF-β and IL-1β modulate microglial activity towards an anti-inflammatory response and stimulate clearance of Aβ plaques (Basu, Krady et al. 2002; Lemere 2007; Shaftel, Kyrkanides et al. 2007).

Furthermore, CCL2 and CXCL10 produced by astrocytes, may modulate activity as well as migration of microglial cells expressing the receptors CCR2 and CXCR3. The importance of the CCL2-CCR2 ligand receptor complex in AD has been investigated in an APP mouse model

lacking CCR2, which demonstrated that together with a reduction in the number of microglia, Aβ clearance was reduced (El Khoury, Toft et al. 2007). Fractalkine (CX3CL1) can be produced by astrocytes and neurons and its receptor CX3CR1 is expressed exclusively by microglia (Cardona, Pioro et al. 2006). Mizuno et al. demonstrated that fractalkine secretion leads to the inhibition of neurotoxicity (Mizuno, Kawanokuchi et al. 2003), and deficiency of CX3CR1 resulted in increased neuronal damage in *in vivo* mouse models of neurotoxicity (Cardona, Pioro et al. 2006).

Min et al. showed that when microglial cultures were treated with astrocyte conditioned media, iNOS expression and ROS production by the microglia was reduced (Min, Yang et al. 2006). They proposed that this effect was created by an increased expression of microglial antioxidant enzymes, such as heme oxygenase-1. The responsible factor in conditioned medium was identified as a small, heat labile active component possibly similar to neutrophil-producing soluble factor (Min, Yang et al. 2006).

2.2.4. Cytokines on APP processing

Several studies investigated the influence of cytokines on the processing of APP by astrocytes. APP synthesis by astrocytes can be stimulated by cytokines such as IL-1β, TNFα, or TGF-β1 through regulation of gene transcription at the promoter level (Amara, Junaid et al. 1999; Ge and Lahiri 2002). Combinations of cytokines such as IL-1β or TNFα and IFN-γ are able to affect the metabolism of APP and to stimulate the production of $A\beta_{40-42}$ peptides (Blasko, Schmitt et al. 1997; Sastre, Dewachter et al. 2003). This effect can be reversed by addition of specific nonsteroidal anti-inflammatory drugs (NSAIDs) (Sastre, Dewachter et al. 2003). The mechanism by which cytokines stimulate Aβ production remains to be elucidated, but it is likely that the maturation of the APP protein is disturbed and β-secretase activity is stimulated (Blasko, Schmitt et al. 1997; Sastre, Dewachter et al. 2003).

2.2.5. Astrocyte senescence

Late-onset AD is mediated by a variety of different factors and little is known about the molecular mechanism that underlie age-related changes of innate immunity and how they affect brain pathology. Aging is characteristically accompanied by a shift within innate immunity towards a pro-inflammatory status. Late-onset AD is not only induced by overproduction of toxic metabolites but the decreased capacity of aged glial cells to degrade toxic products is as well involved. Blasko et al. isolated *post mortem* astrocytes from white matter obtained from seven donors with AD and investigated the capacity of population-doublings (Blasko, Stampfer-Kountchev et al. 2004). Astrocytes of these AD patients proliferated rigorously in culture and underwent 6-8 population doublings (PDs) before proliferation decreased and astrocytes stopped dividing. Growth arrest was reached after 9-10 PDs. Therefore, astrocytes are referred to as 'late passage astrocytes' after 8 PD. Throughout the duration of the cultures, astrocytes constantly expressed the glial fibrillary acidic protein (GFAP), a maker for astrocytes. Late passage astrocytes expressed more GFAP in comparison with early passage astrocytes (Nichols, Finch et al. 1995) which is in agreement with a higher GFAP *in vivo* content in aged rats and mice compared with their young littermates (Kyrkanides, O'Banion et al. 2001). In addition, late passage astrocytes show an increased expression of senescence markers such as p16, p21 and cyclin D1 (Morisaki, Ando et al. 1999; Wainwright, Lasorella et al. 2001). Gray matter astrocytes display different characteristics such as better proliferation

than their white counterparts. The number of PDs reached by grey matter astrocytes was significantly higher than by white matter astrocytes. After reaching the end of their replicative lifespan, astrocytes are prone to undergo apoptosis.

To evaluate whether cellular senescence changes the capacity of astrocytes to respond to cytokines and to produce Aβ, astrocytes were stimulated with TNF-α and IFN-γ. Late passage astrocytes were still capable of producing Aβ peptides, demonstrating that stimulation of Aβ production by inflammatory products does not decrease with aging (Blasko, Stampfer-Kountchev et al. 2004).

2.2.6. Astrocytes secrete anti-inflammatory factors

Since accumulation of Aβ deposits contributes to the AD pathogenesis, a great amount of studies has been done regarding degradation of Aβ by astrocytes. Bard et al. has demonstrated that astrocytes are able to remove and to degrade Aβ without any mediators (Bard, Cannon et al. 2000). Activated astrocytes are able to produce trophic factors such as NGF, basic fibroblast growth factor (bFGF), S100β, the brain derived neurotrophic factor (BDNF), neurotrophin 3 (NT-3) and neurotrophin 4/5 (NT-4/5), which have all shown to have a trophic effect on neurons (Aguado, Ballabriga et al. 1998; Blondel, Collin et al. 2000; Hock, Heese et al. 2000; Mrak and Griffinbc 2001). The high flexibility of astrocytes to express different molecules in response to the environment shows the importance of astrocytes in the brain. The astrocytes in young transgenic animals overexpressing human Aβ peptides produce small amounts of the Aβ-degrading enzyme neprilysine. However, as the animals age and the amounts of the Aβ increase, the expression of neprilysine is stimulated (Blondel, Collin et al. 2000).

Astrocytosis is a morphological feature of the AD brain and represents proliferation of astrocytes in response to replace dying neurons or as a reaction to degrade the increasing amounts of toxic proteins. It is suggested that MCP-1 plays an important role in astrocytosis, since its levels were found to be increased after brain injury in contrast to the levels of pro-inflammatory cytokines (Little, Benkovic et al. 2002). The degradation of Aβ regarding aging is of interest since adult mouse astrocytes do not respond to stimulation with Aβ by increasing their release of the chemokine MCP-1 in the same way as young cells (Wyss-Coray, Loike et al. 2003). In contrast to neonatal mouse astrocytes, adult mouse astrocytes are able to effectively clear surface-bound Aβ and are capable of removing Aβ depositions (Wyss-Coray, Loike et al. 2003).

2.3. Astrocyte receptors

In patients with late-onset AD, inefficient clearance of Aβ seems to be the key event leading to accumulation of Aβ in the brain, rather than increased Aβ production (Selkoe 2001). To clear Aβ from the brain and prevent neurotoxicity, Aβ has to be transported across the BBB. Therefore, accumulation of Aβ in the vessel walls is the primary pathogenic event in AD and results in degeneration of cerebrovascular cells and disruption of the BBB (Davis-Salinas, Saporito-Irwin et al. 1995; Verbeek, de Waal et al. 1997). Vascular Aβ receptors, expressed by endothelial cells, transfer Aβ across the BBB into the circulation and thus mediate clearance of Aβ from the brain (Zlokovic 2004). Aβ receptors may also mediate Aβ clearance via phagocytosis by astrocytes (Nagele, D'Andrea et al. 2003). Farina et al. demonstrated that astrocytes display an array of receptors involved in innate immunity, including TLR, nucleotide-binding oligomerization domains, double-stranded RNA-dependent protein kinase, SRs, mannose receptors and components of the complement system (Farina, Aloisi et al. 2007).

Recently two crucial receptors are found to may act as Aβ receptors, including the low-density lipoprotein receptor (LDLR) and the LDLR-related protein-1 (LRP-1) (Arelin, Kinoshita et al. 2002; Fryer, Demattos et al. 2005). LDLR is shown to regulate apolipoprotein E (ApoE) levels in the CNS and LDLR-deficient mice show learning deficits and increased Aβ accumulation (Cao, Fukuchi et al. 2006), suggesting a crucial role for ApoE in Aβ clearance. LRP-1 is known to bind both Aβ and ApoE-Aβ complexes, which results to clearance from brain to blood (Shibata, Yamada et al. 2000; Zlokovic 2004). In contrast, the receptor for advanced glycation end products (RAGE) binds and transports Aβ from blood to brain (Lue, Yan et al. 2005). Wilhelmus et al. investigated the effect of Aβ receptors in cerebrovascular cells and astrocytes. They showed that astrocytes express several receptors contributing to Aβ transportation across the BBB (Table 1). They reported that LRP-1 and LDLR expression in astrocytes was not increased in response to cerebral Aβ exposure, however astrocytes do internalize Aβ, which in large quantities mediates cell death. Furthermore, in contrast to cerebrovascular cells, both internalization of Aβ and degeneration of astrocytes was not inhibited by receptor activated protein (RAP), which inhibits the function of LRP-1 and LDLR. This suggests that receptor stability in astrocytes of both LRP-1 or LDLR differs from cerebrovascular cells or that Aβ receptors other than LRP-1 or LDLR, or receptor-independent mechanisms are involved in the internalization of Aβ by astrocytes. Thus, the exact roles of these Aβ receptors in Aβ internalization and Aβ-mediated cell death toward astrocytes remain to be elucidated (Wilhelmus, Otte-Holler et al. 2007).

2.3.1. Apolipoprotein E

As mentioned before, ApoE is strongly related to AD from several perspectives. ApoE is a major lipid carrier protein in the brain, which is synthesized and secreted primarily by astrocytes and is involved in brain development and repair (Fagan and Holtzman 2000; Kuo, Kokjohn et al. 2001). In the AD

Table 1.

	Astrocytes
APP	+
MDR1	−
RAGE	+
LRP-1	+
Megalin	+
CD36	+
FPRL1	+
LDLR	+

Table 1. Expression of Aβ receptors in astrocytes. Adapted from Wilhelmus et al. 1993

brain, Aβ deposits are strongly stained for ApoE and are ringed by activated astrocytes. Recent studies suggests that the ability of astrocytes to phagocytize Aβ depends on ApoE, suggesting that ApoE polymorphisms may influence the risk to develop AD by affecting astroglial Aβ phagocytosis (Niino, Iwabuchi et al. 2001). As previously stated, ApoE is implicated in the clearance of Aβ by forming an ApoE-Aβ complex and binding the receptor LRP-1 on astrocytes, mediating phagocytosis. However, the isoform ApoE ε4 is involved in promoting Aβ deposition, which is suggested by the genetic predisposition for AD and the increased Aβ burden in AD brains of patients carrying the ApoE ε4 allele.

LRP-1 plays an important role in the balance between Aβ synthesis and clearance mechanisms (Shibata, Yamada et al. 2000). LRP-1 has been implicated as an ApoE receptor in AD pathophysiology. LRP-1 is a member of the LDL-receptor family which are able to bind multiple ligands, including ApoE and APP. After binding, these ligands undergo endocytosis and degradation. In AD, LRP-1 is upregulated in activated astrocytes and associated with Aβ plaques (Rebeck, Reiter et al. 1993; Donahue, Flaherty et al. 2006). Arélin et al. examined the relationship between LRP-1 and Aβ plaques in both transgenic mice and human AD (Arelin, Kinoshita et al. 2002). They detected strong LRP-1 staining in reactive astrocytes and immunostaining of membrane-bound LRP-1 showed colocalization with fine astrocytic processes surrounding Aβ plaques. They found no LRP-1 present in plaques of young PDAPP transgenic mice or in plaques of ApoE-knockout mice. This data suggests that LRP-1 ligands associated with Aβ plaques in AD brain may play important roles in inducing levels of LRP-1 in astrocytes. This supports the idea that ApoE might be involved in the upregulation of LRP-1 present in astrocytic processes and could act as a local scaffolding protein for LRP-1 and Aβ. The upregulation of LRP-1 would allow increased clearance of LRP-1 ligands as well as clearance of Aβ-ApoE complexes (Arelin, Kinoshita et al. 2002).

The isoforms apolipoprotein ε4 (ApoEε4) and apolipoprotein ε3 (ApoEε3) are a risk factor for developing AD at an earlier age and might contribute to this effect (Fagan and Holtzman 2000). Koistinaho et al. has demonstrated that ApoEε3 promotes astrocyte colocalization and degradation of Aβ deposits. In contrast, they showed that high expression of ApoEε3 in astrocytes disrupts their clearance mechanism of Aβ (Koistinaho, Lin et al. 2004). Furthermore, high expression of ApoEε4 in astrocytes can reduce their response to Aβ deposits (Guo, Wang et al. 2006). Thus, ApoEε4 and ApoEε3 seem to be important in the degradation and clearance of deposited Aβ species by astrocytes in AD. Additional studies demonstrating that ApoEε4 is involved with Aβ deposition comes from studies with APPV717F transgenic mice, in wildtype and ApoE-knockout backgrounds. ApoEε4-knockout APPV717F transgenic mice do not develop fibrillar Aβ deposits and have a lesser amyloid burden, indicating the important role ApoEε4 plays in laying down extracellular Aβ (Holtzman, Fagan et al. 2000). These data suggest that ApoEε4 mediates fibrillar, compact Aβ deposits.

2.3.2. Apolipoprotein E alleles

In most patients AD occurs later in life, suggesting that the disease is clearly not inherited in any simple sense. Several families with the late onset form of the inherited disease exhibited an association gene encoding an isoform of ApoE on chromosome 19 (Corder, Saunders et al. 1993). There are three major alleles of ApoE, ε2, ε3 and ε4. The frequency of the ε4 allele in late-onset familial AD patients is almost four time higher than the general population. People homozygous for ε4 are 8 times more likely to develop AD

compared to individual homozygous for ε3. An increased association of the ε4 allele has been shown in the sporadic form of AD (Corder, Saunders et al. 1993) (Tables 2 and 3). Inheriting the ε4 form of ApoE is not sufficient to cause AD, rather inheriting this gene simply increases the risk of developing AD. In addition, some patients with early-onset forms of familial AD do not have the ε4 allele.

Table 2. Frequency of ApoE alleles in Ad and control cases

	ε2	*ε3*	*ε4*
AD cases	0.02	0.53	0.45
Controls	0.05	0.88	0.07

Table 3. Frequency of ApoE alleles in AD and control cases

	–/–	*–/ε4*	*ε4/ε4*
AD cases	0.321	0.464	0.215
Controls	0.868	0.132	0

Adapted from Chalmers et al. 2003

2.4. Additional evidence

Various additional arguments rise for astrocytic implications in AD, discussed below.

2.4.1. Postmortem studies

Activated microglia and reactive astrocytes are commonly observed in postmortem AD brains (McGeer, Itagaki et al. 1988). McGeer et al. has observed increased expression of specific markers for reactive astrocytes, such as MHC class II and GFAP in postmortem AD brains compared to control brains (McGeer, Itagaki et al. 1988; Wyss-Coray and Mucke 2002). In the human AD brain, activated microglia and reactive astrocytes have been shown to contain Aβ fragments, providing evidence of phagocytic degradation of Aβ (Funato, Yoshimura et al. 1998). Astrocytes that migrated into Aβ plaques are promoted by the presence of MCP-1 (Wyss-Coray, Loike et al. 2003), a chemokine that is released by activated microglia and astrocytes surrounding Aβ plaques in AD brain (Ishizuka, Kimura et al. 1997). As a result, astrocytes are recruited to Aβ plaques and further contribute to degradation and clearance of plaques, which has been demonstrated in several *in vitro* studies (Shaffer, Dority et al. 1995).

2.4.2. A rodent model for AD

Estrogen deprivation and oxidative stress, are closely related to the pathological development of AD (Gandy 2003). These two factors have synergic effects on accelerating the progression of AD (Hua, Lei et al. 2007). Hua et al. have demonstrated that long-term D-galactose injection combined with ovariectomy may serve as a rodent model for AD (Hua, Lei et al. 2008). Intraperitoneal administration of D-galactose (D-gal) for six weeks results in cognitive and memory impairments, forebrain cholinergic deficits, deposition of Aβ and intracellular neurofibrillar tangles in ovariectomized Sprague-Dawley rats (Hua, Lei et al. 2007). Because both estrogen deprivation and oxidative stress could activate astrocytes, it is likely that there are pathological and biochemical alterations of astrocytes in AD development (Lei, Long et al. 2003). Hua et al. addressed the potential contribution of astrocytes to the AD pathogenesis by investigation of the pathological and biochemical alterations of astrocytes (Hua, Lei et al. 2008). Ovariectomized rats injected with D-gal for two weeks showed extensive localization of immunoreactive astrocytes without memory impairments. Ovariectomized rats injected with D-gal for six weeks however, exhibited degeneration of astrocytes accompanied with severe impairments to behavioral test and deficiency of cholinergic terminals. Electron microscopy confirmed pathological alterations of astrocytes, including swollen mitochondria and reduction of glial filaments in the cytoplasm of astrocytic processes in ovariectomized rats injected with D-gal for six weeks. These findings indicate that biochemical and pathological alterations of astrocytes may partially contribute to exacerbating neuronal deficits in the course of AD. Restoring neuroprotective potential of astrocytes may be a useful therapeutic target for AD.

2.4.3. Aquaporin-1 in astrocytes

The aquaporins (AQPs) constitute a family of integral channel proteins that facilitate the osmotic driven bidirectional water transport across the cell membrane (Amiry-Moghaddam and Ottersen 2003). Although AQP1 is expressed widely in cascular endothelial cells outside the CNS, the levels of AQP1 expression are very low in brain endothelial cells, and no expression is found in cultured rodent astrocytes (Dolman, Drndarski et al. 2005). However, increasing evidence indicates that astrocytes express APQ1 in CNS under pathological conditions. A recent study

showed that the expression of AQP1 but not of APQ4 is enhanced in cortical astrocytes at the early stage of AD, suggesting a pathological role of abnormal regulation of water transport in AD (Perez, Barrachina et al. 2007). Misawa et al. investigated the possible relationship between Aβ deposition and AQP1 expression in astrocytes in the cerebral cortex of AD patients (Misawa, Arima et al. 2008). In all cases, AQP1 was expressed exclusively in a subpopulation of multipolar fibrillary astrocytes. The great majority of AQP1 expressing astrocytes were located either on the top of or in close proximity to Aβ plaques in AD brains but not in control cases. They found that cultured human astrocytes constitutively expressed AQP1 and the levels of AQP1 protein were not affected by exposure of Aβ, but were elevated by hypertonic sodium chloride (Misawa, Arima et al. 2008). These observations suggest the possible association of astrocyte AQP1 with Aβ deposition in AD brains.

2.4.4. Prostaglandin transporter in astrocytes

In AD patients, chronic stress stimulates the generation of free radicals and glutamate, triggering inflammatory pathways that lead to increases in chemokines, cytokines and prostaglandins. The inflammatory mediators produced include cyclooxygenase (COX) 1 and 2 and prostaglandins. Thus, it has been reported that COX activity and prostaglandin levels are higher in the brains of AD patients than in control brains (Consilvio, Vincent et al. 2004). Prostaglandins are lipid mediators of inflammation that are produced from arachidonic acid by COX enzymes and are carried across the cell membrane by the prostaglandin transporter (PGT). PGT has been identified and shown to have a role in the release of newly synthesized prostaglandins from cells, the transepithelial transport of prostaglandins, and the clearance of prostaglandins from the circulation of the termination of signaling (Kanai, Lu et al. 1995). Choi et al. have examined the middle frontal gyrus brain tissue from patients diagnosed with AD and that of age-matched control brains to determine the protein expression pattern of PGT and its possible role in modulating neuroinflammation associated with AD. They reported for the first time, that PGT protein was expressed in all brain tissues examined and was localized in neurons, microglia and astrocytes. In addition, the PGT level was significantly less in AD than in age-matched controm brains (Choi, Zhuang et al. 2008). At this point, it is still unclear why the level of PGT protein would be lower in AD, but it is likely that PGT is associated with the neuroinflammatory process.

3. Discussion

Astrocytes fulfill many functions, one of the main being the repairing and scarring process in the brain after a neurodegenerative disease. Various studies demonstrate that astrocytes are highly implicated in the progression of AD. However, much of the found data remains questionable.

For example, although it is generally accepted that Aβ deposition activates astrocytes, this could be an early event in the disease, occurring even in the absence of focal Aβ deposition (Nunomura, Perry et al. 2001). In support of this hypothesis, a clinical study comparing PET and volumetric magnetic resonance imaging of the brain in patients with mild to moderate dementia to healthy individuals suggests that astrocyte activation is an early event in the pathogenesis of AD (Cagnin, Brooks et al. 2001). In parallel, it has been recently reported that focal glial activation precedes amyloid plaque deposition in APPV717I transgenic mice at 3 months of age (Heneka, Sastre et al. 2005). Because these animals show both cognitive deficits and focal glial cytokine production well before Aβ plaque deposition (Moechars, Dewachter et al. 1999), it seems likely that senile plaques are, at least at the beginning of the disease, not the cause of glial activation, but rather a response to Aβ oligomers or protofibrils (White, Manelli et al. 2005). Interestingly, young APPV717I mice show a significant decrease of hippocampal long-term potentiation (LTP), a mechanism essential for memory storage and consolidation. Because cytokines such as TNF-α, IL-1β and IL-6 directly impair neuronal function and suppress hippocampal LTP early focal inflammatory events may contribute to neuronal dysfunction well before neuronal cell death and parenchymal volume reduction become apparent (Murray and Lynch 1998).

Aβ accumulation found in astrocytes could suggest either that astrocytes are involved in the formation or degradation of Aβ plaques. Nagele et al. found that Aβ is degraded in astrocytes (Nestor, Scheltens et al. 2004), while Rossner et al. shows production of Aβ in astrocytes (Rossner, Apelt et al. 2001). Astrocytes are able to release pro-inflammatory and anti-inflammatory in AD brains. This contradicting evidence demonstrates the complexity of astrocytes involved in AD.

4. Conclusion

Activated astrocytes have been found to accumulate around Aβ plaques, suggesting that astrocytes are involved in the processing of Aβ. Nagele et al. has shown that accumulated Aβ by activated astrocytes are degraded in the lysosome (Nagele, D'Andrea et al. 2003). However, too much accumulation Aβ by activated astrocytes results in cell death, releasing intracellular debris and Aβ and contributing to astrocytic plaque formation. Other studies as well reported the astrocytic degrading properties of Aβ (Yamaguchi, Sugihara et al. 1998; Wyss-Coray, Loike et al. 2003).

In addition to Aβ processing, astrocytes are also involved in the immune response of AD brains. Together with microglia and macrophages, astrocytes have been reported to secrete various pro- and anti-inflammatory factors, affecting the production and processing of Aβ. Astrocytes produce ApoE, which is involved in phagocytosis of Aβ by astrocytes. The isofoms of ApoE are a risk factor for developing AD at an earlier age. Recently, astrocytes were found to express AQP1 and PGT in the CNS under pathological conditions (Perez, Barrachina et al. 2007).

These results suggest with regard to the BBB, that BBB disruption in AD is partly due to the altered activity of activated astrocytes. Aβ accumulates in the vessel walls in AD (Davis-Salinas, Saporito-Irwin et al. 1995; Verbeek, de Waal et al. 1997), which suggests that the endothelial cells and perivascular astrocytes are unable to extensively clear the accumulated Aβ.

Astrocytes are unable to efficiently degrade great amounts of Aβ, resulting in lysis and generation of astrocytic plaques. In addition, activated astrocytes are involved in processing APP and generating Aβ plaques (Rossner, Apelt et al. 2001; Nagele, D'Andrea et al. 2003). Isoforms of ApoE disturb the phagocytotic activity of astrocytes and are implicated in accumulation of Aβ (Niino, Iwabuchi et al. 2001). Deposits of these great amounts of Aβ creates a great burden for the BBB, resulting in BBB disruption.

Since Aβ has been considered to play a key role in AD pathogenesis (Walsh and Selkoe 2004), it still remains unclear whether Aβ plaques and neurofibrillary tangles are causative for AD. These doubts are fueled by the finding that the Aβ plaque burden poorly correlates with the progression and severity of dementia in AD. Moreover, transgenic animals that develop widespread mutations show only slight cognitive deficits (Davis and Laroche 2003). Thus, these results suggest that the complexity of the astrocyte contribution in AD is ample and the direct mechanism by which astrocytes contribute to AD progression is unknown. The role of astrocytes in the pathogenesis of AD remains to be determined and may differ on a case to case instance due to dependence on a broad spectrum of interactive events in neurons, astrocytes and microglia.

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