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Blood–brain barrier breakdown in Alzheimer's disease and other neurodegenerative disorders

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Abstract

The blood–brain barrier (BBB) is a continuous endothelial membrane within brain microvessels that has sealed cell-to-cell contacts, and is sheathed by mural vascular cells and perivascular astrocyte end-feet. The BBB protects neurons from factors present in the systemic circulation, and maintains the highly regulated CNS internal milieu, which is required for proper synaptic and neuronal functioning. BBB disruption allows influx into the brain of neurotoxic blood-derived debris, cells, and microbial pathogens, and is associated with inflammatory and immune responses, which can initiate multiple pathways of neurodegeneration. This Review discusses neuroimaging studies in the living human brain, post-mortem tissue and biomarker studies demonstrating BBB breakdown in Alzheimer disease, Parkinson disease, Huntington disease, amyotrophic lateral sclerosis, multiple sclerosis, HIV-1-associated dementia and chronic traumatic encephalopathy. The pathogenic mechanisms by which BBB breakdown leads to neuronal injury, synaptic dysfunction, loss of neuronal connectivity and neurodegeneration are described. The importance of a healthy BBB for therapeutic drug delivery, and the adverse effects of disease-initiated, pathological BBB breakdown in relation to brain delivery of neuropharmaceuticals are briefly discussed. Finally, future directions, gaps in the field and opportunities to control the course of neurological diseases by targeting BBB are presented.

Introduction

The human brain contains ~644 km of blood vessels that supply brain cells with oxygen, energy metabolites and nutrients, and remove carbon dioxide and other metabolic waste products from the brain to the systemic circulation[1,2]. Although representing only 2% of total body mass, the brain consumes ~20% of the body's glucose and oxygen, and can rapidly increase blood flow and oxygen delivery to its activated regions, a process known as

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All authors contributed to the literature search and writing the review. BVZ worked closely with MDS and APS to write the review and design figures and tables.

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neurovascular coupling[2,3]. Capillaries are the smallest cerebral blood vessels (FIG. 1); they account for approximately 85% of cerebral vessel length and are a major site of the **blood–brain barrier** (BBB) (FIG. 1)[1]. In the human brain, capillaries provide approximately 12 m^2 of endothelial cell surface area, which is available for transport of solutes from the blood to the brain, and vice versa. The mean intercapillary distance in the human brain is ~40 μm[4]; solute equilibration is, therefore, almost instantaneous throughout the brain interstitial space once molecules cross the BBB.

The endothelial BBB has tightly sealed cell-to-cell contacts that result in high transendothelial electrical resistance and low paracellular and transcellular permeability[5] (FIG. 2). The endothelial monolayer is sheathed by mural cells (**pericytes** in capillaries and vascular smooth muscle cells in arterioles and arteries) and by astrocyte end-feet[6,7]. In contrast to the highly permeable systemic capillaries[8], brain capillaries exhibit a low rate of transendothelial bulk flow by transcytosis, which together with the tightly sealed endothelium restricts the entry of most blood-derived molecules into the brain, unless they have specialized carriers and/or receptors in the brain endothelium that facilitate their transport across the BBB (FIG. 2).

Maintaining BBB integrity is crucial for tight control of the chemical composition of brain interstitial fluid (ISF), which is critical for proper synaptic functioning, information processing and neuronal connectivity. Loss of BBB integrity results in increased vascular permeability and is associated with reduced cerebral blood flow and impaired haemodynamic responses[2,3,5,7,9]. Breakdown of the BBB enables toxic blood-derived molecules, cells and microbial agents to enter the brain, and is associated with inflammatory and immune responses, which can initiate multiple pathways of neurodegeneration.

In this Review, we first briefly describe the molecular architecture and transport physiology of the BBB, and then examine vascular pathology, and neuroimaging, post-mortem and biomarker studies demonstrating BBB breakdown in several neurodegenerative diseases: namely, Alzheimer disease (AD), Parkinson disease (PD), Huntington disease (HD), amyotrophic lateral sclerosis (ALS), and multiple sclerosis (MS), which is considered to be an autoimmune and neurodegenerative disorder[10], as well as in HIV-1-associated dementia and chronic traumatic encephalopathy (CTE). We focus on the pathogenetic mechanisms by which BBB breakdown leads to **neurodegeneration**, and briefly note the implications of BBB dysfunction for therapeutic drug delivery. Finally, we discuss future directions, gaps in the field, and opportunities to control neurological disease by targeting the BBB. This Review does not examine the role of reduced cerebral blood flow and altered haemodynamic responses in AD and neurodegenerative disorders, nor does it cover BBB disruption in experimental models of AD and neurodegeneration, which have been extensively reviewed elsewhere[2,3,9,11].

The BBB molecular architecture

Brain endothelial cells are connected by tight junctions and adherens junctions. **Tight junctions** involve occludin and claudin-1, claudin-3, claudin-5 and claudin-12, and the membrane-associated guanylate kinases tight junction proteins ZO1, ZO2 and ZO3, whereas

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adherens junctions involve cadherins, platelet endothelial cell adhesion molecule (PECAM-1), and the junctional adhesion molecules (JAMs) JAMA, JAMB and JAMC[6]. A paucity of pinocytosis and bulk flow fluid transcytosis contributes to the limited exchange of solutes across the brain endothelium (FIG. 2), although oxygen and carbon dioxide rapidly diffuse across it. Small arterioles[12] and capillaries[13] are major sources of the brain's oxygen supply. Additionally, small lipid-soluble molecules and compounds with a molecular weight <400 Da or containing <8 hydrogen bonds (such as ethanol), can cross the BBB by simple **transmembrane diffusion**[4].

Solute **carrier-mediated transport** (CMT) facilitates the transport of carbohydrates, amino acids, fatty acids, monocarboxylic acids, nucleotides, hormones, vitamins, organic anions and cations across the BBB. **Receptor-mediated transcytosis** (RMT) enables transendothelial transport of proteins and peptides in both directions: from blood to brain (transferrin and insulin)[4] and from brain to blood (apolipoproteins)[6]. Sodium-dependent lysophosphatidylcholine symporter 1 (NLS1, also known as major facilitator superfamily domain-containing protein 2a), an important transporter, transports essential ω3 fatty acids into the brain[14], which is also critical for BBB formation[15] (FIG. 2).

The sodium pump (Na^+, K^+-ATPase) on the abluminal membrane of the BBB regulates sodium influx into the brain ISF, in exchange for potassium[16]. Other ion transporters regulate the transport of sodium, potassium, chloride and calcium ions, and facilitate the exchange of sodium for hydrogen ions and chloride for bicarbonate ions at the BBB. ATP-binding cassette (ABC) transporters expressed at the luminal side of the BBB prevent brain accumulation of drugs, xenobiotic agents and drug conjugates, via active efflux from endothelium into blood[6,17]. CMT facilitates CNS-to-blood clearance of excitatory amino acids (such as glutamate and aspartate)[18], whereas RMT clearance of amyloid-β (Aβ, some forms of which are associated with AD)[6,19–25] across the BBB keeps brain levels of these potentially toxic substances low (FIG. 2). Much more could be said about the molecular architecture of the BBB and its transport physiology, but only a brief overview is given here as these topics have been reviewed in detail elsewhere[6,7].

Molecules generated by the brain diffuse across brain extracellular spaces, and are cleared from the brain by two mechanisms: trans-vascular transport across the BBB, via the mechanisms illustrated in FIG. 2 (b–d, f–h)[5,6,26]; and perivascular transport of ISF, which travels in the reverse direction to the flow of blood within the basement membranes of arterial vessel walls (FIG. 1)[26–28]. Studies conducted in the 1980s and 1990s showed that solutes carried by the perivascular ISF flow reach the subarachnoid space, which is filled with **cerebrospinal fluid** (CSF) and drains into deep cervical lymph[29,30]. In the past 3 years, further studies have confirmed a role of the dural lymphatic vascular system in clearance of ISF and macromolecules by the meningeal lymphatic vessels[31], which drain into cervical lymph nodes[32–34] (FIG. 1). Under physiological conditions, the perivascular ISF pathway is responsible for 15–20% of the clearance of AD-related forms of Aβ from the mouse brain[19,35], whereas 80–85% is removed by transvascular BBB transport. In 1985, solutes were shown to rapidly distribute throughout the brain by paravascular transport from the subarachnoid space through Virchow–Robin spaces, in the same direction to the flow of blood[36]. Subsequent studies introduced the term 'glymphatic' system to describe this

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paravascular circulation, and suggested that solute transport in the CNS occurs via CSF convective flow through the brain extracellular spaces, in a para-arterial to para-venous direction, regulated by aquaporin-4 (AQP4) water channels on astrocytes[37,38]. The proposed glymphatic mechanism, however, has not been supported by the latest studies[39–42], and the convective, pressure-driven fluid flow of CSF from para-arterial to para-venous extracellular spaces throughout the parenchyma remains unproven[43–45,39,40]. Furthermore, deletion of *Aqp4* in mice and rats does not impair the transport of fluorescent solutes from the subarachnoid space into the brain, which implies that water production by astrocyte end-feet does not have a role in the regulation of solute transport within parenchymal extracellular spaces[39]. Further experimental work is needed to resolve these controversies.

Vascular pathology in neurodegeneration

Cerebrovascular dysfunction and vascular pathology contribute to cognitive decline and neuronal loss in AD, in addition to AD-related Aβ and tau pathology[5,6,46–53]. Many lines of evidence indicate that cerebrovascular dysfunction in AD cannot be simply attributed to comorbid vascular dementia. For instance, in one study of the association between cerebrovascular and neurodegenerative disease, the US National Alzheimer's Coordinating Centre database was used to identify 5,715 patients with an autopsy-based diagnosis of a single neurodegenerative disease (AD, frontotemporal lobar degeneration, α-synucleinopathy, hippocampal sclerosis, prion disease, and cerebrovascular disease)[52]. Within the subgroup of 4,629 patients diagnosed as having AD who had no evidence of mixed dementia, 80% had vascular pathology including cerebrovascular disease, lacunae and multiple microinfarcts indicative of small vessel disease, haemorrhage, atherosclerosis, arteriolosclerosis and **cerebral amyloid angiopathy (CAA)**[52]. The two subgroups of patients with an autopsy-based diagnosis of either AD or cerebrovascular disease exhibited a remarkably similar prevalence of vascular risk factors, such as coronary disease, hypercholesterolemia and diabetes[52].

Cerebral vessel pathology is a major risk factor for AD dementia, and is associated with low scores in most cognitive domains[51]. CAA, which is an important cause of BBB disruption and one of the three pathological hallmarks of AD[54], induces various vascular pathologies that contribute to cognitive decline[26]. Moreover, in preclinical AD, changes in vascular biomarkers occur before the development of cognitive impairment and before detectable increases in standard AD biomarkers, including amyloid deposition, decreased CSF levels of Aβ42, and increased CSF levels of tau and phosphorylated tau[48]. Small vessel disease of the brain is prominent in patients with AD, as discussed below, and contributes to ~50% of all dementias worldwide[3,55–58].

According to the **two-hit vascular hypothesis of AD**, damage to blood vessels is the initial insult, causing BBB dysfunction and diminished brain perfusion that, in turn, lead to neuronal injury and Aβ accumulation in the brain[5,6,47,50]. Cerebrovascular disruption is influenced by lifestyle and might act independently and/or synergistically with Aβ to promote AD pathology, which is accelerated by genetic risk factors, such as carriage of the ε4 allele of apolipoprotein E (***APOE*ε4***), vascular risk factors (such as hypertension, diabetes and dyslipidemia) and environmental risk factors (such as pollution)[47,50].

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Vascular pathology also contributes to other neurodegenerative disorders[52]. For example, cerebrovascular disease plays a part in the pathogenesis of PD[52], the second most common neurodegenerative disorder, which is characterized by accumulation of α-synuclein and degeneration of dopaminergic neurons in the substantia nigra. Vascular disease and vascular risk factors aggravate motor dysfunction and cognitive impairment in PD[59]. Cerebrovascular disease, BBB impairments and neurovascular abnormalities are also found in HD[60,61], an autosomal-dominant neurodegenerative disease with motor, cognitive, psychiatric, and metabolic abnormalities caused by the aggregation of mutant huntingtin protein. BBB disruption and trafficking of T cells, B cells and peripheral macrophages across dysfunctional BBB is a pathological hallmark of MS[33]. BBB disruption has been described in ALS[62], is a feature of CTE[64] and, in HIV-1-associated dementia, enables HIV-1-infected monocytes and macrophages to enter the brain[63].

Neuroimaging evidence of BBB disruption

In this section, we examine recent PET and MRI studies of BBB integrity and function in AD and other neurodegenerative disorders (TABLE 1).

Increased BBB permeability to gadolinium

BBB breakdown in the hippocampus, a centre of memory and learning, has been observed in individuals with mild cognitive impairment (MCI) using **dynamic contrast-enhanced (DCE) MRI**. In this technique, leakage of gadolinium contrast agent into the brain enables the regional CNS BBB permeability constant, K_{trans}, to be quantified using the Patlak analysis method[49,65,66]. A study that compared BBB breakdown in the hippocampus in individuals with MCI compared to age-matched controls found that the extent of BBB breakdown was not affected by vascular risk factors[49], but correlated with increased CSF levels of soluble platelet-derived growth factor receptor-β (PDGFRβ), a marker of pericyte injury[49,67]. BBB breakdown in the hippocampus occurred prior to hippocampal atrophy[49], which is typically seen early in AD[68,69], raising the possibility that BBB breakdown might precede neurodegeneration. This concept is supported by data from experimental models of BBB breakdown, which causes neurodegenerative changes over time[70–73]. Follow-up DCE-MRI studies in patients with early AD confirmed BBB breakdown in several grey matter and white matter regions[46,74–76] (TABLE 1). Consistent with these findings, early contrast-enhanced MRI studies in humans showed increased BBB permeability in the hippocampus in individuals with MCI compared to healthy controls[77], and suggested that contrast agent accumulates in the brains of individuals with probable AD via a blood-to-brain-to-CSF pathway[78].

DCE-MRI studies have detected increased BBB leakage of gadolinium (using the Patlak quantification method[49,65,66]) in the basal ganglia in patients with PD compared to healthy controls[79]. In patients with HD, DCE-MRI analysis reveals a positive correlation between increased BBB permeability in the caudate nucleus and increased disease burden score, as well as increased grey matter arterial cerebral blood volume[60]. DCE-MRI studies have similarly established the presence of increased BBB permeability in white matter in MS[49,80–82], particularly in active MS lesions[83,84] (TABLE 1). Increased matrix

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metalloproteinase-9 (MMP-9) activity in the CSF has been suggested to contribute to BBB breakdown in MS[82,85]. To understand the pathogenetic role of BBB breakdown in the living human brain, future longitudinal DCE-MRI studies are required to investigate the relationships between vascular changes, progression of neurological deficits in AD, PD, HD and MS, and changes in brain structural and functional connectivity. Extending DCE-MRI studies to patients with ALS, HIV-1-associated dementia and CTE will help us to identify whether regional BBB breakdown has a pathogenetic role in neurodegenerative disorders.

Microbleeds

Damage to blood vessels can lead to pronounced BBB breakdown manifested as cerebral microbleeds (microhaemorrhages), which is frequently seen in AD[86–93], MCI[94], and in *APOE*ε4* individuals who have increased genetic risk of AD[94]. CAA is one of the main causes of vascular degeneration and lobar microbleeds in AD, and contributes to BBB breakdown, infarcts, white matter changes and cognitive impairment[26]. The microbleed location relates to its aetiology: CAA causes lobar microbleeds and hypertensive vasculopathy causes microbleeds in the basal ganglia, thalamus, cerebellum and brainstem (reviewed elsewhere[95]). Microbleeds in AD are predominantly lobar[88,92,96–99] (similar to CAA-associated microbleeds) and are mainly found in the occipital lobe[92,98,99]. Amyloid deposition in the brain, as detected by ^{18}F-florbetapir PET, is positively associated with the number of microbleeds in individuals with MCI and AD[99]. Several studies that reported a high prevalence of microbleeds in patients with AD[86–93] or MCI[94] did not perform amyloid-PET imaging[86–93], however, precluding a direct comparison of microbleeds and CAA severity.

Cortical superficial siderosis (that is, detection of subpial deposits of hemosiderin) has been suggested as an alternative imaging biomarker for CAA[100,89,101,102]. The extent of cortical superficial siderosis, lobar microbleeds, and amyloid plaque burden is higher in patients with AD than in cognitively normal controls (as shown by MRI and amyloid-PET studies[94]), and MRI evidence of superficial siderosis was also observed in three individuals with pathologically confirmed CAA. To definitely relate the topography and prevalence of microbleeds and superficial siderosis to CAA in AD, more amyloid-PET and high field strength MRI studies are needed, as discussed below.

Microbleeds are often used as a criterion to define small vessel disease in the brain[103]. Small hypointense regions on **T2*-weighted and susceptibility-weighted imaging (SWI) MRI** are thought to representing blood-derived hemosiderin deposits, probably phagocytosed by macrophages in the perivascular spaces, after microbleeding events[96]. The strength of the MRI magnetic field determines the ability to detect brain microhemorrhages[104]. For example, 3 T MRI studies indicate that approximately 45% of patients with AD[88,90,91,94] and 25% of individuals with MCI[94] develop microhemorrhages, whereas a 7 T MRI study found that 78% of patients with AD have microhemorrhages[87]. Since most current studies involve 1.5T and 3T MRI, the incidence of microhemorrhages in MCI and AD is likely to be underestimated. High-resolution confocal microscopy of brain tissue can detect capillary hemorrhages as small as 20–30 μm in diameter, which are easily missed on 1.5T or 3T MRI[62].

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Cerebral microbleeds have been detected throughout deep grey matter regions (including the caudate, thalamus, putamen and globus pallidus), cortical regions and white matter in patients with PD by T2*-weighted and SWI-MRI. The incidence of microbleeds is higher in patients with PD dementia than in PD patients without dementia and controls, and is associated with the extent of white matter lesions[105,106].

Hypointense areas in the cortex on T2-weighted MRI, suggestive of microbleeds, have also been shown in patients with ALS[62]. Studies using high-resolution T2-weighted 7T MRI have also reported microbleeds in the brains and spinal cords of patients with ALS[107].

Impaired glucose transport

Glucose is a key energy substrate for the brain. Brain uptake of glucose is measured using the radiolabeled glucose analogue, **^{18}F-fluoro-2-deoxyglucose (FDG)**, as a PET tracer[46]. FDG enters the brain via solute carrier family 2, facilitated glucose transporter member 1 (also known as glucose transporter-1 (GLUT1)), which is expressed only in the endothelium of the BBB, not in neurons[6,108]. Besides GLUT1, brain uptake of FDG depends on cerebral blood flow[2,5], which is reduced in MCI and early AD, prior to brain atrophic changes[2].

Although both glucose and FDG are rapidly transported into the brain via GLUT-1, avidly taken up by brain cells and then phosphorylated by intracellular hexokinase[127,128], their subsequent metabolic fate in the brain is completely different[129]. Glucose-6-phosphate is metabolized rapidly in the glycolytic pathway, whereas FDG-6-phosphate is not a substrate for glucose-6-phosphate isomerase and thus cannot be converted into fructose-6-phosphate, which precludes its further metabolism[130,127,131,128]. Therefore, approximately 45–90 min after systemic administration of FDG, 90–97% of this compound persists in the mouse[128] or rat[131,132] brain in the form of FDG-6-phosphate or its epimers; the remainder is FDG. Because brain cells have very low activity of glucose-6-phosphatase, and poor transport of FDG-6-phosphate across cell membranes[133,134], FDG-6-phosphate remains trapped within brain cells[132,135] and is eliminated only slowly from the brain. Since brain uptake of FDG across the BBB depends on GLUT1not direct neuronal uptake, the diminished uptake of FDG in the AD brain points to a vascular deficit (that is, impaired BBB function). Importantly, GLUT1 levels are substantially reduced in brain microvessels in AD[109–112]. Diminished BBB transport and brain uptake of FDG precedes neurodegeneration and brain atrophy in patients with MCI who later convert to a diagnosis of AD, as well as in patients with early AD. This vascular deficit should be considered in staging preclinical AD[136].

FDG-PET studies also indicate that individuals with MCI have diminished glucose uptake in several brain regions (including the precuneus, posterior cingulate, right angular gyrus and bilateral temporal cortices) prior to any detectable neurodegenerative changes, brain atrophy and/or conversion to AD[113]. The reductions in FDG uptake in the posterior cingulate gyri and parietotemporal lobes of patients with AD are observed with and without corrections for partial volume effects, confirming that these decreases are not due to brain atrophy [114]. Longitudinal FDG-PET findings have additionally suggested that reductions in hippocampal glucose uptake during normal ageing can predict cognitive decline years in advance of a clinical AD diagnosis[115]. Similarly, asymptomatic carriers of presenilin-1 (*PSEN1*) mutations associated with early-onset autosomal dominant AD show AD-like reductions in

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FDG uptake in the absence of brain atrophy[116]. Diminished glucose uptake in the hippocampus, parieto-temporal cortex and/or posterior cingulate cortex has been repeatedly shown by FDG-PET in early AD[117,118], in individuals at genetic risk of AD[119,120], with a positive family history of AD[121] and/or MCI, as well as in individuals with no cognitive impairment who went on to develop AD[122,123]. The patterns of FDG brain uptake can also discriminate individuals with normal cognition from those with MCI and AD[117]. FDG-PET changes preceding neurodegeneration are not only found in humans[113–116], but also in transgenic mouse models of AD[124], reflecting reductions in glucose transport across the BBB[125,126].

Moreover, experimental studies in *Slc2a1*$^{+/-}$ mice (which express 50% of GLUT1 levels in cerebral blood vessels compared with their wild-type littermates) have shown rapid BBB breakdown followed by secondary neurodegeneration, which is accelerated by Aβ[108]. No attempts have been made so far to explore whether GLUT1 at the BBB is a therapeutic target in human AD, or whether pharmacological upregulation of this transporter in humans can prevent BBB breakdown, neurodegeneration and cognitive deficits, as it can in animal models. Additionally, the role of glucose transport in other neurodegenerative disorders has not been examined, and should be pursued by future studies.

Impaired P-glycoprotein function

P-glycoprotein (encoded by the *ABCB1* gene) mediates the active efflux of drugs and xenobiotic compounds from the endothelium to blood, thereby preventing their accumulation in the brain[6,17]. P-glycoprotein clears Aβ across the BBB, which requires **LDL receptor-related protein-1 (LRP1)**[137–139]. P-glycoprotein function is clinically assessed by ^{11}C-**verapamil-PET**. Verapamil-PET studies in AD have demonstrated increased uptake of verapamil in frontal, parietal, temporal and occipital cortices, and in posterior and anterior cingulate gyri[140]. Similarly, verapamil-PET studies in patients with mild AD found substantially reduced P-glycoprotein activity in the parietotemporal, frontal, and posterior cingulate cortices and hippocampus[141]. Furthermore, verapamil-PET studies indicated diminished P-glycoprotein activity, indicating BBB dysfunction, in the mid-brain of patients with PD[142]. Collectively, these studies suggest that decreased P-glycoprotein function is involved in the pathogenesis of AD — either by enabling xenobiotic compounds to accumulate in the brain (high levels of which can injure neurons and promote inflammation) and/or by reducing Aβ clearance across the BBB. Thus, P-glycoprotein and LRP1 could be important therapeutic targets in AD, and perhaps also in PD.

CNS leukocyte infiltration

Studies using a radiolabelled MMP as a PET tracer showed increased MMP activity in early MS lesions, which is associated with leukocyte infiltration[143]. Additionally, MS patients show impaired cerebral venous drainage[144] and decreased cerebrovascular reactivity of grey matter, which correlates with grey matter atrophy[145]. As leukocyte infiltration of the CNS also occurs in other neurodegenerative diseases, notably AD[146–149], HIV-1-associated dementia[150] and CTE[151], similar MMP-PET neuroimaging studies would be helpful to identify when in the course of these diseases this cellular infiltration of the CNS takes place. However, as yet, such studies are lacking.

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Postmortem evidence of BBB disruption

In this section, we examine the evidence of BBB disruption derived from analyses of post-mortem tissues from patients with AD and other neurodegenerative disorders. In these studies, BBB disruption is demonstrated by brain capillary leakages, degeneration of BBB-associated cells (including pericytes and endothelial cells), brain infiltration of circulating leukocytes and red blood cells, aberrant angiogenesis and molecular changes (TABLE 2).

Capillary leakages

Several studies of post-mortem brain tissue from patients with AD have found (using various analysis methods: immunohistochemistry, immunoblotting and Prussian blue staining) capillary leakages of blood-derived proteins in the prefrontal and entorhinal cortex and hippocampus, including accumulations of fibrinogen, thrombin, albumin, IgG, and iron-containing proteins such as haemosiderin[146,147,152–157]. These blood-derived proteins are often found co-localized with deposits of AD-associated Aβ[147,153,155]. Evidence of BBB breakdown is most pronounced in individuals carrying the *APOE*ε4* allele, the major genetic risk factor for AD. By contrast, individuals homozygous for the most common allele, *APOE*ε3,* have a reduced risk of AD and show a decreased degree of BBB breakdown[147,152,156,158]. As reviewed elsewhere[11], multiple experimental studies have confirmed that BBB breakdown causes capillary leakage in AD models of β-amyloidosis[159–162] and in *APOE*ε4* transgenic mice[71,163,164].

Post-mortem analysis of brain tissue from patients with PD revealed perivascular deposits of fibrinogen or fibrin[165], IgG[166] and haemosiderin[165,167] in the striatum, which is indicative of BBB breakdown. Fibrin(ogen) deposits around capillaries were also found in brain tissue from patients with HD[60]. Fibrinogen, thrombin, IgG and haemosiderin deposits have been found in brain and spinal cord tissue from patients with sporadic or familial forms of ALS[62,107,168] as well as in a transgenic mouse model of ALS, prior to the onset of motor neuron degeneration[11,169].

Finally, patients with MS develop capillary leakages of fibrinogen within active and inactive lesions, particularly along vessels with abnormal tight junctions[170]. The first post-mortem study of a patient with CTE found cerebral oedema and haemosiderin-laden perivascular macrophages in the Virchow–Robin spaces[171].

Pericyte degeneration

Electron microscopy studies of brain tissue from patients with AD revealed pericyte degeneration in the cortex associated with large accumulations of osmiophilic material. These changes are suggestive of increased phagocytosis of blood-derived proteins, mitochondrial alterations, and an increased number of pinocytotic vesicles[172,173]. Immunostaining for the pericyte marker PDGFRβ revealed reduced pericyte coverage and numbers on brain capillaries in brain samples from patients with AD[155], which showed evidence of a gene-dose effect linked to the number of *APOE*ε4* alleles (compared with homozygosity for *APOE*ε3*)[156]. Immunoassay of AD cortical tissue confirmed loss of the pericyte marker PDGFRβ in the precuneus[157], a region affected early in the course of AD.

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Pericytes maintain BBB integrity[7,176], and their degeneration leads to BBB breakdown[70,174,175]. Additionally, pericytes clear Aβ from the brain, and their loss accelerates the onset and progression of Aβ and tau pathology in mouse models of AD[159].

Immunohistological analysis of spinal cord or brain tissue from patients with ALS also reveals notable pericyte degeneration[62,168]. Post-mortem studies of brain tissue from patients with HIV-1-associated dementia or HIV encephalitis have found evidence of microvascular degeneration and BBB breakdown, including reduced pericyte coverage[177]. Vascular insults, including enlarged perivascular spaces[64,151,178] and mineralization of mural cells of deep penetrating blood vessels[171], have also been found in brain tissue from patients with CTE[64,151,171,178].

Endothelial degeneration

Reductions in capillary length (suggestive of endothelial degeneration), reduced expression of tight junction proteins, and capillary basement membrane changes have been reported in brain tissue from patients with AD[5,155,156,158,173,179,180]. These changes might reflect aberrant brain angiogenesis, caused by the low brain endothelial cell expression of *MEOX2*, encoding homeobox protein MEOX2, a regulator of vascular differentiation in AD[180]. A wide range of proangiogenic factors are also expressed in microvessels isolated from the brains of patients with AD[181], which (in the presence of reduced *MEOX2* expression) leads to reduced brain capillary density and cell death via increased expression of the AFX1 transcription factor, which regulates apoptosis[180]. Pericyte-derived soluble factors that maintain a healthy endothelium might also be lacking in the AD brain owing to pericyte degeneration, which could potentially contribute to endothelial degeneration, as shown in animal models[70].

Endothelial degeneration with microvascular changes (reductions in endothelial cell thickness, length and density), loss of and abnormalities in tight junction proteins, and basement membrane changes have also been reported in brain tissue from patients with PD[166]. Immunohistological analysis of spinal cord or brain tissue from patients with ALS revealed endothelial degeneration with reduced tight junctions, capillary basement membrane changes, and enlarged perivascular spaces[168,182,183], as well as dissociation of astrocyte end-feet from capillaries[107]. Reduced expression of endothelial tight junction proteins claudin-5 and occludin has been shown in HD[60]. Endothelial degeneration with reduced and discontinuous expression of tight junction protein ZO1 has been shown in active and inactive MS lesions, compared with normal-appearing white matter[170]. Post-mortem studies of brain tissue from patients with HIV-1-associated dementia or HIV encephalitis have found evidence of microvascular degeneration and BBB breakdown, including reduced pericyte coverage[177], reduced and disrupted tight junctions[150,184], and capillary basement membrane changes[150].

Cellular infiltration

Extravasation of red blood cells has been found in AD[146], PD[165] and ALS[62]. Infiltration by peripheral macrophages has also been shown in AD[149,172] and in HIV encephalitis[150]. Additionally, neutrophils can cross the BBB in AD[148]. These findings collectively suggest

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that BBB breakdown in AD and other neurodegenerative disorders not only enables extravasation of red blood cells, which causes microbleeds and deposition of haemosiderin (derived from the haemoglobin carried by red blood cells), but also activates the innate immune response in the brain. Whether these immune system responses in non-AD neurodegenerative diseases are directed at identifying and eliminating pathogens that would otherwise enter the brain across the disrupted BBB, (and which in models of AD has been shown to accelerate amyloid deposition in exchange for circumscribing the infection process[185,186]), remains to be determined in future studies.

Aberrant angiogenesis

Increased levels of pro-angiogenic factors have been reported in AD brains[181]. However, successful renewal of lost capillary networks is compromised in AD brains, which is probably due to ongoing pericyte degeneration[7] and low endothelial expression of MEOX2[180], as discussed above. Aberrant angiogenesis, as indicated by changes in markers of angiogenesis, has also been found in the substantia nigra, locus coeruleus, and putamen in PD[187,188]. The effectiveness of deep brain stimulation of the subthalamic nucleus to alleviate motor symptoms in PD might even be attributable to improvements in the microvascular architecture[189,166], such as increased capillary length and density, increased endothelial cell thickness, and increased expression of the tight and adherens junction proteins occludin, claudin-5, ZO1 and vascular endothelial (VE)-cadherin, along with reduced perivascular IgG leakage in post-mortem brain samples from deep brain stimulation-treated compared with untreated PD patients[166].

Increased density of capillaries (vessels 5–10 μm in diameter) and reduced numbers of larger microvessels (10–20 μm in diameter), suggestive of aberrant angiogenesis, was found in HD[60]. Abnormal vascularization in HD was also found in the cortex and substantia nigra[60,61].

Molecular changes

Several studies have shown that AD brain endothelium expresses low levels of GLUT1, a BBB-specific glucose transporter[109–112], which leads to diminished glucose transport across the BBB[108]. AD brain microvessels also show diminished expression of LRP1, a major Aβ clearance receptor at the BBB[19,20,156,190,191] (which change is also present in patients with hereditary cerebral haemorrhage with amyloidosis, Dutch type (HCHWA-D)[20]). Diminished LRP1 expression leads to reduced Aβ clearance from the brain, promoting its accumulation in the brain[20,22,24]. Thus, LRP1 is a key target for enhancing transvascular Aβ clearance[192]. This mechanism could be important for the efficacy of current Aβ clearance therapies based on anti-Aβ antibodies, particularly for therapies with a peripheral Aβ sink mechanism of action, which requires Aβ clearance from brain-to-blood across the BBB[193,194,195] (FIG. 2).

Patients with AD develop increased levels of **receptor for advanced glycation end products (RAGE)** in brain microvessels, both in brain endothelium and mural cells[190,191,196]. RAGE transports Aβ in the opposite direction to LRP1, mediating the re-entry of circulating Aβ into the brain, which promotes inflammation. Experimental studies

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have also identified RAGE as a major therapeutic target in AD[196–199], which led to initiation of an ongoing phase III trial of a RAGE blocker in patients with AD[200].

Compared to controls, patients with AD have increased levels of both cyclophilin A (a proinflammatory cytokine) and matrix metalloproteinase 9 (MMP9) in the brain endothelium and pericytes. These increases are particularly pronounced in *APOE*ε4* carriers[156], findings comparable to those in transgenic *APOE*ε4* mice[71], which suggests that these increases represent activation of a BBB-degrading pathway involving cyclophilin A and MMP9. Activation of this cyclophilin A–MMP9 pathway has been confirmed by CSF analysis in non-symptomatic *APOE*ε4* carriers, in whom it is associated with BBB breakdown[201], and by analysis of cyclophilin A mRNA levels in brain tissue[202]. As the cyclophilin A inhibitor alisporivir has shown promise in a phase III clinical trial as an add-on treatment for hepatitis C[203], these studies raise a possibility that these agents might also be useful in stabilizing the BBB in *APOE*ε4* AD carriers. Whether inhibition of the BBB cyclophilin A–MMP9 pathway can influence the neurodegenerative process in human *APOE*ε4* carriers with AD (as it does in humanized *APOE*ε4* transgenic mice[71]), is an interesting topic for future studies.

Post-mortem studies of patients with HIV-1-associated dementia or HIV encephalitis also report reduced brain endothelial expression of P-glycoprotein[204]. Additionally, in patients with HD, mutant huntingtin aggregates accumulate in brain endothelial cells, perivascular macrophages, vascular smooth muscle cells, and vascular basal lamina[60], and in genetically unrelated fetal neural allografts in the brains of patients with advanced HD[205]. These data suggest that the cerebral vasculature and immune system contribute to the spread of mutant huntingtin as well as the ability of non-neuronal cells including vascular cells to contribute to spreading of HTT mutant.

CSF evidence of BBB disruption

Here we examine CSF biomarkers of BBB breakdown in AD and other neurodegenerative disorders (TABLE 3). Other CSF biomarkers of aberrant angiogenesis, endothelial dysfunction, mural cell injury, inflammatory cytokines and chemokines in AD and other neurodegenerative disorders have been reviewed in detail elsewhere[50,206,207], and are not examined here.

Since albumin is a blood-derived protein, an increase in the ratio of CSF albumin to serum albumin levels, which is known as the albumin quotient (Qalb), is frequently used as a measure of BBB breakdown[50]. Several studies report that Qalb is elevated in individuals with preclinical AD[201], MCI[49], and AD[208–210]. However, other studies did not find any increase in Qalb in patients with AD[211] unless vascular risk factors[211–214] including mild arterial hypertension, diabetes mellitus, ischemic heart disease[211,213] or dyslipidemia[214] were also present. However, the majority of patients with AD have vascular risk factors: 65% of patients aged 65 years and 80% of patients aged 85 years[3,56,57]. Although some studies have not specifically examined the relationship between vascular risk factors and Qalb[201,208], vascular risk factors did not worsen the extent of BBB breakdown, as measured by gadolinium efflux from blood into the brain extravascular-extracellular space on DCE-

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MRI K_{trans} permeability analysis[49]. These observations support the view that BBB breakdown is associated with AD independently from vascular risk factors. Future studies should examine carefully whether ischemic vascular damage from comorbidities and vascular risk factors[3,47,215] can augment BBB breakdown in AD.

However, CSF albumin levels could be influenced by proteolytic cleavage, as well as albumin uptake by brain macrophages, microglia, astrocytes, neurons, and oligodendrocytes (cells that express chondroitin sulfate proteoglycan 4 (also known as NG2))[216–218]. Therefore, Qalb might underestimate the degree of BBB breakdown. On the other hand, decreased CSF reabsorption and/or production could elevate Qalb, leading to false-positive results that might not reflect BBB breakdown[206]. In support of this notion, one study in seven patients with AD found that these individuals had a considerably reduced rate of CSF production[219]. To determine definitively whether diminished CSF turnover underlies increased Qalb in some patients with AD, detailed human studies of CSF dynamics are needed. Sensitive tests of BBB integrity, including DCE-MRI[49], microbleed T2*-weighted MRI[87], and/or measurement of alternative CSF blood-derived biomarkers (such as fibrinogen[220] and plasminogen[221], which have previously been used to detect BBB breakdown in patients with MCI and early AD, respectively) should additionally be considered.

Several important CSF biomarker studies have reported increased Qalb[106,209,222,223] as well as an increased CSF:serum IgG ratio[222] in non-demented patients with early stage PD compared to controls. Four independent studies reported increased Qalb in 55 of 138 (~40%) of patients with ALS, reviewed elsewhere[62]. Increased CSF levels of albumin, IgG and other blood-derived proteins have also been reported in patients with ALS[62,224]. Qalb is also elevated in patients with MS[218], and this change correlates with increased white matter BBB permeability detected by DCE-MRI[80]. In patients with HIV-1-associated dementia, increased Qalb was associated with axonal injury as measured by CSF levels of neurofilament light chain[225]. Finally, increased MMP9 activity in CSF in patients with MS[82,85] and *APOE*ε4* carriers prior to cognitive decline[201] is associated with BBB breakdown.

BBB breakdown and neurodegeneration

The neurodegenerative disorders discussed above share pathological alterations of the vessel wall resulting in BBB disruption. Endothelial degeneration leads to loss of tight junction proteins and/or increased transendothelial bulk flow via transcytosis[5,6]. The associated pericyte degeneration causes BBB breakdown[7,70,174–176] and initiates multiple pathways of neurodegeneration (FIG. 3) owing to the entry of several neurotoxic blood-derived proteins, including plasminogen, thrombin and fibrinogen, which enter different areas of the CNS in different neurodegenerative disorders (TABLES 1–3).

Plasmin, which is generated from circulating plasminogen, degrades the neuronal matrix protein laminin, thereby promoting neuronal injury[226]. High concentrations of thrombin mediate neurotoxicity and memory impairment[227] and accelerate BBB disruption[228]. Fibrinogen leads to axonal retraction[229] and BBB damage that promotes

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neuroinflammation[230]. Additionally, fibrin depletion delays the onset of neuroinflammation and demyelination in transgenic mouse models of MS[231], and treatment with fibrin induced M1-type activation and induction of antigen-presenting genes in both primary microglia and bone marrow-derived macrophages[232]. The role of coagulation and fibrinolysis proteins on development of brain pathology in MS has been reviewed elsewhere[233].

Influx of albumin leads to perivascular oedema that obstructs the brain microcirculation and blood flow. In turn, these hypoxic conditions lead to neuronal injury and impaired haemodynamic responses that contribute to neurodegeneration[2,13]. Extravasation of red blood cells (microbleeds) are seen in almost all neurodegenerative disorders[92,104], and lead to the perivascular accumulation of toxic, iron-containing proteins (such as haemoglobin) that release of free iron (Fe^{2+}) as they are broken down[107,62,73], generating reactive oxygen species (ROS) and subjecting neurons to oxidative stress[234].

In neurodegenerative diseases such as AD, PD, and HIV-1-associated dementia[140–142,204], dysfunction of P-glycoprotein-mediated active efflux transport at the BBB leads to the accumulation of toxic xenobiotic agents (such as environmental pollutants, food additives, pesticides and drugs) in brain. Reduced levels of P-glycoprotein and LRP1 at the BBB[20,25,137], and increased levels of RAGE in brain microvessels[6,190,191,196,199], lead to faulty clearance of toxic Aβ species linked to AD and their accumulation the in brain. Reduced blood flow and increased Aβ levels can both promote tau pathology, another key pathological hallmark of AD[2]. Whether faulty clearance of other proteins — tau in AD and CTE, α-synuclein in PD and/or huntingtin in HD — can also contribute to their respective accumulations in CNS is not clear at present.

Interestingly, experimental studies suggest that α-synuclein is transported into and out of the brain across the BBB as a free peptide[235]. Moreover, systemically administered α-synuclein oligomers, ribbons and fibrils cause distinct synucleinopathies, implying that they can all cross the BBB[236]. In humans, extracellular vesicles containing α-synuclein have been found in the CSF and blood, suggesting bidirectional transport of this protein between the blood and CSF[237,238]. As red blood cells are a source of α-synuclein-containing extracellular vesicles[237], and extravasation of red blood cells into the striatum has been detected in patients with PD[165,166], extravasated red blood cells might contribute to the development of α-synucleinopathy in humans. Since levels of α-synuclein are two orders of magnitude higher in the circulation than in the CNS[238], α-synuclein transport across the BBB might be implicated in the pathogenesis of PD, and could be a novel therapeutic target.

Accumulates of neurotoxic material and reduced blood flow can activate microglia and astrocytes, leading to an inflammatory response with secretion of neurotoxic cytokines and chemokines[47]. Additionally, in some diseases (such as AD) brain infiltration of peripheral macrophages[147,149] and neutrophils[148] suggests activation of an innate immune response. Besides peripheral macrophage infiltration, influx of T and B lymphocytes across the BBB was found in patients with MS, indicating an adaptive immune response[33]. Altogether, these studies suggest that breakdown of the BBB enables the entry of circulating leukocytes into the brain.

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BBB breakdown leads to the generation of several anti-CNS autoantibodies in humans[239], but their roles in the pathogenesis of neurodegenerative disorders have not been fully explored. Additionally, BBB breakdown can enable circulating pathogens to enter the brain and injure neurons, and/or provoke an amyloid response that aggravates β-amyloidosis, as shown in animal models of AD[185,186].

How BBB breakdown affects drug delivery

Successful delivery of therapeutic agents across the BBB requires functionally and structurally healthy blood vessels, normal vascularization, adequate blood flow, and recruitment of solute carrier-mediated transport (CMT) or receptor-mediated transcytosis (RMT) systems to facilitate drug delivery to the CNS (FIG. 4). Strategies that use existing CMT and RMT BBB systems have been explored to increase brain penetration and potency of neurotherapeutic agents (FIG. 2). For example, the large neutral amino acid CMT transporter delivers L-3,4-dihydroxyphenylalanine (L-DOPA) to the brain in PD[240], and the transferrin RMT can deliver therapeutic antibodies to the brain in various neurological conditions[4,241–243]. Other approaches, such as using nanoparticles[244] and/or opening the BBB by focused ultrasound[245,246] to improve the delivery of therapeutic agents to CNS, have been attempted.

Neurologists commonly assume that disease-initiated BBB breakdown might present an opportunity to deliver therapeutic antibodies, proteins, peptides, small molecules, and/or genetic medicines to affected neurons, without further need to manipulate the BBB. However, brain regions affected by neurodegeneration develop a pathological BBB breakdown characterized by functional and structural changes in the blood vessels, which often develop before neurodegeneration and persist as the disease progresses. These vascular changes include endothelial degeneration, reduced expression of tight junctions and adherens junctions at the BBB, increased endothelial bulk flow transcytosis, disrupted BBB transporter expression, pericyte degeneration, perivascular accumulation of toxic products, inflammation, and immune responses (FIG. 3), which all hinder the delivery of therapeutic agents to the brain. Under pathological conditions, blood-derived products, water and electrolytes accumulate in the enlarged perivascular spaces, which interferes with the normal diffusion of solutes across brain extracellular spaces, ISF formation and ISF flow, resulting in impaired distribution of solutes throughout the CNS (FIG. 4). As a consequence of disease-driven BBB disruption, impaired solute transport across parenchymal extracellular spaces and diminished ISF regional flow, therapeutic agents (including antibodies, proteins, peptides and small molecules) are likely to get trapped in pathologically altered brain tissue within the enlarged perivascular spaces, along with other blood-derived debris, preventing them from reaching their neuronal targets. Decreased function of CMT and RMT systems in neurodegenerative diseases additionally complicates their use for therapeutic drug delivery. Therefore, brain regions with healthy blood vessels and/or stabilization of the damaged vasculature in disease-affected brain regions are needed to improve cerebrovascular integrity and re-establish diffusion across extracellular spaces and ISF circulation, factors that are important for the successful delivery of neurotherapeutic agents to disease-affected brain.

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Insight from genetic studies

In this Review, we position BBB breakdown as a key pathogenic feature of neurodegenerative diseases (TABLES 1–3). Importantly, BBB breakdown is also found in (rare), inherited monogenic neurological disorders involving a primary genetic deficit in brain endothelial cells and/or mural cells of the vascular wall[6]. The known genetic aetiology of these diseases offers valuable insights into the shared causal mechanisms underpinning BBB genetic defects and neurodegeneration. For example, mutations in the *SLC2A1* gene (encoding solute carrier family 2, facilitated glucose transporter member 1 (GLUT1)) lead to *GLUT1* deficiency syndrome, which is characterized by onset of seizures in infancy, microcephaly, mild movement disorder, and developmental delay[247] associated with early onset of BBB breakdown[108]. Mutations in the *MFSD2A* gene (encoding sodium-dependent lysophosphatidylcholine symporter 1 (NLS1), the brain endothelial transporter of essential ω3 fatty acids), leads to lethal or nonlethal microcephaly, cognitive disorder, spasticity and absent speech[248,249] and early BBB breakdown[15]. Genetic defects in endothelial monocarboxylate transporter-8 (MCT8; encoded by *SLC16A2*), which transports thyroid hormones into the CNS, leads to Allan–Herndon–Dudley syndrome with severe psychomotor retardation[250]. Mutations in *OCLN* (encoding the tight junction BBB protein occludin) lead to early-onset seizures, microcephaly, and grey matter calcification, whereas mutations in the *JAM3* gene (encoding another tight junction BBB protein, JAMC) lead to brain haemorrhages and subependymal calcifications due to leakage from the BBB[251,252]. Mutations in the *PDGFRβ* gene in pericytes lead to BBB breakdown and cause a primary familial brain calcification characterized by early-onset microvascular calcification in basal ganglia, which induces seizures and motor and cognitive problems[253,254].

Conclusions

By contrast, we have limited knowledge about the molecular mechanisms underlying BBB breakdown in non-monogenic human neurodegenerative disorders (TABLES 1–3). Most mechanistic insights have been gained from animal models of these disorders[6,11]. However, the development of advanced neuroimaging techniques that are capable of interrogating changes in BBB integrity in humans in regions as small as hippocampal subfields[46,49,65,66], and improved imaging techniques for determining regional cerebral blood flow and haemodynamic responses[2], enlarged perivascular spaces[55], and incidence and distribution of microbleeds, using high strength 7T magnets to increase the detectability of these vascular changes[87,104], hold considerable promise for future neurovascular research in humans. The development of new molecular ligands (in addition to the presently used amyloid and tau PET ligands for AD[255] and FDG-PET[113–123] and verapamil-PET[140–142]) for use in other neurodegenerative disorders — for example, ligands to visualize MMP activity at the BBB *in vivo*[143] and/or the activity of other BBB transporters, receptors and/or junctional proteins affected by disease processes), is expected to provide important mechanistic insights into the role of the brain vascular system in neurodegeneration. Development of new biomarkers of vascular injury and/or repair in CSF and blood, and studies to determine how they relate to other systemic and cell-specific biomarkers of the neurovascular unit (including astrocytes, neuronal, oligodendrocytes, microglia and inflammatory biomarkers, and/or standard disease

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biomarkers, such as Aβ and tau in AD[50]), would also advance our understanding of vascular contributions to neurodegeneration and cognitive decline.

Besides the key question — what exactly is the role of the vascular system in the pathogenesis of neurodegenerative disorders, dementia and motor CNS changes — emerging questions relate to the prognostic and diagnostic value of neurovascular imaging and molecular biomarkers in predicting neurodegenerative processes and cognitive decline. If CNS vascular changes drive the initial pathogenic events that contribute to onset and progression of neurodegeneration, loss of brain connectivity, and neuronal injury and loss in complex neurodegenerative disorders, as they do in human monogenic neurological BBB diseases[6], the question persists will therapeutic targeting of BBB arrest and reverse the course of neurological disorder in humans in a way as shown in some animal models[71,73,192,196,199]? How genetics, vascular risk factors, environment and lifestyle influence the BBB functions during normal aging and disease, and how this relates to neurological disorder, is another important focus for future studies.

Findings from this review suggest that healthy brain needs healthy vascular system for its normal functioning. As studies in animal models have begun developing advanced **RNA-seq** molecular atlas of the BBB and its associated cells[6,256], similar studies in humans should be pursued to understand at the molecular level functions of human BBB. Using stem cell technology to develop *in vitro* human BBB models derived from **induced pluripotent stem cells (iPSCs)** from patients with different neurodegenerative disorders carrying genetic risk and from those with sporadic form of disease will advance drug discovery to stabilize vascular function in neurodegenerative disorders and/or develop new drug delivery approaches targeting BBB. Going forward, the BBB should be regarded as an important therapeutic opportunity, in combination with other approaches, to prevent, arrest and ultimately reverse neurodegenerative process and clinical deficits.

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Glossary items

Blood-brain barrier (BBB)
a continuous endothelial membrane of the brain vasculature with sealed cell-to-cell contacts that is sheathed by vascular mural cells and perivascular astrocyte end-feet; functions to separate the circulating blood and brain compartments and strictly regulates blood-to-brain and brain-to-blood transport of solutes

Pericytes
mural cells that wrap brain capillary endothelium and are important for the formation and maintenance of the blood-brain barrier

Neurodegeneration

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progressive neuronal dysfunction causing neuronal degenerative changes and loss of neurons in various regions of the central nervous system in different neurodegenerative diseases

Tight junctions (TJ)
endothelial proteins that tightly connect brain endothelial cells forming the anatomical blood-brain barrier with low paracellular permeability and high transendothelial electrical resistance

Transmembrane diffusion
a type of passive transport across a cellular membrane where the net movement of molecules occurs down their respective concentration gradients

Carrier-mediated transport (CMT)
transport of molecules across the blood-brain barrier down the concentration gradient via specific membrane carrier proteins

Receptor-mediated transcytosis (RMT)
transport of molecules across the blood-brain barrier in a highly specific fashion via membrane receptors that become internalized with the ligand during trans-endothelial transcytosis

Cerebrospinal fluid (CSF)
a fluid continually produced in the choroid plexus that flows throughout the brain's ventricular system; functions as a clearance pathway, maintains intraventricular intracranial pressure in the brain and is often used to measure brain-derived biomarkers of disease

Cerebral amyloid angiopathy (CAA)
amyloid deposition in the vascular wall of small brain arteries and capillaries causing vascular degeneration and lobar microbleeds in Alzheimer's disease, which contributes to blood-brain barrier breakdown, infarcts, white matter changes and cognitive impairment

Two-hit vascular hypothesis of AD
blood vessel damage is an initial insult through which BBB dysfunction and/or diminished brain perfusion lead directly to secondary neuronal injury in an Alzheimer's amyloid-β (Aβ) independent manner, from one hand (hit 1), and Aβ accumulation in the brain due to faulty Aβ clearance and increased Ab production, from the other hand (hit 2)

Apolipoprotein E epsilon 4 (*APOE*ε4*)
the major genetic risk factor for sporadic late-onset Alzheimer's disease

Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI)
dynamic MRI sequence used to quantify regional BBB permeability to a gadolinium contrast agent

T2*-weighted susceptibility-weighted imaging (SWI) MRI
a MRI sequence where haemosiderin deposits yield hypointense signal; allows for the regional *in vivo* measurement of cerebral microbleeds in the living human brain

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^{18}F-fluoro-2-deoxyglucose (FDG)
a ^{18}F (F) radiolabeled analog of glucose, 2-deoxyglucose (2DG) is not metabolized in brain in contrast to glucose; FDG is used in clinic as a surrogate PET ligand for glucose to provide an estimate of glucose uptake by the brain across the blood-brain barrier via GLUT1 glucose transporter

Low density lipoprotein receptor-related protein-1 (LRP1)
major efflux transporter for Alzheimer's amyloid-β at the blood-brain barrier responsible for clearance of Aβ from brain-to-blood

Verapamil-positron emission tomography (PET)
a radioactive ^{14}C-labeled PET ligand allows for the *in vivo* detection of P-glycoprotein function at the blood-brain barrier in the living human brain

Receptor for advanced glycation end products (RAGE)
major influx transporter of Alzheimer's amyloid-β (Aβ) at the blood-brain barrier contributing to Aβ accumulation in brain, inflammatory response, suppression of blood flow and blood-brain barrier breakdown

RNA-sequencing
transcriptomic approach to reveal the presence and quantity of RNA transcripts in a biological sample

Induced pluripotent stem cells (iPSCs)
an adult cell reprogrammed back into an embryonic-like pluripotent state for the purposes of differentiating to a cell type of interest for research studies and/or potential therapeutic efforts

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Key points

- The blood-brain barrier protects neurons from factors present in the systemic circulation, and maintains the highly regulated brain internal milieu, which is required for proper synaptic and neuronal functioning.
- Blood-brain barrier breakdown allows entry into the brain of neurotoxic blood-derived products, cells, and pathogens, and is associated with inflammatory and immune responses, which can initiate multiple pathways of neurodegeneration.
- Neuroimaging studies have demonstrated early blood-brain barrier dysfunction in Alzheimer's disease and other neurodegenerative disorders, also supported by the biomarkers biofluid data, and consistently observed by post-mortem tissue analysis.
- Blood-brain barrier dysfunction in neurodegenerative disorders includes increased blood-brain barrier permeability, microbleeds, impaired glucose transport, impaired P-glycoprotein function, perivascular deposits of blood-derived products, cellular infiltration, pericyte and endothelial cell degeneration.

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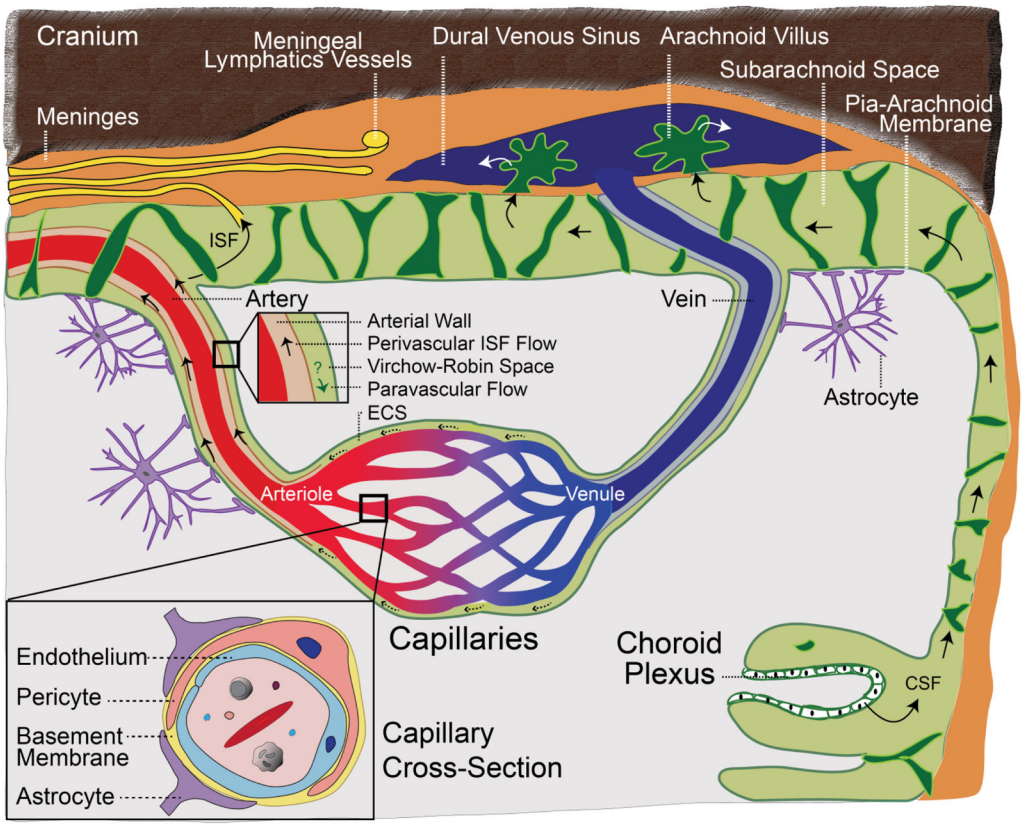

Figure 1. The blood-brain barrier

Brain capillaries are a key site of the blood–brain barrier (BBB). The capillary cross-section (large inset) shows a tightly sealed endothelium, which shares a common basement membrane with pericytes, and astrocyte end-feet wrapping around the capillary wall. The arterial cross section (small inset) shows perivascular flow of interstitial fluid (ISF) through the arterial wall in the opposite direction to blood flow; paravascular flow might also occur in the same direction as blood flow. CSF is produced by the choroid plexus and flows from brain ventricles into subarachnoid spaces, draining into the meningeal lymphatic system and/or venous blood through the arachnoid villi. ISF can exchange with CSF in the ventricles (not shown) and subarachnoid spaces. ECS, extracellular space.

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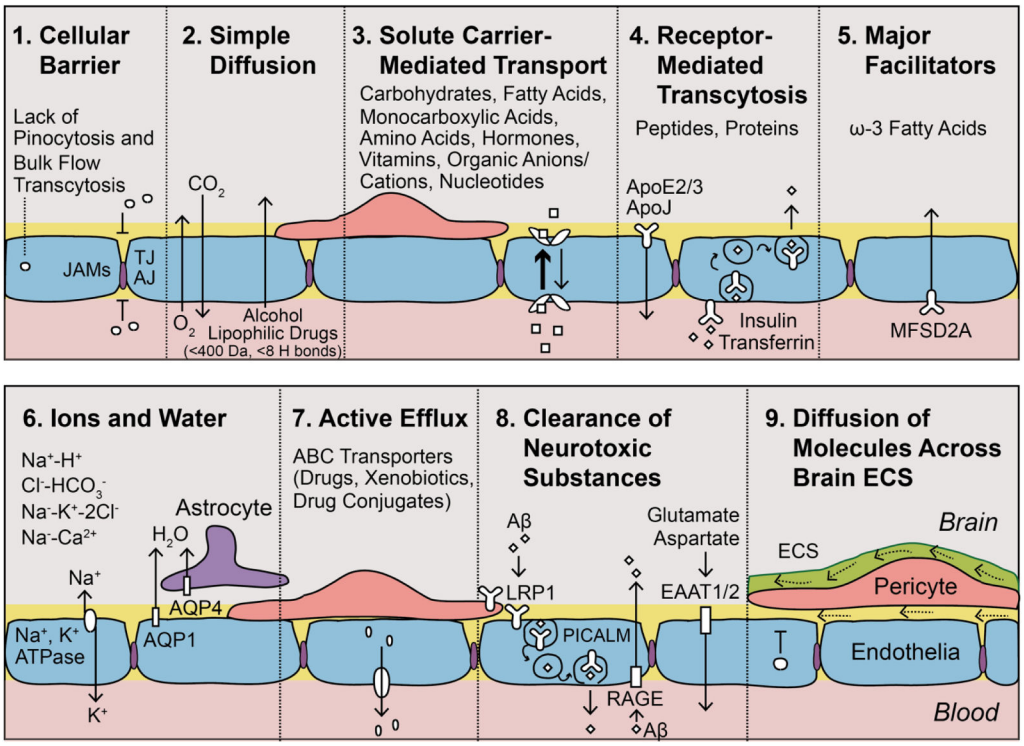

Figure 2. Key transport properties of the capillary endothelium

a | Tight junctions (TJ), adherens junctions (AJ), and junctional adhesion molecules (JAMs) prevent free paracellular exchanges of solutes. Lack of pinocytosis and bulk flow transcytosis contributes to the endothelial barrier function. b | O_2 and CO_2 cross the blood–brain barrier (BBB) by simple diffusion, as do small lipophilic molecules (such as ethanol). c | Solute carrier-mediated transport (CMT) of metabolites, nutrients, vitamins, nucleotides and other substrates, according to substrate specificity and concentration gradient. d | Receptor-mediated transcytosis (RMT) of peptides and proteins. e | NLS1 (sodium-dependent lysophosphatidylcholine symporter 1) transports ω3 essential fatty acids into the brain. f | Ion concentrations are regulated by the abluminal sodium pump (Na^+,K^+ATPase), the luminal sodium-hydrogen exchanger, chloride-bicarbonate exchanger, luminal sodium-potassium-chloride cotransporter, and sodium-calcium exchanger. Water is transported via aquaporin (AQP) receptors: AQP1 on endothelial cells and AQP4 on astrocytic end-feet. g | ATP-binding cassette (ABC) active efflux transporters limit entry of drugs, xenobiotics, and drug conjugates. h | Neurotoxic substances are cleared by phosphatidylinositol binding clathrin assembly protein (PICALM)-mediated transcytosis and LDL receptor-related protein-1 (LRP1), which removes toxic amyloid-β (Aβ) species linked to Alzheimer disease (AD). Excitatory acidic amino acid CMT transporters EAAT1 and EAAT2 clear neurotoxic glutamate and aspartate. However, receptor for advanced glycation end products (RAGE) is upregulated in AD and mediates re-entry of circulating Aβ, which increases brain Aβ levels. i | Solutes diffusing across brain extracellular spaces (ECS) (dotted arrows) are cleared via transvascular transport (c–e, g–i) and by perivascular ISF flow within the arterial wall (solid arrow), in the reverse direction of the blood flow, eventually reaching the CSF-filled

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subarachnoid space and draining into meningeal lymphatic vessels and cervical lymph nodes.

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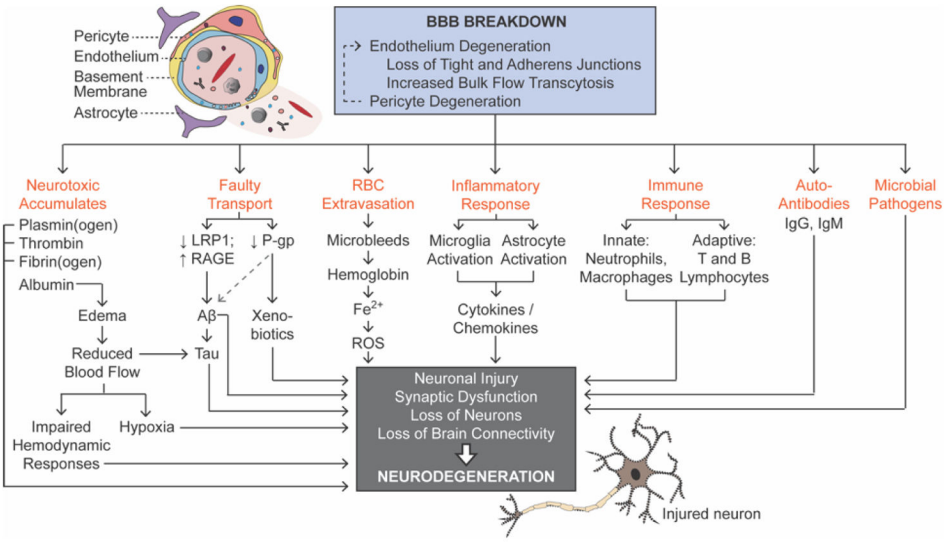

Figure 3. Blood-brain barrier (BBB) breakdown promotes neurodegeneration

BBB breakdown is characterized by pericyte and endothelial degeneration, with loss of tight and adherens junctions and increased bulk flow transcytosis. BBB breakdown leads to the entry of microbial pathogens, accumulation of neurotoxic material faulty BBB transport, red blood cell extravasation and the release of neurotoxic free iron (Fe^{2+}), which generates reactive oxygen species and oxidative stress. Inflammatory and immune responses lead to the generation of autoantibodies. CMT, solute carrier-mediated transport; ECS, extracellular spaces, L-DOPA, L-3,4-dihydroxyphenylalanine; RMT, receptor-mediated transcytosis.

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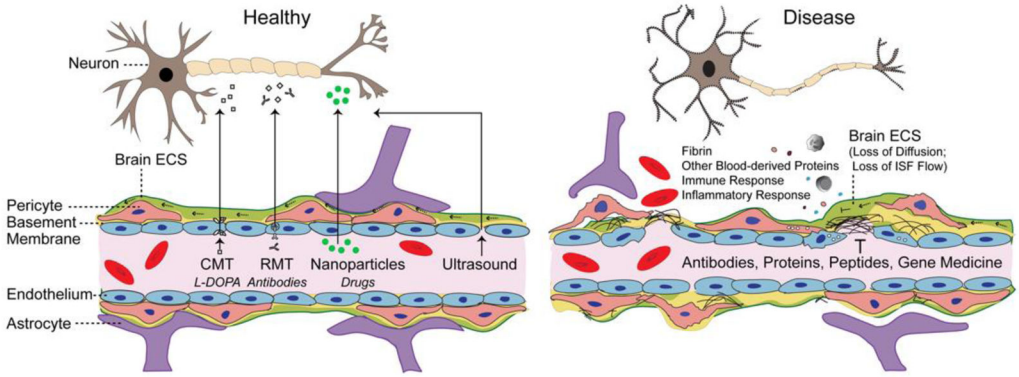

Figure 4. Blood-brain barrier (BBB) dysfunction – implications for drug delivery

In a healthy BBB (*left*), strategies to breach the barrier and deliver neuropharmaceuticals to brain rely on carrier-mediated transporters (CMT), receptor-mediated transporters (RMT), nanoparticles, and/or transient opening of BBB as for example by focused ultrasound. Under pathological conditions (*right*), the disrupted BBB leads to accumulation of blood-derived debris and cells into enlarged perivascular spaces. This blocks normal distribution of molecules throughout the CNS by concentration gradient-driven diffusion across brain extracellular spaces (ECS) and interrupts regionally formation of interstitial fluid (ISF) and ISF flow preventing the therapeutic antibodies, proteins, peptides, gene medicine and other drugs to efficiently reach their neuronal targets. See main text for details.

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Table 1

Blood–brain barrier disruption on neuroimaging in neurodegenerative disorders.

Disease	Region and details	Refs
Increased leakage of gadolinium (DCE MRI; K_{trans}*)		
MCI	Hippocampus	46, 49, 74–75, 77
Early AD	Several grey and white matter regions	46, 74–76
PD	Basal ganglia	79
HD	Caudate nucleus	60
MS	Perivascular growth of lesions in white matter regions	49, 80–84
Microbleeds (T2* and SWI-MRI)		
MCI	25% of patients	94, 104
AD	45–78% of patients	86–94, 104
PD	Several deep and cortical grey matter regions and white matter	105, 106
ALS	Deep cortical layers	107
Diminished glucose transport (FDG-PET)		
Normal cognition with AD genetic or parental risk	Entorhinal cortex, hippocampus, posterior cingulate cortex, whole brain	116, 120–121
MCI	Precuneus, cingulate cortex and temporal cortex (prior to conversion to AD)	115, 117, 118, 122, 123
Early AD	Hippocampus, parietal, temporal and cingulate cortex (prior to development of atrophy and neurodegeneration)	113–115, 117–119, 122, 123
Diminished P-glycoprotein function (verapamil PET)		
Mild AD	Parietotemporal, frontal, and posterior cingulate cortices, and hippocampus	140, 141
AD	Frontal, parietal, temporal and occipital cortices, and posterior and anterior cingulate	140
PD	Mid-brain	142
CNS leukocyte infiltration (MMP inhibitor-PET)		
MS	Leukocyte infiltration (MMP activation) in lesions	143

*The regional CNS blood–brain barrier permeability constant. AD, Alzheimer disease; ALS, amyotrophic lateral sclerosis; DCE, dynamic contrast-enhanced; FDG, fluorodeoxyglucose; FLAIR, fluid-attenuated inversion recovery; HD, Huntington disease; MCI, mild cognitive impairment; MMP; matrix metalloproteinase inhibitor MS, multiple sclerosis; PD, Parkinson disease; SW, susceptibility-weighted imaging.

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Table 2

Blood–brain barrier disruption on post-mortem tissue analysis in neurodegenerative disorders.

Disease	Details	Refs
Brain capillary leakages **§*		
AD	Accumulation of blood-derived fibrinogen, thrombin, albumin, IgG, and haemosiderin in the cortex and hippocampus	146, 147, 152–157
PD	Accumulation of blood-derived proteins in striatum: fibrinogen, IgG, haemosiderin in globus pallidus	165, 166
HD	Leakage of blood-derived proteins: fibrin in the putamen	60
ALS	Leakage of blood-derived proteins: fibrinogen, thrombin, IgG, collagen type IV, iron-containing proteins	62, 107, 168
MS	Leakage of blood-derived proteins: fibrinogen	170
Chronic traumatic encephalopathy	Perivascular haemosiderin-laden macrophages and histiocytes	151, 171
Pericyte degeneration		
AD **‡§*	Ultrastructural changes in the cortex: accumulation of osmiophilic material, mitochondrial changes, pinocytosis, loss of pericyte capillary coverage and numbers in the cortex and hippocampus	155–157, 172, 173
ALS **‡§*	Pericyte loss in the medulla, reduced pericyte capillary coverage and number in the cervical spinal cord	62, 168
HIV-associated dementia ***	Loss of pericyte coverage in the frontal cortex	177
Chronic traumatic encephalopathy ***	Mural cell mineralization in deep penetrating vessels	171
Endothelial degeneration		
AD **‡*	Microvascular reductions, reduced tight junction proteins, capillary basement membrane changes in the cortex and hippocampus	118, 155, 156, 158, 173, 180
PD *‡*	Microvascular degeneration, reduced and disrupted tight junctions, capillary basement membrane changes in the subthalamic nucleus	166
HD *§*	Decreased and disrupted tight junction protein expression in the putamen	60
ALS **‖*	Microvascular degeneration, intracellular vacuolization; reduced or disrupted tight junctions, capillary basement membrane changes, enlarged perivascular spaces in the medulla, cervical spinal cord and lumbar spinal cord	168, 182, 183
MS ***	Decreased and disrupted tight junctions	170
HIV-associated dementia ***	Reduced or disrupted tight junctions	150, 184
Cellular Infiltration ***		
AD	Extravasation of red blood cells, peripheral macrophage infiltration, neutrophils infiltration	146–149
PD	Red blood cell extravasation in striatum	165
ALS	Red blood cell extravasation	62
MS	Leukocyte infiltration	143
HIV-associated dementia	Peripheral macrophage infiltration	150
Chronic traumatic encephalopathy	Lymphocyte infiltration in Virchow–Robin spaces	151
Aberrant angiogenesis ***		
PD	increased endothelial cell number in substantia nigra pars compacta; increased angiogenic endothelial integrin-$\alpha_v\beta_3$ expression in substantia nigra pars compacta, locus coeruleus, putamen	187–188

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Disease	Details	Refs
HD	Increased vessel density, particularly of capillaries in the cortex, caudate or putamen and substantia nigra	60, 61
Molecular changes		
AD *§	Reduced GLUT1 levels (diminished brain glucose uptake); reduced LRP1 levels (diminished Aβ clearance); upregulation of RAGE (increased Aβ re-entry, neurovascular inflammation); activation of proinflammatory cyclophilin A–MMP9 pathway in *APOE4* carriers (blood–brain barrier breakdown owing to degradation of tight junctions and basement membrane proteins); increased levels of angiogenic proteins	19, 20, 109–112, 156, 181, 190, 191, 196
HIV-associated dementia	Reduced P-glycoprotein expression	204
HD *‡	Huntingtin protein aggregation in endothelial cells, perivascular macrophages, vascular smooth muscle cells, and vascular basal lamina in the cortex and putamen	60, 205

*Detected by immunohistochemistry.

‡Detected by electron microscopy.

§Detected by immunoblotting.

//Detected by reverse transcription PCR.

AD, Alzheimer disease; ALS, amyotrophic lateral sclerosis; GLUT1, glucose transporter 1; HD, Huntington disease; LRP1, LDL receptor-related protein 1; MMP, matrix metalloproteinase; MS, multiple sclerosis; PD, Parkinson disease; RAGE, receptor for advanced glycation end products.

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Table 3

Blood–brain barrier disruption on CSF ELISA in neurodegenerative disorders.

CSF ELISA analysis method	Disease	Details	Refs
Albumin	Preclinical AD or MCI	Increased Q_{alb}	49, 50, 201
Albumin	AD	Increased or no change in Q_{alb}	208–210, 212
Albumin	AD with vascular risk factors:	Increased Q_{alb}	211, 213, 214
Albumin	PD	Increased Q_{alb}	106, 209, 222, 223
Albumin	ALS	Increased Q_{alb} in 40% of patients	62, 224
Albumin	MS	Increased Q_{alb}	80, 218
Albumin	HIV-associated dementia	Increased Q_{alb}	225
Plasminogen	Preclinical AD or MCI	Increased CSF levels of blood-derived proteins (plasminogen)	221
Fibrinogen	Preclinical AD or MCI	Increased CSF levels of blood-derived proteins (fibrinogen)	220
IgG	PD	Increased CSF:serum IgG ratio	222
IgG	ALS	Increased CSF levels of blood-derived proteins (IgG)	62

AD, Alzheimer disease; ALS, amyotrophic lateral sclerosis; CSF, cerebrospinal fluid; ELISA, enzyme-linked immunosorbent assay; HD, Huntington disease; MCI, mild cognitive impairment; MMP, matrix metalloproteinase; MS, multiple sclerosis; PD, Parkinson disease; Q_{alb}, CSF:serum albumin ratio.