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REVIEW

Interplay between Brain Pericytes and Endothelial Cells in Dementia

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Prevalence of dementia continues to increase because of the aging population and limited treatment options. Cerebral small vessel disease and Alzheimer disease are the two most common causes of dementia with vascular dysfunction being a large component of both their pathophysiologies. The neurogliovascular unit, in particular the blood-brain barrier (BBB), is required for maintaining brain homeostasis. A complex interaction exists among the endothelial cells, which line the blood vessels and pericytes, which surround them in the neurogliovascular unit. Disruption of the BBB in dementia precipitates cognitive decline. This review highlights how dysfunction of the endothelial-pericyte crosstalk contributes to dementia, and focuses on cerebral small vessel disease and Alzheimer disease. It also examines loss of pericyte coverage and subsequent downstream changes. Furthermore, it examines how disruption of the intimate crosstalk between endothelial cells and pericytes leads to alterations in cerebral blood flow, transcription, neuroinflammation, and transcytosis, contributing to breakdown of the BBB. Finally, this review illustrates how cumulation of loss of endothelial-pericyte crosstalk is a major driving force in dementia pathology. (*Am J Pathol* 2021, 191: 1917–1931; <https://doi.org/10.1016/j.ajpath.2021.07.003>)

Dementia is characterized by a gradual cognitive impairment with additional symptoms, such as depression and changes to balance and gait.¹ Alzheimer disease (AD) is the most common cause of dementia followed by cerebral small vessel disease (cSVD), the leading cause of vascular dementia.² Both diseases can occur in conjunction, thereby worsening the disease risk.³ Mounting evidence supports the role of vascular dysfunction in AD, with many patients also exhibiting signs of cSVD.^{3,4} cSVD encompasses a group of pathologic conditions that affect the perforating cerebral venules, capillaries, and small arterioles, leading to white and gray matter damage in the central nervous system (CNS).⁵ Sporadic forms of AD and cSVD share cardiovascular risk factors,² suggesting that vascular dysfunction is a major contributing factor in both. Vascular dysfunction can take many forms that involve many cells comprising the neurogliovascular unit (NVU) and include disruption of the blood-brain barrier (BBB), which plays a vital role in

maintaining cerebral homeostasis. However, this review focuses on how the complex interactions between endothelial cells lining the blood vessels and pericytes closely apposed to the endothelial cells on the abluminal side contribute to BBB disruption, a central pathologic mechanism in these two important dementias.

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The BBB in Health

Blood vessels throughout the body are normally partly permeable to allow the exchange of nutrients, solutes, and chemical signals between tissue and the blood, which are vital for keeping cells alive. However, the brain microenvironment is different from the rest of the body and requires tighter control provided by the BBB. Substance movement can occur paracellularly (between endothelial cells) or transcellularly (across endothelial cells), and the BBB tightly regulates the two types of substance movement via junctional complexes between endothelial cells and through expression of membrane receptors and pumps, with limited passive diffusion.⁶ Furthermore, the BBB allows separation of peripheral and central neurotransmitters, avoiding potential cross-signaling.⁶ Pathogens, plasma proteins, and immune cells from the blood can have severe detrimental consequences if present within the brain, and disruption to the BBB is a major component of many neurologic diseases, including AD and cSVD.⁷

The BBB comprises various components, commonly referred to as the NVU (Figure 1), including endothelial cells, pericytes, basement membrane, astrocyte end-feet, and surrounding oligodendrocytes and microglia, and links to neurons in a process called neurovascular coupling in which increased neuronal activity leads to increased blood flow to

that area.⁶ The NVU is unique in that its cellular components are in close and sometimes direct contact with one another, allowing for intimate crosstalk.⁶ Endothelial cells line the blood vessels, and pericytes are mesenchymal-derived cells on the brain side of endothelial cells. Pericytes are found encircling capillaries as well as precapillary arterioles and postcapillary venules, whereas vascular smooth muscle cells (VSMCs) are found in larger vessels.⁸ Collectively, pericytes and VSMCs are known as vascular mural cells (VMCs).

In human tissue, pericytes form two subtypes with enriched gene expression of either transmembrane transporters or extracellular matrix (ECM) regulation genes.⁹ In addition, pericyte morphology plays an important role in maintaining BBB integrity because pericytes in the median eminence (ME), where BBB leakage naturally occurs, have a more irregular shape and less prominent nucleus compared with cortical tissue with a functional BBB.¹⁰ A study of pericytes in the mouse cortex found that, depending on their location along the vascular tree, pericytes have different phenotypes.¹¹ Grant et al¹¹ subdivided pericytes into ensheathing pericytes located on larger diameter precapillary arterioles with shorter cell lengths and α -smooth muscle actin (α -SMA), and mesh pericytes and thin-strand pericytes located on smaller diameter capillaries with longer cell lengths, and no detectable α -SMA. They argue that

Cognitively unimpaired Healthy BBB

cSVD and AD BBB Breakdown

Figure 1 Components of the neurogliovascular unit (NVU) and changes that occur in cerebral small vessel disease (cSVD) and Alzheimer disease (AD). The components of the NVU play an important role in maintaining the blood-brain barrier (BBB) in health. In dementia, changes, in particular to the endothelial cells and pericytes, lead to loss of function and BBB breakdown. Subsequently, the surrounding astrocytes, neurons, and oligodendrocytes get damaged, contributing to pathologic findings. Figure generated with Biorender.com (Biorender, Toronto, Ontario, Canada).

ensheathing pericytes are transitional mural cells with characteristics of both VSMCs and pericytes, whereas mesh and thin-strand pericytes comprise the capillary pericytes.¹¹ Despite a morphologic continuum in mice, there appears to be a more distinct difference in gene expression between VSMCs and pericytes in humans.⁹ Much of the literature makes no distinctions between pericyte subtypes because of not assessing morphologic differences. Furthermore, platelet-derived growth factor receptor β (PDGFR- β), which is expressed in all pericyte subtypes,¹¹ is frequently used as a pericyte marker. However, other CNS cell types have also been found to express PDGFR- β , such as perivascular fibroblast-like cells¹² which adds further complexity to distinguishing different pericytes subtypes and from surrounding perivascular cells.

Endothelial cells and pericytes have direct contact through gap junctions, which allow ionic currents to pass between their cytoplasm, and through adhesion plaques, which are involved in tethering pericytes to endothelial cells.¹³ In addition, pericyte-endothelial direct connections are made through peg-and-socket junctions where evaginations interdigitate and provide a form of pericyte-endothelial anchorage.^{10,13} It is the endothelial cells of the NVU that are the mainstay of the BBB, and together the other components of the NVU help regulate it.⁶

Endothelial cells of the BBB possess unique properties reflected in their specialized gene expression profile.¹⁰ It is their junctional proteins in particular that confer the barrier's characteristic properties of limiting paracellular permeability. Brain endothelial cells have a higher expression of certain junctional proteins localized at the cell-cell junctions compared with endothelial cells from nonneural tissues.¹⁴ Endothelial cells express three types of junctional proteins that dimerize between two cells to form junctional complexes: adherens junctions (eg, vascular endothelial cadherin), tight junctions [eg, claudin-5 (CLDN-5) and occludin], and gap junctions (eg, connexins).¹⁵ CLDN-5 is highly expressed in brain endothelial cells¹⁴ and, importantly, is important in limiting movement of small molecules (<800 Da) across the BBB.¹⁶

In rats, the BBB forms at the initial stages of embryonic development, as early as E11 to E17.¹⁷ Already at this stage, endothelial cells and pericytes are seen in association, and both cell types invade the intraneural tissue to begin BBB formation. Endothelial cells initially possess fenestrations, which are lost following migration.¹⁷ The close contact and synchronous migration of endothelial cells and pericytes during BBB formation are suggestive of the essential role of pericyte-endothelial cell interaction in BBB formation. Furthermore, mouse models deficient in pericytes do not survive to birth and have marked cerebral vasculature abnormalities that lack features of a mature functional BBB.¹⁸ After the initial endothelial cell and pericyte migration, the BBB continues to mature with recruitment of other cell types to form the NVU (Figure 1).¹⁷ In addition, endothelial-pericyte

communication in adulthood is essential to maintain a functional BBB as acute ablation of pericytes results in BBB breakdown.¹⁹ A mature BBB is crucial in maintaining brain homeostasis,⁶ and its disruption is involved in numerous diseases.^{7,20}

Clinical Evidence of BBB Disruption in AD and cSVD

The critical role of the BBB and its integrity in health is indicted by the fact that BBB breakdown is a component of many neurologic disorders, ranging from inflammatory to neoplastic to neurodegenerative conditions.^{7,20} Loss of or reduced barrier function allows for ingress of toxic blood components, inflammatory cells, and pathogens, which disrupt the delicate brain environment and lead to pathologic events (Figure 1). In recent years, there has been mounting evidence of the involvement of vascular pathologic mechanisms in addition to the amyloid cascade in the development of AD²¹ (Table 1).^{21–75} For example, a large autopsy study found that 79.9% of patients have vascular pathologic signs.⁴⁶ In addition, genome-wide association studies found a large number of AD-related genes in vascular cells.⁹ Evidence of BBB disruption has been found in postmortem tissue from patients with AD and cSVD.^{28,31} In particular, patients with AD and *APOE4* have increased deposition of the blood component fibrin(ogen) perivascularly,³¹ suggestive of leakage.

Clinical diagnosis of AD or cSVD is supported through visualization of changes on magnetic resonance imaging (MRI) (Table 1), with BBB leakage detectable with dynamic contrast-enhanced MRI, and intravenous injection of gadolinium-based tracers. Gadolinium tracer leakage is seen in AD,⁴² *APOE4* carriers at risk for AD,⁴⁴ and cSVD.⁴⁰ In addition, many of the MRI signs, such as white matter hyperintensities (WMHs), lacunes, microbleeds, and infarcts, are shared between the diseases, emphasizing their overlap.^{3,20,46,76} These lesions, which indicate tissue damage,^{76,77} originate, to a large degree, from the disruption of the BBB.^{40,62}

WMHs on neuroimaging are associated with a threefold increased risk of developing dementia and significantly associated with vascular dementia⁷⁸ and AD.^{3,78} In patients with cSVD, occurrence of BBB breakdown in WMHs is indicated by increased gadolinium leakage compared with that in the surrounding normal-appearing white matter,⁴⁰ and the degree of BBB breakdown positively correlates with WMH volume.⁴¹ Furthermore, disruption to the BBB and leakage has also been found in the normal-appearing white matter of patients with cSVD, indicating more generalized vascular dysfunction⁴¹ and demonstrating the importance of BBB breakdown as a core pathologic mechanism in dementia. Of interest, BBB leakage in the hippocampus of patients with mild cognitive impairment correlates with cerebrospinal fluid (CSF) levels of soluble

Table 1 Evidence of Vascular Pathologic Findings and Crossover in cSVD and AD

Findings	Clinical				Preclinical models			
	cSVD		AD		cSVD		AD	
Amyloid plaques in tissue and cells	No evidence		Evidence	7,21,22	No evidence		Evidence	23
Angiogenic changes and markers (VEGF and TGF- β)								
Tissue and cells	Evidence	24	Evidence	24–26	Unknown		Unknown	
Biofluids	Evidence	27	Evidence	27	Unknown		Unknown	
BBB leakage [fibrin(ogen), IgG, tracer, albumin, ANGPT2]								
Tissue and cells	Evidence	28–30	Evidence	21,24,31,32	Evidence	33,34	Evidence	19,20,35–38
Biofluids	Unknown		Evidence	21,39	Evidence	33	Unknown	
Neuroimaging	Evidence	7,40,41	Evidence	7,21,42–44	Evidence	33,45	Evidence	38
Enlarged PVS in tissue and cells	Evidence	29	No evidence		Unknown		No evidence	
Infarcts								
Tissue and cells	Evidence	46	Evidence	46	Evidence	45	Unknown	
Neuroimaging	Evidence	7,46	Evidence	3	Unknown		Unknown	
Lacunae in tissue and cells	Evidence	29,46	Evidence	46	Unknown		Unknown	
Microbleeds								
Tissue and cells	Evidence	46	Evidence	46	Evidence	45	Evidence	18
Neuroimaging	Evidence	47	Evidence	21,46,48	Unknown		Unknown	
Neuroinflammation (MMPs, CAMs, CypA, and RAGE)								
Tissue and cells	Unknown		Evidence	9,31,49	Evidence	50	Evidence	35,37,38, 51–53
Biofluids	Evidence	54,55	Evidence	31,44,56–58	Evidence	33	Unknown	
Parenchymal changes and WMHs (myelin loss, gliosis, and neuronal loss)								
Tissue and cells	Evidence	29,59	Evidence	21,22	Evidence	33,50,60	Evidence	36,38
Neuroimaging	Evidence	7,40,41, 60,61	Evidence	3,62	Evidence	29,33	Unknown	
Pericyte loss (sPDGFR- β)								
Tissue and cells	Unknown		Evidence	9,32,43	Unknown		Evidence	23
Biofluids	Unknown		Evidence	9,21,43,44	Unknown		Unknown	
Transcytosis in tissue and cells	Evidence	63	Evidence	63	Evidence	64,65	Evidence	53,66
Vasoactive dysregulation and CBF (NO and ET-1)								
Tissue and cells	Evidence	67	Evidence	9,68,69	Evidence	60,70	Unknown	
Neuroimaging	Evidence	61	Evidence	20,21,42,71	Evidence	45,50	Evidence	20,36
Vessel and endothelial cell change (instability, junctional protein changes, and morphologic features) in tissue and cells	Evidence	60	Unknown		Evidence	50,60,64,65, 70,72,73	Evidence	8,18,35,37,38, 53,66,74,75

Examples from selected references of neuropathology are seen in tissue and biofluids, such as serum and cerebrospinal fluid, as well as on neuroimaging, such as magnetic resonance imaging.

AD, Alzheimer disease; ANGPT2, angiotensinogen-converting enzyme 2; CAMs, cellular adhesion molecules; CBF, cerebral blood flow; cSVD, cerebral small vessel disease; CypA, cyclophilin A; ET-1, endothelin-1; MMPs, matrix metalloproteinases; NO, nitric oxide; RAGE, receptor for advanced glycation end products; sPDGFR- β , soluble platelet-derived growth factor receptor β ; TGF- β , transforming growth factor β ; VEGF, vascular endothelial growth factor; WMHs, white matter hyperintensities.

PDGFR- β ,^{43,44} a marker of damaged pericytes,⁴³ which prompts further consideration of their role in dementia.

Loss of Pericyte-Endothelial Signaling and BBB Breakdown

Pericytes directly encircle endothelial cells, and their vascular coverage is thought to positively correlate with barrier strength.⁷⁹ There is much debate about the extent of cerebral pericyte-endothelial cell coverage, primarily because of the wide variety of ways of measuring it. Some studies use electron microscopy to define coverage as a percentage of endothelial cell encircled by pericytes,⁸⁰ although more recent studies use PDGFR- β immunofluorescence to define coverage as a percentage of the vessel area that is PDGFR- β -positive and assess the number of pericytes by counting PDGFR- β -positive cell bodies.^{32,79} In addition, it is likely that coverage varies between organ and tissue location within the organ.^{9,81} A study of retinal and brain cortical tissue found that pericytes covered 85% of the capillary circumference in human and monkey retinas, but coverage was significantly less in monkey cortex.⁸⁰ This study used electron microscopy to determine the extent of pericyte coverage, thereby examining very small areas and perhaps not detecting variation between blood vessels in different regions. Pericytes cover up to 80% of brain capillaries in the human cortex and hippocampus, as indicated by immunofluorescence.³² However, more recently, sequencing showed that the hippocampus has fewer pericytes than cortical tissue.⁹ In three regions of mouse brain, coverage was approximately 80%, whereas in the spinal cord, pericyte coverage varied from 48% to 68%, depending on location.⁷⁹ Pericyte coverage is heterogeneous and dependent on subtype, which relates to vessel diameter, leading to variations in vessel coverage. In mice, ensheathing pericytes cover up to 95% of the vessel, whereas mesh and thin-strand pericytes cover 71% and 51%, respectively.¹¹ However, it is generally accepted that in the CNS a high proportion of endothelial cells are encircled by pericytes, hinting at the vital role they play in the NVU and regulation of the BBB,^{79,81} but the exact figures vary among species, brain regions, and measurement techniques. Of importance, pericyte coverage appears to be related to BBB leakage because naturally leaky capillaries of the ME have a reduced pericyte coverage in mice compared with cortical capillaries.¹⁰

Loss of pericyte coverage is part of normal aging, a major risk factor for dementia, in rodents⁸² and in humans. It is indicated by an increase in soluble PDGFR- β in the CSF,⁴³ which correlates with worse cognitive impairment.⁴³ Loss of pericytes in disease appears greater than that of in normal aging,^{9,32} with patients with AD having up to a 60% reduction in pericyte number and 30% reduction in capillary coverage when compared with controls, as indicated by PDGFR- β immunostaining.³² Furthermore, a recent study

found an overall reduction in VMCs and endothelial cells in patients with AD, in particular a subtype of pericytes involved in regulating the ECM.⁹

The loss of pericytes may occur through disruption of the endothelial sphingosine-1-phosphate (S1P) receptor, which is involved in regulating pericyte vessel coverage^{83,84} by controlling N-cadherin trafficking to the membrane needed for endothelial-pericyte adhesion. A reduction in S1P receptors occurs in AD brain tissue,⁸⁵ and an S1P analogue, a ligand for the S1P receptor, reduces memory impairment in an AD mouse model.⁸⁶ The neuroprotective effect of S1P is mediated by reducing amyloid- β (A β)—induced neuronal apoptosis⁸⁷ and reducing levels of certain S1P isoforms in vascular dementia.⁸⁸ In addition, dysregulation of S1P is implicated in neuroinflammation,⁸⁸ another pathophysiologic component of AD and cSVD.⁸⁹ Therefore, S1P and S1P receptor play a role in the pathophysiologic mechanisms of dementia by altering amyloid-induced apoptosis, neuroinflammation, and endothelial-pericyte adhesion.

Interestingly, recent evidence from a genetic form of vascular dementia, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), has found no reduced pericyte coverage in patients or a mouse model.²⁹ These patients harbor mutations in the *NOTCH3* gene; therefore, the BBB disruption seen may be attributable to the requirement of NOTCH for adhesion of endothelial cells and VSMCs to the basement membrane, affecting vessel stability.⁹⁰ In addition, pluripotent stem cell (iPSC)—derived VMCs in patients with CADASIL have reduced PDGFR- β and can induce apoptosis in neighboring endothelial cells, leading to decreased blood vessel stability. This phenotype is specific to the vascular mural iPSCs because the endothelial iPSCs are able to form normal vessel networks.⁷⁰ Furthermore, differentially expressed genes in the VMCs of patients with AD are similar to those seen in CADASIL, and ECM maintenance in AD involves loss of a pericyte subtype.⁹ Together, these data suggest that BBB disruption in dementia occurs not only through loss of pericyte coverage and endothelial-pericyte contact but also through ECM disruption and/or loss of PDGFR- β signaling (Figure 2).

Platelet-derived growth factor β (PDGF- β)/PDGFR- β signaling is one of the most studied and important pathways in pericyte-endothelial crosstalk. Brain endothelial cells are enriched for and secrete PDGF- β ,⁹¹ which binds to pericyte-specific PDGFR- β .⁹² Most knowledge of the PDGF- β /PDGFR- β signaling pathway stems from studying *Pdgfb*- or *Pdgfrb*-null embryos (because these mice do not survive to birth¹⁸) or postnatally in hypomorphic *Pdgfb*^{ret/ret}- or *Pdgfrb*-deficient models. Of importance, these models have helped further the understanding of possible mechanisms that contribute to BBB breakdown seen in cSVD and AD.^{40,46} As mentioned above, clinical data from patients with dementia indicate an increase in soluble PDGFR- β in the CSF,⁴³ supporting the involvement of PDGF- β /PDGFR- β signaling in disease pathology. Further evidence of the

Figure 2 Loss of pericyte-endothelial crosstalk in cerebral small vessel disease and Alzheimer disease. Disruption to endothelial-pericyte signaling leads to a wide variety of changes: (1) pericyte loss; (2) endothelial cell activation and extravasation of immune cells; (3) loss of balanced amyloid- β (A β) transport caused by increased receptor for advanced glycation end products and loss of low-density lipoprotein receptor-related protein 1 (LRP1), leading to A β build-up in the brain; (4) loss of LRP1 also contributes to loss of inhibition of the cyclophilin A–matrix metalloproteinase (MMP)-9 pathway, leading to extracellular matrix/basement membrane breakdown by MMPs; (5) MMP activation contributes to loss of junctional proteins, changes in organization, and widening junctional gap between endothelial cells; (6) transcriptional changes that lead to a more venous phenotype; (7) reduced major facilitator superfamily domain containing 2a, which contributes to increased transcytosis; (8) BBB breakdown and ingress of neurotoxic substances such as fibrin(ogen) into the brain; (9) dysregulation of vasoactive substances (eg, nitric oxide) and subsequent reduced cerebral blood flow (CBF); and (10) morphologic changes to endothelial cells with cellular hyperplasia and aberrant angiogenesis. Figure generated with Biorender.com (Biorender, Toronto, Ontario, Canada). CAMs, cellular adhesion molecules; CLDN-5, claudin-5; ICAM-1, intercellular adhesion molecule 1; PDGFR- β , platelet-derived growth factor receptor β ; PLVAP, plasmalemma vesicle-associated protein; VCAM-1, vascular cell adhesion molecule 1; ZO-1, zonula occludens protein 1.

clinical importance of disruption to the PDGF- β /PDGFR- β signaling pathway can be seen after surgically-induced large vessel stroke in adult *Pdgfrb* knockout mice, which indicates reduced pericyte coverage, significantly larger stroke lesions, and more BBB leakage, as compared with controls. This phenotype is significantly rescued with the preservation of PDGFR- β expression in pericytes.⁹³ Most importantly, these models allow us to understand the importance of endothelial-pericyte crosstalk in maintaining BBB integrity.

In human AD, brain areas with reduced pericyte coverage of vessels show increased fibrin(ogen) and IgG extravasation demonstrating BBB leakage,³² and similar pathologic changes are seen in cSVD³⁰ (Figure 2). The findings of BBB impairment in patients with reduced pericyte vessel coverage are supported by reduced pericyte coverage in the naturally leaky ME¹⁰ and studies in rodents using pericyte-deficient mice seen by dextran tracer

leakage around pericyte-deficient blood vessels, which worsens with increasing age,³⁵ a major risk factor for cSVD and AD dementias. In these mice, fibrin(ogen) and IgG leakage can occur with as little as 20% reduction in pericyte coverage.³⁵ Pericyte degeneration is also associated with accumulation of fibrin(ogen) in the white matter of pericyte-deficient mice.³⁶ Interestingly, fibrin(ogen) accumulation is much greater in the white matter regions of the brain compared with gray matter. Furthermore, fibrin(ogen) deposition in the white matter leads to loss of myelin, oligodendrocytes, and neuronal axons,³⁶ similar to changes seen in patients with AD and cSVD,⁵⁹ and cSVD rat models.³³ The loss of oligodendrocytes may be, in part, attributable to the reduced vascular density and corresponding reduction in blood flow, leading to increased levels of hypoxia in white matter regions compared with that in the gray matter.³⁶ This study highlights that

regional differences in pericyte coverage within the CNS likely contribute to the differences in tissue susceptibility in dementia.

In addition to overexpression of the A β precursor protein in transgenic mice, pericyte deficiency leads to reduced brain A β clearance mediated by low-density lipoprotein receptor—related protein 1 (LRP1). The reduction of LRP1-mediated clearance is not through changes in pericyte LRP1 expression but through reduction in pericyte numbers,²³ thus demonstrating an important role for pericytes in AD pathology, and in particular, limiting the formation of A β plaques. Endothelial cell LRP1 is important in maintaining BBB integrity; loss of endothelial LRP1 leads to BBB leakage, neuronal loss, and altered cognition in mice. This phenotype is attributable to cyclophilin A (CypA) matrix metalloproteinase (MMP)-9 pathway activation (discussed subsequently) and can be rescued by CypA inhibition or LRP1 reexpression.³⁸ Loss of pericytes as well as changes to pericyte and endothelial protein expression likely affect not only BBB leakage and toxin clearance but also cerebral blood flow.

Pericyte-Endothelial Crosstalk Controls Cerebral Blood Flow

The integrity of the brain vasculature is important in maintaining perfusion to and oxygenation of the brain, which are vital because of a high metabolic demand and low energy storage capacity of the brain.⁹⁴ There is disparity in the literature as to whether pericytes are able to directly control vessel diameter and hence blood flow.^{95–97} It is thought that pericytes lack contractile α -SMA and therefore have the ability to contract in response to stimuli. However, the presence of α -SMA in pericytes could be underreported because of filamentous-actin fixation or low cellular expression.^{11,98,99} Studies have found that retinal and brain pericytes possess α -SMA,⁹⁸ and the ensheathing pericyte subtype is α -SMA-positive.¹¹ Ensheathing pericytes respond to sensory stimuli, and vasodilate before arterioles via nitric oxide and prostaglandin E₂.⁹⁵ Similar results are seen in another mouse model mediated by endothelial-secreted epoxyeicosatrienoates,¹⁰⁰ demonstrating endothelial-pericyte crosstalk in cerebral blood flow control. Disparities in the literature may be attributable to inconsistencies in terminology with first- to fourth-order branches from perforating arterioles termed *capillaries* instead of *precapillary arterioles* and their associated ensheathing pericytes being termed *α -SMA-positive VSMCs*. When different subtypes of pericytes are taken into account, studies appear to agree that precapillary ensheathing pericytes are able to regulate blood flow *in vivo*.¹¹

However, whether the capillary pericytes (mesh and thin-strand pericytes) are able to modulate cerebral blood flow is debatable.^{11,96,101} Cerebral capillary pericytes express the smooth muscle protein, myosin heavy chain, indicating

some contractile ability.¹⁰² Furthermore, recent studies have found that capillary pericytes modulate blood vessel diameter more slowly after direct *in vivo* stimulation compared with surrounding ensheathing pericytes.¹⁰¹ Similar results were seen in retinal capillaries after direct stimulation, but this was not replicated in the cortical capillaries.¹⁰² One hypothesis to explain this is that capillary pericytes require a stronger or different stimulus to respond.^{11,102} Furthermore, imaging techniques may contribute to the ability to detect capillary diameter changes, and regional differences may occur; cerebral *in vivo* studies are currently limited to the cortex. Despite the debate over the ability of capillary pericytes to modulate cerebral blood flow, there are strong data supporting the role of ensheathing pericytes, particularly at vessel junctions,¹⁰² in controlling blood flow response to NVU stimuli.

Reduction in cerebral blood flow occurs in the gray matter of patients with cognitive impairment,^{61,71} who, as mentioned above, have reduced pericyte coverage in these regions.⁴³ The reduction in cerebral blood flow positively correlates with loss of pericytes, demonstrating that pericyte degeneration in dementia^{32,43} is likely to contribute to changes to cerebral blood flow. This theory is supported by data in pericyte-deficient mice in which cerebral blood flow is reduced, particularly in the white matter.³⁶ Furthermore, acute pericyte ablation after diphtheria toxin administration in transgenic mice leads to reduced gray matter blood flow as well as BBB breakdown.¹⁹ Pericyte degeneration in disease likely causes hypoperfusion as vessels lose their contractile ability; however, additional intrinsic differences in endothelial cells and pericytes between brain regions may also be involved.

The hippocampus is one of the first regions of the brain in which BBB leakage and breakdown occur in normal aging and pathologic findings are aggravated in patients with mild cognitive impairment.⁴³ These findings may be attributable to the human hippocampus having fewer pericytes compared with other areas of the brain in addition to the presence of endothelial cells with a more inflammatory signature.⁹ Pericyte-deficient mice have reduced cerebral blood flow and BBB leakage in the hippocampus, albeit to a lesser extent than in the white matter.^{35,36} Nevertheless, hippocampal sensitivity to ischemic injury may occur due to underlying endothelial cell and pericyte differences in addition to reduced pericyte coverage.⁹ In healthy mice, blood flow and oxygenation levels in hippocampal vessels are lower than that in the cortex.¹⁰³ Furthermore, hippocampal capillaries dilate to a lesser extent and frequency than cortical capillaries, possibly because of a longer and less contractile pericyte phenotype. Hippocampal mural cells also have lower expression of ion channels and skeletal muscle actin (*Acta1*). In addition, hippocampal endothelial cells have reduced prostaglandin E synthase (*Ptges*) and *Kir2.1*, an inwardly rectifying potassium channel that propagates the vasodilatory signals from capillaries to arterioles.¹⁰³ Therefore, the hippocampus appears to be

particularly vulnerable to BBB disruption because of regional differences in pericyte morphologic mechanisms and neurovascular coupling, caused by regional heterogeneous endothelial cell and pericyte gene expression. This leads to reduced endothelial-pericyte communication, for example, via prostaglandin,¹⁰³ thereby affecting their ability to control cerebral blood flow.

Endothelial cells are known to secrete vasoactive substances, such as endothelin (ET)-1, which bind to receptors on pericytes. ET-1 from endothelial cells elicits pericyte contraction via inositol phosphate pathways.¹³ Increased ET-1 levels in brain tissue have been found in vascular dementia.⁶⁸ However, ET-1 levels in AD tissue in the literature differ,^{68,69} suggesting that other vasoactive substances, such as nitric oxide, are involved in control of cerebral blood flow, contributing to the hypoperfusion seen in dementia.^{61,71} Dysfunctional endothelial cells secrete less nitric oxide⁶⁰ required for vasodilation via pericyte relaxation via cGMP-dependent pathways.¹⁰⁴ In patients, functional single-nucleotide polymorphisms in *NOS3*, the gene encoding endothelial cell nitric oxide synthase, have different associations with SVD, with single-nucleotide polymorphisms leading to lower nitric oxide plasma levels, indicating a trend to higher SVD risk.⁶⁷ In dementia, the regional difference in endothelial-pericyte gene expression, dysregulation of paracrine signaling via vasoconstrictive and vasodilatory substances, and a loss of pericytes coverage and number contribute to local reduction in brain perfusion^{61,71} (Figure 2). Subsequently, a compensatory angiogenesis in response to hypoxia from hypoperfusion is thought to occur in dementia.²⁵

Endothelial-Pericyte Crosstalk in Angiogenesis

Rodent studies indicate the importance of PDGF- β /PDGFR- β signaling in angiogenesis as well as the formation of the BBB in development,^{53,105} regulating numerous processes such as proliferation and migration.¹⁰⁵ In development, endothelial cells express PDGF- β at areas of angiogenesis, leading to proliferation of PDGFR- β -expressing pericytes.¹⁰⁵ This endothelial cell secretion of PDGF- β is essential for recruitment of pericytes to sites of angiogenesis⁷⁴ because knockout leads to up to a 90% reduction in pericyte number.¹⁰⁶ The requirement of endothelial cell PDGF- β for pericyte recruitment persists into adulthood.¹⁰⁶ The chimeric nature of some knockout models indicates that endothelial cells that express PDGF- β are unable to compensate for those without PDGF- β ,¹⁰⁶ demonstrating local paracrine endothelial cell PDGF- β signaling to pericytes to ensure formation and maintenance of an adequately ensheathed capillary and functional BBB.³⁵ Aberrant angiogenesis has been seen in regions of AD brain tissue²⁶ possibly stimulated by hypoperfusion and subsequent hypoxia, secondary to dysfunction of endothelial-pericyte crosstalk described in the previous

section. Angiogenic dysfunction is seen in AD and vascular dementia with increases in intrathecal angiogenic factors, such as transforming growth factor (TGF)- β in patients,²⁷ which is thought to be involved in initiating or promoting amyloidogenesis in AD.¹⁰⁷

Vascular endothelial growth factor (VEGF), another angiogenic factor, is also elevated in patients with AD and cSVD.²⁷ VEGF stimulates endothelial cell proliferation and is secreted by pericytes and endothelial cells. Pericytes require direct contact with endothelial cells to secrete VEGF via a pathway that also involves TGF- β , promoting endothelial cell survival and allowing vessel maturation by regulating proliferation.^{13,108,109} iPSC mural cells derived from patients with CADASIL have reduced VEGF secretion, leading to vessel destabilization, which can be rescued through VEGF supplementation.⁷⁰ However, increased VEGF has been found in the white matter in patients with cSVD and AD without apparent rescue, suggesting that a more complex signaling mechanism is involved.²⁴ Loss of pericytes leads to increased VEGF-A expression in endothelial cells,¹⁸ which may be a compensatory endothelial cell response to promote cell survival. However, it may also be attributable to loss of pericyte VEGF receptor 1, which regulates VEGF signaling by sequestration¹¹⁰ or a combination of both mechanisms. Endothelial overexpression of VEGF can be detrimental by antagonizing PDGF- β through formation of VEGF receptor 2/PDGFR- β complexes in pericytes, suppressing PDGFR- β signaling required for pericyte recruitment leading to immature leaky vessels as well as inappropriate endothelial cell hyperplasia.^{13,110,111} VEGF is also known to increase paracellular permeability by reducing occludin protein expression.¹¹² In summary, careful calibration of multiple molecular signals that are involved in both promotion and inhibition of endothelial cell proliferation, such as VEGF, is essential to ensure appropriate endothelial cell proliferation while limiting negative regulation of pericyte function.

Furthermore, these vessels may also form abnormally as seen in *Pdgfb*- and *Pdgfr*-null mouse models. Despite having normal microvessel length and density, these mice have pathologic changes in areas with loss of pericyte coverage,¹⁸ for example, microhemorrhages, which are commonly seen in cSVD⁴⁷ and AD.⁴⁸ Endothelial cells without pericyte contact have an altered shape with increased cytoplasmic thickness and luminal projections.¹⁸ The microvessels in the brains of mutant mice are dilated with up to a 25% increase in diameter compared with controls with areas of endothelial cell hyperplasia,^{18,74} which could be attributable to dysfunctional levels of VEGF. Therefore, disrupted pericyte signaling in AD and cSVD may cause secondary changes in endothelial cell morphologic mechanisms, proliferation, and tight junction expression, leading to subsequent BBB disruption (Figure 2). In recent years, the bidirectional relationship between endothelial cells and pericytes required for normal function has further been highlighted with the

use of genetic sequencing to examine the downstream effects of loss of this communication.

Endothelial-Pericyte Signaling Regulates Cellular Transcription

The pericyte-deficient mouse models provide further insight into loss of BBB integrity in dementia through pericyte modulation of endothelial gene expression. Loss of pericyte coverage does not affect BBB-specific endothelial cell genes during development or in adults, indicating an intrinsic expression.^{37,66} However, reports of changes in endothelial cell junctional protein expression and localization differ among studies possibly because of increased loss with age.^{35,53,66} Despite these discrepancies in junctional protein expression levels, researchers who examined endothelial cell junctions saw structural abnormalities in junctional protein alignment in embryos⁵³ or morphologic mechanisms with increased undulations of the endothelial membrane within these junctions in adult mice.⁶⁶ Furthermore, the increase in junctional width between endothelial cells⁶⁶ can be reversed *in vitro* with the addition of pericytes.⁵³ Pericytes may not be directly involved in regulating BBB-specific gene transcription; however, in pericyte-endothelial cell co-culture systems, pericytes increase the barrier strength up to fourfold when measured by trans-endothelial resistance.⁵³ Therefore, the presence of pericytes and crosstalk with endothelial cells is required for the formation of a stronger, perhaps more functional BBB, with correct junctional protein alignment and morphologic features.

A possible explanation for the formation of a more robust BBB in the presence of pericytes was provided by a recent study by Mäe et al.³⁷ Using adult pericyte-deficient mice and sequencing of the endothelial cells, they found alterations in junctional complex alignment in areas of pericyte deficiency. Furthermore, as mentioned above, the endothelial cells retained their BBB-specific genes but had an overall shift in gene expression toward a venous pattern without pericyte contact. In areas of high BBB tracer leakage and pericyte loss, the endothelial cells had altered junctional protein complex distribution with increased expression of plasmalemma vesicle-associated protein (normally expressed in fenestrated capillaries, such as in the ME¹⁰) and low major facilitator superfamily domain containing 2a (MFSD2A) expression, which is involved in suppressing transcytosis, discussed below. Surprisingly, areas of BBB leakage had low expression of angiopoietin-2 (ANGPT2),³⁷ suggesting an interesting role for this protein in maintaining BBB integrity.

ANGPT2 is involved in the angiopoietin/tyrosine protein kinase receptor TIE2 signaling pathway as an antagonist. TIE2 and ANGPT2 are expressed by endothelial cells.^{37,113} ANGPT2 is mainly reported for its autocrine role in blood

vessel destabilization and pathologic angiogenesis through induction of pericyte loss,^{8,37,75} whereas pericytes are involved in paracrine regulation of the TIE2 signaling pathway, reducing vessel permeability in tumor, skin, and BBB models.¹¹⁴ Increased serum ANGPT2 levels have been found in *APOE4* carrier patients with AD with white matter changes,³⁹ and an overall generalized increase in endothelial cell *Angpt2*/ANGPT2 expression is seen in pericyte-deficient mice,³⁷ which fits with its involvement in blood vessel destabilization, leading to the BBB leakage in patients with AD and pericyte-deficient mice. In mice, ANGPT2 was expressed mainly in endothelial cells in pericyte-deficient areas of the brain,^{37,53} supporting the hypothesis that pericytes suppress endothelial cell expression of vessel destabilizing ANGPT2 and thereby maintaining BBB integrity. Therefore, it follows that high ANGPT2 expression is expected in areas of high BBB leakage because of its role in blood vessel destabilization. Surprisingly, Mäe et al.³⁷ found the opposite: low ANGPT2 expression in areas of high BBB leakage. Furthermore, endothelial cell-specific knockout of *Angpt2* in pericyte-deficient mice aggravated BBB leakage instead of the predicted rescue. To investigate this, they examined *Angpt2* knockout mice (without pericyte deficiency) and found abnormal vessel morphologic mechanisms and abnormal CLDN-5 junctional protein staining. Similar abnormal vessel morphologic mechanisms and CLDN-5 distribution were also seen in the pericyte-deficient mice in areas of high leakage and low ANGPT2 expression. Together these data suggest that ANGPT2 exerts a protective effect in endothelial cells at the BBB by regulating junctional protein alignment required for maintenance of BBB integrity, which is particularly important in pericyte loss because *Angpt2* knockout in pericyte-deficient mice worsens BBB leakage.³⁷ This novel vessel stabilizing role for ANGPT2 may be context-dependent because a similar role has been seen in the lymphatic endothelium.¹¹⁵ ANGPT2-TIE2 signaling is involved in trafficking or stabilizing of junctional complexes, but further research is required into ANGPT2-TIE2 at the BBB.³⁷ ANGPT2-TIE2 endothelial-pericyte interaction is complex and requires careful balancing to ensure maintenance of BBB integrity. More generalized changes in BBB permeability with pericyte deficiency have been seen through changes in transcytosis.

Endothelial-Pericyte Signaling in Transcytosis and Increases in BBB Permeability

In pericyte-deficient endothelial cells, transcytosis, movement of substances transcellularly in vesicles, is increased,^{18,53,66,82} suggesting pericyte regulation of endothelial cell transcytosis. Transcytosis occurs through the formation of caveolae, a form of nonspecific transport across the BBB. Caveolae increase with age in mice,

leading to a shift from specific receptor-mediated transport to the nonspecific caveolae uptake.⁸² In particular, pericyte-free endothelial cells have reduced expression of the transferrin receptor (CD71) known to be involved in substrate-specific receptor-mediated transport.⁶⁶ The shift from specific receptor-mediated transport to caveolar nonspecific transport that occurs with normal aging⁸² is thought to occur earlier in dementia, leading to increased BBB permeability.⁶³ The presence of inert tracer molecules within caveolae in BBB endothelial cells devoid of pericytes,⁵³ suggest that increased BBB permeability occurs, in part, through increased transcytosis and not solely paracellularly through tight junction disruption as described above. In rodent models of large vessel stroke, an increase in endothelial caveolae number and size occurred rapidly after stroke induction, coinciding with an initial increase in BBB permeability.^{64,65} A second phase of BBB leakage occurred 48 hours after stroke, which coincided with increased endothelial junctional gaps and tight junction redistribution.^{64,65} Of interest, caveolin-1, a major caveolae component, colocalizes with CLDN-5 *in vitro*.⁷² However, caveolin-1 involvement in redistribution of CLDN-5 requires further study.^{65,72} Disruption to the BBB in dementia most likely occurs through a combination of increased transcytosis and disruption to paracellular junctions. One mechanism through which pericytes may be responsible for regulating transcytosis in endothelial cells of the BBB is via control of *Mfsd2a* expression.

MFSD2A is highly expressed in brain endothelial cells compared to peripheral endothelial cells.¹¹⁶ MFSD2A is a protein involved in transcytosis suppression supported by data from *Mfsd2a* knockout mice that have normal vascular density and branching, tight junction organization, and pericyte coverage but increased endothelial cell transcytosis.¹¹⁶ MFSD2A expression is reduced at sites of BBB leakage and pericyte loss in rodent models.³⁷ Reduced expression is seen only in endothelial cells that lack pericyte contact, implying direct paracrine signaling. Further supporting the importance of pericytes in maintaining healthy BBB function is the reduced expression of *Mfsd2a*/MFSD2A in normal aging accompanying reduced pericyte coverage.⁸² However, *Mfsd2a* suppression may not be solely responsible for the increase in BBB leakage seen in pericyte-deficient models. Contrary to data from Ben-Zvi et al,¹¹⁶ Mäe et al³⁷ did not see any tracer leakage in *Mfsd2a* knockout mice. This discrepancy may be attributable to Ben-Zvi et al¹¹⁶ looking at tracer leakage in embryos and postnatal animals, whereas Mäe et al³⁷ using adult animals in which there may be compensation through other mechanisms. Therefore, loss of endothelial-pericyte crosstalk contributes to *Mfsd2a*/MFSD2A suppression, which may have a role in increased BBB permeability through increased caveolar transcytosis as well as alterations in expression

of other genes, such as those involved in neuroinflammation.

Neuroinflammation and Endothelial-Pericyte Crosstalk

The BBB plays an important role in limiting movement of inflammatory cells across the BBB into the brain, which is thought to be, in part, attributable to the brain's limited ability for regeneration of certain cell types, such as neurons. Disruption to the BBB and subsequent neuroinflammation are components of many neurodegenerative diseases, such as AD and cSVD.⁸⁹ Underlying sensitivity to BBB leakage of the hippocampus in AD⁴³ may be related to higher endothelial cell expression of inflammatory genes in this brain region compared with cortical tissue.⁹ Leukocyte adhesion molecules vascular cell adhesion molecule 1 and intercellular adhesion molecule 1 are expressed on endothelial cells and responsible for leukocyte movement across the BBB. Increased levels of soluble vascular cell adhesion molecule 1 and intercellular adhesion molecule 1 are present in the serum of patients with cSVD and AD compared with age-matched controls^{54,56} and associated with A β plaques in the brain.⁴⁹ Increased intercellular adhesion molecule 1 is present in pericyte-devoid areas of vasculature in pericyte-deficient models, which is accompanied by brain parenchymal leukocyte infiltration.⁵² Increased expression of leukocyte adhesion molecules is also seen at sites of BBB leakage.³⁷ Further supporting a role for pericytes in mediating endothelial leukocyte adhesion molecule expression are *in vitro* studies that showed that co-culture with pericytes is sufficient to reduce intercellular adhesion molecule 1-positive endothelial cells.⁵³ The clinical importance of controlling neuroinflammation is indicated by the correlation of increased soluble vascular cell adhesion molecule 1 levels in AD and cSVD with worsening cognitive impairment.^{54,56} The exact mechanism through which pericytes regulate endothelial leukocyte adhesion molecule expression is not known; however, evidence suggests they play an important role.

Secretion of MMPs is an inflammatory mechanism that leads to BBB breakdown and pathologic findings.⁷ Under normal homeostatic conditions, MMPs from all cell types are required for remodeling of ECM necessary for processes such as angiogenesis or neurogenesis.⁷ However, in inflammation, MMPs are involved in breaking down components of the BBB and NVU, allowing ingress of inflammatory cells into the brain.¹¹⁷ In disease, MMPs are involved in BBB disruption through several mechanisms. Studies have found elevations in serum MMP9 and an association with higher WMH volumes in patients with cSVD.⁵⁵ Furthermore, increased MMP9 staining occurs in leptomeningeal vessels of patients with AD and cerebral amyloid angiopathy from A β deposition and

concurrent intracerebral hemorrhage.⁴⁹ Similar findings are seen in *Pdgfrb*-null mice and a transgenic AD mouse model with increased MMP9 tissue protein levels. There is reduced collagen in these mice, indicating that MMP9 may be involved in the breakdown of the ECM collagen that contributes to BBB disruption and leakage.^{53,118}

Another mechanism through which MMPs act to break down the BBB involves disruption of endothelial junctional proteins (Figure 2). A transgenic AD mouse model with increased MMP9 levels found reduced zonula occludens protein 1, occluding, and CLDN-5 protein levels in brain tissue lysate, indicating disruption to the endothelial cell junctions. The increase in MMP9 was mediated by CypA in pericytes.¹¹⁸ Blockage and/or pharmacologic treatment of MMP9 and CypA reversed BBB leakage, demonstrating that dysregulation of these pathways alone can be responsible for BBB disruption.^{38,118} Activation of the proinflammatory CypA-MMP9 cascade that leads to ingress of neurotoxic substances into the brain is *APOE4* isoform-specific,¹¹⁸ and pericytes and endothelial cells of *APOE4* carrier patients with AD have increased CypA and MMP9 protein levels.³¹ Clinical data indicate that *APOE4* carrier patients with AD who have BBB leakage and increased pericyte damage also have increased CSF CypA and MMP9 levels.⁴⁴

APOE4 isoform-specific CypA-MMP9 activation is thought to occur because *APOE4* only weakly binds pericyte LRP1, whereas *APOE3* binds LRP1 with high affinity, inhibiting the CypA-MMP9 cascade.¹¹⁸ LRP1 is also important in endothelial cells. In a mouse model, loss of endothelial LRP1 led to CypA-MMP9 activation similar to that in the transgenic AD mouse discussed above,¹¹⁸ resulting in loss of tight junctions and subsequent BBB breakdown, neuronal death, and reduced cognitive function. This phenotype was rescued by reexpression of endothelial LRP1 or CypA inhibition.³⁸ Reduced levels of LRP1 have been found in AD brain tissue,⁵⁷ which, together with the increased CypA and MMP9 in CSF,⁴⁴ demonstrate the involvement of this pathway in pathologic findings. Therefore, LRP1 expression and signaling in pericytes and endothelial cells is important in controlling spurious activation of the CypA-MMP9 cascade that leads to BBB breakdown and other features of dementia pathologic findings, such as A β clearance.²³

In addition to BBB breakdown, the activation of the proinflammatory CypA-MMP9 cascade may also be involved in A β pathologic findings. The link between *APOE4* and A β occurs through the receptor for advanced glycation end products in brain capillaries. The receptor for advanced glycation end products is expressed on brain endothelial cells and is responsible for transport of A β across the BBB into the brain.¹¹⁹ An increase in endothelial receptor for advanced glycation end products has been seen in AD brain tissue^{57,58} and together with loss of LRP1,⁵⁷ which traffics A β out of the brain, leads to A β

accumulation seen in AD.⁵⁷ Inhibition of CypA by cyclosporine reduces A β accumulation in *APOE4*-induced mural cells.⁵¹ However, the relationship is likely more complex, with a previous study¹²⁰ finding no effect of CypA inhibition on A β pathologic findings in an *APOE4* mouse model but an involvement in improved BBB integrity, prevention of neuronal loss, and behavioral deficits. Overall, inhibition of CypA appears to improve BBB integrity and reduce neuronal loss and behavioral deficits in several rodent models.^{38,120} In summary, neuroinflammation contributes to pathologic findings of dementia through increased leukocyte trafficking into the brain, CypA-MMP9 disruption to the ECM and tight junctions, and possible increased A β accumulation in the brain, leading to BBB breakdown through abnormal pericyte-endothelial cell-mediated signaling.

Conclusion

The brain environment is delicate and requires careful balance of homeostatic pathways. The BBB is important in maintaining this balance by controlling interactions with the systemic circulation. Disruption to the BBB can lead to pathologic findings and can be the cause or consequence of disease. This review described how endothelial cells and pericytes perform a vital role in maintaining the BBB through intricate crosstalk and how this is altered in both cSVD and AD. It highlighted the overlap of pathologic findings between these two major forms of dementia by focusing on the many ways in which disturbance of pericyte-endothelial cell communication undermines the BBB. There is still much to be understood about endothelial-pericyte crosstalk and many pathways yet to be elucidated. However, understanding the careful signaling and its dysregulation in dementia will allow for the development of future therapies to help with what has become one of the most important diseases in the aging population—dementia.

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