Blood-brain barrier integrity in the pathogenesis of Alzheimer's disease

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Abstract

The blood-brain barrier (BBB) tightly controls the molecular exchange between the brain parenchyma and circulating blood. An increasing volume of evidence from both transgenic animal Alzheimer's disease (AD) models and human AD patients have demonstrated that BBB dysfunction is a major player in AD pathology. In this review, we discuss the role of the BBB in maintaining brain integrity and how this is mediated by crosstalk between BBB-associated cells within the neurovascular unit (NVU). In addition, we discuss the role of the NVU, in particular its endothelial cell, pericyte and glial cell constituents, in AD pathogenesis. The effect of substances released by the neuroendocrine system in modulating the function of BBB and AD pathogenesis is discussed in this context. We perform a systematic review of currently available AD treatments that specifically target pericytes and BBB glial cells. In summary, this review provides a comprehensive overview of BBB dysfunction in AD pathogenesis and a new perspective on the development of therapeutics for AD.

Keywords: blood-brain barrier; neurovascular unit; endothelial cells; Alzheimer's disease; neuroendocrine

1. Introduction

The blood-brain barrier (BBB) is formed by endothelial cells that comprise the primary structure of the capillaries within the microvasculature that are covered by a layer of basement membranes. Capillaries are assembled by a monolayer of brain microvascular endothelial cells (BMEC) that are covered by pericytes and the end-feet of astrocytes (Daneman, 2012; Daneman & Prat, 2015; Langen *et al.*, 2019; Zhao *et al.*, 2015a). These are the smallest cerebral blood vessels, accounting for approximately 85% of the total cerebral vessel length, and is the major site of the BBB. In the human brain, the cerebral capillaries have a total length of approximately 600 km, creating a total abluminal surface area of approximately $20m^2$ that mediates molecular transport and exchange between the blood and the brain (Iadecola, 2017; Kisler *et al.*, 2017).

The BBB plays several crucial functions within the central nervous system (CNS). Firstly, it plays a major role in regulating cerebral blood flow, which is required for CNS homeostasis. Secondly, it regulates the transport of glucose, oxygen and other metabolites from the blood to the brain in order to maintain the proper function of neuronal circuits. Thirdly, the BBB allows the selective removal of metabolic waste from the brain via the brain vasculature (Daneman & Prat, 2015; Zlokovic, 2008b). Apart from these functions, BBB acts as both an endocrine target and an endocrine secretory tissue (Banks, 2019). Substances secreted by the neuroendocrine and endocrine systems influence the function of BBB and in turn, the substances secreted by BBB also influence brain function.

The BBB structure is specifically organized to perform these crucial functions. A physical barrier is created at the BBB structure through tight junctions between endothelial cells; the outer wall of the endothelium is then enclosed by pericytes and the astrocytic end-feet (Daneman, 2012; Daneman & Prat, 2015; Langen *et al.*, 2019; Zhao *et al.*, 2015a). This structure assembles a physically unbroken membrane that partitions the blood away from the brain parenchyma (Reese & Karnovsky, 1967). The BBB forms an important part of the neurovascular unit (NVU), which is created by a network of BMECs together with pericytes, glia and neurons (Daneman, 2012; Daneman & Prat, 2015). Each

individual constituent of the NVU performs individually distinct physiological roles in maintaining normal BBB functions (Daneman, 2012). This ensures that the BBB can restrict the passage of neurotoxic molecules and harmful pathogens into the CNS (Winkler *et al.*, 2011). It therefore serves as the key homeostatic regulator that connects the CNS to the blood circulation.

Several neurodegenerative diseases such as Alzheimer's disease (AD) possess significant vascular pathophysiology such as the disruption of BBB integrity and function (Kapasi & Schneider, 2016; Sagare *et al.*, 2012; Yarchoan *et al.*, 2012; Zlokovic, 2011). AD is one of the most common causes of age-related neurological and cognitive functional decline (Hebert *et al.*, 2013). AD is primarily characterized by the aggregation of extracellular Aβ peptides into plaques (Ellis *et al.*, 1996; Shinohara *et al.*, 2016) and the accumulation of hyper-phosphorylated tau-forming neurofibrillary tangles (NFTs) (Heneka *et al.*, 2015; Serrano-Pozo *et al.*, 2011; Spires-Jones & Hyman, 2014). Progressive cognitive and behavioral deficits are major clinical manifestations in AD patients, mainly due to the damage to the hippocampus and neocortex. BBB dysfunction in AD leads to increased vascular permeability and the inability to remove neurotoxic substances from the CNS. BBB dysfunction also allows toxic blood-derived molecules and microbial pathogens to enter the brain causing neuroinflammatory responses which may further contribute to neurodegeneration (Daneman, 2012; Sagare *et al.*, 2012; Zenaro *et al.*, 2017; Zlokovic, 2005; Zlokovic, 2008b; Zlokovic, 2011).

In this review, we will firstly discuss the role of the NVU in maintaining normal BBB characteristics and function. Secondly, we will discuss the link between BBB dysfunction and AD pathogenesis with an emphasis on the role of pericytes and glial cells. We will also review the crosstalk between these cells in AD pathology. Furthermore, we discuss the influences of neuroendocrine system as well as sex difference on the function of BBB and the pathogenesis of AD. Finally, we will summarize the current evidence for potential therapeutic approaches that target pericytes and glial cells which are urgently needed to effectively treat patients with AD.

2. Physiological functions of BBB-related cells

While endothelial cells predominantly control BBB integrity and function, other components of the NVU including pericytes, astrocytes and microglia are also critical for the maintenance of proper BBB function (Daneman & Prat, 2015; Erickson & Banks, 2013; Zenaro *et al.*, 2017; Zlokovic, 2005; Zlokovic, 2008a). We will discuss the key cellular and molecular pathways that underlie the formation and maintenance of the BBB below. (See also Figure 1)

2.1. Physiological function of endothelial cells in the CNS

Endothelial cells are the fundamental elements of the vascular system, and are important for precise regulation of molecular transport across the BBB. They are connected by both tight and adherens junctions which limit the permeability between luminal and abluminal compartments (Daneman & Prat, 2015; Deeken & Loscher, 2007).

Four major classes of tight junction proteins are highly expressed in the BMECs of the CNS: Occludin, Tricellulins, Claudins, and junctional adhesion molecules (JAMs). Occludin (encoded by *Ocln*) was the first transmembrane protein shown to localize exclusively to tight junctions (Furuse *et al.*, 1993). The function of Occludin is not entirely understood; phenotypically, *Ocln*$^{-/-}$ mice develop normal tight junctions, but display neurological phenotypes such as abnormal brain calcification (Martin-Padura *et al.*, 1998; Saitou *et al.*, 2000). One possibility for this phenotypic disparity is that Tricellulins, which are structurally similar to Occludin, may partially compensate the function of Occludin (Ikenouchi *et al.*, 2005). Claudin has more than 20 orthologs in the mammalian genome. Among these members, Claudin-3, -5, and -12 have been found to be localized at the BBB. Claudin-5 (encoded by *Cldn5*) is the most highly enriched in the BMECs (Morita *et al.*, 1999). *Cldn5* knockout mice demonstrate perinatal fatality, however, these mice display normal vascular development and show no overt morphological abnormalities in peripheral tissues. However, *Cldn5*$^{-/-}$ mice also exhibit a size-selective increase in barrier permeability: the BBB against small molecules (with a molecular

weight less than 800 Da) was severely affected in Cldn5$^{-/-}$ mice (Nitta *et al.*, 2003). In normal circumstances, small molecules less than 400 Da are able to cross the BBB (Pardridge, 2015).

Endothelial cells allow the diffusion of oxygen from the blood circulatory system into the brain, and carbon dioxide from the brain into the blood. This process is crucial for nutrient and pH homeostasis in the brain (Pardridge, 2015). However, the permeability of other molecules across the BBB is tightly regulated by four major endothelial transportation mechanisms: 1) active efflux, 2) carrier-mediated transportation, 3) receptor-mediated transportation, and 4) major-facilitator-superfamily-facilitated movement (Zhao *et al.*, 2015a). The barrier function of endothelial cells is highly sensitive to inflammatory mediators and reactive oxygen species (ROS) that cause neuroinflammation and oxidative stress, which results in CNS disorders (Sanchez del Pino *et al.*, 1995; Vorbrodt & Dobrogowska, 2003).

2.2. Physiological function of pericytes in the CNS

Pericytes (also known as mural cells) are embedded in the basement membrane surrounding the blood vessels. Their morphology are highly variable, dependent on their position along blood vessels (Sweeney *et al.*, 2016). Although pericytes were first described 150 years ago (Rouget, 1873), their function has only been investigated in recent years. Specific markers for pericytes are still unavailable, but pericytes can be identified by the co-staining of Neural/glial antigen 2 (NG2) and Platelet-derived growth factor receptor β (PDGFRβ), and by the absence of alpha-smooth muscle actin (α-SMA) (Armulik *et al.*, 2011). In the NVU, pericytes are positioned between the endothelial cells, astrocytes, and neurons (Attwell *et al.*, 2016; Hartmann *et al.*, 2015; Sweeney *et al.*, 2016). They can communicate directly with endothelial cells through gap junctions and also with other pericytes via 'peg-and-socket' contacts (Armulik *et al.*, 2005; Winkler *et al.*, 2011).

Pericytes carry out important physiological roles including angiogenesis, the removal of toxic cellular byproducts, regulating immune cell entry, regulating cerebral blood flow, as well as self-proliferation and migration in response to injury (Armulik *et al.*, 2010). Pericytes are necessary for embryonic

brain blood vessel formation (Bell *et al.*, 2010). In addition, they are crucial for adult brain angiogenesis by secreting factors such as vascular endothelial growth factor (VEGF) and notch homolog protein (Notch) ligands (Ribatti *et al.*, 2011). Some studies suggested that pericytes play a role in blood vessel regeneration, and are capable of differentiating into other cell types in the NVU in response to ischemic stress (Nakagomi *et al.*, 2015; Sakuma *et al.*, 2016).

Pericytes play a crucial role in barrier maintenance. In pericyte-ablated adult mice, retina barriers are more likely to disintegrate under stress and injury (Park *et al.*, 2017). In adult *Foxf2* deficient mice, CNS pericyte-specific transcriptional factor PDGFRβ signaling was decreased, which leads to severe BBB leakage (Reyahi *et al.*, 2015). Additionally, pericytes contain contractile fibers and can restrict capillary blood flow by contraction, which leads to increased BBB permeability (Rucker *et al.*, 2000).

2.3. Physiological function of astrocytes at the BBB

Astrocytes, the 'star-shaped' cells, are the most abundant glial cells in the brain. Their morphology also varies according to their location and their interacting cell types (Agulhon *et al.*, 2008). Astrocytes also have several other important functions in the CNS outside of the BBB, such as providing nutrients to the neurons, maintaining extracellular ion balance, regulating the release of neurotransmitters, and controlling synaptic transmission (Abbott *et al.*, 2006; Mookherjee *et al.*, 2011; Shtrahman *et al.*, 2017; Sofroniew, 2015). In the NVU, astrocytes are positioned between the cerebral endothelial cells and neurons where astrocyte end-feet cover most of the capillary vessels (Filosa *et al.*, 2016; Sosunov *et al.*, 2014). Astrocytes are unlikely to contribute to BBB formation during development as they appear after the BBB has been sealed (Yang *et al.*, 2013b). However, they play an important role in BBB maintenance. Astrocyte-derived Sonic hedgehog (Shh) signaling stimulates the expression of tight junction proteins such as Claudin-5 and Occludin in endothelial cells *in vitro* (Alvarez *et al.*, 2011; Wang *et al.*, 2014). In addition, an *in vivo* study has shown that laminins specifically secreted by the astrocytes are important for maintaining BBB integrity; the deletion of astrocytic laminins leads to decreased Aquaporin4 (AQP4) expression in astrocytes and reductions in

tight junction protein levels in endothelial cells (Yao *et al.*, 2014). Astrocytes express high levels of gap junction protein connexin 43 and 30 (Cx43 and Cx30). Astroglial Cx43/Cx30 double knockout mice have abnormal morphology in their end-feet, which leads to weakened BBB integrity (Ezan *et al.*, 2012). Furthermore, fatty acid-binding protein 7 (FABP7) secreted by astrocytes can improve BBB integrity (Rui *et al.*, 2019).

The crosstalk between the astrocyte end-feet and endothelial cells in the brain is essential for the maintenance of vascular integrity throughout adulthood (De Bock *et al.*, 2014; Gordon *et al.*, 2011; Tanigami *et al.*, 2012). The secretion of growth factors from astrocyte end-feet contributes to vascular integrity also by upregulating nutrient transporters in endothelial cells (Alvarez *et al.*, 2013).

2.4. Physiological functions of microglia at the BBB

Microglia are the resident "macrophages" in the brain, which play an important role in immune response and phagocytosis in the CNS in the event of injury and disease (Alliot *et al.*, 1999). Microglia are also an important component of the NVU as they play a vital role in angiogenesis and regulating BBB function (Fantin *et al.*, 2010). Microglia promote the stabilization and fusion of endothelial cells during fetal vascularization in the CNS. Specific depletion of microglia by clodronate liposomes (CL2MDP-lip) leads to decreased vessel density in mice (Espinosa-Heidmann *et al.*, 2003).

3. BBB function and dysfunction in AD pathology

While the BBB is vital for CNS homeostasis maintenance, it is also susceptible to neurodegenerative pathologies such as AD. The characteristics of BBB dysfunction in AD include increased BBB permeability, microbleeds, reduced expression of tight junctions, impaired BBB transporter expression, perivascular accumulation of blood-derived products, and degeneration of pericytes and endothelial cells (Sweeney *et al.*, 2018). When aggregation of Aβ, one of the key pathogenic peptides

in AD, occurs in and around the cerebral blood vessels, this can lead to cerebral amyloid angiopathy (CAA) which results in an increased BBB permeability in the AD brain (Erickson & Banks, 2013; Hashimura *et al.*, 1991; Kimura *et al.*, 1991; Roher *et al.*, 2003; Zlokovic, 2008a). In addition, the accumulation of perivascular tau protein, another AD pathogenic protein, can also promote BBB leakage (Blair *et al.*, 2015). This leakage enables blood-derived toxic molecules, cells, and pathogens to enter the brain, which further triggers inflammatory responses that leads to disease progression.

Aβ peptides or aggregates undergo clearance from the brain through several pathways: 1) receptor-mediated transcytosis across the BBB, 2) interstitial fluid (ISF) bulk flow into the cerebrospinal fluid (CSF), 3) extracellular Aβ-degrading enzymes, and 4) glial phagocytosis (Ries & Sastre, 2016; Xin *et al.*, 2018). The majority of Aβ is cleared from the brain primarily by receptor-mediated transcytosis across the BBB (Bell *et al.*, 2007; Deane *et al.*, 2004; Shibata *et al.*, 2000). This process requires specific transporter proteins, such as low-density lipoprotein receptor-related protein 1 (LRP1), very low-density lipoprotein receptor (VLDLR) and P-glycoprotein (P-gp)(Xin *et al.*, 2018). We have summarized the key cellular and molecular pathways that underlie Aβ transcytosis across the BBB and Aβ phagocytosis by microglia and astrocytes in Figure 2.

LRP1, an apolipoprotein E (ApoE) receptor, is expressed mainly at the abluminal side of the BMEC and mediates internalization of soluble Aβ (Bell *et al.*, 2007; Deane *et al.*, 2004; Zhao *et al.*, 2015b). LRP1 interacts with the ApoE/Aβ complex, which initiates Aβ transcytosis across the BBB. Aβ transcytosis is regulated by phosphatidylinositol-binding clathrin assembly protein (PICALM), which guides Aβ-containing endocytic vesicles to fuse with Rab5+ early endosomes and then Rab11+ sorting endosomes, ultimately leading to Aβ exocytosis across the luminal part of the BBB into the blood (Zhao *et al.*, 2015b). Endothelium-specific deletion of *Lrp1* (Storck *et al.*, 2016) or *Picalm* (Zhao *et al.*, 2015b) leads to accelerated Aβ pathology in amyloid precursor protein (APP)-overexpressing $APP^{sw/0}$ mice. Similarly, other cell types in NVU, such as astrocytes and pericytes, also clear deposited Aβ via the LRP1/ApoE pathway (Liu *et al.*, 2017; Ma *et al.*, 2018).

There are three major ApoE isoforms (E2, E3 and E4) in humans (Zlokovic, 2011). Extracellular ApoE isoforms associate with Aβ and differentially regulate Aβ metabolism and transport at the BBB. For instance, ApoE2/Aβ and ApoE3/Aβ complexes mediate rapid BBB clearance by interacting with LRP1. On the other hand, ApoE4/Aβ complexes interact with VLDLR resulting in slower Aβ clearance, which may contribute to Aβ accumulation in the brain (Bell *et al.*, 2007; Deane *et al.*, 2008). Transgenic mice overexpressing the human ApoE4 gene have been associated with vascular injury in neurodegenerative diseases (Bell *et al.*, 2012; Zlokovic, 2005; Zlokovic, 2011). Conversely, ApoE2 is considered to be a protective variant against AD (Huang *et al.*, 2019), whereas the ApoE4 allele is considered the best established genetic factor for sporadic, late-onset AD (Al-Majdoub *et al.*, 2019; Hediger *et al.*, 2013; Pardridge, 2012; Tanzi, 2012).

P-glycoprotein, P-gp, is an efflux transporter expressed on both sides of the BBB, and has been proven to transport Aβ out of brain (van Assema *et al.*, 2012; Wang *et al.*, 2016). Several other proteins have also been shown to regulate BBB-mediated Aβ clearance. Glucose transporter 1 (GLUT1, encoded by *SLC2A1*), a transmembrane protein responsible for transport of glucose across the plasma membranes, regulates LRP1-dependent Aβ clearance by increasing the expression of LRP1 (Winkler *et al.*, 2015). In addition, transport of GLUT1-mediated glucose into the brain is also beneficial to maintaining the integrity of the BBB, thereby ensuring the normal transport of Aβ from brain into blood (Winkler *et al.*, 2015). Furthermore, it has also been shown that absence of *Abcb1a/b* (which encodes P-gp) from the endothelial cells leads to accelerated Aβ deposition in Tg2576 mice (Cirrito *et al.*, 2005). It has been shown that in the endothelium of AD brains, the levels of GLUT1 (Mooradian *et al.*, 1997), PICALM (Zhao *et al.*, 2015b), and P-gp (Chiu *et al.*, 2015; Wijesuriya *et al.*, 2010) are reduced, which impairs clearance of Aβ peptides, and subsequently speeds up Aβ accumulation in the brain.

As animal studies have shown, BBB dysfunction may be a consequence of AD pathology and contribute to disease progression. Increasing numbers of clinical studies have confirmed that neurovascular dysfunction contributes to the onset and progression of AD, and have proposed a link

between cerebrovascular changes and neurodegeneration (Sagare *et al.*, 2013a; Sagare *et al.*, 2012; Zlokovic, 2005; Zlokovic, 2011). For instance, recent studies using advanced dynamic contrast-enhanced magnetic resonance imaging (MRI) have shown that patients with mild cognitive impairment (MCI) at early stages of AD display signs of BBB leakage as a result of CAA prior to brain atrophy or dementia (Montagne *et al.*, 2015; Montagne *et al.*, 2016; van de Haar *et al.*, 2016). Disrupted BBB function could also decrease cerebral blood flow, resulting in neurofibrillary tangles and neuronal loss (Sagare *et al.*, 2013a), which is increasingly recognized as a primary contributor to AD pathophysiological development (Erickson & Banks, 2013; Zenaro *et al.*, 2017; Zlokovic, 2005).

In conclusion, the BBB plays a key role in the clearance of toxic Aβ from the CNS. In the AD brain, BBB dysfunction due to the exacerbation of Aβ plaques and tau pathophysiology further worsens neurodegeneration and neuroinflammatory responses (Zlokovic, 2005; Zlokovic, 2008a).

4. The function of other NVU elements in the AD brain

A dysfunctional NVU means that its constituent components may undergo significant structural and functional changes that ultimately lead to neural inflammation, neurodegeneration, and cognitive deficits that are the hallmarks of AD pathology. In this section, we will discuss BBB dysfunction in AD brains in the context of the various NVU elements, highlighting the individual roles of the pericytes, astrocytes and microglia.

4.1. Pericytes in the AD brain

Taking into consideration the importance of pericytes in normal BBB function, their dysfunction unsurprisingly results in BBB disruption, which leads to neurodegeneration and cognitive impairment (Nikolakopoulou *et al.*, 2019; Sagare *et al.*, 2013a).

Within pericytes, PDGFRβ signaling activated by BMEC-secreted PDGF-BB is important for pericyte survival, proliferation, and migration (Stratman *et al.*, 2010). Therefore, aberrant PDGFRβ shedding by ADAM10 (A Disintegrin and Metalloproteinase 10) leads to the loss of pericytes (Nation *et al.*, 2019; Stratman *et al.*, 2010). *Pdgfrβ*$^{+/-}$ pericyte-deficient mice have the characteristics of BBB dysfunction, which leads to secondary neurodegeneration (Bell *et al.*, 2010). In a murine model of AD (APP$^{sw/0}$), such pericyte reduction is correlated with BBB dysfunction, which is associated with an accelerated buildup of Aβ and tau pathology (Sagare *et al.*, 2013a). In clinical studies, the loss of pericytes is evident in the hippocampus and cortex in AD patients, which is a primary contributor to BBB disruption in these brain areas (Sengillo *et al.*, 2013). Loss-of-function mutations in PDGFβ in pericytes lead to idiopathic primary familial brain calcification as well as motor and cognitive impairment (Keller *et al.*, 2013; Nicolas *et al.*, 2014).

Recent studies on biomarkers in CSF have found an elevation of soluble PDGFRβ (sPDGFRβ) in the CSF associated with hippocampal BBB leakage in patients with MCI and at early stages of AD (Miners *et al.*, 2019; Montagne *et al.*, 2015). sPDGFRβ was shed from injured pericytes by ADAM10. CSF sPDGFRβ level can be used as a marker to predict cognitive decline independently from Aβ or tau levels in the CSF (Nation *et al.*, 2019). However, it is unclear if sPDGFRβ regulates BBB function.

It has been shown that pericytes also play a role in Aβ clearance. Pericytes can internalize different Aβ peptides via the LRP1 pathway for lysosomal degradation (Sagare *et al.*, 2013b; Wilhelmus *et al.*, 2007). In the brain of AD patients, pericytes can internalize the accumulated Aβ peptides (Saint-Pol *et al.*, 2012); however, prolonged exposure to high concentrations of Aβ peptides leads to pericyte cell death (Sagare *et al.*, 2013b; Wilhelmus *et al.*, 2007). This accelerates Aβ deposition in the brain by reducing Aβ clearance through the LRP1 pathway (Sagare *et al.*, 2013b). Another study suggests that pericyte dysfunction due to Aβ accumulation leads to cerebral hypoperfusion and compromised Aβ peptide clearance in the AD brains (Dalkara *et al.*, 2011). In addition to LRP1, studies from post-mortem AD brains suggest that other Aβ-binding receptors, such as the low-density lipoprotein receptor (LDLR), receptor for advanced glycation end products (RAGE), and the class B scavenger

receptors (CD36), are implicated in AD-related pericyte loss (Park *et al.*, 2013; Wilhelmus *et al.*, 2007).

As previously mentioned, ApoE4 is an ApoE variant that has been correlated with BBB impairment in AD. ApoE4 is also implicated in the functional deficits in pericytes. Overexpression of the human ApoE4 gene in mouse activates the pro-inflammatory cyclophilin A (CypA)-nuclear factor κB (NFκB)-metalloproteinase-9 (MMP-9) pathway in pericytes; activated MMP-9 subsequently leads to enzymatic degradation of endothelial cell junction complexes resulting in BBB dysfunction, and increased influx of blood-derived neurotoxins, such as thrombin, fibrin, and hemoglobin-derived iron (Bell *et al.*, 2010). Consistent to this, post-mortem studies using human brain tissues have found that ApoE4 accelerates pericyte loss in AD (Halliday *et al.*, 2016). ApoE4 leads to a higher accumulation of CypA and MMP-9 in pericytes and endothelial cells compared to ApoE3, which is associated with more severe BBB dysfunction in AD brains (Halliday *et al.*, 2016).

In addition, pericytes may also mediate the leukocyte recruitment to the CNS in AD. Aβ triggers pro-inflammatory responses and promotes leukocyte recruitment (Honig *et al.*, 2018; Logovinsky *et al.*, 2016; Town *et al.*, 2005). During inflammation, pericytes regulate the trafficking of leukocytes by increasing adhesion molecules and releasing chemo-attractants (Daneman *et al.*, 2010; ElAli *et al.*, 2014; Hill *et al.*, 2014; Hurtado-Alvarado *et al.*, 2014; Stark *et al.*, 2013). Mice with a defect in pericyte development have higher levels of intercellular adhesion molecule 1 (ICAM-1) (Daneman *et al.*, 2010). Thus, pericytes may facilitate leukocyte infiltration by ICAM-1-dependent pericyte-leukocyte interactions (Zenaro *et al.*, 2015). However, more research will be required to determine the pathways that mediate pericyte dysfunction and its influence on the inflammatory responses during AD development.

4.2. Astrocytes in the AD brain

In the AD brain, astrocytes undergo a series of morphological changes accompanied with expression profile changes and are activated into a reactive astrocyte state, which is one of the primary hallmarks

of AD pathology (Cai *et al.*, 2017). Astrocytes play opposing roles during the pathogenesis of AD; they can function in the traditional neuroprotective role which leads to Aβ clearance and barrier formation around plaques. On the other hand, astrocytes also have a neurotoxic effect. Cross-talk between astrocytes and microglia triggers a chronic inflammatory response and the release of gliotransmitters, which contribute to neurodegeneration (Perez-Nievas & Serrano-Pozo, 2018; Yi *et al.*, 2016). Reactive astrocytes in AD also show reduced glutamate transporter 1 (GLT1) expression, and/or mis-localization of GLT1, which undermines glutamate reuptake at synapses, leading to neuronal damage (Hefendehl *et al.*, 2016; Li *et al.*, 1997; Scimemi *et al.*, 2013; Scott *et al.*, 2011). Furthermore, loss of GLT1 has also been linked to cognitive function decline in AD patients (Masliah *et al.*, 1996).

During AD pathogenesis, astrocytes are involved in the production and clearance of Aβ peptides (Kodam *et al.*, 2019; Liu *et al.*, 2017). Internalization of Aβ occurs through the RAGE and LRP1 pathways (Guenette, 2003; Liu *et al.*, 2016; Ueno *et al.*, 2014), but additionally, astrocytes secrete several extracellular Aβ degrading enzymes, including neprilysin (NEP) (Yamamoto *et al.*, 2016; Yamamoto *et al.*, 2017; Yamamoto *et al.*, 2014), insulin-degrading enzyme (IDE) (Son *et al.*, 2016; Son *et al.*, 2015), and MMP-2 and -9 (Yin *et al.*, 2006). ApoE, a protein crucial for Aβ clearance through BBB, is also mainly expressed and secreted by astrocytes (Suidan & Ramaswamy, 2019; Zhang *et al.*, 2016).

However, upon Aβ deposition, abnormal astrocytic activity leads to the breakdown in these functions, leading to deficits in Aβ clearance, which results in the formation of amyloid plaques and neurofibrillary tangles in AD brains (Olabarria *et al.*, 2010). For instance, impaired RAGE/LRP1-dependent Aβ clearance by astrocytes leads to an imbalance between the generation and clearance of Aβ (Guenette, 2003; Liu *et al.*, 2016; Ueno *et al.*, 2014). Dysfunctional ApoE synthesized by astrocytes can promote AD pathology by reducing Aβ clearance through the BBB (Suidan & Ramaswamy, 2019). Therefore, loss of normal astroglial functions is also one of the major contributors to aging and neurodegenerative diseases (Rodriguez-Arellano *et al.*, 2016).

In addition, astrocyte end-feet depolarization in AD brains may contribute to the deterioration of BBB integrity (Yang *et al.*, 2011). Studies on cortex biopsies from AD brains have identified morphological changes in astrocytes near the Aβ deposits in both the brain parenchyma and cerebral blood vessels (Wisniewski *et al.*, 1989). In human AD patients and in murine AD models associated with CAA, astrocyte end-feet which surround vascular Aβ deposits undergo several structural changes, such as swelling, retraction, and detachment. Astrocyte-dependent cerebral vascular activity has also been reported to be compromised in amyloid-laden vessels in a mouse model with CAA (Kimbrough *et al.*, 2015).

The pathways mediating the crosstalk between astrocytes and the BBB are not well understood. One of the key astrocytic proteins related to BBB is AQP4. AQPs are a group of plasma membrane water-channel proteins that act as a cellular regulator for water content. Among AQPs, AQP4 is the most abundant protein in the CNS and is highly enriched at the astrocyte end-feet, the interface between the CNS and blood vessels (Moftakhar *et al.*, 2010; Nagelhus *et al.*, 2013). AQP4 plays an important role in regulating water transport across the BBB under pathological conditions; for example, brain edema caused by water intoxication and cerebral ischemia is reduced in AQP4 deficient mice (Bonomini & Rezzani, 2010). AQP4 at astrocyte end-feet has also been shown to be important in the regulation of Aβ clearance and degradation (Hoshi *et al.*, 2012; Yang *et al.*, 2012). In AD patients, an increased AQP4 expression has been found in brain temporal lobes (Hoshi *et al.*, 2012). Animals with astrocytic AQP4 deficiency fail to remove Aβ peptides, suggesting a functional role for AQP4 in Aβ clearance from the brain (Iliff *et al.*, 2012).

Different murine models have been used to study the role of astrocytic AQP4 in AD brains. In the Arc/SweAβ AD mouse model, Aβ aggregation in blood vessels hampers the proper formation of the perivascular sheath of astrocytes by disrupting the anchoring of perivascular AQP4, resulting in astrocyte depolarization. AQP4 is relocated from the astrocytes end-feet membranes at perivascular Aβ deposits to other membrane domains in the neuropil-surrounding Aβ plaques (Yang *et al.*, 2011). Similarly, in the transgenic 5xFAD mouse model, astrocyte polarization was impaired, which was

concomitant with reduced expression of both AQP4 and basement membrane component laminin α2 (Park *et al.*, 2014). Loss of AQP4 in the APP/PS1 mouse accelerates cognitive impairment by promoting Aβ deposition in the brain. The deletion of AQP4 in cultured astrocytes shows reduced activation and impaired Aβ uptake, which might account for the increased Aβ deposition *in vivo* (Xu *et al.*, 2015).

In addition, AQP4 might also be involved in glymphatic clearance of toxic proteins. In post-traumatic brains, AQP4 knockout can exacerbate the impairment of the glymphatic clearance system, and this therefore accelerates the development of neurofibrillary tau tangles (Iliff *et al.*, 2014). This finding is however controversial. A recent study in *Aqp4* gene deletion mice and rats has shown that *Aqp4* gene deletion did not impair transport of solutes from the sub-arachnoid space to the brain in mice or rats, suggesting that tracer movement within the brain parenchyma was size-dependent and consistent with the classical view of diffusive solute transport (Smith *et al.*, 2017). Further investigation will be required to resolve the precise roles of AQP4 in tracer distribution in the perivascular and parenchymal compartments.

4.3. Microglial cells in the AD brain

Microglia act as the first line of defense in the brain. They become activated upon brain injury or pathogen invasion (Kettenmann *et al.*, 2011; Kreutzberg, 1996), but the resulting uncontrolled neuroinflammation is often implicated in exacerbating the progression of neurodegenerative diseases (McGeer & McGeer, 1995) and correlated with BBB impairment (Lassman *et al.*, 2012; Zipser *et al.*, 2007). A recent study has shown that vessel-associated microglia initially protect BBB integrity via the expression of the tight-junction protein Claudin-5 during inflammation. However, with prolonged inflammation, microglia display an increasingly activated phenotype leading to impaired BBB function (Haruwaka *et al.*, 2019).

Similarly, in the AD brain, microglia are activated around Aβ deposits where they release inflammatory cytokines, such as interleukin (IL)-1, IL-6, tumor necrosis factor (TNF)-α and TNF-β,

which play a causal role in the development and progression of AD (Perlmutter *et al.*, 1990; Wisniewski *et al.*, 1992; Wyss-Coray, 2006; Zhou *et al.*, 2012). These inflammatory cytokines such as IL-1β, can compromise BBB integrity and increase BBB permeability (Wang *et al.*, 2014), resulting in increased neutrophil trafficking through the BBB into the brain (Allen *et al.*, 2012; Zenaro *et al.*, 2015). The activated microglial cells also lead to the production of ROS and reactive nitrogen species, resulting in neurotoxicity and BBB impairment (Block, 2008; Sumi *et al.*, 2010).

In addition, the Aβ aggregates can be phagocytized by microglia through an ensemble of cell surface receptors, including class A scavenger receptor (SR-A) (El Khoury *et al.*, 1996; Paresce *et al.*, 1996), CD36, CD14, CD47 and Toll-like receptor (TLR) 2, TLR4, TLR6 and TLR9 (Bamberger *et al.*, 2003; Reed-Geaghan *et al.*, 2009; Stewart *et al.*, 2010).

4.4. The pericyte-glia crosstalk in the AD brain

The communication between astrocyte end-feet and pericytes in the brain is essential for the maintenance of vascular integrity during adulthood (Herland *et al.*, 2016; Paolinelli *et al.*, 2011). They are both crucial for angiogenesis. During angiogenesis, the pericytes, assisted by astrocytes, synthesize and deposit basal lamina components that provide the mechanical support required for cell attachment and serve as the substratum for capillaries (Balabanov & Dore-Duffy, 1998; del Zoppo, 2010). In the AD brain, astrocytes may exacerbate pericyte proliferation by increasing PDGFRβ levels (Montagne *et al.*, 2015; Vinukonda *et al.*, 2010). Overall, the crosstalk between pericytes and glia under both physiological and pathological conditions still remains largely unknown. A better understanding of this interaction will help to better understand the pathophysiology of pericyte-glia associated neurodegenerative phenotypes, which can lead to the development of a novel angle in therapeutic strategies for AD.

5. Neuroendocrine regulation of BBB function and AD pathogenesis

A fundamental part of neuroendocrinology encompasses the regulated secretion of hormones, neurotransmitters, or neuromodulators by specialized cells. In this section, we will first review the involvement of BBB-related neuroendocrine systems in AD. In addition, we will review how sex differences, which has been the subject of intensive study on its influence on neurodegenerative disease over various years, may play a role in AD pathogenesis, which may be influenced by sex hormones. Therefore, the second and the third part of this section will focus on our current understanding of sex differences in AD, as well as the implications of sex hormones on BBB function.

5.1. Involvement of neuroendocrine system in BBB function in AD

The BBB acts as both an endocrine target and an endocrine secretory tissue (Banks, 2019); the substances secreted by neuroendocrine and endocrine systems can affect the function of the BBB and in turn, substances secreted by the BBB can also affect the brain function.

Somatostatin (encoded by the SST gene), a growth hormone inhibiting factor synthesized in neurons and in pancreas delta cells, acts as a neurotransmitter/neuromodulator. Somatostatin promotes BBB integrity and can resist Aβ-induced toxicity (Basivireddy *et al.*, 2013; Paik *et al.*, 2019). Decreased levels of somatostatin in the brain has been reported in the aging brain (Lu *et al.*, 2004) and in the AD brain (Davies *et al.*, 1980; van de Nes *et al.*, 2002). Somatostatin may also regulate NEP activity in neurons and facilitate Aβ clearance. Consequently, NEP activity was found to be decreased in *Sst* knockout mice and led to Aβ accumulation in the brain (Saito *et al.*, 2005). Treatment with the somatostatin analogue octreotide may hence have an impact on improving the memory of AD patients by reversing Aβ-induced pathophysiologies (Craft *et al.*, 1999).

BBB-related cells are also able to secrete several substances. Of these cells, BMECs are the most widely studied. BMECs are known to release nitric oxide (NO), prostaglandins, and adrenomedullin (Banks, 2019). NO is involved in several important functions in the CNS, including modulation of neurotransmission and the regulation of local blood flow (Benarroch, 2011). NO signaling is also involved in controlling neuroendocrine cells in the hypothalamus. For example, NO tonically inhibits

vasopressin-secreting magnocellular neurons of the supraoptic and paraventricular nucleus, whereas it activates neurons in the medial preoptic area secreting gonadotrophin releasing factor, facilitating reproductive processes (Bellefontaine *et al.*, 2011; Pyner, 2009). The role of NO in AD pathogenesis is multifaceted. NO can confer neuroprotective properties through its induction of the cGMP pathway (Ditlevsen *et al.*, 2007; Kohgami *et al.*, 2010) but NO also exhibits neurotoxic effects when it is converted to peroxynitrite (Malinski, 2007; Nathan *et al.*, 2005).

Adrenomedullin, another hormone released by the BMECs, was first isolated from human pheochromocytoma (Kitamura *et al.*, 1993). Subsequently, endothelial cells were found to express high levels of adrenomedullin, and the level of adrenomedullin was increased in endothelial dysfunction (Ishihara *et al.*, 1997). Adrenomedullin plays multiple physiological functions during nervous system injury and disease. Recent studies have shown that adrenomedullin is increased in postmortem human AD brains and transgenic mouse models of AD (Fernandez *et al.*, 2016; Ferrero *et al.*, 2018). Mid-regional proadrenomedullin is also encoded by the same gene as adrenomedullin (*ADM*). The level of mid-regional proadrenomedullin was increased in MCI patients, and mid-regional proadrenomedullin has been proposed as a progression biomarker from predementia into full-fletched AD (Buerger *et al.*, 2011; Verweij *et al.*, 2013). However, its involvement in AD progression remains to be elucidated.

5.2. Sex differences in AD

Growing evidence suggests that sex influences the etiology, diagnosis, progression, and treatment outcomes of many diseases (Regitz-Zagrosek, 2012). However, the influence of sex on AD is controversial. Over the past 20 years, several studies have identified differences between the sexes across the full spectrum of clinical manifestations of AD and throughout the continuum of disease. Several studies have demonstrated that more women than men are living with a diagnosis of AD, and that women also have a greater lifetime risk of developing AD (Alzheimer's, 2020; Chene *et al.*, 2015; Seshadri *et al.*, 1997). The influence of sex on the prevalence or incidence of AD appears to be

dependent on the time period and geographic region where studies have been conducted. A higher incidence of AD in men before the age of 75 (Letenneur *et al.*, 1999; Matthews *et al.*, 2016) and a higher incidence in women after this age(Fratiglioni *et al.*, 1997; Letenneur *et al.*, 1999). However, most studies of AD incidence in the United States have found no significant differences between men and women when examining the proportion of each sex developing AD or other dementias at any given age (Evans *et al.*, 2003; Hebert *et al.*, 2001; Rajan *et al.*, 2017; Tom *et al.*, 2015) . Similarly, analysis by sex demonstrated that the prevalence of AD amongst women is significantly greater than that amongst men in some, but not all geographical regions (Prince *et al.*, 2013); a recent review and meta-analysis of over 22 studies across the world did not find that the prevalence of AD was significantly higher in women as compared to men (Fiest *et al.*, 2016).

Apart from AD, sex differences also affect the rates of cognitive decline. Cognitive deterioration is faster in women than men over a 1-year period and becomes twice as fast over an 8 year period (Holland *et al.*, 2013; Lin *et al.*, 2015). The faster cognitive decline observed in women may result from the later diagnosis of AD in women as compared to men. However, this possibility will need to be confirmed in further studies.

Based on these results which are somewhat controversial, a commonly held view is that the observed sex differences in AD frequency can largely be explained by the longer life expectancy of women, even after the diagnosis of AD. However, longevity does not wholly explain the higher frequency and lifetime risk in women. Some evidence suggests that conditions related to pregnancy and menopause are female-specific risk factors for AD. Pre-eclampsia has been associated with higher risks of cognitive impairment later in life and also with protein misfolding leading to defective amyloid processing (Buhimschi *et al.*, 2014; Fields *et al.*, 2017). In addition, a higher risk of cognitive decline, dementia and levels of AD neuropathology have been associated with early, surgically induced menopause (Bove *et al.*, 2014; Rocca *et al.*, 2007). This indicates that menopause before the age of 40–45 represents a female-specific risk factor for AD or dementia. These reports suggest that intrinsic

sex differences in the endocrine transition states may contribute to the higher prevalence of AD amongst women.

Mechanistically, BBB-related cell functions are susceptible to sex-associated differences, which may hence be a factor in influencing the sex-dependency of AD incidence. For instance, sex affects microglia more profoundly in adulthood than during development, and the increased expression of immune-related genes in microglia of adult mice is stronger in females than in males (Thion *et al.*, 2018). Sex-related differences in BBB permeability can be found in rats with seizures where BBB leakage is found to be more pronounced in female rats brains compared to male rats after bicuculline-induced epileptic seizure (Oztas *et al.*, 1992).

5.3. Effect of sex hormones on BBB function in AD

With the view that sex differences have a profound impact on AD onset and progression and BBB function, it is reasonable to consider that hormonal difference between men and women might play a role in these processes.

As we have earlier discussed, menopause is a significant risk factor in the onset and disease progression of AD in women, suggesting a possible involvement of estrogen in AD. Clinical studies have shown that estrogen replacement in perimenopausal females may reduce AD incidence, though the effect of estrogen replacement is time-dependent (Resnick & Henderson, 2002; Zandi *et al.*, 2002). It has been shown that estrogen can reduce Aβ-induced neural cell death (Green *et al.*, 1996; Green *et al.*, 2000). Estrogen-depleted mice show increased Aβ accumulation in the brain, which can be reversed by estrogen reintroduction (Zheng *et al.*, 2002). However, there is also evidence showing that estrogen has no effect on Aβ deposition in the PDAPP murine AD model (Green *et al.*, 2005). To our knowledge, no animal experiment has been done to analyze if estrogen treatment improves BBB function in any AD mouse model. However, it has been shown that estrogen treatment can promote Occludin expression in BMECs (Kang *et al.*, 2006). Yet, the functional implication of this finding remains to be investigated. Nevertheless, studies in other disease models provide evidence

that estrogen treatment can protect BBB function (Kuruca *et al.*, 2017; Maggioli *et al.*, 2016; Xiao *et al.*, 2018). However, the effect of estrogen on BBB function is still controversial, and may be dependent on factors such as age (Bake & Sohrabji, 2004; Sohrabji, 2007).

Recent studies of the effect of testosterone, a predominant male steroid hormone, on AD has been well reviewed by Lei et al. (Lei & Renyuan, 2018). Several clinical studies show that men with AD display lower serum testosterone level, while in men with MCI, serum testosterone levels has been shown to inversely correlated with serum Aβ levels (Gillett *et al.*, 2003; Hogervorst *et al.*, 2003; Moffat *et al.*, 2004; Paoletti *et al.*, 2004). In addition, testosterone withdrawal by androgen deprivation therapy in men with prostate cancer is associated with an increased risk of cognitive impairment and dementia, suggesting a potentially beneficial role for testosterone in AD (Nead *et al.*, 2017; Sun *et al.*, 2018). In vivo experiments have shown that depletion of testosterone may lead to BBB leakage and inflammation in male mice, which is accompanied with an decreased expression of BBB tight junction proteins Claudin-5 and zonula occludens-1 (ZO-1) (Atallah *et al.*, 2017). A study has demonstrated that testosterone may also prevent neuronal and endothelial senescence via the eNOS/SIRT1 pathway (Ota *et al.*, 2012).

In conclusion, the influence of the neuroendocrine system on BBB function and AD pathogenesis still remains to be elucidated. A better understanding of the neuroendocrine system will help to further our understanding of these processes in order to develop novel therapeutic strategies for AD. Sex is an important factor in phenotypic variability in AD and should not be neglected in clinical practice or in preclinical studies. Sex hormones, including estrogen and testosterone, may have beneficial effects on BBB function and cognitive performance, yet their therapeutic potential requires further investigation.

6. Influence of BBB dysfunction on drug delivery in AD

The BBB is the main barrier that impedes pharmaceuticals from entering the brain (Pardridge, 2012); neurologists therefore propose that BBB leakage as a result of AD present a unique opportunity for the delivery of therapeutic agents such as antibodies, peptides, small molecules to affected neurons without further manipulation of the BBB.

However, pathological BBB dysfunction induced by neurodegeneration is characterized by functional and structural changes to the blood vessels. These vascular changes include endothelial degeneration, reduced expression of tight junctions and adherens junctions, impaired BBB transporter expression, increased endothelial bulk flow transcytosis, pericyte degeneration, and perivascular accumulation of toxic products, which collectively impede the delivery of therapeutic agents to the brain (Sweeney *et al.*, 2018). Under pathological conditions, blood-derived toxic products, water and electrolytes accumulate in enlarged perivascular spaces, and interfere with the normal diffusion of solutes across brain extracellular spaces, ISF formation and ISF flow (Kisler *et al.*, 2017). This results in an impaired distribution of solutes throughout the CNS. As a consequence of disease-driven BBB dysfunction, impaired solute transport across parenchymal extracellular spaces and diminished ISF regional flow, therapeutic agents are likely to get trapped in pathologically-altered brain tissue within enlarged perivascular spaces along with other blood-derived debris, preventing them from reaching their neuronal targets (Sweeney *et al.*, 2019).

The drug delivery to CNS is also mediated by carrier-mediated transport and receptor-mediated transcytosis systems. The BBB transporters generally belong to one of two large gene families: the Solute Carrier (SLC) family and the ATP-binding cassette (ABC) family (Hediger *et al.*, 2013). In these two families, SLC2A1 (which encodes GLUT1) and ABCB1 (which encodes P-gp) have the highest level of expression in the BBB (Al-Majdoub *et al.*, 2019). However, the levels of these two proteins are reduced in the AD brain (Chiu *et al.*, 2015; Mooradian *et al.*, 1997; Wijesuriya *et al.*, 2010). Therapeutic agents also show limited brain penetration; anti-amyloid monoclonal antibodies, for instance, have very limited BBB penetration. It is estimated that less than 1.5% of an administered dose enters the brain, and high dosage treatment increases the risk of adverse events (Honig *et al.*,

2018; Ostrowitzki *et al.*, 2017; Vandenberghe *et al.*, 2016; Zott *et al.*, 2019). The current slew of failed clinical trials using these drugs may possibly be explained by their limited brain penetration. As a result, healthy blood vessels and BBB integrity in brain regions are important for the successful delivery of neurotherapeutic agents to brain tissue.

7. Potential for therapeutic targeting of the BBB in AD

To date, there are no effective treatments available to halt or reverse the progression of AD. We have discussed that one of the major reasons for this is that drug delivery into the brain is often inefficacious.

The components of the NVU in the BBB such as the pericytes, astrocytes and microglia cells act together to maintain neuronal homeostasis and dysfunction of these NVU components contributes to AD pathology. Therapeutic targeting of these NVU components is a plausible alternative treatment for AD. One study has found that progressive inhibition of the CypA-NFκB-MMP-9 pathway using specific inhibitors and genetic manipulation of CypA can ameliorate both vascular and neuronal dysfunction in ApoE4 mutant mice (Bell *et al.*, 2012). Pericytes contain a remarkable concentration of acid phosphatase–positive lysosomes, indicating that pericytes have the capability to eliminate cellular debris and metabolic waste (Broadwell & Salcman, 1981). This evidence, taken along with its crucial role in maintaining BBB integrity, suggests that pericytes represent another therapeutic avenue for exploration.

Studies have also shown increased levels of N -methyl-D-aspartate (NMDA) receptors and reduced GLT1 expression in the astrocytes of AD brains. Drugs to increase astrocytic glutamate transporter expression and function are possible potential treatment options to improve AD brain function. Memantine is the currently approved AD drug that reduces excitotoxicity by blocking NMDA receptors; however, this drug only has limited clinical effects (Esposito *et al.*, 2013). Interestingly, one study has shown that the impairment of synaptic plasticity and hippocampal memory deficits due

to AQP4 deficiencies and the GLT1 stimulator Ceftriaxone can rescue AD phenotypes (Yang *et al.*, 2013a).

Furthermore, Minocycline has been recognized as a microglial inhibitor, and it reduces BBB dysfunction by preventing microglial production of glutamate, MMPs and IL-1β (Huang *et al.*, 2014; Kobayashi *et al.*, 2013). The inhibition of microglial activation by Minocycline results in an increase in the levels of differentiated oligodendrocyte precursors and immature oligodendrocytes. These cells are responsible for the remyelination of neurons, and can be a potential target to treat AD through the manipulation of remyelination mechanisms (Li *et al.*, 2005; Miron *et al.*, 2013). Another drug, Dipyridamole, a phosphodiesterase inhibitor, is able to attenuate the expression of TLR-induced cytokines and chemokines in human microglia by reducing microglial activity. This consequently results in the reduction of IL-1β, TNF-α and IL-6 expression, which are all responsible for increased BBB permeability (Sloka *et al.*, 2013). A recent study has shown that activated protein C analogs can enhance the function of endothelial membranes and protect BBB integrity, which slows down the pathological process in the 5×FAD mouse model (Lazic *et al.*, 2019). Collectively, all these findings suggest that pharmacological modulation of NVU components may halt or reduce the impairment of the BBB in AD, which in turn can lead to the gradual slowing down of AD progression.

8. Conclusion

The role of BBB dysfunction during AD is being increasingly recognized as a major contributor to AD pathology. The primary mechanisms by which BBB dysfunction occurs are through influencing Aβ clearance and endothelium-mediated transport, impairment of endothelial cell and pericyte functions, modification of tight junction integrity, activation of glial cells which facilitates the recruitment of leukocytes in the brain. In this review, we have discussed the current state of the art in the understanding of BBB function which suggests that pericytes and glial cells play an important role in regulating BBB function both in physiological and pathological conditions in particular in AD.

Impairment of pericytes and glial cells may hence engender AD pathology. We have also discussed the effects of substances released from neuroendocrine system on the function of the BBB and AD pathogenesis. In addition, we have reviewed currently available AD treatments specifically targeting pericytes and BBB glial cells. In conclusion, we hope that a better understanding of these mechanisms at the BBB level will offer avenues for novel therapeutic approaches for the treatment of AD.

Author Contributions

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Conflicts of Interest

The authors declare no conflicts of interest.

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Abbreviations

Aβ	Amyloid-β
AD	Alzheimer’s disease
ADAM10	
Apo	Apolipoprotein
APP	Amyloid precursor protein
AQP	Aquaporin
BBB	Blood-brain barrier
BMEC	Brain microvascular endothelial cell
CAA	Cerebral amyloid angiopathy
CD	Cluster of differentiation
CNS	Central nervous system
CSF	Cerebrospinal fluid
CypA	Cyclophilin A
EAAT	Excitatory amino acid transporter
FABP7	Fatty acid-binding protein 7
GLT1	Glutamate transporter 1
GLUT1	Glucose transporter 1
ICAM-1	Intercellular adhesion molecule 1

IDE	Insulin-degrading enzyme
IL	Interleukin
ISF	Interstitial fluid
LDLR	Low density lipoprotein receptor
LRP	Lipoprotein receptor-related protein
JAM	Junctional adhesion molecule
MCI	Mild cognitive impairment
MCT1	Monocarboxylate transporter 1
MMP	Matrix-metalloproteinase
MRI	Magnetic resonance imaging
NEP	Neprilysin
NFκB	Nuclear factor Kb
NG2	Neural/glial antigen 2
NFT	Neurofibrillary tangles
NMDA	N -methyl-D-aspartate
NO	Nitric oxide
NVU	Neurovascular unit
PDGFRβ	Platelet-derived growth factor receptor-β

P-gp	P-glycoprotein
PICALM	Phosphatidylinositol-binding clathrin assembly protein
RAGE	Receptor for advanced glycation end products
ROS	Reactive oxygen species
α-SMA	alpha-smooth muscle actin
TNF	Tumor necrosis factor
TLR	Toll-like receptor
VEGF	Vascular endothelial growth factor
VLDLR	Very low-density lipoprotein receptor
ZO-1	Zonula occludens 1

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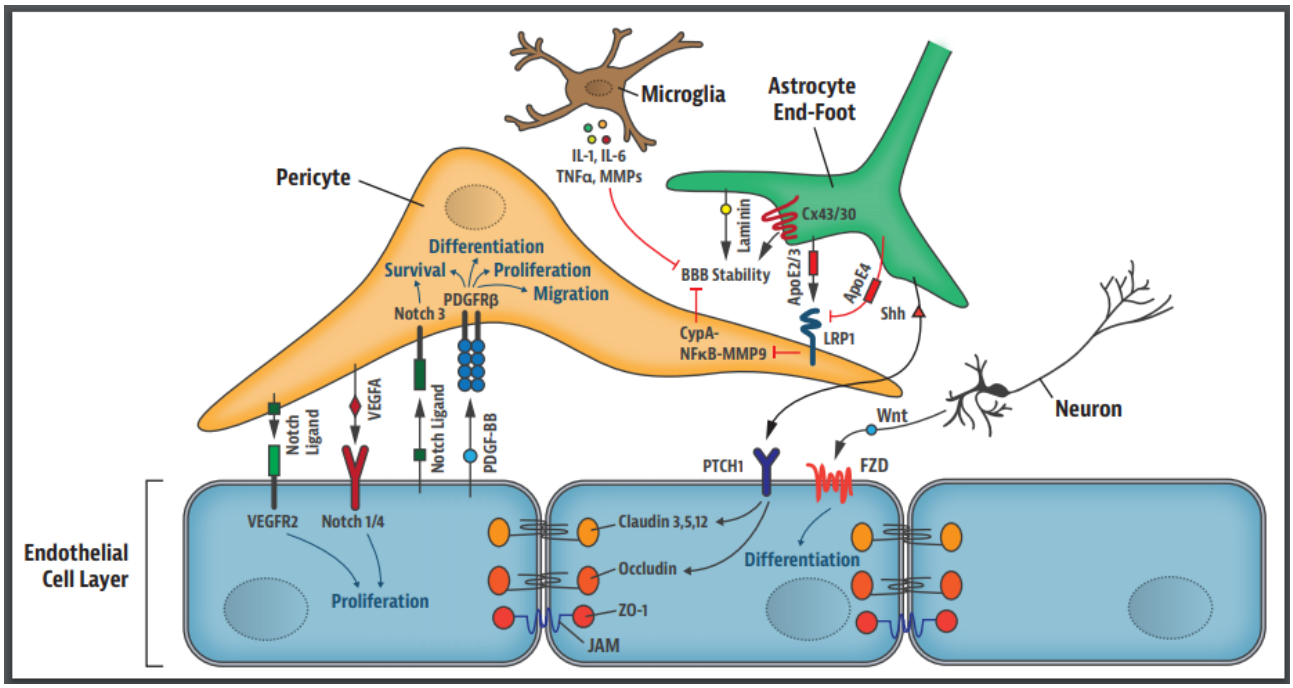

Figure 1. Key cellular and molecular pathways regulating BBB integrity.

BBB integrity is maintained by the tight junction proteins at the co-interfaces of endothelial cells and by low-level bulk-flow transcytosis. Pericyte-endothelial cells crosstalk occurs through Notch ligands binds to Notch3 receptor on pericytes which promotes pericyte survival. Platelet-derived growth factor-BB (PDGF-BB) binds to PDGFRβ on the pericyte surface, which leads to pericyte differentiation, proliferation, and migration. Vascular endothelial growth factor-A (VEGFA) binds to endothelial VEGF receptor-2 (VEGFR2) which mediates endothelial survival and proliferation. Pericyte-derived notch ligands bind to endothelial Notch-1/4 receptor, which mediates BBB stability and endothelial cell proliferation. Astrocyte-endothelial cells crosstalk occurs through astrocyte-secreted ApoE2 and ApoE3. This contrasts with the ApoE4 isoform, which suppresses the pro-inflammatory signaling CypA - NF-κB - matrix metalloproteinase-9 (MMP9) pathway in pericytes in order to maintain BBB stability. Additionally, astrocyte-produced laminin and gap junction proteins Cx43 and 30 on the astrocyte surface maintain BBB stability. Sonic hedgehog (Shh) secreted by astrocytes interact with Patched-1 (PTCH1) at the endothelia surface to further promote BBB stability by increasing tight junction protein expression. Microglia-endothelial cells crosstalk occurs

through the microglia-secreted pro-inflammatory cytokines IL-1, IL-6, TNFα and MMPs, which disrupts BBB stability. Neuron-endothelial cells crosstalk occurs primarily through neuron-secreted Wnt, which acts as a ligand for Frizzled (FZD) at the endothelium which leads to endothelial cell differentiation.

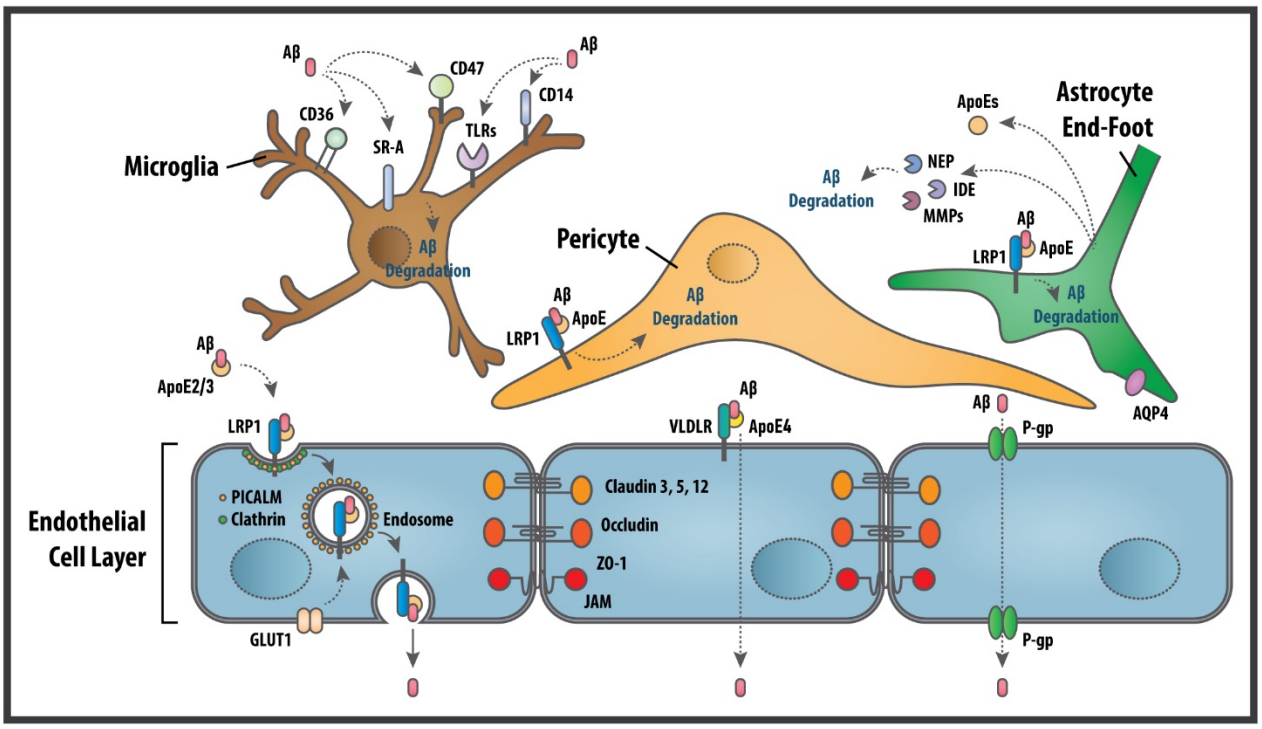

Figure 2. **Aβ transcytosis across the BBB mediated by microglia and astrocytes.**

Aβ clearance in the BBB is mediated following pathways: (1) Brain-derived Aβ is cleared across the BBB primarily by low-density lipoprotein receptor-related protein 1 (LRP1)-mediated transcytosis. Free Aβ forms a complex with apolipoprotein E2 (ApoE2) or ApoE3, and the ApoE2/Aβ and ApoE3/Aβ complexes bind to LRP1 at the abluminal side of the BBB, which leads to a rapid PICALM/clathrin-dependent endocytosis of Aβ-LRP1 complexes. PICALM continues to guide intracellular trafficking of Aβ-containing endocytic vesicles for fusion with Rab5+ early endosomes followed by Rab11+ sorting endosomes, ultimately leading to Aβ exocytosis across the luminal side of the BBB into the blood. (2) Other cell types of the neurovascular unit, for example, pericytes and

astrocytes, clear deposited Aβ via the LRP1/ApoE pathway. (3) Aβ is also bound to ApoE4, and the Aβ/ApoE4 complex interact with VLDLR, resulting in slower Aβ clearance. (4) Aβ can be exported into the blood by P-glycoprotein (P-gp) expressed on the BBB. (5) The Aβ aggregates can be phagocytized by microglia through an ensemble of cell surface receptors, including class A scavenger receptor (SR-A), the class B scavenger receptors CD36, CD14, CD47 and Toll like receptor (TLR) 2, TLR4, TLR6 and TLR9. (6) The Aβ aggregates can be degraded by the enzymes secreted by astrocytes, including neprilysin (NEP), insulin-degrading enzyme (IDE), and matrix metalloproteinases (MMPs)-2 and -9.