Astrocytes: Implications for Neuroinflammatory Pathogenesis of Alzheimer's Disease

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Abstract: Alzheimer's disease (AD) is a neurodegenerative disease with major clinical hallmarks of memory loss, dementia, and cognitive impairment. Neuroinflammation is involved in the onset of several neurodegenerative disorders. Astrocyte is the most abundant type of glial cells in the central nervous system (CNS) and appears to be involved in the induction of neuroinflammation. Under stress and injury, astrocytes become astrogliotic leading to an upregulation of the expression of proinflammatory cytokines and chemokines, which are associated with the pathogenesis of AD. Cytokines and related molecules play roles in both neuroprotection and neurodegeneration in the CNS. During early AD pathogenesis, amyloid beta (Aβ), S100B and IL-1β could bring about a vicious cycle of Aβ generation between astrocytes and neurons leading to chronic, sustained and progressive neuroinflammation. In advanced stages of AD, TRAIL secreted from astrocytes have been shown to bind to death receptor 5 (DR5) on neurons to trigger apoptosis in a caspase-8-dependent manner. Furthermore, astrocytes could be reactivated by TGFβ1 to generate more Aβ and to undergo the aggravating astrogliosis. TGFβ2 was also observed to cooperate with Aβ to cause neuronal demise by destroying the stability of lysosomes in neurons. Inflammatory molecules can be either potential biomarkers for diagnosis or target molecules for therapeutic intervention. Understanding their roles and their relationship with activated astrocytes is particularly important for attenuating neuroinflammation in the early stage of AD. The main purpose of this review is to provide a comprehensive insight into the role of astrocytes in the neuroinflammatory pathogenesis of AD.

Keywords: Alzheimer's disease, astrocyte, inflammation, cytokine, chemokine, amyloid beta.

INTRODUCTION

Alzheimer's disease (AD) is an irreversible, progressive, neurodegenerative disease. Memory loss is one of the earliest symptoms of AD, along with a gradual decline of other intellectual abilities and most notably changes in personality and behavior. People suffering from AD exhibit dementia, which is classified as a group of brain disorders that seriously impair cognition [1-3]. There are approximate 50 brain diseases that can cause dementia, of which AD is the most common for the elderly population, accounting for around 50-70 percent of all dementia cases [1, 4]. The prevalence of dementia rises with age, doubling every 5 years between the ages of 60 and 90. An estimated 35.6 million people worldwide suffered from AD and this number will quadruple by 2050 (World Alzheimer Report 2009, http://www.alz.co.uk/research/worldreport).

Three major neuropathological hallmarks have been identified in the brain of AD patients, amyloid plaques, neurofibrillary tangles (NFTs), and astrogliosis [3, 5]. Based on previous amounting studies, researchers have postulated four main hypotheses for AD pathogenesis, cholinergic deficiency, amyloid deposition, tau protein hyperphosphorylation, and neuroimmunomodulation. Cholinergic deficiency was the oldest hypothesis, but has not been strongly supported by clinical and experimental investigations [6-8]. Although amyloid and tau hypotheses could explain the neuropathological alterations during the late stage of AD, they provide little insight into the early events in AD. There have been strong evidences demonstrating that distress accumulated in cells could initiate an early activation of the innate cellular immunity and the inflammatory cascade [1]. These events might account for the early triggering events in AD pathogenesis and contribute to neuroinflammatory impairment in AD pathology [1, 9]. It forms the basis of the neuroimmunomodulatory hypothesis.

More recent investigations have revealed an active interaction of the CNS with the immune system through cytokines and other inflammatory factors [10]. However, the cellular sources of cytokines have not been clearly defined. Among the many brain cells, microglia and astrocytes are considered the best candidates. They could be activated by various stimuli and insults to secrete cytokines that modulate

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the survival of neurons in the brain and have been found to be closely associated with amyloid plaques. The secreted proinflammatory factors from these cells have been implicated in the chronic neuroinflammation that mediates AD neurodegeneration. Astrocytes outnumber microglia in the brain and its reactivation has been believed to last longer than microglia, suggesting that astrocytes may have a more important and sustained role than microglia in the enduring neuroinflammatory process in AD. In this review, we focused on the neuroinflammatory role of astrocytes in AD pathogenesis, with emphasis on some cytokines and chemokines that are known to be secreted by astrocytes.

ASTROCYTES AND AD

Astrocytes have traditionally been thought to serve others in the nervous system and to regulate and optimize the environment within which neurons function. We have summarized their functions in Table **1**. Astrocytes maintain tight control on local ion and pH homeostasis, deliver glucose and provide metabolic substrates to neurons. They also clean up neuronal wastes, which include not only metabolic byproducts but also neurotransmitters released during synaptic transmission through active uptake. In other words, they are multifunctional housekeeping cells. Recent advances in research on astrocytes instigated us to reconsider astrocytes as dynamic regulators of neuronal activities; i.e., astrocytes, in addition to just cleaning up the neuron's mess, might also be telling them what to do [11-14]. The major role played by astrocytes in the molecular and cellular pathogenesis of many specific degenerative diseases in the CNS was further confirmed by the recent identification of astrocytes carrying mutated superoxide dismutase I (SOD1) as the cause of death of motor neuron in some cases of amyotrophic lateral sclerosis [15] (Table **1**).

One characteristic of astrocytes that deserves to be emphasized here is their capability to repair and form scar following traumatic and inflammatory injuries (Table **1**). Astrocytes are activated to display "reactive astrogliosis" around site of injury, a process that leads to excessive scar formation and interferes with the neuronal recovery process. Astrogliosis is probably a response to stress stimulators, such as anomalous amount of glutamate, blood lipid disorders, folic acid deficiency, K^+ efflux and oxidative stress [1, 16-18]. Reactive astrocytes would become hypertrophic and hyperplastic, concomitant with anomalous functions of astrocytes, including glutamate uptake [2, 19]. Other noticeable changes in reactive astrocytes include an elevation of expression of glial fibrillary acidic protein (GFAP) and an increase in actin polymerization and cytoskeletal protrusions [20]. In the AD brain, it has been shown that Aβ could trigger astrocytes reactivation. Moreover, these reactive astrocytes are the main cellular components found to surround and penetrate the senile plaques with their projections and processes replacing the dead or dying neuronal cells [21]. We previously used a scratch wound model and an ischemia model to investigate the fate of some proinflammatory factors in astrocytes in primary culture. These *in vitro* models allowed experimenters to study the pure response of a single cell type to physical or metabolic injuries without the exogenous influence of blood borne factors in the *in vivo* trauma and ischemia models [22-27]. Cytokines were detected in the astrocytes and its culture medium [28]. These findings provided the first evidence that astrocytes, without the influence from other cell types, could produce and release interleukin-1α (IL-1α), interleukin-6 (IL-6), tumor necrosis factor α (TNFα), and interferon γ (IFN-γ) following mechanical and ischemia insults [28] (Table **2**). Reactive astrocytes are known to be different from quiescent astrocytes. During the reactivation process, they are induced to release and/or overproduce many different factors including growth factors, cytokines, chemokines and so forth [1, 18, 29] (Table **2**). One of the main functions of these chemokines relates to the recruitment of leukocytes to inflammatory sites. It is therefore especially important that the astrocytes in the blood brain barrier (BBB) start to release cytokines and chemokines; as these chemokines would attract leukocytes across the BBB to initiate neuroinflammation in the CNS [1, 12, 30].

Astrocytes have been shown to be activated by many pathogenic factors to overproduce many proinflammatory cytokines and chemokines, such as S100B, IL-1β, IL-6, TNFα, interferon-γ-inducible protein-10 (IP-10), and transforming growth factor (TGF). These factors could be involved in the neuroinflammatory processes of AD [1, 31-34] (Fig. **1**, Table **2**). The sensitive reactivation process and the inducible overproduction of proinflammatory mediators make astrocyte a very important contributor to the neuroinflammation in the early stage of AD pathogenesis.

AMYLOID BETA AND ASTROCYTES

Amyloid precursor protein (APP) is an integral transmembrane protein expressed in the CNS and has been found to be concentrated in the synapses between neurons (Fig. **1**). It serves as a receptor on the surface of neurons and has substantial physiological roles in neuritic growth, synaptic formation and neuronal repair. Therefore, the wellbeing of APP is concomitant with the enhancement of memory performance and long-term potentiation [35, 36]. APP can be cleaved by α-secretase to produce APPα (Fig. **1**), which is secreted in its soluble form and can protect neurons from excitotoxic, metabolic, and oxidative insults [37]. APP can also be cleaved sequentially by β- and γ-secretases to generate soluble APPβ and Aβ [3, 5] (Fig. **1**). The physiological role of APPβ has not been clarified yet. Aβ is a 39- to 42-amino acid peptide located in the membrane-spanning domain of APP [38, 39]. The most common isoforms of Aβ are Aβ40 and Aβ42 whereas Aβ42 is a neurotoxic fibrillar peptide and the primary component of amyloid plaques. Aβ42, more amyloidogenic compared to Aβ40, was found to exist as a tangle of insoluble amyloid fibrillary aggregates that elicit inflammatory responses, tau protein aggregation and oxidative stress in AD neuropathology [40-43]. Aβ, therefore, is considered as one of the major causative agents in the development of AD.

Aβ could be generated from both astrocytes and neurons through the stimulation with various cytokines [17, 44, 45]. Neuron is the predominant source of Aβ production in the brain due to the specific localization of APP to the neuronal membrane surface [17, 45]. However, astrocytes could also be induced by proinflammatory stimuli to produce Aβ. It has been demonstrated that IFN-γ in combination with TNFα or IL-1β could induce primary human astrocytes or astrocytoma

Table 1. The Functions of Astrocyte

Astrocyte functions		
	Physiological functions [References]	**Pathological functions [References]**
Widely accepted functions	• Support and isolation [154] • Metabolic support [155] • Sequestration and/or redistribution of K^+ during neural activity [154] • Removal of glutamate and GABA at synapses [156] • Synthesis of precursor for glutamate and GABA production [157] • Ammonium detoxification [158] • Promoting neuronal survival and maturation [159, 160] • Regulation of synaptogenesis and angiogenesis [154, 161] • Blood brain barrier (BBB) induction and maintenance [154]	• Alexander disease (the formation of Rosenthal fibers) [162] • Cytotoxic brain edema (phagocytosis function) [163] • Glioma formation (astrocyte differentiation) [164] • Failure of extracellular glutamate homeostasis [165]
Probable and emerging functions	• Providing energy to neurons [166] • Sharing energy substrate derived from glycogen with neurons [167] • Brain water homeostasis [168] • Sensing neuronal activity [169] • Regulation of blood flow [170] • Regulation of extracellular pH [171] • Modulation of excitatory and inhibitory synapses [172] • Control of BBB permeability, metabolism and vascular tone [173, 174] • Dynamic control of synaptic structure [175] • The stem cells in the adult neurogenic zone [176] • Regulation of neurogenesis in adult brain (e.g. hippocampus) [177] • Detoxification of brain free radical species [178] • Fast modulation of synaptic transmission [179]	• Hepatic encephalopathy [180] • Modulation of stroke outcome (scavenging free-radical, glutamate homeostasis, expression connexins, releasing inflammatory factors) [14] • Trophic modulation of post-injury neural repair and axon regrowth [181] • Release of cytokines and chemokines [182] • Neuroinflammation [183] • Forming "glial scar" after injury [184] • Playing roles in various neurodegenerative disorders: Alzheimer's disease (degrading Aβ protein, releasing kinds of cytokines and chemokines, astrogliosis and interaction with activated microglia) [51, 185, 186] Parkinson's disease (neuroinflammation) [187] Huntington's disease (astrogliosis and neuroinflammation) [188] Amyotrophic lateral sclerosis (astrocytes express mutated SOD1, astrogliosis) [15, 189] Multiple sclerosis (neuroinflammation and interaction with activated microglia) [122, 190] Epilepsy (altering expression of glutamate and GABA transporters, decreasing glutamate reuptake) [191-193] HIV-associated dementia (enhancing adhesion molecules expression, upregulating production of various chemokines and cytokines) [194-196]

cells to produce Aβ [44]. The astrocyte-secreted proinflammatory factors could promote the expression level of secretases, thereby enhancing the conversion of APP on the membrane of neurons into neurotoxic insoluble fibrillary Aβ [3, 5] (Fig. **1**). Exogenous Aβ could induce astrocytes to produce more inflammation-like glial responses that could sustain neurodegenerative injuries, including progressive neuronal loss, enhanced astrogliosis, amyloid plaques formation, NFT formation, dystrophic neurite growth, and excessive tau protein phosphorylation [1, 16, 18, 46, 47]. The neurotoxic Aβ could also activate microglia to produce TNFα and reactive oxygen species (ROS), which in turn stimulate astrocytes and microglia to release more IL-1β [18, 32, 34]. These IL-1β in turn binds to IL-1β receptors (IL-1βR) on the membrane of astrocyte to induce the Aβ-triggered astrocytes to undergo further reactivation [2, 19].

It has been shown that Aβ induces astrogliosis through enhancing phosphorylation of extracellular signal-regulated kinase (ERK) in astrocytes of AD brain [2, 48-52]. An elevated level of phosphorylated ERK (pERK) was observed in the midfrontal cortex of patients during all stages of AD development in both clinical and neuropathological studies. Immunocytochemical and Western blot analysis detected intense astrogliosis with elevated level of pERK in the astrocytes of white matter from patients with early AD [2, 53]. In contrast, pERK immunoreactivity was found in neuronal cell bodies and dystrophic neurites around the plaques in patients with more advanced AD [49, 53]. Moreover, others have found that the activation level of pERK in astrocytes was strongly correlated with cognitive performance and the severity of AD neuropathology [2, 53]. These findings suggest that Aβ-activated ERK in astrocytes may be an important early event in the onset of AD.

Evidences have shown that Aβ could elevate the expression of cytokines and chemokines in astrocytes, which could in turn cause the astrocytes *per se* to reactivate [1, 33, 34, 50, 54, 55]. These reactivated astrocytes could secret more cytokines and chemokines to upregulate the APP mRNA and protein levels in neuron, thereby leading to an enhanced Aβ generation from the APP on the neuronal membrane surface

Table 2. Factors Released by Reactive Astrocyte

Kinds of Molecules	Factors Released by Reactive Astrocyte	[References]
Cytokines	G-CSF (CSF-3), GM-CSF (CSF-2), IFN-α/β, IFN-γ, IL-1α/β, IL-3, IL-5, IL-6, IL-15, M-CSF (CSF-1), MIP-1α, TNF-α	[28, 109, 197-200]
Chemokines	Fractalkine (CX3CL1), Gro-α (KC/CXCL1), I-309 (CCL2), I-TAC (CXCL11), IL-8, IP-10 (CXCL10), MCP-1 (CCL2), MIP-1β (CCL4), MIP-3α (CCL20), MIP-3β (CCL19), RANTES (CCL5), SDF-1α/β (CXCL12)	[11, 109-111]
Chemokine receptors	BOB/GPR15, CXCR3, CXCR4, CCR1, CCR2, CCR3, CCR5, CCR8, V28/CX3CR1	[11, 109-111]
Growth factors	BDNF, CNTF, FGF, GDNF, IGF-I, LIF, NGF, NT-3, PDGF, TGF-β	[24, 109, 200-204]
Glial trophic substances	Cholesterol, S100B, TSP	[63, 205, 206]
Complement proteins	C3, C4, C6, C7, C8, C9, Clusterin, Factor B, Factor I, MCP, Vitronectin	[200]
Coagulation factors	t-PA, u-PA	[200, 207]
Proteases	α-1-ACT, Cathepsin G, MMP	[200, 207]
Protease inhibitors	α-2M, PN-1	[200]
Matrix proteins	CS-PGs, DS-PGs, Fibronectin, Heparan, Laminin, N-cadherin, Tenascin	[200, 207]
Transport proteins	Apolipoprotein E, GLT-1	[200, 208]
Adhesion factors	ICAM-1, ICAM-2, NCAM, VCAM-1	[200]
Reactive N_2 intermediate	Nitric oxide	[200]
Mitogen and morphogen	EET	[207]
Gliotransmitters	ATP, D-serine, GABA, Glutamate	[209-211]

Abbreviations: BDNF Brain-derived neurotrophic factor, bFGF Basic fibroblast growth factor, CNTF Ciliary neurotrophic factor, CS-PGs Chondroitin-sulfate proteoglycans, DS-PGs Dermatan-sulfate proteoglycans, EET Epoxyeicosatrienoic acid, FGF Fibroblast growth factor, GABA Gamma-aminobutyric acid, G-CSF Granulocyte-colony stimulating factor, GDNF Glial cell-derived neurotrophic factor, GLT-1 Glutamate transporter subtype 1, GM-CSF Granulocyte macrophage-colony stimulating factor, Gro-α Growth related oncogene-α, ICAM-1 Intercellular adhesion molecule-1, ICAM-2 Intercellular adhesion molecule-2, IFN-α/β Interferon-alpha/beta, IFN-γ Interferon-gamma, IGF-I Insulin like growth factor-I, IP-10 Interferon-γ-inducible protein-10, LIF Leukemia inhibitory factor, MMP Matrix metalloproteinases, MCP Membrane cofactor protein, MCP-1 Monocyte chemoattractant protein-1, M-CSF Macrophage-colony stimulating factor, MIP-1α Macrophage inflammatory protein-1α, MIP-1β Macrophage inflammatory protein-1β, MIP-3α Macrophage inflammatory protein-1α, MIP-3β Macrophage inflammatory protein-3β, NCAM Neural cell adhesion molecule, NGF Nerve growth factor, NT-3 Neurotrophin-3, PDGF Platelet-derived growth factor, PN-1 Protease nexin 1, RANTES Regulated on activation normal T cell expressed and secreted, SDF-1α/β Stromal cell-derived factor-1alpha/beta, I-TAC IFN-gamma-inducible T cell alpha chemoattractant, TGF-β Transforming growth factor-beta, TNF-α Tumor necrosis factor-alpha, t-PA Tissue-type plasminogen activator, TSP Thrombospondins, u-PA Urokinase-type plasminogen activator, VCAM-1 Vascular cell adhesion molecule-1, α-1-ACT α-1-antichymotrypsin, α-2M α-2-macroglobulin.

[17, 56] (Fig. **1**). Thus, Aβ, astrocytes, cytokines and neuronal APP could speculatively bring about a vicious cycle in AD immunoneuropathology (Fig. **1**). Furthermore, this relationship was elucidated by an interesting study in which lipopolysaccharide (LPS) was found to induce IL-6 and TNFα production in glial cells. The results also demonstrated that Aβ could amplify the LPS-induced production of both IL-6 and TNFα in astrocytes, but not in microglia [55]. During the early stage of AD, Aβ-triggered astrocytes reactivation, rather than microglia activation, initiates a neuroinflammatory cascade in which the proinflammatory cytokines and chemokines exert autocrine and paracrine effects through interactions between astrocytes, neuron and microglia [42, 50, 55]. Thus, the Aβ-reactivated astrocytes might be the predominant player in the cytokine-mediated inflammation in early AD pathogenesis.

CYTOKINES AND ASTROCYTES IN AD

Chronic neuroinflammation mediated by cytokines released from activated astrocytes and microglia has been identified as one of the major mechanisms in AD neuropathology [1, 18, 29]. It has been shown that cytokines could deter learning and memory in acute cognitive deficits induced by LPS through the peripheral innate immune system [33, 55]. Along with the neuroinflammatory progression, astrocytes remain activated and secret various immunoinflammatory factors [1, 47, 57] (Table **2**). Among the many factors secreted by reactive astrocytes, we shall focus on a few cytokines and chemokines on which the well-established studies on their interaction with Aβ and AD development have already been conducted.

S100B

S100B is a neurotrophic factor and neuronal survival protein that stimulates neurite outgrowth, modulates long-term synaptic plasticity, and promotes neuronal survival and development [45, 58, 59]. Under physiological conditions, it is only expressed by NG2-expressing cells and a subtype of mature astrocytes that ensheath blood vessels, and not by all astrocytes [58]. During injury, S100B could also be upregulated in astrocytes [60]. Thus, it could serve as biomarker for certain astrocytes. Since the level of S100B was elevated in

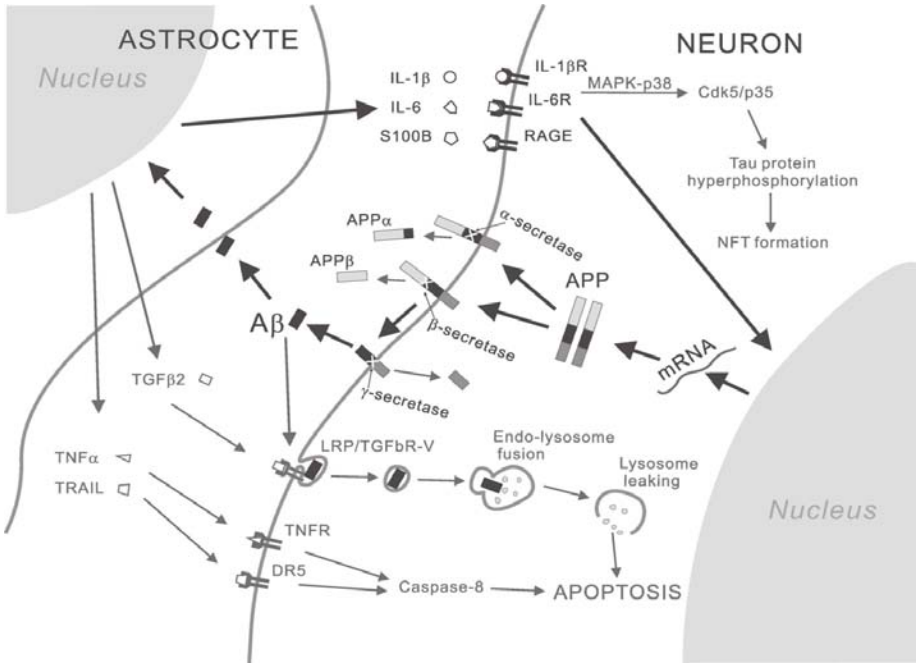

Fig. (1). The enhancive feedback of Aβ generation and the neuronal apoptosis induced by cytokines. The overproduced Aβ in neuron activates astrocytes to release proinflammatory cytokines (IL-1β, IL-6, S100B, TGFβ2, TNFα and TRAIL), which could increase hyperphosphorylation of tau protein and promote the transcriptional activity of APP mRNA. These lead to a vicious cycle of Aβ generation through cleavage by β- and γ- secretases. Aβ could be uptaken by neuron and delivered to lysosome by the complex of TGFβ2 and LRP/TGFbR-V, which brings about neuronal apoptosis via lysosome leaking. TNFα and TRAIL bind to TNFR and DR5 respectively to initiate neuronal apoptosis synergistically through caspase-8-dependent pathway.

Abbreviations: APP Amyloid precursor protein, Aβ Amyloid beta, IL-1β Interleukin-1β, IL-1βR IL-1β receptors, IL-6 Interleukin-6, RAGE receptor for advanced glycation end products, TNFα Tumor Necrosis Factor α, TNFR TNF receptor, TRAIL TNF-related apoptosis-inducing ligand, DR5 death receptor 5, TGFβ2 Transforming Growth Factor β2, MAPK-p38 mitogen-activated protein kinase-p38, Cdk5 cyclin dependent kinase 5, LRP Low-density lipoprotein receptor-related protein, TGFbR-V TGFβ receptors V.

activated astrocytes, it could also serve as a clinical marker for astrocyte injury in addition to GFAP [60, 61]. The amount of S100B-positive astrocytes and the S100B level could be elevated experimentally in rat brains injected with the recombinant IL-1 [62, 63]. In addition, IL-1β has been shown to remarkably upregulate S100B and S100B mRNA in the astroglioma cell line C6. Likewise, IL-1β could induce the production of S100B secreted by the plaque-associated activated astrocytes in primary cortex [59]. S100B secreted by astrocytes has been shown to promote the synthesis of APP mRNA and APP in neurons, which could serve as a source for additional accumulation of Aβ [45, 59, 62] (Fig. **1**). These evidences illustrate that the upregulated expression of IL-1β in the activated astrocytes in AD patients could drive the astrocytes *per se* to overproduce S100B, which could in turn promote neuron to generate more APP and Aβ [45, 64]. More astrocytes could then be reactivated by these newly made Aβ to further upregulate their expression of S100B via IL-1β (Fig. **1**). Hence, S100B constitutes part of the regenerative feedback loops of self-propagation provoked by Aβ and IL-1β in astrocytes, leading to chronic, sustained and progressive neuroinflammation that results in neurodegeneration.

Interleukin-1

The IL-1 super family members includes IL-1α, IL-1β, and IL-1 receptor antagonist (IL-1RA). Both IL-1α and IL-1β are proinflammatory cytokines involved in the inflammation and immune defense against infection. They could be produced by several kinds of cells, including astrocytes. It has been reported that the level of IL-1β was elevated in the serum and cerebrospinal fluid (CSF) of patients suffering from AD and other forms of dementia [32, 65, 66]. In astrocytes of the cortex and hippocampus, IL-1β level was dramatically increased by Aβ [34]. The IL-1β could then be released and bind to IL-1 receptors on the membrane of astrocytes to further induce IL-1β secretion. Moreover, IL-1β secreted by astrocytes could also stimulate neurons to increase the production of APP and neurotoxic Aβ [32, 34, 56, 67, 68] (Fig. **1**). The tissue levels of APP were significantly elevated when IL-1 was injected into the cerebral hemisphere [64]. This feedback loop of IL-1β secretion resulted in an elevation of Aβ production which, as mentioned above, is important in the progression of chronic neuroinflammation and activation of astrocytes in AD patients.

In addition to the vicious feedback loop, the effect of IL-1β on promoting neuronal degeneration and astrogliosis has

been verified by clinical and experimental studies [47, 68]. The IL-1β induced activation of mitogen-activated protein kinase (MAPK)-p38 in neurons has been implicated in the hyperphosphorylation of tau protein, a major component of NFT in AD brain [2, 68, 69]. This notion was supported by reports that IL-1β is markedly overexpressed at the neuroinflammatory sites and promotes MAPK-p38 generation in the AD brain [68-70]. IL-1β has also been revealed to induce the Aβ-treated astrocytes to undergo astrogliosis by binding to IL-1β receptors on their membrane [2, 19]. The upregulation of IL-1β in the activated astrocytes around the senile plaques contributes not only to neuroinflammation, but also to the production of neurotoxic free radicals through a synthesis partially mediated by the expression of inducible nitric oxide synthase (iNOS) [71]. The expression of iNOS might have resulted from the initial induction by IL-1β as IL-1RA which could inhibit nitrite accumulation [71]. Likewise, alpha-phenyl-N-tert-butyl nitrone, a nitrone-based free radical trapper, significantly suppressed the activation of MAPK-p38 by IL-1β in rat astrocytes in primary culture as well as decreased the amount of the IL-1β-induced ROS produced in mitochondrial respiration [70, 71]. These observations confirmed that IL-1β expressed by the activated astrocytes leads to nitric oxide (NO) accumulation around the Aβ plaques in AD brain [71, 72]. Thus, the studies indicated a possible additional proinflammatory role of IL-1β in the progression of neuronal degeneration, since it could have upregulated the expression of iNOS in Aβ-activated astrocytes.

Interleukin-6 (IL-6)

IL-6 is a potent pleiotropic interleukin. It is evidenced that astrocyte is one of the main sources of synthesis and secretion of IL-6 in the CNS [33, 67, 73]. IL-6 and its receptors (IL-6R) are localized in several regions of the normal brain, including hippocampus, striatum, hypothalamus, neocortex, and brainstem [73]. It is interesting to find a significantly elevated level of IL-6 in the CSF and serum of AD patients [65, 74, 75]. The incremental level of IL-6 in the CSF could be used as one of the useful parameters for screening AD patients in the elderly population [74]. Group comparisons accounting for multiple testings revealed that the level of IL-6 was consistently elevated in AD brain tissue. Immunohistochemistry staining confirmed this increase. IL-6 was also found to be localized in both astrocytes and neurons [29]. The level of IL-6 in astrocytes was greatly increased by Aβ [55, 76]. In the amyloid plaques penetrated by IL-6-immunoreactive astrocytes, IL-6 was overproduced by astrocytes, neurons and microglia, and secreted into the extracellular space [29, 55, 67, 77, 78]. IL-6 knock-out mice are refractory to the LPS treatment that induced impairment in working memory in the wild-type mice [79]. Furthermore, LPS could induce a significant increase in mRNA levels of IL-1β and TNFα in hippocampal tissue of IL-6 wild-type mice. Such effects of LPS, however, were greatly attenuated or entirely absent in IL-6 knock-out mice [79]. These results suggested that IL-6 plays a role in mediating the production of the proinflammatory IL-1β and TNFα in AD.

IL-6 secreted from astrocytes mediated tau protein hyperphosphorylation in neuron by activating the neuronal protein kinase cdk5/p35 complex [80]. The activation of this complex also involved MAPK-p38 signaling pathway, JAKs/STATs pathway and NMDA receptors [80]. Further, it was confirmed that IL-6 could not induce hyperphosphorylation of tau protein in neurons treated with phosphokinase inhibitor (butyrolactone-I) [80]. It was therefore proposed that IL-6 from astrocytes could induce a calcium influx in neurons through the NMDA receptors and activate the JAKs/STATs pathway, subsequently leading to the tau protein hyperphosphorylation and NFT formation [81]. These results implied additional neuropathologic roles of IL-6 and astrocytes in agitating the neuroinflammatory response and the neuronal degeneration in AD.

Tumor Necrosis Factor α and TNF-Related Apoptosis-Inducing Ligand (TRAIL)

TNFα is a proinflammatory cytokine and could be induced by Aβ peptides, glutamate and LPS to be expressed in astrocytes [5, 55, 82]. In the presence of Aβ, TNFα induces activation of astrocytes and also mediates the neuronal loss and the chronic neuroinflammatory pathogenesis of AD via TNF receptors (TNFR) on the neuronal membrane [1, 79, 83, 84]. Recent clinical investigation displayed a significantly higher concentration of TNFα in the CSF and serum of AD patients [31, 74, 85]. In the CSF of AD patients, TNFα increased while Aβ42 level decreased [74]. Under normal physiological conditions, there is a homeostasis between neurotoxic factors (i.e. TNFα, IL-1β) and survival factors (i.e. nerve growth factor, NGF). Aβ, however, could break this homeostasis by causing the over production and secretion of TNFα, eventually resulting in AD neuroinflammation. Thus, variations in the levels of TNFα in the CSF and serum of AD patients could be used as potential biomarkers for future diagnosis of AD and for monitoring the effects of anti-inflammatory and/or neurotrophic drugs.

TRAIL is a novel cytokine that functions as a ligand binding to DR5 on neuron in the CNS. The binding would initiate the process of apoptosis in a caspase-8-dependent manner (Fig. **1**). DR5 has been demonstrated to be a key factor in TRAIL death pathway [41, 86]. Immunohistochemical staining showed that TRAIL was present in the cerebral cortex and the proximity of Congo-red-positive amyloid plaques of AD patients but completely absent in a healthy brain [87]. Rat astrocytes and human SH-SY5Y neuronal cell line expressed both TRAIL receptor mRNAs and proteins when exposed to Aβ [88]. TRAIL was also able to induce its own expression in astrocytes. However, it failed to induce the apoptosis of astrocytes per se, since astrocytes simultaneously express decoy receptors (DcR1, 2, 3) that lack intact death domains in their intracellular regions [86, 87, 89]. The extracellular domains of decoy receptors compete with those of DR5 for TRAIL binding, thereby neutralizing the effect of TRAIL death pathway in astrocytes [89]. Further experiments demonstrated that TRAIL/DR5-mediated apoptosis is involved in the Aβ-dependent neuronal loss, and is probably responsible for the AD related cognitive impairment and memory loss [41, 85, 88].

Transforming Growth Factor (TGF)

Transforming growth factor is a super family of cytokines that consists of two classes of polypeptide growth factors, TGFα and TGFβ. These two classes of TGFs are not

structurally or genetically related to one another, and they act through different receptor mechanisms. TGFα was found to be produced by the maturing astrocytes from the cerebral cortex, hypothalamus and cerebellum of newborn rats. It cooperated with the epidermal growth factor (EGF) receptor signaling pathway to influence the postnatal differentiation of astrocytes through upregulating the expression of GFAP [90, 91]. However, there is a lack of direct evidence on the role of TGFα in AD pathogenesis [91, 92].

TGFβ is a protein secreted by many cell types, including astrocytes in the brain. It consists of three isoforms TGFβ1, TGFβ2, and TGFβ3. The isoforms are encoded by different genes but have high structural homology. In addition, they have similar cellular signaling targets on the TGFβ receptors (TGFbR) which exists in I, II, III, and V subtypes [57]. TGFβ is implicated in AD and other chronic inflammatory diseases. Level of TGFβ1 was found to be elevated in the CSF, serum, and brains of AD patients [38, 57, 93-95]. All three isoforms of TGFβ can increase the accumulation of Aβ in pyramidal neurons in hippocampal slice cultures under Aβ treatment [96]. TGFbR-I and II immunoreactivity was increased in reactive astrocytes in the AD brain [97]. Thus, TGFβ seems to be involved in the neuroinflammation underlying the immunopathogenesis of AD and could also serve as an early biomarker for AD [98].

TGFβ1 protein is not expressed in the normal adult CNS [99]. However, it has been observed to increase immediately following the CNS injury [100]. Experimental reduction of TGFβ1 has been shown to partially reduce astrocyte reactivation [99, 100]. TGFβ1 was found to exert a dual effect on AD. It not only increases Aβ levels in astrocytes, but also activates microglia to remove the neurotoxic Aβ from the brain into blood vessels [101, 102]. TGFβ1 was able to increase Aβ immunoreactive plaque-like deposits in the rat brain. It could also induce an overproduction of APP and soluble APPβ in astrocytes, but not in neurons [96, 101]. It has been shown that an overproduction of TGFβ1 can trigger an inflammatory cascade that results in AD-like cerebrovascular amyloidosis and prominent perivascular astrocytosis in transgenic mice with low astrocytic TGFβ1 expression [102]. These findings indicated that TGFβ1 could specifically enhance Aβ production in astrocytes and exacerbate astrogliosis, thereby leading to the formation of senile plaques in AD brain.

In the CNS, TGFβ2 mRNA and protein are predominantly expressed in astrocytes of white matter [103]. It has been shown that TGFβ2 expression could be induced by Aβ in astrocytes and neurons [104]. Moreover, an increase in the immunoreactivity of TGFβ2 was shown in reactive astrocytes and NFT-bearing neurons in AD patients [97]. The effects of Aβ on neuronal death was suppressed by both the neutralizing antibodies to TGFβ2 and the recombinant soluble extracellular domain of APPα generated by α-secretase [104]. TGFβ2 appeared to be collaborating with Aβ to mediate both neuronal death and astrogliosis in AD.

Although there is no doubt for TGFβ2 to be implicated in AD pathogenesis, the underlying mechanisms remain elusive. Research has demonstrated that TGFβ2 could bind to APP to activate an APP-related death pathway *via* heterotrimeric G protein, c-Jun N-terminal kinase, NADPH oxidase, and related caspases [105]. In the cortical neurons, TGFβ2 altered the morphology and increased the number of lysosomes [106]. It also increased the uptake of Aβ into lysosomes and accelerated the Aβ degradation. At the same time, this caused instability and leakage of the lysosomal membranes leading to the activation of cell death pathways, gliosis and demise of neuronal cells [106, 107] (Fig. **1**). Both TGFβ1 and TGFβ2 could bind to low-density lipoprotein receptor-related protein (LRP), acting as an endocytic receptor and a signaling receptor. LRP has recently been shown to have identical functions as TGFbR-V. TGFβ2 upregulated the mRNA and protein levels of LRP/TGFbR-V in the cortical neurons in cultures [106]. Furthermore, TGFbR-V antagonist prevented Aβ/TGFβ2-induced memory retention deficits by attenuating the cellular targeting of Aβ [107]. Therefore, TGFβ2 appeared to participate in the signaling pathway cascade triggered by Aβ in the early AD neuroinflammatory pathogenesis.

CHEMOKINES AND THEIR RECEPTORS IN AD

Chemokines are a family of chemotactic cytokines with relatively smaller molecular weights (8-10 kDa) and are secreted by a variety of cell types [108]. Chemokines are produced during immune responses and function mainly as chemoattractants for leukocytes. They recruit monocytes, neutrophils and lymphocytes from blood to sites of infection or tissue damage by binding to chemokine receptors on the target cells. Many chemokines have been shown to be involved in the chronic Aβ-induced neuroinflammation in AD [29].

Among 47 chemokines, astrocytes have been shown to secret about a dozen of them [108, 109] (Table **2**). Several chemokine receptors are also expressed on the membrane of astrocytes [11, 109-111] (Table **2**). The upregulation of some major chemokines, including macrophage inflammatory proteins (MIP)-1α (MIP-1α), MIP-1β, monocyte chemoattractant protein-1 (MCP-1), interleukin 8 (IL-8), and IP-10 has been observed in Aβ-activated astrocytes [18, 110]. TNFα and IL-1β have been found to promote astrocytes to produce MIP-1α, MIP-1β, MCP-1, RANTES and CCL20, and to significantly upregulate BOB/GPR15, CCR2, and V28/CX3CR1 mRNA levels [11, 110, 111] (Table **2**). Among these chemokines, MCP-1 and RANTES were shown to be produced by astrocytes under Aβ stimulation. This was supported by the study on the co-cultures of astrocytes and macrophages in which Aβ stimulated higher levels of MIP-1α, MIP-β and MCP-1 secretion than control [18].

MIP-1 has α and β isoforms. MIP-1α is principally secreted in neurons in both the normal and AD brains. The significantly higher serum levels of MIP-1α in AD patients have been shown to associate with a bi-allelic dinucleotide microsatellite repeat (TA repeat) polymorphism, known as the MIP-1α -906 (TA)(6)/(TA)(6) genotype, which has been shown to exacerbate neuroinflammation. This genotype, when combined with the ApoE ε4 allele, acts as a genetic risk factor for AD [112, 113]. On the other hand, the MIP-1β-bearing astrocytes have been shown to surround plaques with amyloid deposits in AD brain [114]. Immunohistochemical analysis confirmed that MIP-1β is predominantly present in reactive astrocytes and significantly more widespread in the brains of AD patients than control groups.

The MCP-1 levels were found to increase significantly in the CSF and serum of the patients with mild cognitive impairment (MCI) and mild AD, which has been considered to be an early event in AD pathogenesis [29, 115-118]. Thus, MCP-1, together with the other cytokines mentioned above, may serve as biomarkers in the CSF for AD clinical diagnosis. The level of MCP-1 mRNA was shown to be elevated in the peripheral blood mononuclear cells, in parallel with its level in the serum. In the CNS, astrocytes have been discovered to be the major MCP-1-producing cells [111] (Table **2**). A SNP occurring at position -2518 of the MCP-1 gene promoter was found to be a critical genetic factor which strongly influences the serum levels and biological activity of MCP-1 in AD patients [116]. The MCP-1 gene polymorphism was revealed to occur at a significantly higher frequency in the AD group than in the age-matched control group. The presence of this genotype was also found to strongly correlate with higher levels of MCP-1 in the serum [118].

IL-8 secretion has been shown to increase significantly in U-373 MG astrocytoma cells under Aβ treatment [39]. The Aβ induced neuronal damage and astrogliosis could be attenuated by a nonselective K^+ channel blocker 4-aminopyridine through regulating the generation of IL-8 [29, 119]. As one of the IL-8 receptors, CXCR2 is expressed at high levels by subsets of neurons in diverse regions of the brain and spinal cord, and has been found to exist in the neuritic portion of plaques surrounding amyloid deposits in pathologic brain tissues of AD patients [120].

IP-10 is a member of the chemokine family and is induced in a variety of cells in response to interferon gamma (IFN-γ) and LPS [53, 110, 111, 121]. Astrocytes have been shown to be the major source of IP-10 in the CNS [110, 111, 121, 122] (Table **2**). The expression of IP-10 was significantly elevated in the astrocytes surrounding the amyloid plaques in AD brains [53]. Moreover, immunohistochemistry staining indicated that IP-10 and its receptor, CXCR3, existed on neurons in various cortical and subcortical regions of the normal and AD brains. Otherwise, there were co-localizations of Aβ positive plaques with IP-10 that are strongly expressed in the cerebral cortex and hippocampus in the brains of transgenic mice APP SWE [121].

REMARKS AND CONCLUSION

AD is a complex disease. To date, there is no single "magic bullet" identified that could prevent or cure it. The U.S. Food and Drug Administration has approved several drugs to treat AD. However, none of them has been proven to stop AD [123-125]. Most current treatments focus on helping people to maintain mental functioning, managing behavioral symptoms, or slowing down the progression of AD. This is all because research only developed to a point to look beyond treating symptoms hoping to delay or prevent AD. Most significant of all, the etiopathogenesis of AD still remains poorly understood.

Nowadays, none of the therapeutic modalities used on AD patients are especially effective and could not reach satisfactory treatment outcomes. One of the reasons for this is the lack of understanding of the early disease process and the lack of early diagnosis tool that would allow us to identify patients in the presymptomatic stages. The identification of IL-1β, IL-6, MCP-1, TGFβ and TNFα in the CSF and plasma or serum of AD patients could be translated into potential early biomarkers for AD diagnosis. The levels of these inflammatory molecules, when analyzed in combination with clinical symptoms, functional magnetic resonance imaging and the levels of biomarkers in the CSF with the highest diagnostic accuracy (such as Aβ42, total tau protein and phosphorylated tau protein) for AD, could improve the quality of the early diagnosis of AD and help the tracking of the disease progression. The levels of these cytokines in the plasma or serum, however, remain somewhat controversial. It is possible that the severity of AD could influence the expression of cytokines in the brain or that the accumulation of inflammation molecules within the AD brain is not reflected in the serum or plasma [126-128]. Most therapeutics developed today are based on four current hypotheses of AD development. The cholinergic hypothesis, the oldest of the four, has not been widely supported because medications treating acetylcholine deficiency have not been very effective [129-132]. The amyloid hypothesis postulated that Aβ deposits are the fundamental cause of the disease [133, 134]. An experimental Aβ vaccine was found to clear the amyloid plaques but did not have any significant effect on dementia [135]. The tau hypothesis proposed that tau protein abnormalities initiate the disease cascade [134]. We have discussed above that both Aβ and tau abnormalities appeared at a later stage of AD long after the initiation of neuroinflammation triggered by proinflammatory factors in the CNS. Lastly, Herpes simplex virus type 1 has also been proposed to play a causative role in AD pathogenesis in people carrying the susceptible versions of the ApoE gene [136].

Among all these hypotheses, neuroinflammation is most likely to play a pivotal role during the earliest stage of AD pathogenesis. Therefore, understanding the mechanisms of neuroinflammation might reveal the mysterious causes of AD and would allow us to develop therapies or drugs with anti-inflammatory property aimed specifically at early AD treatment. Some anti-inflammation therapies have been tested in AD patients. Estrogen could reduce the response of astrocytes, microglia and neuron to various insults, including those from Aβ, through modulating the expression of proinflammatory cytokines [137-139]. The clinical trial of active AN-1792 (purified Aβ42 peptide, as an Aβ vaccine) immunotherapy showed an improvement in cognitive abilities compared with the control group [140, 141]. Although AN-1792 immunization resulted in clearance of amyloid plaques in patients with AD, phase II clinical trials were halted due to the failure of AN-1792 to prevent neurodegeneration progression and the occurrence of meningoencephalitis in a significant proportion of treated patients [141]. Future research might develop ways to eliminate these side effects. Nonsteroidal anti-inflammatory drugs (NSAIDs) have been studied for the treatment and prevention of AD for more than two decades. A number of epidemiological studies have demonstrated that patients with histories of NSAIDs treatments had significantly lower risks of developing AD [123, 142-145]. NSAIDs have been shown to decrease the production of Aβ through suppression of amyloidogenic activities [123, 125, 146]. Based on laboratory, preclinical and limited clinical studies, they also have been found to significantly repress the

activation of astrocytes and microglia, and the expression of proinflammatory factors [18, 123, 124, 146, 147]. However, significant effectiveness of NSAIDs for AD treatment has not yet been verified from the clinical trials performed [147, 148]. Hence, NSAIDs are not considered a promising treatment for patients with late stage of AD.

One focus of therapeutics in neurodegenerative disease has been strategies for the replacement of neurotransmitters. However, neurotransmitter replacement has had only very limited success in AD patients with mild-to-moderate dementia, and has failed in patients with severe dementia [149, 150]. A large body of work has been devoted to neuroprotective strategies, with enormous basic science and clinical efforts dedicated to treatments that are effective in neuronal cultures [150-153]. With our recent advances in the understanding of the relationship between nerve cells, targeted therapeutics for neuroinflammatory pathways in which astrocytes have a prominent position, as outlined above in this review, need to be developed. It is also likely that therapeutics effective at neuroprotection also enhance astrocyte-neuron interactions. However, this possibility has not been fully investigated. Astrocytes should not be considered only as supporting cells for neurons anymore, but should also be viewed as neuroinflammatory cells in the CNS. Astrocytes secreting cytokines and chemokines in the early stage of AD development make a new potential therapeutic target and offer the possibility of developing new medicines to cure AD by intervening neuroinflammation.

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