Alzheimer’s disease (AD) and vascular dementia are the major causes of cognitive disorders worldwide. They are characterized by cognitive impairments along with neuropsychiatric symptoms, and that their pathogeneses show overlapping multifactorial mechanisms. Although AD has long been considered the most common cause of dementia, individuals afflicted with AD commonly exhibit cerebral vascular abnormalities. The concept of mixed dementia has emerged to more clearly identify patients with neurodegenerative phenomena exhibiting both AD and cerebral vascular pathologies—vascular damage along with β-amyloid (Aβ)-associated neurotoxicity and hyperphosphorylation. Cognitive impairment has long been commonly explained through a ‘neuro-centric’ perspective, but emerging evidence has shed light over the important roles that neurovascular unit dysfunction could have in neuronal death. Moreover, accumulating data have been demonstrating astrocytes being the essential cell type in maintaining proper central nervous system functioning. In relation to dementia, the roles of astrocytes in Aβ deposition and clearance are unclear. This article emphasizes the multiple events triggered by ischemia and the cytotoxicity exerted by Aβ either alone or in association with endothelin-1 and receptor for advanced glycation end products, thereby leading to neurodegeneration in an ‘astroglial-centric’ perspective.

Keywords: Alzheimer’s disease; astrocytes; ET-1; ischemia; RAGE; vascular dementia

INTRODUCTION

Alzheimer’s disease and vascular dementia

Alzheimer’s disease (AD) has constituted a major public health challenge for the elderly population worldwide. The prevalence of AD increases significantly with age and comprises 50–70% of all dementia cases.1,2 AD is histopathologically characterized by the formation of β-amyloid (Aβ) and neurofibrillary tangles, leading to neuropathological events and subsequent synaptic dysfunction, glial activation and eventual neuronal demise.3–6 Vascular dementia (VaD) is another common cause of dementia, accounting for 15–25% of all dementia cases.1 It is characterized by cerebrovascular dysregulations such as a decrease in the cerebral blood flow (CBF), breakdown of the blood brain barrier (BBB), upregulation of inflammatory processes and the production of reactive oxidative species (ROS), leading to multiple lacunar infarcts, leukoencephalopathy, and hemorrhagic and ischemic lesions, thereby resulting in neurodegeneration.7 VaD is characterized into three main subtypes: cortical VaD—also referred to as multi-infarct or post-stroke dementia, subcortical VaD or small-vessel dementia and strategic-infarct dementia. Other subtypes include hypoperfusion dementia, hemorrhagic dementia and hereditary VaD. Cortical VaD is characterized by multiple cortico-subcortical infarcts related to large vessel disease and major hemodynamic events. Subcortical VaD is related to small-vessel disease and is characterized by small infarcts causing lacune development and ischemic white matter lesions with incomplete ischemic injuries. Strategic-infarct dementia is characterized by ischemic lesions that occur in specific regions critical for higher cognitive functions.8–10 Cerebrovascular abnormalities are often found to co-exist in the AD brains, thereby blurring the distinction between AD and VaD. Mixed dementia (MixD)—individuals having both AD and VaD pathologies—has therefore been conceptualized.11–15 The clinical criteria being used to identify AD, VaD and MixD, including the Consortium to Establish a Registry for AD, Alzheimer Disease Diagnostic & Treatment Center, National Institute of Neurological Disorders and Stroke and Association Internationale pour la Recherche et l’Enseignement en Neuroscience, among others, show inconsistency, thereby yielding highly variable prevalence rates of 2 to 60% for MixD.16–19 Some of the major vascular pathological features found in AD with cerebrovascular abnormalities include cerebral amyloid angiopathy, intracerebral Aβ-deficient clearance through the BBB and ischemic stroke lesions, suggesting the significant involvement of the cerebral vasculature in AD pathophysiology.20,21 The neurovascular unit (NVU) can be considered as the smallest functional component of the neurovasculature, comprising of endothelial cells, pericytes, vascular smooth muscle cells and with the astrocytes end-feet being the direct links between neurons and the cerebral vasculature.22 The NVU functions to control CBF, BBB exchange, transmigration of leukocytes in brain
infection and the trophic interaction between the endothelial cells, neurons and glia.23

Research has shown that intracerebral Aβ is transported outside by the low-density lipoprotein receptor-related protein 1 and transported into the brain by the receptor for advanced glycation end products (RAGE), with both receptors being localized on the BBB.24 Interestingly, disruption of NVU homeostasis secondary to chronic ischemia could lead to a decrease in CBF, disrupting the BBB integrity, thereby leading to the formation of Aβ plaques, trophic support impairment, also triggering oxidative stress and inflammation.24-28 Taken together, NVU dysfunction is likely to be an important early pathological event that leads to neurodegeneration as observed in AD and MMD.

ASTROCYTE: MULTIPLE FACETS

Comprehensive literature has depicted the pivotal roles of astrocytes in the neurological disorders over the previous decades. They have been implicated in a number of neurodegenerative diseases, including AD, amyotrophic lateral sclerosis, Wernicke encephalopathy, frontotemporal dementia, frontotemporal lobar degeneration, thalamic dementia and Huntington’s disease.29 They appear to have significant, yet controversial, roles in the central nervous system (CNS) pathophysiology.

Anatomical features

Astrocytes are star-shaped cells that exist in well-distributed, non-overlapping fashion in the CNS, each confined in its own discreet territory.30,31 Interdigitation primarily occurs at the end tips of their diversified processes, forming gap junctions with adjacent astrocytes, establishing close contact with neurons and blood vessels, and enveloping thousands of synapses.32 The two main classical subtypes are the protoplasmic and fibrous astrocytes.33 Protoplasmic astrocytes are found in the gray matter with many fine processes enveloping synapses, whereas fibrous astrocytes are within the white matter, extending longer but with fewer processes to the nodes of Ranvier.32 Further morphological studies have provided more diversified subdivisions, including Bergmann glia, Müller glia, tanyocytes, radial, velate and perivascular astrocytes.34

Physiological functions of astrocytes

Initially regarded as supportive elements in the brain, astrocytes are now known to have multiple neurophysiological roles. Astrocytes participate in the development of the neuronal circuitry by being actively involved in synaptic formation, maturation and pruning. Astrocytes also secrete several synaptogenic molecules, including thrombospondin, brain-derived neurotrophic factor (BDNF) and glycinan.35 They are responsible for the uptake and release of numerous transmitters important for information signaling, such as glutamate, γ-aminobutyric acid, purines, D-serine, acetylcholine, prostaglandins and eicosanoids.36 Moreover, astrocytes are able to establish bidirectional communication with neurons, modulating synaptic information transmission, presynaptic and postsynaptic events, and are commonly known as the tripartite synapse.37

Modulation of Ca2+ signaling is another central task for astrocytes. Regulation of CBF by astrocytes is attributed by either the release of cyclooxygenase 1 metabolites through activation of phospholipase A2, or an alternative pathway involving the release of K+ through the Ca2+-activated K+ channels into the extracellular space between astrocytic end feet and the arteriole in order to induce vasodilation.38,39 Elevation of intracellular Ca2+ concentration is likewise needed for the release of excitatory and inhibitory ‘gliotransmitters’ such as glutamate and adenosine, respectively.40 In addition, excess of glutamate in the extracellular space is also neurotoxic to the brain, and astrocytes constitute the primary CNS cell type for glutamate clearance from the extracellular space through its uptake by the excitatory amino acid transporters 1 and 2, and convert it to glutamine by the enzyme glutamine synthetase.41

Astrocytes also accomplish many other responsibilities in the CNS, including anatomically ensheathing the BBB, influencing its permeability by inducing junction formation and the upregulation of several transporter enzymes on the BBB such as glucose transporter 1 and P-glycoprotein.42 Water and K+ homeostasis in the brain is also achieved by the coupling of both aquaporin 4 and rectifying K+ channels localized on astrocytic end-feet.43

Astrocytic reactivity

Astrogial responses in CNS pathologies are often mediated by astrogliosis, a process that leads to astrocyte morphological changes characterized by an augmented expression of intermediate filaments, proliferation and astrocytic hypertrophy surrounding the affected area.44 The main intermediate filaments of the astrocytic cytoskeleton that are increased in astrogliosis are glial fibrillary acidic protein (GFAP), vimentin and nestin.45 Hyper trophy is resulted from the somata and processes thickening, and it appears that interdigitation is minimal and domain overlap does not occur.46 However, this is only partially true depending on the experimental model being studied; loss of domain architectural structures is reported in the brains of epileptic mice, whereas this is not the case for AD animal models.47 Interestingly, recent research has discovered that distance from the lesion has a crucial role in determining the morphological and physiological responses of astrocytes toward induced spinal cord injuries. Specifically, newly generated astrocytes in glial scar borders after injury present an elongated morphology with extensive domain overlaps and interdigitation, whereas reactive astrocytes derived from matured astrogliia distant to the injury would retain their ‘tile’ domain architecture and stellate shape, with minimal contact of their processes. Moreover, signal transducer and activator of transcription 3 is critically involved in the formation of astroglial scar, which could affect the process of interdigitation.48

Reactive astrocytes exert both beneficial and detrimental effects, initially protecting and enhancing neuronal survival from oxidative stress, inflammation and hypoxic/ischemic episodes. Ablation of astrocyte reactivity has been shown to increase leukocyte trafficking into the CNS, BBB repair deficiency with vasogenic brain edema production, demyelination and neuronal degeneration.49,50 Conversely, prolonged astrocytic activation can inhibit axonal regeneration by inducing the production of persisting mature glial scars; furthermore, reactive astrocytes can release proinflammatory and cytotoxic cytokines.51,52 The generation of ROS, nitric oxide (NO) and Aβ toxicities have been hypothesized to be facilitated by astrocytes.53,54

Astrocytic activation is a complicated process with highly variable outcomes. Recent evidence proposes the existence of different subtypes of reactive astrocytes that are dependent on the type of stimuli received. For example, differences in gene expression profiling were elicited between astrocytes subjected to ischemic stroke and neuroinflammation. Middle cerebral artery occlusion generated reactive astrocytes with upregulated levels of thrombospondins and growth factors, which are important for synaptic repair, hence indicating a neuroprotective role. Lipopolysaccharide injection, however, would induce astrocytic activation with the upregulation of classical complement cascade proteins, leading to synaptic loss and neuronal degeneration.55 Furthermore, it appears that reactive astrocytes, as a single entity, can have different reactions to the same stimuli, thereby generating markedly heterogeneous responses. It has been reported that the effects of inflammatory mediators on individual reactive astrocyte can generate fluctuating expression levels of GFAP, interleukin 6 (IL-6) and chemokine ligand 7.56
Astrocytes and ischemia

Evidence shows that chronic ischemia contributes to a series of pathophysiological conditions in the development of neurodegenerative diseases, including AD. Vascular damage, as a result of inflammation and oxidative stress, would lead to CBF interruption and subsequent oxygen and energy deprivations, thereby causing the eventual neuronal demise. In addition, increase in Aβ deposition and τ-phosphorylation occurrences also contribute to neurodegeneration.60-64

Astrocytes, being the principal glycogen storage in the CNS, are more resilient in surviving energy deprivation in anaerobic conditions, with the ability to increase the expressions of antioxidant enzymes such as glutathione and superoxide dismutase, and anti-apoptotic protein levels such as Bcl-2.65 Moreover, astrocytes undergo astrogliosis within the injured area, enhancing neuronal plasticity and regenerative purposes.61 Reactive astrocytes are capable of supplying energy to dying neurons, preventing inflammation from spreading, restoring normal pH conditions, uptaking excess glutamate, repairing tissue damage, and upregulating neurotrophic and growth factors such as transforming growth factor-β1 and endothelin-1 (ET-1).62,63 Under more severe ischemic insult, however, in addition to astrocytic apoptosis and autophagy, a series of pathogenic scenarios would occur, resulting in neuronal regeneration inhibition and ultimately to neuronal demise.33,64-67 Among these events include the release of excitotoxic glutamate, production of proinflammatory cytokines and chemokines, formation of brain edema by the upregulation of aquaporin 4 water channels and the production of C-terminal of amyloid precursor protein (APP) that contributes to the eventual increase in total Aβ burden.

Other glial cells in ischemia

Microglia constitutes the CNS-resident immune cells. They are responsible for immune surveillance, elimination of dead cells and synaptic pruning during neuronal development. During an injury, they rapidly surround the affected site and become activated.68 In cerebral ischemia, neuronal glutamate release would trigger microglial activation.69 Activated microglia release several neurotoxic factors—BDNF, nerve growth factor—and help to restore homeostasis in the post-ischemic brain; however, in the presence of persisting insult, activated microglia would participate in neuroinflammatory response by releasing a number of neurotoxic molecules such as free oxygen radicals, NO, tumor necrosis factor-α (TNF-α) and IL-1β, and stimulate astrogliosis.70 In both activated microglia and astrocytes, superoxide and NO can react to produce peroxynitrite, which is a highly toxic radical species that can cause significant oxidative injury. Moreover, astroglial inducible NO synthase-mediated NO release during ischemic condition would compromise mitochondrial electron transfer process.71,72

Oligodendrocytes are the myelin-producer cells in the CNS, enveloping the axon in a thick layer of myelin. Myelination acts as an insulator to accelerate action potential, thereby allowing faster communication. Demyelination often occurs after an ischemic episode. Unlike astrocytes, oligodendrocytes are particularly susceptible to oxidative stress and energy deprivation.70

AMYLOIDOGENESIS THROUGH AN ASTROCYTIC PERSPECTIVE

Pathophysiology of Aβ peptide

Aβ is derived from the APP, which is an integral membrane protein that has been localized at the Golgi apparatus, endoplasmic reticulum, endosomes, lysosomes, mitochondria and plasma membranes.73 In order to produce Aβ, APP is cleaved by β-secretase—as also known as β-site APP cleaving enzyme 1 (BACE1)—at its N-terminal, followed by γ-secretase at its C-terminal, generating Aβ fragments of 40, 42 or 43 residues.74,75 Alternatively, APP can be cleaved by α-secretase instead of BACE1, thereby precluding the formation of Aβ.76

It has long been thought that fibrillar Aβ found in the extracellular space as insoluble aggregated depositions are responsible for the observed neurotoxicity in AD. However, mounting data over the past two decades are supporting the notion that the soluble oligomeric Aβ is as neurotoxic and is found to be significantly increased in the AD brains.77,78 Comparing between the oligomers and fibrils, oligomers can induce a different repertory of proinflammatory molecules secreted by activated microglia and jeopardize neuronal viability by tenfold greater than fibrils.79,80

In a number of studies, amyloid oligomers located intracellularly in neurons and astrocytes have been reported to disrupt Ca2+-homeostasis, leading to neurodegeneration.73,81-83 Several research groups suggest that the mechanism through which this is accomplished involves the ability of intracellular Aβ to permeabilize membranes and to activate the N-methyl-D-aspartate receptors.84,85 These pathological events generate intracellular cytosolic Ca2+ elevation, thereby triggering mitochondrial dysfunction, nicotinamide adenine dinucleotide phosphate oxi-dase activation, ROS generation, glutathione depletion, CBF dysregulation and excessive neurotoxic glutamate accumulation in the extracellular space. These mechanisms lead to subsequent oxidative injury and synaptic dysfunction.86,87

Recent studies have reported the existence of ‘pore-like structures’ of Aβ formed by the circularization of amyloid pre-fibrillar oligomers subunits, referred to as amyloid annular protofibrils (APFs), localized on plasma membranes, cellular vesicles in neurons and inside activated astrocytes.88,89 It has been suggested that the pathogenicity of prefibrillar oligomers reside in their ability to permeabilize the membrane, adhering to it and forming a calcium-permeable channel, resulting in the disruption of intracellular Ca2+-homeostasis.90 In congruence with the APF-proposed pathogenicity hypothesis, other protofibrillar structure molecules such as α-synuclein in Parkinson’s disease, Aβ1 amyloid subunit in the familial British dementia and islet amyloid polypeptide in type II diabetes have been implicated to exert the same membrane permeabilization effect.91-93

Amyloid plaque formation is the consequence of fibril accumulation in the brain extracellular space. Fibrils have been commonly assumed to be derived from oligomers in a pathway involving Aβ APFs as intermediates, which lead to the belief that the existence of amyloid plaques could be used as a reliable biomarker of AD progression. However, newer evidence suggests that Aβ APFs are products of a distinct pathway that does not end in the subsequent fibrils formation.94 This provides a possible explanation that fibrillar plaques are found in non-demented patients and emphasize the importance of Aβ APFs as the oligomeric cytotoxic conformation in AD.95

Aβ could also induce neurotoxicity by promoting τ-phosphorylation through the cyclin-dependent kinase 5 pathway, which is often accompanied by ROS increase, along with activations of the caspase 3, JUN kinase and nuclear factor κ-light-chain-enhancer of activated B cells (NFκB) pathways.96,97 In oligodendrocytes, Aβ activates the neutral sphingomyelinase-ceramide pathway, causing DNA fragmentation and mitochondrial functional impairment, resulting in oligodendrocytic apoptosis and necrosis.98,99 Both astrocytes and microglia have phagocytic activity and can clear Aβ. On the other hand, activated microglia found in the periphery of Aβ plaques could also release pro-inflammatory cytokines, thereby increasing oxidative stress.100,101

Astrocytic Aβ peptide

Accumulating data show the importance of astrocytes in Aβ pathogenesis in AD. Numerous debates have discussed whether astrocytes uptake Aβ from the extracellular space or they could synthesize Aβ de novo. Moreover, there is incongruent evidence
toward the activation of astrocytes by Aβ. The notion that astrocytes phagocytose Aβ from the extracellular space is supported by several studies, which demonstrated that Aβ was taken up by astrocytes for lysosomal degradation in order to maintain Aβ homeostasis.53 Studies have shown that astrocytes clear preferentially N-terminal-truncated Aβ, and subsequently concluded that the Aβ was of neuronal origin.102,103 Other reports indicated that astrocytes were more inclined to phagocytose oligomeric Aβ1–42 than fibrillar Aβ1–42, and that Aβ oligomers do activate astrocytes.103,104 It is worthwhile to note that one of the studies used human astrocytes from AD patients, whereas the other used primary astrocytes derived from postnatal wild-type mice. The difference between species and tissue healthiness could explain the disparity of results in astrocytes responsiveness. In another study, a lower responsiveness to Aβ in human astrocytes from AD cases than in non-AD cases was demonstrated, suggesting that amyloid plaque formation in the extracellular space in AD cases is secondary to insufficient clearance of Aβ by astrocytes.105 APP and BACE1 overexpression in astrocytes may lead to Aβ synthesis de novo. Compelling studies demonstrate APP immuno-reactivity in astrocytes and its subsequent upregulation in the post-ischemic brain along with Aβ deposition appearances.106–108 It has been recently suggested that APP has a physiological role in astrocytic Ca2+ oscillations, APP silencing leads to dysfunctional store-operated Ca2+ channels.109 One study reported an enhanced BACE1 production in activated astrocytes after stroke, thereby suggesting that Aβ accumulation in the extracellular space could be due to its production and release by activated astrocytes.110 Furthermore, astrocytic BACE1 and APP expressions were found to have increased significantly upon stimulation with TNF-α and interferon-γ.110,111 It has also been suggested that astrocytes may have a dual role in clearing and producing Aβ.112

The ability for astrocytes to degrade Aβ relies on the expression of proteases such as nephrilysin, insulin-degrading enzyme, ET-converting enzyme, angiotensin-converting enzyme, plasminogen activators and matrix metalloproteinases.112 In addition, secretion of apolipoprotein E (APOE) by astrocytes is suggested to be part of the neuronal repair as much as part of the Aβ clearance mechanisms. However, different isoforms of APOE were suggested to have different functions in AD, for example, APOE ε4 isoform has been reported to be a major risk factor, whereas APOE ε2 isoform exerts a protective role.113 It is worthwhile to note that APOE has been implicated in vascular dysregulation, but much controversy still exists in this area.114,115

ET-1 and RAGE participation in an ischemic AD brain
Endothelin-1

ET-1 is a potent vasoconstrictor first characterized from the porcine aortic endothelial cells, generally recognized for its role in blood pressure regulation and inflammatory mediation.116–118 In astrocytes, ET-1 is an important growth factor that can regulate its proliferation, survival, migration, and functional properties of astrocytes. The ET-1 overexpression in astrocytes is associated with the inhibition of gap junctional communication, cellular migration through upregulation of matrix metalloproteinase-9, as well as activation after brain injury via G-protein-coupled endothelin A receptor and endothelin B receptor.119–122 ET-1 can also induce intracellular Ca2+ concentration increase through the activation of these receptors. Endothelin A receptor and endothelin B receptor trigger phospholipase C, which can hydrolyze phosphatidylinositol 4,5-biphosphate to generate inositol 1,4,5-triphosphate (IP3). The binding of IP3 to its receptor in the endoplasmic reticulum induces Ca2+ release into the cytoplasm.123

During ischemia, ET-1 is upregulated in both endothelial cells and astrocytes.124 In astrocytic cultures, ET-1 shows a protective function against hypoxic-ischemic insults.55 In an animal model, however, ET-1 overexpression due to transient middle artery occlusion is followed by increases of ROS, NO and proaggregin levels, matrix metalloproteinase-2 expression and aquaporin 4 translocation. These changes would lead to the generation of oxidative and nitrosative stress, inflammation, BBB breakdown and other deleterious effects leading to the cerebrovascular dysfunction.125–130 ET-1 preconditioning of neuronal cultures appear to exert a protective role from inflammation and oxidative stress.131 Inflammation constitutes an important pathological event in ischemia. Recent evidence has reported that the additions of inflammatory mediators transforming growth factor-β1, lipopolysaccharide and interferon-γ to astrocytes can alter the expression of endothelin A and B receptors among other G-protein-coupled receptors, resulting in the modulation of astrocytic calcium signaling.56

In relation to AD, it is known that ET-1 precursor ‘pre-proendothelin-1’ is cleaved by the ET-converting enzyme—2 present in neurons, astrocytes and microglia—that coincidentally is also one of many proteases that contribute to Aβ degradation. It has been proposed that Aβ overexpression upregulates ET-converting enzyme-2, synchronously enhance ET-1 production, which then leads to a decrease in CBF in AD owing to the ET-1-mediated vasocostriction, thus suggesting ET-converting enzyme-2 as an important factor involved in AD pathology.132 In an attempt to study the relationship between cerebral ischemia and cognitive impairment in AD, one study showed that a combination of overdosed ET-1 and Aβ infusion, mimicking cerebral ischemia in an AD animal model, led to a more severe decline in cognition.12 The overdosage of ET-1 and Aβ generated an increased proinflammatory response. Aβ deposition, neuronal loss, t-phosphorylation and astrocytes activation. Moreover, it has also been demonstrated that influx of intracerebral Aβ through the BBB by RAGE induced the expression of proinflammatory cytokines such as IL-6 and TNF-α, along with ET-1 production in endothelial cells.133 The increased ET-1 production led to a decrease in CBF. As ET-1 levels remain unchanged in the RAGE knockout mouse model, but upregulated in RAGE-associated Aβ transportation, it was concluded that ET-1-mediated vasocostriction is RAGE-dependent.

RAGE and cerebral ischemia

In cerebral ischemia, RAGE activation can trigger increased oxidative stress and inflammation.134–136 As mentioned earlier, RAGE appears to be involved in the Aβ-ET-1 interaction at the BBB during brain ischemia. RAGE expression can trigger multiple downstream signaling cascades to induce chronic inflammation, involving the activation of NFκB.137 RAGE was first described to bind advanced glycation end products on the cell surface of endothelial cells in hyperglycemia cases.138 It is now known that RAGE is localized on the cell membrane as receptor to multiple ligands, such as advanced glycation end product, Aβ, a family of S100/calgranulins, β2-integrins and high mobility group box 1 protein. RAGE is found in various cells, including microglia, astrocytes, endothelial cells, neurons and oligodendrocytes.139

Research in AD has shown that RAGE is responsible of Aβ influx from circulating plasma into the brain through the BBB.12 RAGE overexpression in endothelial cells can cause BBB leakage by decreasing F-actin stress fibers through β-catenin increment, thus preventing membrane resealing. Without an intact BBB, the influx/exflux homeostasis of biomolecules between the blood and the brain is interrupted, leading to a cascade of pathological events contributing to neuronal demise.140 RAGE expression is not limited to the endothelial cells in the BBB. Studies have explored its role in neurons, microglia and astrocytes. Aβ–RAGE interaction induces mitochondrial dysfunction in cortical neurons through the activation of p38 mitogen-activated protein kinase. Furthermore, it was shown that Aβ is
internalized in cortical neurons through a RAGE-dependent pathway. On astrocyte surface, the same Aβ–RAGE ligand–receptor interaction leads to extracellular signal-regulated kinases 1/2 and calcium-dependent phospholipase A2 phosphorylation, along with ROS production through a different mechanism than in cerebral endothelial cells. In microglia, overexpression of RAGE causes an enhanced production of IL-1β, TNF-α and amyloid accumulation. Moreover, synthesis of Aβ in neurons has been shown to be mediated by a RAGE-dependent pathway, involving the activation of the nuclear factor of activated T cell 1 (NFAT1) through Ca²⁺ concentration increment, which in turn upregulated BACE1 expression after RAGE activation.

In studying other factors that are influenced by RAGE expression, one study reported that the secretion of the proinflammatory cytokines IL-6 and TNF-α in astrocytes on S100B stimulation was through a RAGE-dependent manner. Furthermore, both extracellular glutamate and S100B have been shown to enhance RAGE expression independently in cortical neurons, thus providing evidence of the importance of S100B—a factor enhanced and secreted on astroglial stimulation—in RAGE activation.

**MECHANISM UNDERLYING Aβ PRODUCTION IN ASTROCYTES**

The previous sections have discussed the potential importance of vascular lesions in the development of neurodegeneration as observed in AD. Moreover, the NVU and astrocytes are likely to have pivotal roles in the initiation of the early pathologies that lead to the eventual neuronal demise.

In attempt to elucidate one of the mechanisms leading to Aβ production in astrocytes during ischemic conditions, we hypothesize that the increase of ET-1 as a result of astrocytic hypoxic-ischemic stress, ET-1 acts in an autocrine manner to activate astrocytic responsiveness. S100B, as a result of astrocytic reactivity, binds to its receptor RAGE in an autocrine manner. S100B–RAGE complexes are internalized to the cytosol, Figure 1. β-Amyloid (Aβ)–peptide generation in astrocytes. Under hypoxic-ischemic conditions (1), healthy astrocytes increase endothelin-1 (ET-1) production and secretion (2). ET-1 autocrine interaction with its receptors endothelin A receptor (ETₐR) and endothelin B receptor (ETᵦR) activates astrocytes (3), upregulating glial fibrillary acidic protein (GFAP), vimentin and S100B production. On extracellular S100B interaction with its receptor for advanced glycation end product (RAGE), both are translocated to the cytosol by endocytosis, but RAGE is recycled to the membrane (5), whereas S100B binds to calcium, causing intracellular calcium raise, disrupting calcium homeostasis (6). On intracellular calcium increment, phosphorylated nuclear factor of activated T cell 1 (pNFAT1) is dephosphorylated and translocated to the nucleus (7), binding to β-site amyloid precursor protein (APP) cleaving enzyme (BACE1) gene promoter and activating BACE1 transcription (8). APP cleavage by BACE1 will be favored, generating more Aβ oligomers (9). Extracellular Aβ may form fibrils that will consecutively lead to dense core plaques formation (10a), whereas intracellular Aβ may form amyloid annular protofibrils (APFs) that consecutively will lead to membrane disruption through the formation of a calcium-permeable channel (10b).
Astrocytes

Stroke (ET-1 is overexpressed)

Astrogliosis (S100B is upregulated)

S100B binds to RAGE and is translocated to the cytosol

Calcium homeostasis is disrupted and NFAT1 is translocated to the nucleus

NFAT1 acts as transcription factor for BACE1 (BACE1 expression is upregulated)

Amyloidogenic pathway is favoured

Aβ is produced

Figure 2. Astrocytic β-amyloid (Aβ)-peptide synthesis in stroke. After an ischemic episode, endothelin-1 (ET-1) is upregulated, inducing astrogliosis. S100B expression will be enhanced after astrocytes reactivation and will be translocated to the cytosol on its binding to receptor for advanced glycation end product (RAGE). As a result, calcium homeostasis will be disrupted by S100B interaction with calcineurin. Phosphorylated nuclear factor of activated T cell 1 (pNFAT1) will be dephosphorylated and translocated to the nucleus, where it might bind to a gene promoter region of β-site amyloid precursor protein (APP) cleaving enzyme (BACE1), upregulating BACE1 expression. With more BACE1 to cleave APP, the amyloidogenic pathway is favored producing more Aβ.

but RAGE is then recycled to the membrane. The sudden increment of S100B in the cytosol causes calcium homeostasis disruption, raising intracellular calcium concentrations, activating NFAT1 translocation to the nucleus. NFAT1 acts as a transcription factor for the BACE1 gene, favoring APP cleavage to Aβ production nuclear factor of activated T-cells. Aβ could then go through two different pathways, forming either Aβ fibrillary plaques or Aβ APFs. Under ischemic condition such as stroke, the described putative events would lead to increased Aβ production, thereby leading to the neurodegeneration observed in MixD and VaD (Figure 2).

SUMMARY

Increasing number of studies in the literature has pointed out the significant involvement of vascular lesions in AD patients, thereby leading to the conceptualization of MixD as a distinct dementia etiology. Some researchers argue that MixD is in fact the most common form of dementia. Although the MixD concept remains unclear, it is generally accepted that cognitive decline in these cases is resulted from vascular damage in conjunction with amyloid deposition and neurofibrillary tangles formation. As the NVU represents the functional unit in maintaining cerebral vascular and transport homeostasis, the disruption of its proper functioning could be important in the neurodegenerative processes that occur in MixD and VaD. Furthermore, astrocytes, being an essential component of the NVU, form the intimate link between the endothelium and neurons. During an ischemic insult, astrogliosis acts as a ‘double-edged sword’ in restoring the brain microenvironment but could also lead to ROS-mediated and inflammatory damages. In relation to AD and MixD, astrocytes appear to be closely linked to Aβ pathogenicity. The interactions between RAGE, ET-1 and astrocytic activation in ischemia likely have a critical role in amyloidogenesis. In view of the high prevalence of AD/MixD and the lack of thorough understanding of AD/MixD mechanisms, further pursuit of knowledge with respect to astrogliosis and NVU dysfunction in dementia pathogenesis is highly warranted.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Molecular Psychiatry (2014), 159 – 167


