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Review Article

Pericytes and the Neurovascular Unit: The Critical Nexus of Alzheimer Disease Pathogenesis?

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Abstract

Alzheimer disease (AD) has been viewed as the quintessential neurodegenerative disorder, and has defied decades of extensive research to find safe and effective disease-modifying treatment approaches. However, over the last 15–20 years, a new focus has developed on the role of vascular dysfunction in AD. Key to this approach is the consideration of the non-neuronal cells and other structural elements comprising the neurovascular unit (NVU), in particular pericytes. This review will examine the role of pericytes and the NVU in AD pathogenesis and the manner in which they interact with traditional factors, such as neuroinflammation, amyloid-beta, and apolipoprotein E. Based on the emerging evidence of the unique properties of pericytes, these "forgotten cells" might represent a crucial nexus for solving the mysteries of AD.

Introduction

Alzheimer disease (AD) is the pre-eminent enigma in clinical neuroscience. The disorder was first described in 1907 by the German psychiatrist and neuropathologist Alois Alzheimer.[1,2] To date, AD has resisted sustained efforts to develop effective disease-modifying therapies. From the first-generation cholinesterase inhibitor, tacrine,[3] to second-generation agents, including donepezil[4] and memantine,[5] to the development of monoclonal antibodies, such as solanezumab[6] and aducanumab,[7] investigators have failed to achieve satisfactory clinical endpoints. Some therapies have proved to be dangerous and serious adverse events have been observed, such as hepatotoxicity,[8] meningoencephalitis,[9] and cerebral edema.[10]

From its initial description, AD has been conceptualized as representing the quintessential, pure neurodegenerative disorder. However, this perspective of AD belies its complexities and represents one of the false dichotomies that remain in the neurosciences. The most notable of these might be the outdated (yet still widely taught in medical schools and residency programs) distinction between "organic" versus "functional" disorders in neurology and psychiatry. As neuroscience researchers have expanded the knowledge base, the array of disordered functions at the molecular, cellular, behavioral, and cognitive levels has been revealed.

The previous view of AD pits it against vascular dementia (VD). Diagnostic classification systems of dementia syndromes have traditionally assumed that a clear and reliable distinction exists between AD and VD. In recognition of its own complexities, the conceptualization of VD has undergone revision. The original terminology—multi-infarct dementia[11]—was changed to its current and more inclusive nosology, which reflects the fact that small-vessel disease contributes more frequently to cognitive decline than the accrual of large-vessel infarctions.[12] Throughout these changes in thinking and terminology, VD has continued to be viewed as the prime example of dementia caused by vascular pathology.

Accumulating evidence has compelled researchers and clinicians to reconcile these findings and develop more refined and comprehensive hypotheses. It is now understood that the pathophysiology of AD is not limited to the classical view of neuronal degeneration. It also involves the full complement of glia, and cells that comprise the cerebral vasculature and other structural components, which are collectively referred to as the neurovascular unit

Keywords: Pericyte; Neurovascular unit; Blood-brain barrier; Alzheimer disease; Neurodegenerative disorders; Amyloid-beta; Apolipoprotein E.

Abbreviations: Aβ, amyloid-beta; ACH, amyloid cascade hypothesis; AD, Alzheimer disease; AGE, advanced glycation end products; APOE, apolipoprotein E; APP, amyloid precursor protein; BBB, blood-brain barrier; CAA, cerebral amyloid angiopathy; CVD, cerebrovascular disease; ET-1, endothelin-1; IL, interleukin; LRP-1, low-density lipoprotein receptor-related protein-1; NOX, nicotinamide adenine dinucleotide phosphate oxidase; NVU, neurovascular unit; PDGF, platelet-derived growth factor; PDGFR-β, platelet-derived growth factor-beta; RAGE, receptor for advance glycation end products; ROS, reactive oxygen species; TNF, tumor necrosis factor.

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(NVU). This review presents the evolution of knowledge on AD pathogenesis, beginning with the initial identification of the principal risk factor for sporadic AD, the discovery of a range of autosomal dominant mutations that cause familial AD, and the insights obtained from those rare cases. Then, it will discuss the increased prominence of the role of vascular mediators for AD and consider the specific processes through which the new data on the blood-brain barrier (BBB), pericytes, and other components of the NVU could provide a basis to integrate previously disparate factors into a comprehensive modern viewpoint.

The classical view of Alzheimer disease pathophysiology: background and context

Factors that were initially identified as principal contributors to AD pathophysiology were amyloid-beta (Aβ) protein, tau protein, and apolipoprotein E (APOE). Each will be considered individually. APOE was the first established risk-mediating variable associated with sporadic AD.[13] In particular, the ε4 allele of APOE (APOE4) confers an elevated risk of sporadic AD. APOE4 operates dose-dependently. Heterozygosity for ε4 results in a 2–5 fold increased risk for the development of AD; ε4 homozygosity increases AD risk by approximately 5–10 fold.[13–15] In contrast, the APOE ε2 allele is protective, whereas the ε3 allele is neutral for AD risk.

Despite the evidence that APOE confers substantial risk of AD, questions remained regarding the nature of its contribution to AD pathogenesis until recently. Ultimately, investigators determined that APOE interacts with Aβ by functioning as a chaperone or catalytic protein. APOE2 inhibits the polymerization of Aβ monomers to form toxic oligomeric species but APOE4 promotes β polymerization,[16,17] findings that are consistent with epidemiological studies of AD risk. In addition, APOE is an important component that regulates the clearance of Aβ from the extracellular space. The APOE4 variant is the least efficient at clearing Aβ.[18]

Around the time that the importance of APOE was recognized, advances in genetic sequencing analyses allowed for the rapid identification and characterization of mutations associated with clinical presentations of early-onset familial AD (EOFAD). The first was identified in 1991, affecting amyloid precursor protein (APP), and accounts for approximately 15%–20% of all EOFAD cases.[19] The gene for APP resides on chromosome 21. Overexpression of APP explains the high incidence of AD among individuals with trisomy 21—Down syndrome—due to the extra chromosome.[20] Symptom onset typically occurs in the late fourth to fifth decade of life among people affected by Down syndrome, which reflects the dose-dependent nature of APP translation.

APP is a single-pass transmembrane protein that is involved in synapse production and maintenance and cellular signaling.[21] APP undergoes sequential cleavage by the gamma (γ)-secretase complex, which yields a specific set of polypeptide products.[22] The most important is Aβ, one of the principal markers of AD pathology. When APP undergoes γ-secretase processing, Aβ monomers are released primarily into the extracellular space. Aggregation of Aβ begins when amyloidogenic monomers attract and attach to additional colocated Aβ monomers to form oligomers. Aβ oligomers appear to be the most toxic form of Aβ, particularly the production of 40- and 42-residue isoforms via the amyloidogenic pathway. In addition to their propensity to aggregate, Aβ oligomers exert direct neurotoxic effects.[23]

Accumulation of oligomers leads progressively to the production of insoluble Aβ fibrils, which self-propagate to form plaques[24] that are highly resistant to proteolysis and clearance. Regions of Aβ seeding then continue to propagate along interconnected neuroanatomic pathways,[25] in a manner similar to the process that occurs in prion diseases.[26] Aβ fibrils and mature plaques induce an inflammatory response that is mediated by astrocytes and activated microglia, which generate reactive oxygen species (ROS) and leads to oxidative stress and apoptosis.[27] In addition to increased production, dysfunctional clearance of Aβ has been implicated in AD pathophysiology.[28] Aβ clearance occurs via several mechanisms: glial endocytosis, proteolytic enzymatic degradation, transport across the BBB mediated by low-density lipoprotein family receptors, activation of the complement arm of the immune response, and passive drainage through interstitial perivascular spaces and specialized lymphatic vessels, referred to as the glymphatic system.[29,30] Events subsequent to Aβ deposition and aggregation—neuroinflammation, generation of ROS, excitotoxicity, tau hyperphosphorylation, microtubule disruption, and ultimately neuronal apoptosis—are referred to as the amyloid cascade hypothesis (ACH).

The ACH initially came under criticism when studies showed that the correlation between cerebral Aβ burden and cognitive function was weak[31] and when evidence of neuronal injury was observed to precede Aβ deposition, such as in a murine model that over-expresses Aβ.[32] These findings raised doubts that the ACH was sufficient to account for AD pathophysiology. Accordingly, increased attention turned toward the other major marker of neuropathology in AD, tau protein. Microtubules are composed principally of the protein tubulin; together with tau, these proteins form a critical structural component of neurons. Tau is a soluble phosphoprotein that acts to stabilize microtubules,[33] and performs several other important functions which include providing intracellular axonal transport, regulating synaptic plasticity, and supporting the structural integrity of intraneuronal signaling pathways.[34]

Under pathological conditions, tau becomes hyperphosphorylated, which decreases the affinity of tau toward tubulin and leads to the dissociation of tau from microtubules, thereby resulting in their destabilization and the formation of insoluble tau aggregates. These events contribute to structural degradation and lead to neuronal death.[35] The characteristic pathological marker of tau dysfunction is the flame-shaped neurofibrillary tangle that, when colocated with aggregations of Aβ, are referred to as neuritic plaques. In contrast to the weak relationship between Aβ levels and cognitive impairment, tau density is strongly correlated with dementia staging.[36]

Subsequent research revealed that, although tau protein is an important mediator of AD pathogenesis, Aβ was a necessary condition for the development of the cognitive syndrome of AD.[37] For example, when Aβ was absent, there was no association between tau binding and hippocampal volume. In contrast, in the presence of Aβ, tau binding was greater and was associated with lower hippocampal volume.[38] Apart from modifications to the ACH, investigators believed that other more fundamental factors contributing to AD pathogenesis were likely to play an important role.

Relationships between Alzheimer disease and vascular risk

The early conceptualization of AD focused heavily on the neurodegenerative aspects of its pathophysiology. In addition, cerebrovascular disease (CVD) and related dementia syndromes, as exemplified by VD, were viewed as occupying two poles of a single spectrum, with neurodegeneration at one end and vascular pathology at the other. However, overlaps between AD and CVD have been uncovered; among the earliest research to demonstrate an overlap was the Rotterdam study.[39] Of note, Alois Alzheimer

found[1] that neurofibrillary tangles co-existed with cerebral microvascular arteriosclerotic disease,[40] the first clue into the role of vascular pathology in neurodegeneration.

Several factors identified as promoting risk for CVD have been shown to confer risk for AD. These include hypertension, hyperlipidemia, type 2 diabetes, and cigarette smoking.[41–45] These shared risk variables raised new questions about the processes that underlie the generation of AD pathology. This view coincided with an increased awareness of the relevance of changes that involve the cerebral microvasculature in AD,[42,46–47] and suggested that rather than existing as discrete entities, neurodegeneration and microvascular disease constituted two ends of a common spectrum of pathology.[48] Therefore, research into factors that mediate vascular damage in AD began.

The most direct relationship between AD-type pathology and CVD manifests as an intramural deposition of Aβ within cerebral arteries, arterioles, and capillaries, as well as meninges, a condition that is called cerebral amyloid angiopathy (CAA).[49] The functional effect of Aβ infiltration is a weakening of vessel walls, which makes them susceptible to leakage, results in microhemorrhage,[50] and less frequently, rupture causing large-vessel hemorrhage. Accordingly, CAA is a major risk factor for stroke.[51] Although CAA and AD have overlapping features, they are considered distinct diagnostic entities. As with AD, most CAA cases are sporadic. Increased production and diminished clearance of Aβ have been implicated as underlying mechanisms of sporadic CAA.[29] CAA and AD share APOE4 as the primary risk factor for sporadic disease.

However, there are important distinctions between these diseases.[52] Aβ40 is the predominant species deposited in CAA as opposed to Aβ42, which dominates in AD. In addition, APOE2 is protective in AD, but it is associated with an enhanced risk of blood vessel breakdown in CAA. Familial, or hereditary CAA (HCAA) is caused by a variety of rare autosomal dominant mutations. Six HCAA variants have been identified to date, and only the Dutch-type is caused by a mutation to APP on chromosome 21, as in familial AD. All other HCAA variants consist of mutations that code for amyloid proteins other than Aβ.

The BBB and neuroinflammation

The BBB is composed of specialized endothelial cells that form the walls of all cerebral vessels. These cells are interconnected by tight junctions that strictly limit permeability bidirectionally and serve to compartmentalize the parenchyma from the blood. The BBB is a component of a larger, integrated set of elements that comprise the NVU, which will be discussed in the following sections.

Dogma once held that the brain was immune-privileged; that is, it was assumed that innate and adaptive immune system components were sequestered from the brain by the BBB. However, the BBB is neither impenetrable nor impervious as previously understood. B lymphocytes migrate from the periphery across the BBB,[53] where they become activated and perform immune regulating functions within the central nervous system (CNS).[54] These activated microglia secrete interleukin-6 (IL-6) within the CNS compartment.[55] In addition to local cytokine production, circulating IL-1α and tumor necrosis factor-alpha (TNF-α)[46,56] enter the CNS via active transport.

Complex links between neuroinflammation and BBB function have been discovered. Endothelial cells secrete pro-inflammatory cytokines. When exposed to Aβ40 species *in vitro*, cultured human brain endothelial cells respond by up-regulating gene expression for inflammatory cytokines IL-1β and IL-6.[57] Endothelial cells are primary regulators of Aβ influx into the brain[58] via receptors for advanced glycation end products (RAGE),[59] a process that contributes to the propagation of the inflammatory response. Pro-inflammatory cytokines IL-2 and IL-6,[60,61] regulate BBB permeability. Evidence indicates that age-related BBB disruption is more pronounced among individuals with cognitive dysfunction.[62] Similarly, levels of IL-1β, IL-6, and TNF-α in endothelial cells are higher in AD patients than in cognitively intact individuals.[63]

Pericytes and the neurovascular unit: overview

The NVU consists of three major components: neurons, glia, and vascular cells. The vascular cells and their interactions with the other components have been the target of recent research. In addition to the endothelial cells, the basal lamina (also referred to as the basement membrane), microvascular smooth muscle cells, and pericytes comprise the vascular component of the NVU. The endothelium forms the first structural layer of the NVU and is in direct contact with plasma and other blood components. The second cellular layer of the NVU consists of the end feet of astrocytes, the basal membrane, which is an extension of astrocyte end feet, and the pericytes. Perictyes envelop cerebral capillaries, and precapillary arterioles and venules, and make anatomical contact with endothelial cells (Fig. 1).[64] A host of disturbances that involve pericytes have been shown to occur during physiological aging. Disturbances of pericyte function might represent a unifying feature of disparate lines of evidence that accounts for age-related neuronal loss, neurodegeneration, and the pathophysiology of AD (Fig. 2).

Pericytes and aging

One consequence of physiological aging is the increased production and diminished scavenging of various ROS, in particular, the free radical superoxide anion (O_2^-). Increased ROS levels affect many organ systems, including the cerebral microvasculature. The accumulation of ROS species gradually leads to mitochondrial dysfunction, DNA damage, and apoptosis. In addition, O_2^- breaks down nitric oxide (NO), which is a regulator of vascular tone in its role as a vasodilator. Aging is associated with reduced bioavailability of NO in multiple organ systems.[65] Perictytes are among the cells that are subject to the cumulative deleterious effects of age-associated ROS overexpression.

Aging pericytes develop certain deviations of their ultrastructural elements; these include intracellular inclusions, pinocytotic vesicles, enlarged lipid granules, and mitochondrial abnormalities, all of which suggest cellular dysfunction, degeneration, or both.[66] Alterations of desmin protein filaments in pericytes have been observed,[67] which suggests a disturbance of their cellular structure. In human elders, pericytes become depopulated[68] and show a significant reduction in their area of capillary coverage.[69]

The precise roles that pericytes play under optimal physiological conditions and during normal aging have not yet been fully elucidated. Research over the past 10–15 years, however, has revealed several key functions that these cells perform during the development and maintenance of cerebral microcirculation. Pericytes control microvascular blood flow directly via contraction, which causes capillary constriction[70] and, under pathological conditions, ischemia which might lead to localized hypoxemia.[71] They play an important role in angiogenesis[72]; associated properties include migration and variability in phenotype, alignment, and endothelial cell contact. Pericytes guide and determine the direc-

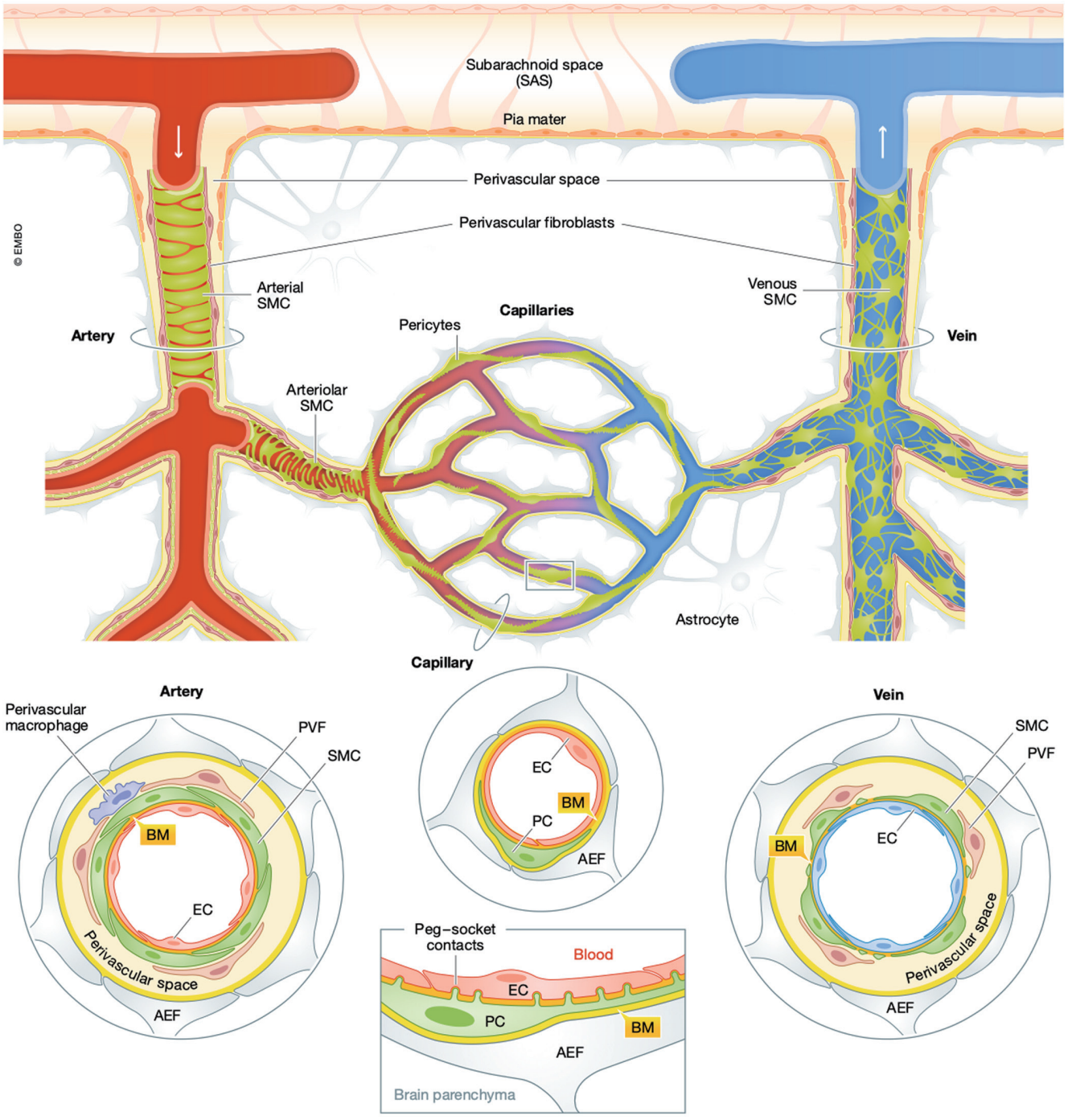

Fig. 1. Schematic representation of the neurovascular unit.[64] Penetrating arteriole branches into arterioles and capillaries, which then drain via venules into veins, returning to subarachnoid space. Schematic cross sections of arterial, capillary, and venous levels are shown, each with its vessel-associated cell types. The box depicts how pericytes establish direct connections with endothelial cells through peg-socket contacts. AEF, astrocyte endfoot; BM, basement membrane; EC, endothelial cell; PC, pericyte; PVF, perivascular fibroblast; SMC, smooth muscle cell. Source: Lendahl *et al.* 2019, Figure © EMBO. Reproduced under the terms of the Creative Commons Attribution 4.0 License which permits use, distribution, and reproduction in any medium, provided the original work is properly cited.

tion and branching of newly formed blood vessels,[73] prevent vessel regression,[74] and promote endothelial cell survival.[75] Pericytes in the hypothalamus serve an important role in the regulation of glucose levels through insulin signaling. In a murine model, pericytes increased insulin sensitivity in hypothalamic neurons in a dose-dependent fashion; neither astrocytes nor vascular smooth muscle cells contributed to that process.[76] This finding suggests that hypothalamic pericyte loss might be implicated specifically

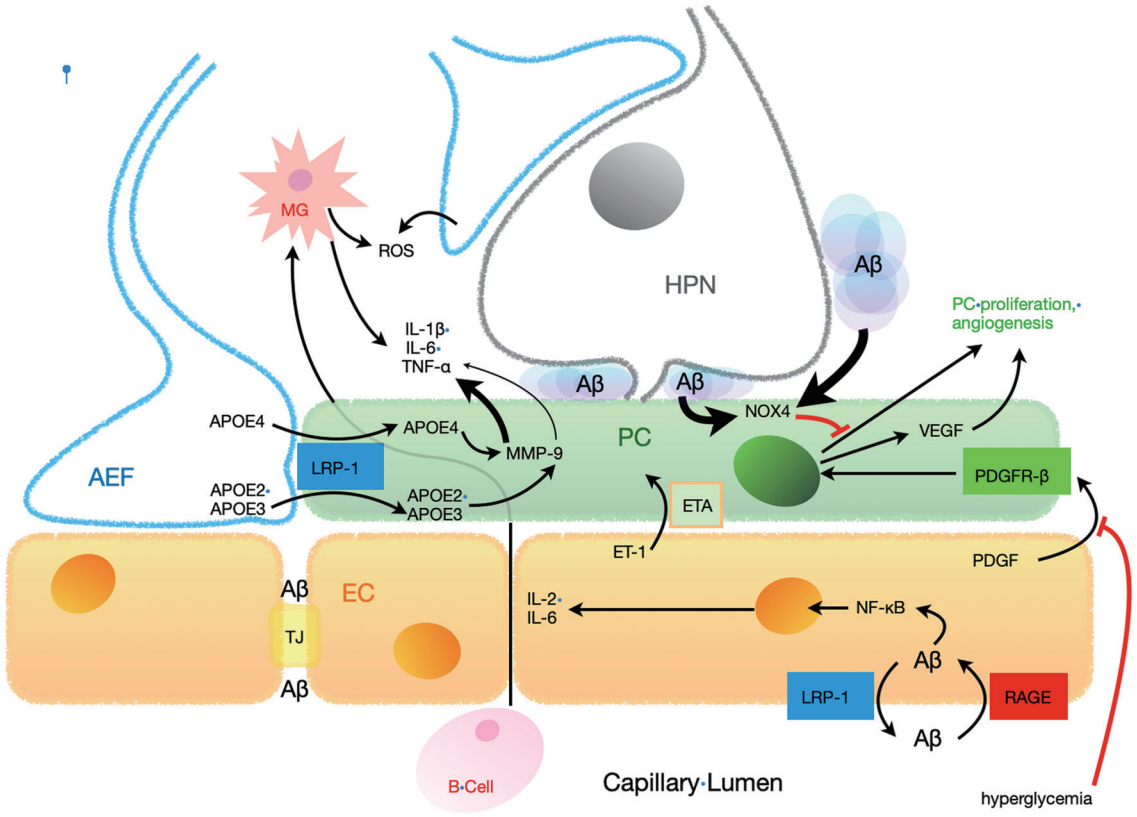

Fig. 2. Idealized longitudinal cross section of the neurovascular unit summarizing molecular mechanisms of pericyte regulation and their relationships with AD pathophysiology. Microglia release ROS and inflammatory factors in the presence of Aβ aggregates. Accumulating extracellular Aβ is transported into endothelial cells and perictyes via RAGE, triggering transcription factors, such as NF-κB and MMP-9 to produce IL-x and TNF-α, setting up a feed-forward loop of accelerating Aβ production and neuroinflammation. APOE4 secreted by astrocytes is taken up by perictyes via LRP-1 and further promotes release of inflammatory factors, but APOE2 and APOE3 inhibit that pathway. Aβ induces a significant increase in NOX4 that inhibits pericyte proliferation and downstream angiogenesis. Aβ interferes directly with tight junction proteins, increasing BBB permeability. ET-1 secreted by endothelial cells binds to ETA receptors on perictyes, causing them to contract, leading to capillary constriction. Hyperglycemic states inhibit the interaction between PDGF secreted by endothelial cells and its receptor PGDFR-β found on perictyes, contributing to pericyte apoptosis, endothelial proliferation, capillary remodeling and BBB breakdown. Aβ, amyloid beta; AEF, astrocyte endfoot; APOEx, apolipoprotein Ex; BBB, blood-brain barrier; EC, endothelial cell; ET-1, endothelin-1; ETA, ET-1 type A receptor; HPN, hippocampal pyramidal neuron; IL-x, interleukin-x, LPR-1, low-density lipoprotein receptor-related protein-1; MG, microglial cell; MMP-9, matrix metalloproteinase-9 complex; NF-κB, nuclear-factor kappa B; NOX4, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase 4; PC, pericyte; PDGF, platelet-derived growth factor; PDGFR-β, platelet-derived growth factor receptor-beta; RAGE, receptor for advanced glycation end products; ROS, reactive oxygen species; TJ, tight junction; TNF-α, tumor necrosis factor-alpha; VEGF, vascular endothelial growth factor. Figure © Steven P. Cercy.

in the dysregulation of insulin sensitivity, which is a fundamental aspect of diabetes.

The molecular mechanisms that drive the formation and maintenance of the cerebral microvasculature are centered on intracellular signaling. Platelet-derived growth factor (PDGF) is secreted by endothelial cells and binds to platelet-derived growth factor receptor-beta (PDGFR-β), which is expressed by pericytes. This ligand-receptor complex activates signal transduction pathways that regulate migration and proliferation of pericytes toward endothelial cells that compose the vascular wall.[77] Experimental models of diabetic retinopathy that used pericyte-deficient mice revealed that chronic hyperglycemia resulted in diminished PDGFR-β signaling, which leads to pericyte apoptosis.[78] Pericyte loss induces endothelial cell proliferation with increased numbers of abnormal acellular capillaries,[74,79] rather than endothelial apoptosis.[80] Downstream events that are related to pericyte changes include significant capillary remodeling, which is characterized by vessel dilation and tortuosity, as well as basal lamina hypertrophy.[67,81]

Subsequent studies revealed the particular importance of pericytes in microvascular regulation; they are the only cells of the NVU that express PDGFR-β to enable a response to PDGF.[80] Moreover, soluble PDGFR-β has been identified as a specific marker of pericyte injury.[82] In addition to PDGF, vascular endothelial growth factor is secreted by pericytes under hypoxic conditions, which stimulates proliferation and migration of additional pericytes.[83] Nuclear factor-kappa B (NF-κB), an important transcription factor that mediates the inflammatory response, is activated in a subpopulation of pericytes in response to exercise, which promotes angiogenesis.[84]

In concert with their angiogenic properties, pericytes are crucial to maintain the integrity of the BBB. Vascular remodeling that occurs with pericyte apoptosis contributes to capillary destabilization,[67] which causes the breakdown of the BBB. Pericytes regulate the formation of endothelial tight junctions that modulate vascular permeability. Pericyte structural deterioration and apoptosis via any mechanism results in reduced contact with and coverage of endothelial cells[67,85] and the breakdown of the BBB, consequently permitting the influx and accumulation of serum proteins includ-

ing the key coagulation macromolecules thrombin, fibrinogen, and fibrin—which thrombin cleaves from fibrinogen—as well as plasmin and hemoglobin.[80]

Elevated thrombin in AD[86] contributes to neuronal loss, vascular injury, and cognitive impairment.[87] Fibrinogen exacerbates the neurotoxic effects of Aβ.[88] Moreover, fibrinogen leakage from injured cerebral microvessels activates resident microglia bearing CD-11b receptors, which then prune neuronal dendritic spines and whole dendrites.[89] Protein influx from all sources contributes to cerebral edema that might cause further capillary compression.[90] The NF-κB signaling pathway is a fundamental link between thrombosis and inflammation.[91] Free iron from degraded hemoglobin generates ROS.[92] All of these aberrant processes contribute to secondary neuronal degeneration.[90,93]

Pericytes and AD pathophysiology

Based on the foregoing research findings, the issues to be determined are to what extent pericyte dysfunction contributes to or interacts with classical AD pathophysiological processes, and by what mechanisms those interactions occur. Pericyte degeneration begins early in AD, particularly in hippocampal neurons, which are among the first cells involved in AD pathology. Reductions in pericyte coverage correlate inversely with evidence of BBB permeability,[62] as measured by hippocampal levels of plasma proteins, including immunoglobulin G and fibrin.[94] Elevated soluble PDGFR-β in cerebrospinal fluid is an early biomarker of cognitive dysfunction, which is independent of Aβ and tau levels.[82,95] This shows that pericyte-specific dysfunction, which is characterized by vascular regression and disrupted vascular permeability, is associated with key attributes of AD pathology.[93]

Pericytes, APOE and Aβ

As APOE and Aβ functions have been further elucidated, their relationships with pericytes and other elements of the NVU have come into focus. APOE2 and APOE3 are secreted by astrocytes and taken up by pericytes via low-density lipoprotein receptor-related protein-1 (LRP-1), where they inhibit a key pro-inflammatory pathway, the cyclophilin A-nuclear factor B-matrix metalloproteinase 9 complex. However, APOE4 promotes that pathway,[66,96] which directly increases pericyte injury[97] and impairs the formation of basement membranes.[98] Thus, APOE4 accelerates pericyte loss relative to carriers of APOE2 and APOE3 alleles.

It is well known that in AD, cerebral capillary constriction is provoked by Aβ via the production of ROS.[99] However, the mechanisms of this and locus of action remained unclear. Aβ enhances the activity of nicotinamide adenine dinucleotide phosphate oxidases (NOXs) to form reactive superoxides. NOX4 is found in pericytes and endothelial cells. In a transgenic mouse model, Aβ increased NOX4 levels sevenfold, and the blockade of NOX4 prevented capillary constriction following the application of Aβ. In contrast, NOX2, which is found in macrophages, increased by only twofold in the presence of Aβ, and blocking NOX2 produced a substantially attenuated effect on capillary relaxation. Similar findings were elicited using human brain slices treated using comparable methods.[89]

RAGE, which is expressed by neurons, glia, vascular smooth muscle cells, endothelial cells, and pericytes, has a crucial role in the influx of peripheral Aβ into the parenchyma. RAGEs bind to Aβ and a broad range of compounds that are referred to as advanced glycation end products (AGEs), also known as glycotoxins. These compounds are produced as a normal consequence of lipid and protein metabolism and are present in foods that are cooked at high temperatures. During physiological aging, AGEs accumulate progressively within all cells. However, in AD and diabetes, this normal process is accelerated. AGEs are present within Aβ plaques and neurofibrillary tangles of patients with AD. Accumulated AGEs contribute to the induction of oxidative stress by glial cells.[100] Increases in RAGE protein and RAGE-expressing microglia occur in AD, and correlate with disease severity.[101]

Pericytes are crucial for regulating Aβ trafficking between the BBB and parenchyma via the LRP-1 and RAGE pathways described previously. They assist with Aβ clearance by phagocytosis and promote Aβ efflux from parenchyma via the LRP-1 pathway.[102] Despite this role, pericytes remain susceptible to Aβ toxicity.[103] In AD, the accumulation of Aβ within pericytes leads to dysmorphic remodeling[104] and apoptosis. In a transgenic model of pericyte-deficient viable mice overexpressing APP, degeneration and loss of pericytes resulted in elevated levels of Aβ40 and Aβ42 in the brain, as well as increased levels of tau not typically observed in this transgenic model. Reinforcement of this destructive cycle occurs when reduced cerebral blood flow leads to further synthesis and accelerating burden of Aβ, worsening pericyte apoptosis, progressive microvascular injury, and cognitive impairment.[102]

Endothelin-1 (ET-1), which is a powerful vasoconstrictor that is secreted by endothelial cells, also appears to play an important role in Aβ-mediated vascular insult. Levels of ET-1 are elevated in individuals with AD, and are up-regulated by Aβ.[105] Subsequent research revealed that Aβ oligomers induced the release of ROS in pericytes, which enhances transcription and release of ET-1,[106] which then binds ET-1 type A (ETA) receptors. It is this ligand-receptor complex that causes pericytes to contract, which leads to capillary constriction, and results in hypoxic ischemia, local hypoglycemia, and neuronal loss in affected microregions. Constriction of capillaries worsens with increasing Aβ load.[107] Furthermore, BBB disruption is directly related to the influx of circulating Aβ into brain parenchyma.[94]

The intramural deposition of Aβ within the walls of the cerebral vasculature that occurs in CAA is an important yet overlooked component of AD pathology.[108] Cultured human brain pericytes exposed *in vitro* to the Dutch-type mutation that affects the Aβ40 species is the predominant species that induces the formation of Aβ fibrils at the cell surface, which leads to pericyte degeneration. Insulin appears to inhibit Aβ fibril formation in a dose-dependent manner, further suggesting that insulin might be involved in the regulation of Aβ fibrillization and might mitigate Aβ-induced pericyte loss in AD.[109] In humans with CAA, dystrophic neurites are found within the perivascular spaces of capillaries in occipital association cortex, more so than in primary occipital cortex. In addition, the density of dystrophic neurites correlates with Aβ levels.[108] Relationships among insulin regulation, Aβ fibril formation, and the deposition of Aβ in intramural and perivascular spaces have implications for diabetes and the risk it poses for the development of sporadic AD.

Aβ interferes directly with the integrity of endothelial tight junctions, which are regulated by pericytes and leads to an inappropriate increase in BBB permeability.[60] In addition, Aβ stabilizes fibrin clots;[110] increased fibrin deposition has been observed in vessels that are affected by CAA.[74] Of note, age-dependent vascular damage in pericyte-deficient mice precedes neuroinflammation, neurodegeneration, and consequent impairments in learning and memory.[80] This is consistent with cerebral hypometabolism outweighing the degree of volume loss among humans who are presymptomatic EOFAD mutation carriers.[111]

Pericytes and tau

In addition to the deleterious influence of Aβ on the BBB that was identified recently, tau plays a significant but less understood role in the regulation of BBB integrity. In a transgenic mouse model, age-related increases in tau were associated with increased BBB permeability against erythrocytes, peripheral T lymphocytes, and immunoglobulin G. This effect was first observed in the hippocampus,[112] where the earliest tau aggregation occurs in AD. The migration of cells and molecules of the immune system, which is facilitated by a leaky BBB, helps to drive neuroinflammation.[113] However, detectable neuroinflammation and neurodegeneration do not occur until well after the initiation of BBB deterioration.[112] One mechanism through which tau might influence BBB permeability occurs through its interactions with tight junctions and adherens junctions between endothelial cells and their actin cytoskeleton proteins. Disrupting this association appears to result in tau-induced neurotoxicity.[114] Pericyte deficiency in a transgenic model of AD in mice led to increased phosphorylation of tau in the hippocampus and cortex.[102] Collectively, these processes could potentially cause a synergistic effect between pericyte loss, tau generation, Aβ accumulation, and neurodegeneration.

Future directions

Based on recent and emerging evidence, pericytes present a novel opportunity to shift the focus of disease-modifying therapies away from failed efforts to break the link between amyloid deposition and cognitive decline. AD pathology appears to develop 15–20 years prior to symptom onset. One likely reason for the failure of available therapies could be that the development of pathology in AD has advanced too far to be amenable to treatment once individuals become symptomatic. Thus, the main thrust of AD research over the last decade has been to identify early markers of AD well in advance of symptom onset.[115]

One potential approach entails a renewed focus on neuroinflammation. However, rather than using traditional NSAIDs, inflammation that is mediated by IL-1β, IL-6, and other pro-inflammatory cytokines could be specifically targeted in an attempt to block Aβ-mediated generation of ROS in pericytes.[116] The Aβ-RAGE-cyclophilin A-nuclear factor B-matrix metalloproteinase 9 cascade, which is an important mechanism of AD pathogenesis via BBB disruption might be a promising potential target for AD therapies.[60]

Agents that inhibit the release of ET-1 or antagonize the ETA receptors on pericytes are worthy of consideration. In a murine model, C-type natriuretic peptide reversed the effects that were mediated by ET-1 and interrupted Aβ-provoked capillary constriction.[105] Depletion of fibrinogen reduces CAA and cognitive decline in transgenic AD mice,[110] and therefore, could represent an important therapeutic target.

Finally, because of their unique properties, in particular, their ability to withstand hypoxic conditions, perictyes should be considered as a viable option for cell-based therapies. In one study, mesenchymal stem cells that had differentiated into pericytes were stereotactically injected into the brains of mice genetically modified as an animal model for AD. The mice showed improved microcirculation and reduced levels of insoluble cortical and hippocampal Aβ,[117] which suggested that pericyte implantation might provide a novel approach to the management of AD. Additional data indicate that pericytes harvested from temporal neocortex and cerebral ventricular zone proliferate readily in culture and are robust under storage.[118,119]

In summary, research into the NVU, in particular, the so-called "forgotten"[120] cell—the pericyte—has yielded valuable insights into the pathogenesis of AD. With this knowledge, there is potential to develop promising avenues for treating what is perhaps the most relentless and refractory disease known to neuroscience.

Conclusions

Converging evidence has revealed that vascular pathology, rather than reflecting collateral or ancillary damage, is central to the pathophysiology of AD. Moreover, the markers for AD pathology that had been considered evidence of pure neurodegeneration, are mediators of vascular pathology. Pericytes, with their unique attributes as a locus for the interaction of multiple factors that contribute to neuronal integrity and stability, are perhaps positioned as a crucial nexus to resolve the pathophysiology of AD and establish a basis for the first effective disease-modifying interventions.

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Conflict of interest

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Author contributions

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