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The Blood-Brain Barrier in Alzheimer's Disease

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Abstract

The accumulation of neurotoxic amyloid-beta (Aβ) in the brain is one of the characteristic hallmarks of Alzheimer's disease (AD). Aβ-peptide brain homeostasis is governed by its production and various clearance mechanisms. The blood-brain barrier provides a large surface area for influx and efflux mechanisms into and out of the brain. Different transporters and receptors have been implicated to play crucial roles in Aβ clearance from brain. Besides Aβ transport, the blood-brain barrier tightly regulates the brain's microenvironment; however, vascular alterations have been shown in patients with AD. Here, we summarize how the blood-brain barrier changes during aging and in disease and focus on recent findings of how the ABC transporter P-glycoprotein (ABCB1/P-gp) and the receptor low-density lipoprotein receptor-related protein 1 (LRP1) play a role in Aβ clearance from brain.

Keywords

Alzheimer's disease (AD); Amyloid-beta (Aβ); Blood-brain barrier; Clearance; LRP1; Neurovascular unit; P-gp/ABCB1

Alzheimer's disease (AD), the most common form of dementia, is a devastating neurodegenerative disorder (Prince et al. 2013). In 1906, the German physician and neuropathologist, Alois Alzheimer, was the first to describe amyloid plaques and neurofibrillary tangles in the brain of his deceased patient who suffered from severe memory problems (Alzheimer 1906, 1907). To this date, plaques and tangles are considered the two characteristic hallmarks of the disease. Much effort has been devoted to study the underlying pathophysiological mechanisms of the disease. Despite tremendous effort, more than a century later, there is no treatment available, no cure in sight (Iadecola 2016).

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On a molecular level, the presence of intracellular hyper-phosphorylated tau protein and extracellular aggregates of amyloid-beta (Aβ) protein in the brain of AD patients suggest that both proteins play a key role in disease pathogenesis. However, it is unclear whether these protein abnormalities are causative or rather incidental changes of the disease. Nevertheless, it is generally accepted that high levels of Aβ are toxic, not only to neurons, but also to other brain cells and lead to neurodegeneration, neuroinflammation, and neurovascular dysfunction resulting in behavioral changes (Iadecola 2004; Zlokovic 2005, 2011). Studies in animal AD models propose that Aβ acts upstream of tau, since amyloid models show tauopathies, whereas tau models do not display amyloid pathology (Bloom 2014). Recently, it was shown in humans that cerebrospinal fluid (CSF) levels of tau increase in response to Aβ deposition in the early stages of AD further suggesting that both pathogenic proteins are somehow linked (Mattsson-Carlgren et al. 2020). There is no generally accepted hypothesis on how AD develops or progresses. Over the years, researchers have developed multiple hypotheses. For example, in 1993, the "*Vascular Hypothesis of Alzheimer's Disease*" was formulated by de la Torre and his colleagues. This hypothesis is based on findings that two common medical conditions, chronic hypertension and carotid atherosclerosis, appear to be independently associated with AD (Iadecola 2016). The hypothesis postulates that vascular risk factors responsible for persistent cerebral hypoperfusion are key factors for converting normal aging into dementia. Ample epidemiological studies have shown that hypertension and cardiovascular risk factors are strongly associated with AD (Iadecola 2016). A recent multifactorial, data-driven analysis of PET and MR images of over 7,700 individuals showed that vascular alterations are one of the earliest events in AD pathology (Iturria-Medina et al. 2016). These changes are later followed by characteristic AD features such as Aβ brain accumulation, metabolic dysfunction, functional impairment, and structural atrophy with significant volume loss of grey and white matter (Iturria-Medina et al. 2016). In 2004, Zhu et al. proposed the "*Two-Hit Hypothesis*" which states that although either oxidative stress or abnormalities in mitotic signaling can independently serve as initiators, both processes are necessary to propagate disease pathogenesis (Zhu et al. 2004). In 2011, this concept was modified and the "*Two-Hit Vascular Hypothesis*" was formulated to explain why vascular pathology often coincides with Aβ and tau pathology. This hypothesis states that vascular changes represent the first insult (*hit 1*), which is then followed by the second insult (*hit 2*) consisting of Aβ and tau accumulation in the brain (Zlokovic 2011). Several recent studies support the hypothesis, that vascular alterations precede neurodegeneration and cognitive decline (Iturria-Medina et al. 2016; Montagne et al. 2015; Nikolakopoulou et al. 2019).

Over the last decades, there is increasing evidence that the interfaces – blood-brain barrier, blood-CSF barrier, and meninges – that separate the central nervous system (CNS) from the periphery and tightly regulate brain homeostasis, play a major role in AD pathology. For example, the blood-brain barrier is involved in Aβ clearance from the brain, regulates infiltration of macrophages and other immune cells into the brain, and mediates inflammation (Zlokovic 2005). The reduction of neurotoxic Aβ brain levels is currently a major treatment strategy in AD either by targeting the generation or the removal of Aβ (Long and Holtzman 2019; Storck and Pietrzik 2017). It has been shown that in cases of sporadic AD and some form of familial AD, Aβ accumulation is the result of reduced

brain clearance instead of overproduction (Bateman et al. 2006; Mawuenyega et al. 2010). The blood-brain barrier with its large surface area provides a big potential for therapeutic intervention (Storck and Pietrzik 2018). In this chapter, we focus on the role of blood-brain barrier function in Aβ clearance from the brain and how these processes could be used for therapeutic targeting.

1 Amyloid Dynamics at the Blood-Brain Barrier

The blood-brain barrier keeps neurotoxic blood-derived components, xenobiotics, cells, and pathogens out of the brain. At the same time, it supplies the brain with nutrients and oxygen and facilitates the clearance of waste products and neurotoxins out of the brain (Bell and Zlokovic 2009). The apicobasal polarized endothelial cells comprising the blood-brain barrier are equipped with specialized transport systems for nutrients, energy metabolites, signaling molecules, and waste products for transport in either direction. These include solute carrier-mediated transport of carbohydrates (e.g., glucose), amino acids, vitamins, fatty acids, hormones, nucleotides, and monocarboxylic acids (e.g., lactate); ion transporters, ATP-binding cassette transporters specialized for efflux of xenobiotics, and receptor-mediated transporters for peptides (Bell and Zlokovic 2009).

In AD patients, it has been shown that decreased Aβ clearance from the brain is a key event in AD pathogenesis (Bateman et al. 2006; Mawuenyega et al. 2010). In a healthy individual, Aβ clearance through different clearance mechanisms in the brain exceeds its production (Bateman et al. 2006). In AD, however, this balance appears to be dysfunctional and the brain can no longer efficiently remove Aβ. To date, we have no understanding of how much each mechanism contributes to the overall clearance of Aβ from the brain. Several different mechanisms have been identified and impairment of any of these in mouse models of AD worsens Aβ pathogenesis. Aβ clearance mechanisms include extracellular enzymatic degradation, intracellular degradation by cerebral cells, transport into the periphery across the blood-brain barrier or the choroid plexus, glymphatic flow, lymphatic, or perivascular drainage (Da Mesquita et al. 2018; Storck and Pietrzik 2017; Tarasoff-Conway et al. 2015). Several independent studies suggest that the blood-brain barrier significantly contributes to Aβ clearance by translocating Aβ from the interstitial fluid (ISF) into blood (Cirrito et al. 2005; Shibata et al. 2000; Storck et al. 2016; Van Gool et al. 2019). Recently, the glymphatic system which is believed to act by water influx through astrocytic aquaporin-4 water channels has received attention. Glymphatic influx of CSF into the brain parenchyma along arteries is thought to create an ISF bulk flow between arteries and veins. This bulk flow drains nonspecific solutes including Aβ from the ISF into the CSF of para-venous spaces (Iliff et al. 2012; Kress et al. 2014). This hypothesis contradicts the traditional belief of ISF flow into the CSF relying mainly on diffusion along the perivascular route. Further studies need to be conducted to test whether distinct pathways exist, or whether the same pathway may give rise to different results depending on differing physiological or experimental conditions (Abbott et al. 2018; Iliff and Simon 2019; Smith and Verkman 2019; Tarasoff-Conway et al. 2015). Once in the CSF, Aβ seems to be eliminated by meningeal lymphatics (Da Mesquita et al. 2018) or can reenter the brain parenchyma by glymphatic-mediated CSF influx and access all the various clearance routes including blood-brain barrier-mediated clearance again.

Although it is believed that under homeostatic conditions proteins cannot passively cross the blood-brain barrier, a recent study in Aβ-overexpressing mice shows an involvement of a paracellular pathway under pathological conditions (Keaney et al. 2015). Keaney and colleagues showed that pathophysiological Aβ accumulation in the brain causes a transient downregulation of claudin-5 and occludin protein expression, allowing for its own auto-regulated paracellular passage across the blood-brain barrier. Interestingly, tight junction dysfunction has been described in patients with AD and/or with cerebral amyloid angiopathy (Biron et al. 2011; Carrano et al. 2011; Hartz et al. 2012). Under homeostatic conditions, Aβ is transported like other peptides via fast and efficient receptor- and/or transporter-mediated mechanisms across the CNS endothelium.

2 P-gp- and LRP1-Mediated Aβ Transport Across the Blood-Brain Barrier

Reducing Aβ transport across the blood-brain barrier in mouse models of AD results in accumulation of Aβ in the brain accompanied by cognitive deficits in learning and memory (Cirrito et al. 2005; Storck et al. 2016). The most studied clearance proteins are LRP1 (low-density lipoprotein receptor-related protein 1) and P-glycoprotein (P-gp), an ATP-binding cassette (ABC) transporter also known as ABCB1 or MDR1. While neither of them has been an obvious hit in genome-wide association (GWAS) studies, recent evidence has shown that Phosphatidylinositol binding clathrin assembly protein (PICALM), a highly-validated candidate found in these studies (Harold et al. 2009; Jun et al. 2010; Lee et al. 2011), regulates both LRP1 and P-gp trafficking in endothelial cells and therefore their function (Storck et al. 2018; Zhao et al. 2015). Another study recently found PICALM mRNA to be reduced in aged blood-brain barrier endothelial cells suggesting impaired Aβ transcytosis during aging (Yang et al. 2020).

LRP1 is a member of the low-density receptor (LDLR) family. Its expression decreases in aging and in AD, both in total brain tissue and brain capillaries (Kang et al. 2000; Osgood et al. 2017; Shibata et al. 2000; Silverberg et al. 2010a, b). Decreased blood flow in AD patients (Roher et al. 2012) could affect LRP1 as it has been shown that LRP1 protein expression is decreased during hypoxia (Bell et al. 2009; Kanekiyo et al. 2012). The membrane receptor can be shed from the cell surface and is then released as a soluble form (sLRP1) in the ISF or the bloodstream. Shedding of membrane-bound LRP1 in endothelial cells can influence brain clearance of Aβ (Shackleton et al. 2016; Zandl-Lang et al. 2018). Furthermore, sLRP1 in blood plasma is believed to drive Aβ clearance from the brain by binding Aβ in the blood and therefore acting as a peripheral sink (Sagare et al. 2007). Interestingly, AD patients show significantly higher amounts of oxidized sLRP1 with lower efficiency to bind Aβ (Sagare et al. 2007).

LRP1 is highly expressed in all cells that constitute the neurovascular unit (Kanekiyo et al. 2012; Liu et al. 2017; Storck et al. 2016) so that transcytosis from ISF through the cellular layer into the periphery is possible. Due to its fast endocytosis rate compared to other members of the LDLR family (Li et al. 2001), LRP1 rapidly removes soluble Aβ from the ISF. Immunohistochemical analyses in human brain capillaries suggest that LRP1 expression is restricted to the abluminal, brain-facing side of the endothelium (Zhao et al. 2015). For the longest time, it has been believed that LRP1 fully transcytoses Aβ through

endothelial cells by binding the protein at the brain-facing side and releasing it into the blood at the luminal side. Recent studies, however, suggest that LRP1 needs luminal ABC transporters such as P-gp for transcytosis (Storck et al. 2018) for the actual release of Aβ into the vessel lumen. Although the exact transcytosis mechanism is not fully understood yet, studies suggest that in endothelial cells Aβ triggers LRP1 and P-gp internalization and localization to sorting endosomes, where LRP1-bound Aβ dissociates from the receptor and binds to P-gp (Storck et al. 2018; Zhao et al. 2015). It is thought that P-gp is then directed back to the luminal side and releases Aβ into the blood, whereas LRP1 is redirected to the abluminal side of the endothelial cell. Both LRP1 and P-gp internalization and therefore their function seem to be regulated by the late-onset AD risk factor PICALM. This proposed mechanism could explain how luminal P-gp gains access to brain-derived Aβ in order to be able to efflux it. The exact mechanism of P-gp-mediated Aβ remains unclear.

In addition to LRP1 and P-gp, other members of the LDLR family (e.g., LRP2), other ABC transporters (ABCC1, ABCG2, and ABCG4) as well as the neonatal Fc receptor (FcRn) have been described to assist in Aβ clearance from the CNS (Bell et al. 2007; Deane et al. 2005; Do et al. 2012; Krohn et al. 2011; Lamartiniere et al. 2018). To what extent these transport systems contribute to homeostasis, whether these also act in concert with others, or if these transporters compensate for Aβ clearance when other systems fail is still unknown.

Recent findings further promote the role of LRP1 in AD pathogenesis. For decades, there had been no visible link between the pathogenic proteins that accumulate in AD patients' brains. Now, several recent reports provide insights into the pathways linking tau, Aβ, and neurodegeneration. A recent report now shows that LRP1 is also endocytosing the protein tau (Rauch et al. 2020). Another study in mice and humans shows that CSF levels of tau increased in response to Aβ deposition in the early stages of AD implicating that the clearance of LRP1 ligands is impaired when a certain threshold is reached (Mattsson-Carlgren et al. 2020). To our knowledge, there are no reports on LRP1-mediated transport of tau across the BBB. However, tau in blood is currently considered to be a new biomarker of disease progression (Barthelemy et al. 2020). All these novel findings help to understand that the trafficking of pathogenic proteins in and out of the brain is far more complex than initially thought and that future research is crucial to unravel the complex interactions in AD pathology.

3 RAGE-Mediated Aβ Transport Across the Blood-Brain Barrier

Studies suggest that receptor-mediated entry of Aβ into the brain is mediated by the receptor for advanced glycosylation end products (RAGE) that – in contrast to LRP1- is mainly expressed at the luminal side of the capillary endothelium (Deane et al. 2003; Deane et al. 2012; Zhao et al. 2015). Therefore, RAGE mediates Aβ (re)-uptake from blood into brain. RAGE-mediated endocytosis is enriched within caveolae microdomains on the apical plasma membrane of endothelial cells and the receptor has been shown to colocalize with caveolin-1 (Stitt et al. 2000). Co-immunoprecipitation analysis revealed an interaction between caveolin-1 and RAGE, but not LRP1. Further, phosphorylation of caveolin-1 has been described to modulate the function of a number of membrane transporter proteins including RAGE and P-gp (Barakat et al. 2007; Zhu et al. 2018). Based on the recent finding

that brain-to-blood transcytosis of Aβ via LRP1 requires ABC transporter function of P-gp (Storck et al. 2018), one could speculate that RAGE-mediated blood-to-brain transcytosis is dependent on/or restricted by similar mechanisms. Endothelial transcytosis is believed to be mediated by a so-called common recycling endosome that connects basolateral and apical endocytosis events and directs cargo to different cellular compartments (Thuenauer et al. 2017). In this scenario, in health, when P-gp is high, the ABC transporter could efflux RAGE-mediated uptake of Aβ into the endothelium and thus limit RAGE-mediated Aβ transcytosis from blood into the brain. Dysregulation of this compensatory mechanism, either by downregulation of P-gp or upregulation of RAGE as seen in AD patients (Deane et al. 2003; Deo et al. 2014) would therefore promote brain uptake of peripheral Aβ by reducing the rate of efflux from intracellular vesicles. Evidence for an involvement of RAGE in AD pathophysiology in humans has been supported by its upregulation in the vasculature, astrocytes, and microglial cells in the hippocampus of AD patients compared to healthy individuals (Yan et al. 1996). Moreover, inhibition of RAGE-Aβ interaction, using either a soluble form of RAGE or an anti-RAGE antibody, results in reduced neuroinflammation, improved cerebral blood flow (CBF), reduced brain Aβ levels, and a lower rate of cognitive decline in Tg2576mice (Deane et al. 2003, 2012). Based on these findings, a clinical Phase 2/3 trial with a RAGE blocker in patients with mild AD and impaired glucose tolerance (NCT03980730 June 2019–July 2023) has been recently initiated. In addition to RAGE, organic-anion-transporting polypeptide transporters (OATPs) such as Oatp1a4 have been reported to be involved in transporting Aβ from the periphery into the brain (Do et al. 2013). More studies are needed to establish the role of OATPs in Aβ clearance and AD pathology.

4 Transmission of Aβ Protein Pathology Via Peripheral Blood Sources

Data from several studies suggest that the blood-brain barrier might also play a role in the uptake of peripheral Aβ from blood into the brain (Bu et al. 2017; Eisele et al. 2010; Purro et al. 2018). Studies in mouse models have shown that small amounts of peripherally applied pathological oligomeric Aβ can act as a seed and accelerate Aβ pathology and its spreading within the brain (Eisele et al. 2009, 2014, 2010). Moreover, animal studies also suggest that medical and surgical procedures might pose a risk of transmission of AD. For example, Purro and colleagues showed that batches of human cadaveric pituitary-derived growth hormone (c-hGH) contaminated with Aβ and tau proteins can lead to Aβ plaques and cerebral amyloid angiopathy following intracerebral inoculation in mice (Purro et al. 2018). In a parabiosis experiment, Bu et al. recently demonstrated that surgical union of a human Aβ-overexpressing mouse with a wild-type mouse for several months resulted in the accumulation of human Aβ in the brains of wild-type animals. In this study, human Aβ was deposited in amyloid plaques and triggered tau phosphorylation, inflammation, and neurodegeneration in the wild-type mouse. These findings implicate that human Aβ produced in brains of AD mice was translocated from the brain into the periphery, entered the conjoined blood circulation, crossed the brain barriers in the wild-type animals, possibly by crossing the blood-brain barrier, and triggered aggregation and neuropathology in the brain of the wild-type animals (Bu et al. 2017). The exact route of brain entry, its mediators, and relevance to humans is unclear and therefore more research is needed to understand cellular and molecular mechanisms underlying this process.

Overall, little is known about the relevance of transmission of AD pathology via peripheral blood sources in humans. Data from a retrospective study with 1,465,845 patients who received blood transfusions between 1968 and 2012 provided no evidence for the transmission of AD (Edgren et al. 2016). Therefore, further studies are needed to investigate the possibility of transmission of Aβ pathology via peripheral amyloidogenic proteins in humans.

5 Changes of Blood-Brain Barrier Function in Alzheimer's Disease

During aging and in disease state many of the described receptors, transporters, and carriers are dysregulated suggesting that Aβ clearance across the blood-brain barrier is altered. A recent study shows that there is an age-related shift in transport from ligand-specific receptor-mediated transport to nonspecific caveolar transcytosis in brain endothelium (Yang et al. 2020). The authors of this study found many receptors such as LDLR, leptin receptor, transferrin receptor (TfR), or insulin receptor downregulated with aging on RNA levels suggesting changes in specific trafficking of solutes into and out of the brain. No significant changes were detected for *Lrp1*. Interestingly, the authors found a significant downregulation of intracellular adaptor protein PICALM which is required for LRP1 and P-gp trafficking. *Picalm* reduction in mice reduces LRP1-mediated Aβ clearance without affecting LRP1 or P-gp protein levels (Zhao et al. 2015). Taken together, these studies nicely show that age-related changes in brain endothelium occur that affect Aβ clearance even when it seems that the RNA levels of transporters seem unaffected. Accumulation of Aβ in the brain triggers neurovascular stress (Deane et al. 2012), downregulates tight junction proteins (Keaney et al. 2015; Park et al. 2014), and allows the entry of blood-derived substances and immune cells penetrating the CNS which disturbs the tightly controlled homeostasis of the brain. In mouse models of AD, it has been shown that Aβ deposition in blood vessels, also known as cerebral amyloid angiopathy (CAA), causes blood vessels to degenerate which disturbs blood flow and angiogenesis throughout the brain (Meyer et al. 2008). Therefore, Aβ accumulation in the brain has a massive impact on cerebral blood vessel function and their distribution and consequently on brain health. Clinical studies have shown impaired P-gp transport function in AD patients (Deo et al. 2014). As P-gp acts as a gatekeeping efflux pump, P-gp impairment leads to reduced brain clearance of Aβ and potentially also to increased brain uptake of xenobiotics in AD.

Recent studies in mice show that meprin β, a protease upregulated in AD brain (Schlenzig et al. 2018), not only generates Aβ (Schonherr et al. 2016) but also affects blood-brain barrier integrity by cleaving tight junction proteins (Gindorf et al. 2020). In humans, it has been shown that the blood-brain barrier becomes leaky even before the onset of hippocampal atrophy typically seen in early AD (Montagne et al. 2015). Cognitive impairment occurred regardless of Aβ or tau pathology (Montagne et al. 2020) – the hallmarks of AD. Moreover, blood-brain barrier leakage was enhanced in patients carrying the *APOE4* allele, the strongest genetic risk factor for AD. These findings again support the hypothesis that vascular abnormalities precede neuronal degeneration. Many other vascular alterations have been reported in the brain of AD patients. Microbleeds or microhemorrhages often coincide with AD leading to extravasation of blood-derived substances into the brain causing neuroinflammation and immune responses (Cullen et al. 2005). Blood-brain barrier transport

of glucose, the most important energy source of the brain, has been demonstrated to be impaired in AD due to a lower endothelial expression of glucose transporters and reduced blood flow (Kalaria and Harik 1989). In line with an age-related shift to nonspecific caveolar transcytosis (Yang et al. 2020) along with an entry of serum proteins into the brain are other studies that report that an age-related dysfunction of the blood-brain barrier allows an influx albumin into the brain (Milikovsky et al. 2019; Senatorov Jr. et al. 2019). Albumin, then, activates Transforming growth factor-beta (TGF-β) receptors, overstimulates neuronal networks, and impairs cognition. The reports show that an opening of the blood-brain barrier correlated with localized slowing of cortical activity in epilepsy, AD patients, as well as in mouse models of AD (Milikovsky et al. 2019; Senatorov Jr. et al. 2019).

However, endothelial cells are not the only cell type of the neurovascular unit affected by changes occurring in AD. Pericytes, that are in direct contact with endothelial cells are critical regulators of blood-brain barrier function, are equally affected by AD pathology (Ben-Zvi et al. 2014; Daneman et al. 2010). It has been shown that pericyte numbers are decreased in brain samples from human subjects with AD (Sengillo et al. 2013) and that reduced pericyte expression correlates with barrier dysfunction in AD cases. The apolipoprotein E (APOE) is a major risk factor of AD. APOE 4 not only affects blood-brain barrier-mediated Aβ clearance (Bell et al. 2007; Deane et al. 2008), it also seems to have a direct effect on barrier integrity (Bell et al. 2012). In subjects with AD, it has been suggested that APOE 4 accelerates the loss of pericytes and causes microvascular reductions (Halliday et al. 2016; Sengillo et al. 2013). Studies in mice have shown that pericyte ablation leads to acute circulatory failure and rapid loss of neurons and behavioral deficits within days (Nikolakopoulou et al. 2019). The authors of the study postulate that circulatory stress, together with the loss of brain pericyte-derived neurotrophic support, is responsible for the observed neurodegenerative and behavioral phenotypes in these mice. Recently, it was shown that an age-related shift in receptor-mediated transcytosis to unspecific caveolar transcytosis occurs alongside a specific loss of pericyte coverage (Yang et al. 2020).

In the last decades, researchers have moved away from a neuro-centric view of AD and appreciated that other cell types of the brain contribute to AD pathology. As such, it has been recognized that neurons are not the only Aβ-producing cell type in the brain. To date, we know that also other cerebral cells such as astrocytes (Berkenbosch et al. 1990; Zhao et al. 2011), oligodendrocytes (Walter et al. 2019), vascular smooth muscle cells (Frackowiak et al. 2005), endothelial cells (Devraj et al. 2016; Kitazume et al. 2010), as well as peripheral cells (Citron et al. 1994; Gowert et al. 2014; Jiang et al. 2003) contribute to Aβ production and might affect brain levels of Aβ. Therefore, the neurovascular unit could not only be an important mediator of Aβ clearance but also contribute to Aβ production.

However, not only production but also factors modulating Aβ clearance or Aβ deposition are increasingly recognized. As such, it has been suggested that pericytes play a significant role in the development of vascular deposition of Aβ, also known as CAA (Blanchard et al. 2020). Blanchard and colleagues recently devolved a humanized in vitro model that combined endothelial cells, astrocytes, and pericytes in a three-dimensional gel. The endothelial cells formed capillaries with tight junctions that had high electrical resistance mimicking the neurovascular unit in vivo. When pericytes in this model expressed the AD

risk factor *APOE4*, they activated a transcriptional pathway leading to enhanced ApoE secretion, which, in turn, led to amyloid deposition on capillary walls. However, blocking this pathway prevented deposition.

6 Current Treatment Approaches

Currently, several cholinesterase inhibitors approved by the US Food and Drug Administration (FDA) are being used to treat AD, with some clinical improvement in cognition (Raina et al. 2008). However, these drugs can only improve cognitive symptoms of AD for a certain period and cannot modify the disease course. Therefore, we are in the need of new drugs that slow down disease progression or even prevent their onset.

Many of the current treatment strategies for AD are targeting elevated Aβ levels in the brain. Although the blood-brain barrier provides a potential for its therapeutic interaction, the physical barrier is at the same time an obstacle for drug delivery (Storck and Pietrzik 2017, 2018). One possible solution for this problem is a temporal opening of the blood-brain barrier by focused ultrasound which might allow the delivery of larger amounts of therapeutics, such as antibodies, to the brain (Meng et al. 2019). Interestingly, in mice focused ultrasound alone, without the need for any additional therapeutic agent such as anti-Aβ antibody, was efficient to reduce brain amyloid suggesting focused ultrasound to be a noninvasive method with therapeutic potential in AD (Leinenga and Gotz 2015). In humans, in a small study, focused ultrasound opened the barrier in the cortices of five patients with early AD with no obvious adverse long-term effects (Lipsman et al. 2018). Overall, focused ultrasound transiently affected neurologic function, but the functional organization was restored the next day and remained unchanged 3 months after. These first in human data have implications for the development of focused ultrasound as a drug delivery platform to the brain and its potential use. However, larger trials have to determine the method's efficacy and potential side effects. In mice, it has been shown that drug delivery works. A combination of an anti-tau antibody analog plus scanning ultrasound restored memory better than either the antibody or ultrasound alone (Nisbet et al. 2017).

We are just beginning to understand other possibilities that might enhance drug delivery to the brain. The expression of many blood-brain barrier genes is highly controlled by the circadian clock. For example, the expression of the drug efflux transporter P-gp (Zhang et al. 2018) and the tight junctional protein claudin-5 (Hudson et al. 2019) are highly dynamic and claudin-5 protein levels change up to 30–40% dependent on the time of day. Both proteins are tight controllers of blood-brain barrier passage and thus one could imagine that timing of drug administration in patients can alter the efficacy for brain delivery, even without physically altering the blood-brain barrier.

To date, targeting Aβ with anti-Aβ antibodies are a major treatment strategy. Just recently, it was announced that Biogen submitted a biologics license application to the FDA for aducanumab (Schneider 2020), a therapeutic antibody developed to clear amyloid plaques from the brain. While its efficacy in a robust improvement of cognitive decline is still debated due to inaccuracies in the conduction of the Phase 3 trial and therefore inconclusive findings, aducanumab's ability to remove brain amyloid is unquestioned and the medical

need enormous. If successful, aducanumab would be the first anti-amyloid treatment – and the first new drug for AD in more than 16 years in the US, since Namenda was approved in 2003. Other pharmaceutical companies are also trying to develop anti-Aβ antibodies, which are currently tested in clinical trials. An improvement of brain uptake is facilitated by targeting receptors at the blood-brain barrier such as the TfR.

A recent study reports the development of Anti-BACE1 antibodies with an engineered fragment in the constant region of a human IgG1 (Kariolis et al. 2020). This engineered fragment binds to the apical domain of the human TfR at a site that is distinct from its binding sites for transferrin, a natural ligand for TfR and FcRn. Thus, these novel anti-BACE1 antibodies with engineered constant regions showed substantially enhanced brain uptake and decreased Aβ levels in mice and non-human primates.

Several therapeutic approaches target neuroinflammation in the AD brain. The abovementioned RAGE antagonists, currently tested in clinical trials, are not only believed to prevent the reuptake of circulating plasma Aβ into the brain but also regulate multiple other effects, including inflammatory processes, oxidation stress, and cerebral blood flow (Burstein et al. 2018). Other strategies target the triggering receptor expressed on myeloid cells 2 (TREM2) in microglia. By activation of this receptor, enhanced removal of amyloid plaques should be achieved. The TREM2-activating antibody AL002, developed by Alector and Abbvie, entered early clinical trials in 2019. In recent years, peroxisome-proliferator-activated receptor gamma (PPARγ) has come to be a target of interest for AD drug development. PPARγ activation has been shown to increase microglial Aβ phagocytosis and decrease cytokine release (Mandrekar-Colucci et al. 2012; Yamanaka et al. 2012). AD-4833 (Pioglitazone), a PPARγ agonist, is currently tested in clinical trials.

Similar to Aβ, tau is targeted by either passive immunotherapy or by trying to prevent its aggregation with similar strategies. However, until today none of the clinical trials have led to a major breakthrough.

Looking at current targets for AD treatment, it seems that the blood-brain barrier with all its potential for therapeutic intervention is neglected. Up until recently, tools to modify specific gene expression at the blood-brain barrier had been lacking. However, using novel techniques, recently identified brain endothelial-specific targets or ways to manipulate the brain endothelium, which might be useful for the development of treatment strategies in the future. However, even today, we still do not know the causes of AD, which is crucial for the development of efficient treatment strategies. Therefore, it should be the major goal to improve our general understanding of pathophysiological pathways underlying disease progression. In this chapter, we have mainly focused on amyloid transport across the blood-brain barrier but also highlighted that AD is a multifaceted disease. The complexity and the various pathways that contribute to disease progression are beyond current understanding. It has been shown that in most AD cases, clearance pathways are affected. Moreover, in recent years, many novel AD risk factor genes have been discovered that affect Aβ clearance.

It remains uncertain whether targeting the blood-brain barrier will help to treat, prevent, or slow down AD pathology. However, the barriers of the CNS are crucial mediators for proper

brain function. Therefore, neutralizing the alterations during aging and disease progression are likely to be beneficial for brain health.

7 Summary and Future Perspectives

The different cell types comprising the neurovascular unit act in concert as a gatekeeper and regulator of brain homeostasis ensuring proper brain function. In AD, however, this delicate system is disturbed and changes occurring at the blood-brain barrier, in astrocytes, pericytes, and neurons result in neurovascular dysfunction which has a massive impact on brain health. There is increasing evidence that neurovascular function is important for information processing, neuronal connectivity, and synaptic function suggesting a link between neurovascular dysfunction and memory decline seen in AD. To date, it is unknown whether barrier dysfunction is a cause or consequence of AD. As described in this chapter, studies support the hypothesis that vascular impairment precedes neurodegeneration suggesting that targeting the blood-brain barrier has the potential to slow the progression of the disease. In this regard, novel approaches are needed that specifically target mechanism(s) underlying blood-brain barrier dysfunction in AD.

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