Alzheimer’s disease: A matter of blood–brain barrier dysfunction?

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The blood–brain barrier (BBB) keeps neurotoxic plasma–derived components, cells, and pathogens out of the brain. An early BBB breakdown and/or dysfunction have been shown in Alzheimer’s disease (AD) before dementia, neurodegeneration and/or brain atrophy occur. However, the role of BBB breakdown in neurodegenerative disorders is still not fully understood. Here, we examine BBB breakdown in animal models frequently used to study the pathophysiology of AD, including transgenic mice expressing human amyloid-β precursor protein, presenilin 1, and tau mutations, and apolipoprotein E, the strongest genetic risk factor for AD. We discuss the role of BBB breakdown and dysfunction in neurodegenerative process, pitfalls in BBB measurements, and how targeting the BBB can influence the course of neurological disorder. Finally, we comment on future approaches and models to better define, at the cellular and molecular level, the underlying mechanisms between BBB breakdown and neurodegeneration as a basis for developing new therapies for BBB repair to control neurodegeneration.

Introduction

The blood–brain barrier (BBB) is formed by a tightly sealed monolayer of brain endothelial cells, which keeps neurotoxic plasma–derived components, RBCs, leukocytes, and pathogens out of the central nervous system (CNS; Zlokovic, 2011). The capillary length in mouse and human brain is 0.6 and 650 km, respectively, which accounts for >85% of total cerebral blood vessel length, providing the largest endothelial surface area for solute transport exchanges between blood and brain, and vice versa (e.g., ∼120 cm²/g of brain; Zlokovic, 2008; Pardridge, 2015). Endothelium allows free, rapid diffusion of oxygen and carbon dioxide across the BBB. Specialized endothelial transport systems carry energy metabolites, nutrients, and regulatory molecules across the BBB from blood to brain and metabolic waste products and potentially neurotoxic molecules from brain to blood. This includes solute carrier–mediated transport of carbohydrates (e.g., glucose), amino acids, vitamins, hormones, nucleotides, and monocarboxylic acids (e.g., lactate); receptor-mediated transport (RMT) of peptides and proteins; a major facilitator transporter of essential omega-3 fatty acids; ATP-binding cassette active efflux of xenobiotics and drugs; and multiple ion transporters.

The BBB transport systems and cellular junctions have been recently reviewed elsewhere (Zhao et al., 2015a).

The mean distance between the BBB and neurons is ∼8 µm, allowing rapid diffusion of molecules across the brain interstitial space from capillaries to neurons, and vice versa (Pardridge, 2015). The BBB controls the composition of neuronal internal milieu, which is essential for proper neuronal and synaptic functioning (Zhao et al., 2015a). The BBB-associated mural cells (pericytes) play a key role in the formation and maintenance of the BBB (Armulik et al., 2010; Bell et al., 2010; Daneman et al., 2010; Sweeney et al., 2016). BBB breakdown has been shown in Alzheimer’s disease (AD; Zlokovic, 2011) and is associated with cerebral blood flow (CBF) reductions and impaired hemodynamic responses (Iadecola, 2013, 2017; Kisler et al., 2017a). Recent imaging and biomarker studies suggest an early BBB breakdown and vascular dysregulation in AD detectable before cognitive decline and/or other brain pathologies (Montine et al., 2014; Montagne et al., 2015, 2016; Snyder et al., 2015; Sweeney et al., 2015; Iturria-Medina et al., 2016; van de Haar et al., 2016a; van de Haar et al., 2016b; Kisler et al., 2017a). Neuropathological studies also support early contribution of cerebral vessel disease to AD (Toledo et al., 2013; Arvanitakis et al., 2016).

Here, we briefly summarize key findings from human studies demonstrating BBB breakdown and dysfunction in AD and examine BBB breakdown in transgenic models frequently used to study pathophysiology of AD. We concentrate on animal models carrying major autosomal–dominant AD (ADAD) mutations in human amyloid-β precursor protein (APP) and presenilin 1 (PSEN1) genes (Zhao et al., 2015a),...
models associated with BBB breakdown, such as PGRN integrity and they degenerate in AD (Sweeney et al., 2016) pericyte-deficient models, because pericytes maintain BBB (Park et al., 2013b; Sagare et al., 2013). Other models associated with BBB breakdown, such as PGRN (progranulin)-deficient mice modeling loss-of-function mutations in the PGRN gene linked to frontotemporal dementia (Jackman et al., 2013), deficiency in endothelial sphingosine-1-phosphate receptor 1 (Yanagida et al., 2017), and vascular risk factors such as diabetes and hypertension (Girouard and Iadecola, 2006; Iadecola, 2013; Nelson et al., 2016), have been summarized elsewhere.

We focus on CNS capillaries that maintain the largest surface area of the BBB. Additionally, we examine changes in other vascular segments, including arteries and small arteries, which when disrupted also contribute to a loss of blood-to-brain vascular integrity. We discuss the role of BBB breakdown and dysfunction in the neurodegenerative process, pitfalls in BBB measurements, and how targeting the BBB can influence the course of a neurological disorder. We also suggest future cellular and molecular studies to investigate the underlying mechanisms between BBB breakdown and neurodegeneration as a basis for developing new therapeutic approaches for BBB repair to control neurodegeneration.

**BBB breakdown in AD**

Recent neuroimaging studies in individuals with mild cognitive impairment (MCI) and early AD have shown BBB breakdown in the hippocampus (Montagne et al., 2015) and several gray and white matter regions (van de Haar et al., 2016a,b, 2017), respectively, before brain atrophy or dementia. These neuroimaging studies used advanced contrast-enhanced magnetic resonance imaging (MRI) to determine BBB permeability to the contrast agent Gadolinium. Increased CNS cerebral microbleeds reflecting loss of cerebrovascular integrity have also been shown by MRI studies in 25% of individuals with MCI and 45–78% of individuals with early AD before dementia (Brundel et al., 2012; Heringa et al., 2014; Yates et al., 2014; Shams et al., 2015; Poliakova et al., 2016; Shams and Wåhlund, 2016).

The BBB breakdown in AD has been confirmed by more than 20 independent postmortem human studies showing brain capillary leakages and perivascular accumulation of blood-derived fibrinogen, thrombin, albumin, immunoglobulin G (IgG) and hemosiderin deposits, pericyte and endothelial degeneration, loss of BBB tight junctions, and RBC extravasation, as recently reviewed (Nelson et al., 2016). An early cerebrovascular disorder (Toledo et al., 2013; Arvanitakis et al., 2016), vascular dysregulation (Iturria-Medina et al., 2016), ischemic vascular damage from comorbidities and vascular risk factors (Iadecola, 2013; Faraco et al., 2016; Nelson et al., 2016), and small vessel disease of the brain (Warlaw et al., 2013; Snyder et al., 2015; Hachinski, 2016) may introduce additional vascular components contributing to BBB breakdown in AD.

The identification of peripheral macrophages (Hultman et al., 2013) and neutrophils (Zenaro et al., 2015) in the brain in human AD suggests BBB breakdown to circulating leukocytes and their influx into the brain. A novel matrix metalloproteinase radioactive positron emission tomography (PET) ligand was recently used to visualize leukocyte penetration across the BBB and infiltration into the brain in patients with multiple sclerosis (Gerwien et al., 2016). Similar studies, however, have not yet been performed in AD patients, and it remains unclear whether leukocyte infiltration is a cause or a consequence of BBB breakdown.

Multiple studies with [18F]Fluoro-2-deoxy-d-glucose (FDG-PET) in individuals with MCI and early AD showed impaired regional brain uptake of glucose before brain atrophy, neurodegeneration, or conversion to AD, suggesting reduced glucose brain utilization caused by diminished glucose transport across the BBB via endothelial-specific glucose transporter 1 (GLUT1), as recently reviewed (Nelson et al., 2016). 2-Deoxy-d-glucose (2DG) was originally developed by Sokoloff as a surrogate molecule to study brain glucose uptake and utilization (Sokoloff et al., 1977). Similar to glucose, 2DG is transported across the BBB by GLUT1, and after crossing the BBB, it is taken up by different cell types in the brain, including neurons and astrocytes (Cummane et al., 2011). 2DG is rapidly phosphorylated by intracellular hexokinase (Sols and Crane, 1954) and converted in the brain into 2DG-6-phosphate (2DG-6P; Sokoloff et al., 1977; McDougal et al., 1990). However, unlike glucose–6-phosphate, which is metabolized further in the glycolytic pathway, 2DG-6P is not a substrate for glucose–6-phosphate isomerase and therefore cannot be converted into fructose–6-phosphate, a necessary step to enter the glycolytic pathway or Krebs cycle, as shown by multiple independent studies (Sols and Crane, 1954; Sokoloff et al., 1977; McDougal et al., 1990; Rokka et al., 2017). For example, studies in the mouse brain using enzymatic assays indicated that 1 h after 2DG systemic administration, ~90% is converted into 2DG-6P and 10% remains as 2DG, with no other significant metabolites found (McDougal et al., 1990). This has been confirmed by another study in the mouse brain using thin-layer chromatography and digital autoradiography, which indicated that after 1 h of [18F]-DG administration, 97% of the [18F]-DG is converted into [18F]-DG-6P (Rokka et al., 2017). Similarly, at longer time points, such as 90 min after [18F]-DG administration to rats, 90% of the [18F]-DG in the brain is converted into [18F]-DG-6P and/or its epimers that are not metabolized further, whereas 10% remains as [18F]-DG (Southworth et al., 2003). Because of minimal glucose–6-phosphatase activity in the brain (Hers and De Duve, 1950; Sokoloff et al., 1977) and low 2DG-6P membrane permeability, 2DG-6P remains trapped in brain cells (Huang and Veech, 1985; Southworth et al., 2003) and is eliminated very slowly from the brain.

P-glycoprotein (Pgp) dysfunction in BBB active efflux transport of xenobiotics and drugs in individuals with early...
AD has also been shown using verapamil (Pgp ligand)-PET (van Assema et al., 2012; Deo et al., 2014). Implications of BBB breakdown and dysfunction in BBB transport systems for development of brain pathology based on findings in AD models are discussed next.

**BBB breakdown in APP transgenic models**

Transgenic models expressing mutations in human APP gene linked to early ADAD have been frequently used to study the pathophysiology of AD and/or develop treatments to control AD-related cerebral β-amyloidosis. Here, we examine BBB breakdown in APP transgenic models and its relationship with Alzheimer’s amyloid-β (Aβ) pathology, neurodegeneration, and behavioral deficits (Table 1).

Several independent studies have shown BBB breakdown in APP<sup>sw/0</sup> mice harboring the Swedish double APP mutation (KM670/671NL) driven by the hamster prion gene promoter (Hsiao et al., 1996). This mutation increases abnormal cleavage of cellular APP by β-secretase, causing Aβ overproduction. The BBB breakdown has been demonstrated by capillary leakages of blood-derived fibrinogen, IgG, and albumin; BBB leakage of intravenously administrated Evans blue; and immunohistological and electron microscopy studies showing degeneration and loss of brain capillary pericytes, endothelial cells, vascular smooth muscle cells (VSMCs), loss of endothelial tight junction proteins, and cerebral microhemorrhages (Kumar-Singh et al., 2005; Paul et al., 2007; Biron et al., 2011; Park et al., 2013b; Sagare et al., 2013; Table 1). Previous studies have shown that leakage of molecules across the BBB could be caused by a loss and/or misalignment of the tight junction proteins and/or enhanced bulk flow fluid transcytosis across the BBB (Zhao et al., 2015a). Additionally, pericyte degeneration has been shown to lead to BBB disruption at the level of brain capillaries (Armulik et al., 2010; Bell et al., 2010; Daneman et al., 2010). VSMC degeneration is associated with the breakdown of vascular integrity at the level of small arterioles and arteries, often causing microhemorrhages (Holtzman et al., 2000; Bell et al., 2009). Brain endothelial degeneration is typically seen with advanced damage to the BBB at the capillary level (Zlokovic, 2008).

Studies examining the time course between BBB breakdown and other pathologies indicated that BBB breakdown develops early in APP<sup>sw/0</sup> mice (at 1, 3, and 6 mo of age; Ujie et al., 2003; Paul et al., 2007; Sagare et al., 2013) before Aβ deposition, cerebral amyloid angiopathy (CAA), and/or behavioral deficits that develop later (at 7–10, 9–12, and 6–9 mo of age, respectively; Domnitz et al., 2005; Webster et al., 2014). The underlying mechanisms of early BBB disruption in APP<sup>sw/0</sup> mice remain elusive but likely involve direct vasculotoxic effects of oligomeric Aβ and/or other APP-mediated toxic effects on vasculature.

The role of BBB breakdown in the development of brain pathologies has been supported by findings showing that loss of pericytes causing an accelerated BBB breakdown also accelerates parenchymal Aβ deposition and CAA and leads to tau pathology and neuronal loss, which is not seen in APP<sup>sw/0</sup> mice but has been shown in double-transgenic APP<sup>sw/0; Pdgfβ<sup>−/−</sup></sup> (platelet-derived growth factor receptor β) mice with accelerated pericyte loss (Sagare et al., 2013).

Aberrant expression of BBB transporters and/or receptors in cerebral microvessels has also been shown in APP<sup>sw/0</sup> mice and other APP models. This includes low levels of low-density lipoprotein receptor–related protein 1 (LRP1), a major clearance receptor for Aβ toxin at the BBB (Deane et al., 2004). Its diminished expression in brain capillaries precedes onset of Aβ pathology and behavioral deficits in APP<sup>sw/0</sup> mice and triple-transgenic APP<sup>sw/0; Pdgfβ<sup>−/−</sup></sup> mice carrying three human APP mutations (Swedish, Dutch, and Iowa) under the control of the mouse Thy1 neuronal promoter (Deane et al., 2004). Genetic approaches have demonstrated that increasing or diminishing LRP1 levels in cerebral blood vessels slows down or accelerates Aβ pathology and CAA in APP<sup>sw/0</sup> and APP<sup>sw/0; Pdgfβ<sup>−/−</sup></sup> mice, respectively (Bell et al., 2009; Table 1). Genetic deletion of LRP1 from brain endothelium also accelerates Aβ pathology in 5xFAD mice (Storck et al., 2016) carrying three APP mutations (Swedish, Florida, and London) and two Presenilin 1 human mutations (PSEN1 M146L and PSEN1 L286V; Oakley et al., 2006) causing ADAD (Karch et al., 2014).

Expression of the receptor for advanced glycation end products (RAGE), a major Aβ influx receptor at the BBB, is increased in brain microvessels in APP<sup>sw/0</sup> and 5xFAD mice, which accelerates reentry of circulating Aβ into the brain, causing Aβ accumulation, CBF reductions, and a neuroinflammatory response (Deane et al., 2003) and BBB breakdown (Kook et al., 2012), respectively. Blockade of RAGE slows down Aβ pathology and behavioral deficits in PD-hAPP mice expressing a mutant form of human APP encoded by a minigene containing several substitutions from ADAD (V717F, K670M, and N761L) under the control of platelet-derived growth factor B (PDGF-B) chain promoter (Deane et al., 2003; Table 1).

**APP<sup>sw/0</sup>** mice develop an early GLUT1 BBB dysfunction that reduces brain glucose uptake (Niwa et al., 2002). Moreover, diminished GLUT1 expression in brain endothelium leads to BBB breakdown and transcriptionally inhibits LRP1. This accelerates Aβ pathology and leads to secondary neurodegenerative changes, loss of neurons, and brain atrophy, as shown in double-transgenic Skc2a1<sup>−/−</sup>; APP<sup>sw/0</sup> mice with GLUT1 endothelial-specific haploinsufficiency (Winkler et al., 2015). Loss of brain endothelial Pgp efflux transporter also diminishes LRP1 expression at the BBB and leads to brain accumulation of Aβ, as shown in triple-transgenic APP<sup>sw/0; mdr1a/b<sup>−/−</sup></sup> (Pgp-null) mice (Cirrito et al., 2005). Additionally, Pgp endothelial dysfunction diminishes active efflux at the BBB, leading to brain accumulation of potentially neurotoxic xenobiotics such as environmental pollutants, food additives, pesticides, and drugs (Mokgokong et al., 2014).

Recent studies have shown that brain endothelial deficiency in the phosphatidylinositol-binding clathrin assembly
### Table 1. BBB breakdown in APP, PSEN1, Tau, and pericyte-deficient transgenic models

| Mouse line, construct, promoter | Findings | Age | Region | Reference | Brain Aβ | CAA | Neurodegenerative and behavior changes | Reference |
|---------------------------------|----------|-----|--------|-----------|----------|-----|----------------------------------------|-----------|
| **In APP models**               |          |     |        |           |          |     |                                        |           |
| APPSw/0 (Tg2576), hAPP695, K670M/N671I, hPrP | Fibrin perivascular deposits, BBB leakage of Evans blue | 6 and 12 mo; not studied before 6 mo | Cortex, hippocampus | Paul et al., 2007 | 7–10 mo | 9–12 mo | 6–9 mo | Hsiao et al., 1996; Domnitz et al., 2005; Kumar-Singh et al., 2005; Webster et al., 2014 |
|                                 | Loss of BBB tight junctions, LRP1 endothelial expression, Pdgfrβ+ pericytes, VSMCs | 18 mo; not studied before 18 mo | Cortex | Park et al., 2013a |
|                                 | IgG perivascular deposits, loss of CD11b+ pericytes, endothelial degeneration | 1, 3, 6, and 9 mo | Cortex, hippocampus | Sagare et al., 2013 |
|                                 | IgG perivascular deposits, albumin perivascular deposits, microhemorrhages, endothelial degeneration, pericyte degeneration, VSMC loss, swollen astrocytic end-feet | 17 and 25 mo; not studied before 17 mo | Cortex, hippocampus, thalamus | Kumar-Singh et al., 2005 |
|                                 | BBB leakage of Evans blue, BBB leakage of exogenous tracers | 4 and 10 mo | Cortex | Ujiie et al., 2003 |
|                                 | Loss of BBB tight junctions | 18 and 24 mo; not studied before 18 mo | Cortex, hippocampus | Biron et al., 2011 |
|                                 | Loss of LRP1 endothelial expression | 4 and 6 mo | Cortex, hippocampus, thalamus | Deane et al., 2004 |
|                                 | Increased RAGE vascular expression | 9 mo; not studied before 9 mo | Cortex, hippocampus | Deane et al., 2003 |
|                                 | Genetically increased LRP1 expression in brain microvessels | 16 and 24 mo; not studied before 16 mo | Cortex | Bell et al., 2009 | Slowed down | Slowed down | Not studied | Bell et al., 2009 |
| APPSw/0; Pdgfrβ−/− | IgG perivascular deposits, loss of CD11b+ pericytes | 1, 3, 6, and 9 mo | Cortex, hippocampus | Sagare et al., 2013 | Accelerated, +tau pathology and loss of neurons | Accelerated | Accelerated | Sagare et al., 2013 |
| APPSw/0; Sic2a1+/−, GLUT1 endothelial haploinsufficiency | Fibrin perivascular deposits, IgG perivascular deposits, and loss of BBB tight junctions, LRP1 endothelial expression, GLUT1 endothelial expression | 2 wk and 1, 8–10, 12, and 16 mo | Cortex, hippocampus | Winkler et al., 2015 | Accelerated, +loss of neurons | Accelerated | Accelerated | Winkler et al., 2015 |
| APPSw/0; mdr1a/b−/− | Loss of Pgp endothelial expression; BBB leakage of Pgp substrate; loss of LRP1 endothelial expression | 2 and 3 mo | Striatum, cortex, hippocampus | Cirrito et al., 2005 | Accelerated | Not studied | Not studied | Cirrito et al., 2005 |
Table 1. BBB breakdown in APP, PSEN1, Tau, and pericyte-deficient transgenic models (Continued)

| Mouse line, construct, promoter | Findings | Age | Region | Reference | Brain Aβ | CAA | Neurodegenerative and behavior changes | Reference |
|--------------------------------|----------|-----|--------|-----------|----------|-----|---------------------------------------|-----------|
| APPSwD0; Picalm+/-, PIC ALM endothelial haploinsufficiency | Loss of PICALM endothelial expression | 3 mo | Cortex, hippocampus | Zhao et al., 2015b | Accelerated | Accelerated | Accelerated | Zhao et al., 2015b |
| APP V717F (PDAPP), hAPP full-length, V717F, PDGFb | Fibrin perivascular deposits, BBB leakage of Evans blue | 6 and 12 mo; not studied before 6 mo | Cortex, hippocampus | Paul et al., 2007 | 6–9 mo | 10–12 mo | 6 mo | Paul et al., 2007; Webster et al., 2014 |
| PD-hAPP, hAPP minigene, V717F/ K670M/N671L, PDGFb | Blockade of RAGE | 9 mo | Cortex, hippocampus | Deane et al., 2003 | Slowed down | Not studied | Not studied | Deane et al., 2003 |
| APPSwI (TgCRND8), hAPP695, K670M/ N671L/V717F, P45P | Fibrin perivascular deposits, BBB leakage of Evans blue | 6 and 12 mo; not studied before 6 mo | Cortex, hippocampus | Paul et al., 2007 | 3 mo | 6–7 mo | 3 mo | Domnitz et al., 2005; Webster et al., 2014 |
| Fibrin perivascular deposits | 6 mo; not studied before 6 mo | Cortex | Chen et al., 2017 |
| APPSwD1, hAPP770, K670M/N671L/ E693D/E694N, mThy1 | Albumin perivascular deposits | 6 mo; not studied before 6 mo | Dorsal subiculum | Kruyer et al., 2015 | 3–6 mo | 6 mo | 3–6 mo | Davis et al., 2004; Deane et al., 2004 |
| Loss of LRP1 endothelial expression | 4 and 6 mo | Cortex, hippocampus, and thalamus | Deane et al., 2004 |
| Genetically decreased LRP1 expression in brain microvessels | 16 and 24 mo; not studied before 16 mo | Cortex | Bell et al., 2009 | Accelerated | Accelerated | Not studied | Bell et al., 2009 |
| APPSw (APP23), hAPP751, K670M/ N671L, mThy1 | Microhemorrhages, increased BBB permeability to iodinated contrast agent | 16 and 30 mo; not studied before 16 mo | Cortex, thalamus | Beckmann et al., 2011 | 6 mo | 12 mo | 3 mo | Webster et al., 2014 |
| TetO-APPSwI, hAPP695, K670M/ N671L/V717F, tetracycline-responsive promoter | Microhemorrhages, increased BBB permeability to iodinated contrast agent | 14 mo; not studied before 14 mo | Cortex, hippocampus, thalamus | Tanifum et al., 2014 | 2–6 mo | 14 mo | Not studied | Tanifum et al., 2014 |
| APPSwArc (ArcAβ), hAPP695, K670M/ E693D/E694N, mThy1 | Microhemorrhages and increased BBB permeability to gadolinium contrast agent | 9 and 21 mo; not studied before 9 mo | Cortex, hippocampus, olfactory bulb | Klohs et al., 2011, 2013 | 6 mo | 9–15 mo | 6 mo | Klohs et al., 2012 |
| APPSwD0; PSEN1ΔE9, m/hAPP695 K595N/M596L × hPSEN1ΔE9, mPP | Microhemorrhages, increased BBB permeability to gadolinium contrast agent | 9 mo; not studied before 9 mo | Cortex | Kelly et al., 2015 | 6–7 mo | 6 mo | 6 mo | Duff et al., 1996; Webster et al., 2014 |
| Fibrin perivascular deposits | 10 mo; not studied before 10 mo | Hippocampus, periventricular zone | McManus et al., 2017 |
| APPSwD0; PSEN1M146L, hAPP695 K670M/ N671L × PSEN1 M146L, hPP | Microhemorrhages, increased BBB permeability to gadolinium contrast agent, immunoglobulin extravasation, endothelial degeneration | 5 and 11 mo | Cortex, hippocampus, thalamus | Kumar-Singh et al., 2005 | 3–6 mo | 10 mo | 3 mo | Kumar-Singh et al., 2005 |

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| Mouse line, construct, promoter | Findings | Age | Region | Reference | Brain Aβ | CAA | Neurodegenerative and behavior changes | Reference |
|---------------------------------|----------|-----|--------|-----------|----------|-----|---------------------------------------|-----------|
| APPSwFlLo; PSEN1M146L/L286V (5xFAD or Tg6799), hAPP695, K670M/ N671Q/L74V/ V717L, mThy1, PSEN1 M146L/L286V, mThy1 | IgG perivascular deposits | 9 and 10 mo; not studied before 9 mo | Cortex | Park et al., 2017 | 2 mo | Not studied | 4-5 mo | Oakley et al., 2006; Webster et al., 2014 |
|                                  | Loss of LRP1 endothelial expression | 7 mo; not studied before 7 mo | Cortex | Storck et al., 2016 | |
|                                  | Loss of GLUT1 endothelial expression, increased RAGE vascular expression, increased MMP-9 vascular expression, loss of BBB tight junctions | 8 mo; not studied before 8 mo | Cortex | Kook et al., 2012 | |
| Tg PSEN1 models | PSEN1–/– | Microhemorrhages, endothelial degeneration | E18.5 | Neocortex | Wen et al., 2005 | Not studied | Not studied | Not studied (perinatal lethality) | Wen et al., 2005 |
| PSEN1M146V | Microhemorrhages, basement membrane degeneration | 10 and 37 mo | Cortex, hippocampus | Gama Sosa et al., 2010 | 24 mo | No CAA | Not studied | Chen et al., 2000 |
| Tg tau models | TetO-TauP301L (rTg4510), hMAPT P301L, tetracycline-responsive promoter | BBB leakage of Evans blue, IgG perivascular deposits, microhemorrhages, leukocyte infiltration | 9 and 12 mo | Cortex and hippocampus | Blair et al., 2015 | No Aβ pathology; Tau pathology | 12 mo | Blair et al., 2015 |
| To pericyte-deficient models | Pdgfrβ+/- | Loss of pericytes, IgG perivascular deposits, fibrin perivascular deposits, thrombin and plasmin brain extravasation, loss of BBB tight junctions, brain extravasation of exogenous tracers | 1, 6, 8, 14, and 16 mo | Cortex, hippocampus | Bell et al., 2010 | Not studied | Not studied | 6-9 mo, +neurodegenerative changes, loss of neurons | Bell et al., 2010; Kisier et al., 2017b |
| PdgfrβF7/F7 | Loss of pericytes, IgG perivascular deposits, fibrin perivascular deposits, thrombin and plasmin brain extravasation, loss of BBB tight junctions, brain extravasation of exogenous tracers | 6 and 8 mo; not studied before 6 mo | Cortex, hippocampus | Bell et al., 2010 | Not studied | Not studied | 6-9 mo, +neurodegenerative changes, loss of neurons | Bell et al., 2010 |
|                             | Fibrin perivascular deposits | 1, 2, 3, 4, 8, and 12 mo | Cortex, hippocampus, striatum | Nikolakopoulou et al., 2017 |

APP, amyloid precursor protein; CAA, cerebral amyloid angiopathy; GLUT1, glucose transporter 1; H2P, hamster prion promoter; IgG, immunoglobulin G; LRP1, low-density lipoprotein receptor-related protein 1; MAPT, microtubule-associated protein tau; MMP-9, matrix metalloproteinase-9; mPrP, mouse prion promoter; mThy1, mouse thymus cell antigen 1 promoter; PDGFβR, platelet-derived growth factor receptor β; Pgs, P-glycoprotein; PICALM, phosphatidylinositol binding clathrin assembly protein; PSEN1, presenilin 1; RAGE, receptor for advanced glycation end products; VSMC, vascular smooth muscle cell.

1Loss of BBB tight junctions as shown by high-resolution confocal microscopy analysis.
1Microhemorrhages (hemosiderin deposits) at the capillary level.
1Microhemorrhages (hemosiderin deposits) at the arteriolar level.
1Loss of BBB tight junctions as shown by immunoblotting of isolated brain capillaries.
1The exact vascular location is difficult to determine.
1Loss of BBB tight junctions as shown by electron microscopy analysis.
(PiCALM) protein encoded by the PiCALM gene, a highly validated genetic risk factor for human AD (Harold et al., 2009; Lambert et al., 2009; Huang et al., 2017), accelerates Aβ deposition in APPSw/0 mice, consistent with its critical role in regulating LRPI-mediated Aβ internalization at the abluminal side of the BBB and Aβ transcytosis and clearance across the BBB via Rab5- and Rab11-guided vesicular trafficking (Zhao et al., 2015b; Table 1).

Several studies using other APP transgenic models, such as APP V717F, APPSw/3 (Swedish, Indiana), APPSw/DI, and APPSw/Arc (Swedish, Arctic), and APP mice crossed with PSEN1 ADAD mutants, confirmed BBB breakdown, including fibrinogen, IgG, and albumin perivascular deposits; BBB leakage of Evans blue and contrast imaging agents; microhemorrhages; leukocyte infiltration; loss of tight junctions; and diminished GLUT1 and LRPI expression at the BBB (Table 1). Collectively, these data provide strong experimental support for the role of BBB breakdown and dysregulated BBB transport in AD pathophysiology.

**BBB breakdown in PSEN1 transgenic models**

PSEN1 ADAD mutations lead to elevated Aβ production (Tanzi, 2012; Potter et al., 2013; Karch et al., 2014; Elbert et al., 2015), neuronal dysfunction (Lee et al., 2010; Karch and Goate, 2015), and major cerebrovascular pathology, including BBB breakdown; pericyte degeneration; Aβ capillary, arteriolar, and arterial deposits; and decreased BBB glucose uptake, as shown by human postmortem studies (Dermaut et al., 2001; Mann et al., 2001; Armstrong, 2008) and imaging studies in the living human brain (Bateman et al., 2012; Benzinger et al., 2013; Fleisher et al., 2015), respectively. Consistent with these findings, PSEN1-knockout mice (Wen et al., 2005) and mice expressing PSEN1 mutations driven by the neuronal Thy1 promoter (Gama Sosa et al., 2010) develop BBB breakdown and microhemorrhages and loss of BBB integrity and reductions in microvascularity, suggesting loss-of-function vascular phenotype in the absence of CAA and/or other Aβ-related pathology. Vascular and BBB changes are also seen in PSEN1 models crossed with APP mice (Kumar-Singh et al., 2005; Kelly et al., 2015; Table 1).

**BBB breakdown in Tau transgenic mice**

BBB leakage of Evans blue, IgG perivascular deposits, microhemorrhages, and leukocyte infiltration indicating BBB breakdown have been shown in hTau.P301L mice before significant accumulation of tau pathology (Blair et al., 2015; Table 1) and in the absence of CAA and/or other Aβ pathology. These mice carry a Pro to Leu mutation at codon 301 of the tau gene driven by an inducible Tet-on promoter. In humans, this tau mutation leads to early-onset frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17) that is characterized by extensive tau pathology (Lewis et al., 2000). Whether the underlying mechanisms of BBB breakdown involve disrupted tau clearance across the BBB or direct vasculotoxic effects of tau remains unclear at present. Interestingly, Tau P301L mice actually outperform wild-type littermates in behavioral tests within the first 2–3 mo of age (Boekhoorn et al., 2006), before the onset of BBB breakdown, raising the possibility that other pathologies, including BBB breakdown, may contribute to dementia and motor symptoms. Because tau pathology is seen in AD, these findings are relevant to AD dementia as well. Future studies should determine the role of BBB breakdown and tau clearance across the BBB in models of dementias caused by tau pathology.

**BBB breakdown in Pdgfra−/− deficient transgenic mice**

Pericyte-deficient transgenic mice, including Pdgfra+/− mice and Pdgfra−/− mice with disrupted Pdgfra signaling, develop an early and progressive BBB breakdown (Bell et al., 2010; Daneman et al., 2010; Nikolakopoulou et al., 2017) and impaired hemodynamic responses (Kisler et al., 2017b) beginning at 1 mo of age. These vascular changes lead to secondary neurodegeneration, loss of cortical and hippocampal neurons, and behavioral deficits at 6–9 mo of age (Table 1). Loss of pericytes in mice with diminished PDGF-BB bioavailability also leads to an early BBB breakdown (Armulik et al., 2010) and calcium deposition in the basal ganglia detectable at 1 yr of age (Keller et al., 2013; Vanlandewijck et al., 2015). Because pericytes degenerate in human AD and other neurodegenerative disorders (Sweeney et al., 2016), these findings support vascular-mediated neurodegeneration independent of Aβ and tau pathology.

**BBB breakdown in APOE transgenic models**

The apolipoprotein E (APOE) gene is the strongest genetic risk factor for AD (Verghese et al., 2011; Zlokovic, 2013; Liao et al., 2017). One and two APOE ε4 alleles increase risk by ∼3.8- and ∼12-fold, respectively, compared with ε3/ε3 genotype, respectively. The effect of one ε4 allele on AD risk is stronger in females than in males. One copy of APOE ε2 allele decreases risk by ∼0.6-fold relative to ε3/ε3 genotype. Additionally, APOE4 increases the risk of CAA.

APOE exerts its toxic effects on the cerebrovascular system (Zlokovic, 2013) and neurons (Mahley and Huang, 2012) and influences Aβ clearance, amyloid deposition, and tau-related neurodegeneration in an allele-dependent manner (ε4>ε3>ε2; Holtzman et al., 2012; Liao et al., 2017). Despite the fact that APOE has such a strong effect on AD risk, we still lack a comprehensive understanding of how the effects of APOE on the cerebrovascular system and other aspects of its function contribute to AD. We also do not have an effective ApoE-based therapy for AD.

Apoε−/− mice lacking mouse apolipoprotein E (Apoε) develop BBB breakdown, as shown by accumulation of perivascular IgG, fibrinogen, thrombin, and hemosiderin deposits; leakage of Evans blue, different exogenous tracers, or MRI contrast agents; penetration of N-methyl-D-aspartate receptor antibodies; loss of tight junctions; basement membrane degeneration; and loss of perivascular pericytes (Fullerton et al., 2001;
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| Mouse line | Findings | Age | Region | Reference |
|------------|----------|-----|--------|-----------|
| Apoe<sup>−/−</sup> | BBB leakage of Evans blue, IgG perivascular deposits | 2 and 3 mo | Cortex, cerebellum, spinal cord | Fullerton et al., 2001 |
| | BBB leakage of Evans blue, BBB leakage of peroxidase | 1.5 and 2 mo | Cortex | Methia et al., 2001 |
| | IgG perivascular deposits, brain leakage of exogenous tracer (sodium fluorescein) | 11 mo | Hippocampus, cerebellum | Mulder et al., 2001 |
| | BBB leakage of Evans blue | 3 and 4 mo | Cortex, cerebellum | Hafezi-Moghadam et al., 2007 |
| | BBB extravasation of exogenous tracers (Dextran and Cadaverine), fibrin perivascular deposits, thrombin perivascular deposits, IgG perivascular deposits, hemosiderin deposits, loss of BBB tight junctions,<sup>3</sup> increased MMP-9 vascular expression | 6 mo | Cortex, hippocampus | Bell et al., 2012 |
| | Fibrin perivascular deposits, loss of PDGFR<sub>β</sub> pericytes, basement membrane degeneration | 9 mo | Cortex | Soto et al., 2015 |
| | BBB leakage of extracted immunoglobulin fractions directed against NMDAR, behavior alterations after injection of immunoglobulin fractions directed against NMDAR | 3 and 4 mo | Cortex, hippocampus, cerebellum, brainstem, spinal cord | Hammer et al., 2014; Castillo-Gomez et al., 2016 |
| TR-APOE<sub>4</sub> and GFAP-APOE<sub>4</sub> | BBB extravasation of exogenous tracers (Dextran and Cadaverine), fibrin perivascular deposits, thrombin perivascular deposits, IgG perivascular deposits, microhemorrhages (hemosiderin deposits),<sup>3</sup> loss of CD13+/PDGFR<sub>β</sub> pericytes, loss of BBB tight junctions, increased MMP-9 vascular expression | 2 wk and 4, 6, 8, 9, and 18 mo | Cortex, hippocampus | Bell et al., 2012 |
| | BBB extravasation of exogenous tracers (Dextran and Cadaverine), fibrin perivascular deposits, thrombin perivascular deposits, IgG perivascular deposits, microhemorrhages (hemosiderin deposits),<sup>3</sup> loss of CD13+/PDGFR<sub>β</sub> pericytes, loss of BBB tight junctions, increased MMP-9 vascular expression | 2 wk and 4, 6, 8, 9, and 18 mo | Cortex, hippocampus | Bell et al., 2012 |
| | BBB leakage of Evans blue, BBB leakage of peroxidase, fibrin perivascular deposits, loss of BBB tight junctions, increased MMP-9 vascular expression | 9 mo | Cortex | Soto et al., 2015 |
| | BBB leakage of extracted immunoglobulin fractions directed against NMDAR, behavior alterations after injection of immunoglobulin fractions directed against NMDAR | 3 and 4 mo | Cortex, hippocampus, cerebellum, brainstem, spinal cord | Hammer et al., 2014; Castillo-Gomez et al., 2016 |
| TR-APOE<sub>4</sub> | BBB extravasation of exogenous tracers (Dextran and Cadaverine), fibrin perivascular deposits, thrombin perivascular deposits, IgG perivascular deposits, microhemorrhages (hemosiderin deposits),<sup>3</sup> loss of CD13+/PDGFR<sub>β</sub> pericytes, loss of BBB tight junctions, increased MMP-9 vascular expression | 2 wk and 4, 6, 8, 9, and 18 mo | Cortex, hippocampus | Bell et al., 2012 |
| | BBB leakage of Evans blue, BBB leakage of peroxidase, fibrin perivascular deposits, loss of BBB tight junctions, increased MMP-9 vascular expression | 9 mo | Cortex | Soto et al., 2015 |
| | BBB leakage of extracted immunoglobulin fractions directed against NMDAR, behavior alterations after injection of immunoglobulin fractions directed against NMDAR | 3 and 4 mo | Cortex, hippocampus, cerebellum, brainstem, spinal cord | Hammer et al., 2014; Castillo-Gomez et al., 2016 |
| TR-APOE<sub>4</sub> | BBB extravasation of exogenous tracers (Dextran and Cadaverine), fibrin perivascular deposits, thrombin perivascular deposits, IgG perivascular deposits, microhemorrhages (hemosiderin deposits),<sup>3</sup> loss of CD13+/PDGFR<sub>β</sub> pericytes, loss of BBB tight junctions, increased MMP-9 vascular expression | 2 wk and 4, 6, 8, 9, and 18 mo | Cortex, hippocampus | Bell et al., 2012 |
| | BBB leakage of Evans blue, BBB leakage of peroxidase, fibrin perivascular deposits, loss of BBB tight junctions, increased MMP-9 vascular expression | 9 mo | Cortex | Soto et al., 2015 |
| | BBB leakage of extracted immunoglobulin fractions directed against NMDAR, behavior alterations after injection of immunoglobulin fractions directed against NMDAR | 3 and 4 mo | Cortex, hippocampus, cerebellum, brainstem, spinal cord | Hammer et al., 2014; Castillo-Gomez et al., 2016 |
| APOE<sub>4</sub> knock-in | Microhemorrhages (hemosiderin deposits)<sup>4</sup> | 6 and 7 mo | Cortex | Cacciottolo et al., 2016 |
| | BBB leakage of Evans blue | 6 mo | Cortex, cerebellum | Nishitsuji et al., 2011 |

APOE, apolipoprotein E; GFAP, glial fibrillary acidic protein; GLUT1, glucose transporter 1; IgG, immunoglobulin G; MMP-9, matrix metalloproteinase-9; NMDAR, N-methyl-D-aspartate receptor; PDGFR<sub>β</sub>, platelet-derived growth factor beta; RAGE, receptor for advanced glycation end products; TR, targeted replacement.

<sup>1</sup>Loss of BBB tight junctions as shown by high-resolution confocal microscopy analysis.
<sup>2</sup>Loss of BBB tight junctions as shown by immunoblotting of isolated brain capillaries.
<sup>3</sup>Microhemorrhages (hemosiderin deposits) at the capillary level.
<sup>4</sup>Microhemorrhages (hemosiderin deposits) at the capillary and arteriolar level.

Studies using transgenic mice with targeted replacement of mouse Apoe with each human APOE isoform (TR-APOE) or mice expressing each human APOE isoform under control of the astrocyte-specific glial fibrillary acidic protein (GFAP) promoter on an Apoe-null background, have shown that expression of APOE4, but not APOE2 and APOE3, leads to BBB breakdown (Nishitsuji et al., 2011; Bell et al., 2012) and cerebral microhemorrhages (Cacciottolo et al., 2016; Table 2). These studies indicate that Apoe is essential for maintaining BBB integrity.

Genetic ablation, siRNA silencing, and pharmacological studies in transgenic APOE4 mice revealed that activation of the proinflammatory cyclophilin A (CypA)–matrix metalloproteinase 9 (MMP-9) pathway in pericytes leads to degradation of tight junction and basement membrane proteins causing BBB breakdown (Bell et al., 2012). Interestingly, neuropathological findings in AD APOE4 carriers compared with noncarriers also demonstrate elevated levels of CypA and MMP-9 in brain endothelium and pericytes that correlate with elevated IgG and fibrinogen capillary leakages, suggesting activation of the CypA-MMP-9 BBB degrading pathway (Halliday et al., 2016). Cerebrospinal fluid analysis in APOE4 nonsymptomatic carriers compared with noncarriers confirmed that activation of the CypA-MMP9 pathway correlates with BBB breakdown (Halliday et al., 2013), which has been also corroborated by tissue CypA mRNA analysis (Conejero-Goldberg et al., 2014). An early and progressive BBB breakdown detectable in TR-APOE4 and GFAP-APOE4 mice between 2 and 6 wk of age precedes changes in sensory-evoked neuronal functioning, loss of neuritic density, and loss of pre- and postsynaptic...
aptic proteins that develop at 4 mo of age (Bell et al., 2012). Importantly, pharmacologic inhibition of the CypA–MMP-9 BBB pathway in addition to repairing the BBB also slowed down and reversed neurodegenerative changes in APOE4 and Apoe<sup>−/−</sup> mice. Similarly, genetic inhibition of the CypA–MMP-9 pathway at the BBB reversed neurodegenerative changes in Apoe<sup>−/−</sup> mice (Bell et al., 2012). Because Apoe<sup>−/−</sup> (Raber et al., 1998; Bell et al., 2012; Lane-Donovan et al., 2016) and APOE4 transgenic mice (Bell et al., 2012; Salomon-Zimri et al., 2014, 2015; Liu et al., 2015) develop behavioral deficits at 4–6 mo of age after BBB breakdown, collectively, these findings suggest that BBB breakdown (Table 2) not only contributes to neuronal changes in these models but also is an important therapeutic target.

Several studies have established that human APOE isoforms differentially regulate brain Aβ clearance (Castellano et al., 2011), as reviewed elsewhere (Vergheese et al., 2011; Liao et al., 2017). Consistent with these findings, APOE isoforms differentially regulate Aβ clearance across the BBB (Deane et al., 2008; Zhao et al., 2015b). Studies in animal models have shown that APOE3 and APOE2 mediate Aβ clearance across the BBB via LRP1, whereas APOE4 preferentially binds to very-low-density lipoprotein receptor (VLDR) at the mouse BBB, which clears ligands across the BBB at much slower rate than LRP1, contributing to brain accumulation of Aβ (Deane et al., 2008).

**Potential pitfalls in BBB permeability measurements**

High-resolution confocal microscopy analysis and multiphoton microscopy imaging of various endogenous and exogenous tracers, studies using molecular biomarkers of BBB cellular breakdown and/or dysfunction, electron microscopy studies, and MRI studies strongly support BBB breakdown in animal models of AD and Apoe<sup>−/−</sup> and APOE4 transgenic mice (Tables 1 and 2). Some studies, however, did not detect BBB breakdown in Apoe<sup>−/−</sup> and TR-APOE4 mice using exogenous circulating antibodies (e.g., IgG and anti-β-secretase 1 [BACE1]) but showed significant brain uptake of the specific therapeutic anti–transferrin receptor–BACE1 antibody, which uses the Trf receptor to cross the BBB (Bien-Ly et al., 2015). Interestingly, this study also did not detect BBB breakdown in a superoxide dismutase–1 SOD<sub>1</sub>G93A model of amyotrophic lateral sclerosis (ALS) using control IgG and anti–BACE1 antibodies (Bien-Ly et al., 2015). In contrast, multiple independent studies in SOD<sub>1</sub>G93A mice and other SOD1 ALS mutants reported an early BBB breakdown using different techniques and approaches (Garbuzova-Davis et al., 2007a,b; Zhong et al., 2008, 2009; Nicaise et al., 2009; Miyazaki et al., 2011; Winkler et al., 2014). Here, we briefly discuss potential reasons for discrepancy between different studies and possible factors that could interfere with interpretation of BBB measurements in animal models.

In brief, the use of systemically administered exogenous macromolecules such as nonimmune antibodies to determine BBB permeability requires extensive vascular perfusion of the brain with cold saline at the end of experiment to eliminate antibodies from cerebral blood vessels (Bien-Ly et al., 2015). However, this perfusion step can also remove tracers from the brain, particularly nonfixable ones such as exogenous nonimmune IgG, which tends to underestimate brain uptake values. This could be particularly critical in models with focal and/or rather discrete BBB changes, when tracer concentration is determined in brain homogenates or lysates. To overcome this problem, some studies used tissue-fixable tracers, such as Cadaverine-Alexa Fluor 555 (Armulik et al., 2010; Bell et al., 2012) or lysine fixable dextrans (Ben-Zvi et al., 2014), which after crossing the disrupted BBB remain bound to brain tissue and therefore are easily detectable by either histological or biochemical analysis.

Besides technical challenges, BBB breakdown in models of neurodegenerative disorders (Zlokovic, 2011) and in humans with these disorders such as AD (Montagne et al., 2016; Nelson et al., 2016) typically starts focally (Montagne et al., 2015) and then spreads throughout several gray and white matter regions (van de Haar et al., 2016a,b, 2017). In contrast, BBB breakdown in models of neuroinflammation, such as experimental allergic encephalitis, is more widespread and robust (Wang et al., 2016; Shaw et al., 2017), similar to multiple sclerosis in humans, where BBB breakdown develops more rapidly and is typically an order of magnitude greater than in neurodegenerative disorders such as AD (Montagne et al., 2016). Thus, experimental approaches that rely solely on biochemical analysis of the exogenous tracer’s concentration in brain lysates without additionally using tissue imaging analysis might run the risk of missing subtle changes in BBB integrity around brain capillaries first affected by the disease process, which are otherwise detectable by high-resolution confocal or multiphoton microscopy analysis (Paul et al., 2007; Bell et al., 2010, 2012).

It is not completely unexpected, therefore, that studies using systemically administered radiolabeled tracers (e.g., dextran and albumin) and assaying their concentration in different CNS tissue lysates were able to readily detect BBB permeability increases in models of neuroinflammation, but not in models of neurodegenerative disorders (Bien-Ly et al., 2015). Because multiple independent studies by different groups showed BBB breakdown in models of neurodegeneration using imaging techniques (Tables 1 and 2), the question persists whether radiotracer methods alone are sensitive enough to detect focal and less pronounced BBB changes. A recent neuroimaging study in individuals with MCI also suggests that even within a single brain region in humans, as for example in the hippocampus, some subregions may have intact BBB, whereas others such as CA1 and dentate gyrus show BBB breakdown (Montagne et al., 2015). Combining different methods and approaches is therefore highly recommended to ascertain whether given animal models develop BBB breakdown.
Blood–brain barrier breakdown in early Alzheimer’s | Montagne et al.

Targeting BBB to control neurodegeneration

Fig. 1 illustrates various pathways showing how BBB breakdown and dysregulated BBB transport to AD pathophysiology based on findings in animal models, as shown in Tables 1 and 2. BBB breakdown (left) leads to perivascular accumulation of blood-derived neurotoxic products in the brain, such as red blood cell (RBC)–derived hemoglobin (Hb) and free iron (Fe^{2+}) generating reactive oxygen species (ROS) and oxidant stress to neurons; potentially toxic plasma proteins such as fibrinogen, plasminogen, thrombin, and/or autoantibodies, which could lead to neuronal injury, cell death, and inflammatory response; and albumin contributing to the development of edema, hypoperfusion, and tissue hypoxia. Pericyte detachment, degeneration, and loss leads to BBB breakdown. Apolipoprotein E (APOE) isoforms differentially regulate pericyte metabolism and BBB integrity. APOE2 and APOE3, but not APOE4, act via low-density lipoprotein receptor–related protein-1 (LRP1) on pericytes to inhibit the proinflammatory cyclophilin A (CypA)–matrix metalloproteinase-9 (MMP-9) pathway. When activated by APOE4, this pathway leads to MMP-9–mediated degradation of BBB tight junction and basement membrane proteins causing BBB breakdown. Dysregulated BBB transport (right) leads to a loss of equilibrium between Alzheimer’s amyloid β (Aβ) influx and influx across the BBB, which is a key mechanism that maintains brain Aβ homeostasis. Aβ influx is normally mediated via its receptors on brain endothelium, including LRP1, which works closely with phosphatidylidyinositol-binding clathrin assembly protein (PICALM) to clear Aβ monomers, oligomers, and aggregates from brain across the BBB; LRP2, which clears Aβ in a form of complexes with APOJ (clusterin); and P-glycoprotein (Pgp), which mediates active efflux of Aβ from brain endothelium to blood. LRP1 and Pgp BBB levels are reduced in AD models before Aβ deposition, which contributes to Aβ accumulation in the brain. Aβ influx from blood to brain is mediated by the receptor for advanced glycation end products (RAGE), which also triggers an inflammatory response. RAGE expression at the BBB is increased in AD models, which contributes to Aβ accumulation in the brain and inflammatory response. APOE isoforms differentially regulate Aβ clearance. Aβ complexes with human APOE2 and APOE3 isoforms are cleared across the BBB by LRP1. APOE4 has lower affinity for LRP1 and binds to the very-low-density lipoprotein receptor (VLDLR), which slowly transports its ligands across the BBB, including the APOE4–Aβ complex, causing its accumulation in the brain. BBB GLUT1 transporter delivers glucose to the brain across the BBB and is down-regulated in AD models. Its reduction accelerates BBB breakdown and Aβ pathology and leads to tau pathology and neuronal loss. BBB MFSD2a (major facilitator superfamily domain–containing protein 2) transports essential ω-3 fatty acids into the brain, which is essential for brain development, cognition, and maintenance of BBB integrity. Aβ can also accumulate in the perivascular space (PVS) between astrocyte end-feet and the vessel wall because of inefficient drainage along the perivascular route.
lization of the cytoskeleton and/or down-regulating MMP-9 at the BBB, thus protecting the neuronal environment from systemic influences (Zlokovic and Griffin, 2011). Whether APC can exert similar beneficial effects in AD models with disrupted BBB (Tables 1 and 2) remains to be seen. Because 3K3A-APC analogue has completed a phase 2 clinical trial for stroke (NCT02222714), this approach holds promise to translate to patients with other neurological disorders, including AD. It also remains to be determined whether inhibition of the CypA–MMP-9 pathway at the BBB with CypA inhibitors can seal the BBB and exert beneficial effects on cognitive function in human APOE4 carriers, as it does in transgenic APOE4 mice (Bell et al., 2012; Fig. 1). Again, this approach is attractive, because CypA inhibitors are currently being tested in a phase 3 trial for hepatitis C (NCT01318694).

Besides sealing the BBB, eliminating the consequences of BBB breakdown has been investigated. When the BBB is open, plasma proteins enter the neuroglial space and become neurotoxic; therefore, neutralizing toxic accumulates represents a valuable therapeutic approach for neurodegenerative diseases such as AD that are associated with BBB pathology. In fact, depleting accumulated fibrinogen from the brain with ancred, a defibrinogenating agent, or by genetic manipulation attenuated both neuroinflammation and vascular pathology in APP mice (Paul et al., 2007) and in a model of multiple sclerosis (Davalos et al., 2012). On the other hand, BBB damage causing RBC extravasation and brain accumulation of free neurotoxic iron causing oxidant stress can be successfully controlled by iron chelators and/or antioxidant treatment, as shown in SOD1G93A ALS model (Winkler et al., 2014; Fig. 1).

Other approaches, such as using RAGE blockers to inhibit Aβ influx across the BBB and the neurovascular inflammatory response (Deane et al., 2003, 2012), have advanced from animal models to a phase 3 trial in AD (NCT02916056). Targeting BBB clearance in AD is an emerging therapeutic approach to restore the balance between Aβ production and clearance. LRP1 minigene delivery to the BBB by viral vectors facilitates Aβ clearance and mitigates Aβ pathology (Winkler et al., 2015). The PICALM–dependent transcytotic machinery at the BBB can also be targeted therapeutically by gene therapy (Zhao et al., 2015a). Additionally, current Aβ clearance treatments with anti–Aβ antibodies to remove Aβ from brain would benefit from intact Aβ transvascular transport and clearance across the BBB, particularly for the antibodies acting mainly through peripheral Aβ sink action (NCT02008357). Therefore, repairing BBB and Aβ BBB clearance mechanisms is critical for success of these treatments as well.

The vascular drainage pathway along the perivascular spaces of CNS vessels contributes to the clearance of molecules from brain extracellular spaces (ECSs), including Aβ (Tarasoff-Conway et al., 2015; Bakker et al., 2016), and is connected with the cerebrospinal fluid (CSF) compartment and lymphatic vessels within the dura matter of the brain, which drain to the peripheral lymph nodes (Louveau et al., 2015; Engelhardt et al., 2017). Additionally, it has been proposed that convective, “glymphatic” flow of CSF through the ECSs from the para-arterial to the paravenous spaces plays a role in solute transport exchanges in parenchymal ECSs (Iliff et al., 2012; Jessen et al., 2015). However, recent studies have not supported the proposed glymphatic mechanism of convective solute transport in brain parenchyma and ECSs (Asgari et al., 2015, 2016; Jin et al., 2016; Holter et al., 2017; Smith et al., 2017). The current view states that metabolic waste products and endogenous molecules generated by the brain diffuse away through brain ECSs and are eliminated from the brain by transvascular transport across the BBB (Zlokovic, 2011; Zhao et al., 2015a) and perivascular transport along the vessel walls in the direction opposite the blood flow (Tarasoff-Conway et al., 2015; Bakker et al., 2016), as originally proposed by physiologists decades ago (Milhorat, 1975; Bradbury et al., 1981). This clearance pathway extends to toxic metabolites such as Aβ, which in normal mice contributes to ~15–20% of Aβ clearance from the brain (Shibata et al., 2000; Xie et al., 2013); the remaining 80–85% is removed from the brain by transvascular transport across the BBB into the blood. Whether ECSs and perivascular clearance of Aβ can be targeted therapeutically in AD and AD models to remove excess Aβ remains to be explored in future studies.

**Future directions**

Multiple studies have shown BBB breakdown and dysregulated BBB transport in AD models, establishing their roles in neurodegeneration and development of Alzheimer’s Aβ and tau pathology. BBB breakdown and dysfunction have also been reported in rare inherited monogenic human neurological disorders with genetic defects affecting exclusively BBB cells, which directly supports the link between BBB breakdown and neurological disorders (Zhao et al., 2015a). For example, loss-of-function mutations in the human GLUT1 gene encoding the BBB glucose transporter result in GLUT1-deficiency syndrome with early-onset seizures and microcephaly (Wang et al., 2000). Glut1−/− mice not only phenocopy human pathology but also develop BBB breakdown, causing microcephaly and neurodegeneration (Winkler et al., 2015). The role of diminished GLUT1 expression at the BBB in human AD (Nelson et al., 2016) and animal models is still not fully understood; in particular, it is unclear whether pharmacologically reversing GLUT1 expression repairs BBB integrity and reverses neurodegenerative changes, cognitive decline, and behavioral deficits, as suggested by gene therapy studies in Sk2α1−/−; APPsw/w mice with endothelial-specific GLUT1 haploinsufficiency (Winkler et al., 2015).

Loss of tight junction proteins causing BBB breakdown has been found both in human AD and animal models. However, their role in disease process remains elusive. In contrast, mutations in the OCLN (occludin) gene encoding the tight junction protein occludin (O’Driscoll et al., 2010) or the
junctial adhesion molecule-C (JAM-C) gene encoding junctonal molecule JAM-C (Wyss et al., 2012; Akawi et al., 2013) are causatively related to BBB breakdown, which leads to early-onset seizures, microcephaly, and band-like calcification with simplified gyration in case of OCLN mutations or hemorrhages and hydrocephalus, as shown in JAM-C−/− deficient mice, and/or hemorrhagic destruction of the brain, subependymal, calcification, and congenital cataracts in humans with homozygous mutations of JAM-C. Is there any relationship between these findings and findings in complex neurodegenerative disorders such as AD?

Inactivating mutations in major facilitator superfamily domain−containing protein 2α (MFSD2a) gene encoding the BBB transporter for essential ω-3 fatty acids cause a lethal microcephaly syndrome (Guemez-Gamboa et al., 2015). Mfsd2a-deficient mice exhibit impaired brain uptake of ω-3 fatty acids (Bethsholtz, 2014; Nguyen et al., 2014), as well as dysregulated caveolae-mediated transcellular trafficking across the BBB and develop BBB breakdown (Ben-Zvi et al., 2014; Zhao and Zlokovic, 2014; Andreone et al., 2017) resulting in microcephaly, neuronal loss, and cognitive deficits. Essential ω-3 fatty acids found in fish oil help reduce the risk of cardiovascular disease and have beneficial effects on cognition. However, the role of the MFSD2a BBB transporter as a possible therapeutic target in AD models and human AD remains underexplored.

Loss-of-function mutations in the PDGFRB gene in pericytes lead to idiopathic primary familial brain calcification and motor and cognitive impairment (Keller et al., 2013; Nicolas et al., 2013). Similarly, Pdgfrb+/− mice with severe pericyte loss and BBB breakdown develop deep brain calcification (Keller et al., 2013), whereas Pdgfrb−/− and Pdgfrb−/− pericyte−deficient mice develop BBB breakdown, leading to secondary neurodegeneration (Bell et al., 2010). Pericytes degenerate in AD (Farkas and Luiten, 2001; Baloyannis and Baloyannis, 2012; Sengillo et al., 2013; Halliday et al., 2016) and AD models (Tables 1 and 2), but at present, we know very little about whether cell therapies directed at re-placing pericytes will have the same beneficial effects in AD models or AD as shown in other models, such as SOD1G93A ALS (Coatti et al., 2017).

Another example is CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy). This is an inherited autosomal-dominant ischemic stroke syndrome that progresses to dementia, and it is caused by mutations in the NOTCH3 gene, which is expressed only in vascular smooth muscle cells and pericytes (Chabriat et al., 2009). Mice carrying the CADASIL Notch3-R169C mutation also show accumulation of NOTCH ectodomain in pericytes, pericyte degeneration, and BBB breakdown (Ghosh et al., 2015). Small-vessel disease, on the other hand, is prominent in AD (Iadeola, 2013, 2017; Wardlaw et al., 2013) and contributes to ~50% of all dementias worldwide, including AD (Wardlaw et al., 2013; Montine et al., 2014; Snyder et al., 2015; Hachinski, 2016). Thus, it would be interesting to study whether there is a common vascular mechanism predisposing for the development of small ischemic strokes and Alzheimer’s pathology in animal models of small-vessel disease.

Finally, as the RNA-sequencing and proteomic studies in animal models have begun to provide new insights into the molecular composition of the BBB and the associated cell types (Lu et al., 2008; Daneman et al., 2010; Zeisel et al., 2015; He et al., 2016), our understanding of the cellular and molecular mechanisms of the BBB transport functions will continue to expand. Hopefully, future large-scale single-cell transcriptomic studies of brain endothelial cells, brain capillary pericytes, and smooth muscle cells on arterioles, arteries, and venules will characterize more precisely cell-specific regional expression and zonation of key endothelial transporters on brain capillary, arteriolar, and venular endothelium and determine to which extent the associated mural cells, including pericytes, contribute to the overall transport of solutes exchanges across the BBB.
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