THE VASCULAR DIMENSION

Endothelial VCAM-1, the Aged Systemic Milieu, and the Neurovascular Substrate of the Collapse Trilogy

Zlokovic · Montagne · Nation · Sweeney · Bell
Wyss-Coray · Yousef · Villeda · Iadecola
Nedergaard · Iliff · Mestre · Greenberg
Sperling · van Veluw · Daneman · de la Torre · Kisler

a first-principles companion to the Convergent Synaptic, Homeostatic Microglial, and Bioenergetic Collapse theses

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Dr. James Truchard & Benjamin Aaron Gustafsson

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Fourth-Substrate Chapter of the Collapse Trilogy

Contents

Abstract

The Collapse Trilogy treats late-onset Alzheimer's disease as a sequence of cellular failures internal to the neural parenchyma — bioenergetic exhaustion of the locus coeruleus, loss of the Butovsky-maintained homeostatic microglial signature, and digestion of the parvalbumin perineuronal net by matrix metalloproteinases. The trilogy's account of the central nervous system, however, treats the cerebrovasculature as a passive conduit and the blood-brain barrier as a static partition between parenchyma and periphery. This treatment is anatomically incorrect and mechanistically misleading. The cerebrovasculature is a continuously active homeostatic system whose component cells — endothelial cells, pericytes, smooth muscle cells, perivascular macrophages, and the astrocytic endfeet that complete the neurovascular unit — execute a daily quantitative load of solute transport, blood flow regulation, immune surveillance, and waste clearance comparable to that of any parenchymal compartment. The cerebrovasculature is, moreover, the first cellular substrate at which the systemic aging program imposes a load on the central nervous system: the brain endothelium is the point of contact between an aged plasma proteome and the parenchymal cells that the trilogy treats, and the brain endothelium is the cellular locus at which that aged plasma is read, transduced, and converted into parenchymal signals. This paper develops the case that the cerebrovasculature is the fourth substrate of late-onset Alzheimer's pathogenesis, parallel and prior to the three substrates of the trilogy, and that any account of disease initiation that omits the vascular dimension is structurally incomplete. The central molecular thesis of the paper is that vascular cell adhesion molecule-1 (VCAM-1), an inducible immunoglobulin-superfamily adhesion molecule expressed on brain endothelial cells in a strictly age-dependent and inflammation-dependent fashion, is the proximate molecular gateway through which the aged systemic milieu accesses the parenchymal compartment and through which the trajectory of brain aging is set. The Yousef–Wyss-Coray work establishes VCAM-1 as the most age-upregulated soluble protein in mammalian plasma, identifies brain endothelial VCAM-1 as the necessary mediator of aged-plasma toxicity to the hippocampal neurogenic niche, and demonstrates that endothelial-specific VCAM-1 ablation or antibody blockade reverses microglial reactivity, restores neurogenesis, and rescues cognition in aged mice. The CSF VCAM-1 elevation across the preclinical, prodromal, and dementia stages of Alzheimer's disease, the correlation of VCAM-1 with tau and cortical thinning, and the BACE2-specific shedding of VCAM-1 that links the molecule to amyloid metabolism collectively establish that VCAM-1 is the integrative molecular node of the vascular dimension and the appropriate molecular focus of any account that treats vascular aging as causally upstream of parenchymal failure. The paper develops the architecture of the neurovascular unit, the cellular biology of the blood-brain barrier, the pericyte-endothelial axis articulated by Berislav Zlokovic's two-hit vascular hypothesis, the VCAM-1 mechanism in detail, cerebral amyloid angiopathy and the ARIA constraint that vascular pathology imposes on anti-amyloid monoclonal antibody therapy, neurovascular coupling and the bioenergetic bottleneck, glymphatic clearance failure

and the sleep-vascular interaction, small vessel disease and the vascular-AD continuum, APOE4 as a vascular genotype, and the integration of the vascular substrate with each of the three theses of the trilogy. The central claim is that the vascular dimension is not a comorbidity or a secondary contributor to Alzheimer's pathogenesis but the first cellular substrate at which the disease can be detected, the cellular substrate at which the aged systemic milieu meets the parenchyma, and the appropriate locus of intervention for the Phase 0 stage that precedes the bioenergetic ignition of the brainstem and the microglial transition of the parenchyma.

1. Introduction: Why the Vasculature Belongs in the First Principles

The architecture of the Collapse Trilogy locates the cellular substrates of late-onset Alzheimer's pathogenesis within the neural parenchyma. The Bioenergetic Collapse thesis traces the disease's earliest substrate to the locus coeruleus and to the cellular biology of catecholamine metabolism, with NAD depletion under sustained PARP-1 hyperactivation as the proximate driver of brainstem aminergic failure across the third through fifth decades. The Homeostatic Microglial Collapse thesis traces the disease's principal middle-phase substrate to the parenchymal microglia of the hippocampus and cortex, with the loss of the Butovsky-defined TGF-β/SMAD-maintained homeostatic signature as the proximate driver of disease-associated microglial trajectories across the fifth through seventh decades. The Convergent Synaptic Collapse thesis traces the disease's principal late-phase substrate to the parvalbumin-positive interneuron populations of the hippocampus and cortex, with the matrix metalloproteinase digestion of perineuronal nets and the consequent loss of gamma-frequency drive as the proximate executor of structural disintegration across the seventh decade and beyond. The three theses together specify the cellular substrates, the proximate drivers, and the temporal sequence of late-onset Alzheimer's pathogenesis with a mechanistic precision that has been the principal contribution of the Organic Network Synthesis methodology to the prize corpus.

The architecture has, however, a structural assumption that has not been examined explicitly in any of the three theses individually nor in the integrative companion papers that have been produced to bridge them. The assumption is that the cellular substrates of disease initiation are parenchymal — that the disease begins, in each of the three theses, within a neuronal or glial population whose internal biology is the appropriate focus of the analysis. This assumption treats the cerebrovasculature, in effect, as a passive conduit through which oxygen, glucose, and metabolic substrates pass to the parenchyma and through which the waste products of parenchymal metabolism pass back to the systemic circulation. The cerebrovasculature is, on this treatment, a plumbing system whose only relevance to disease pathogenesis is its eventual failure as a

downstream consequence of parenchymal pathology — capillary degeneration secondary to amyloid deposition, blood-brain barrier compromise as a consequence of inflammation, microbleeds as the iatrogenic cost of anti-amyloid monoclonal antibodies. The vasculature is, on this treatment, a downstream substrate whose pathology is interpretable only in light of the upstream parenchymal events.

This treatment is anatomically and physiologically untenable. The human cerebrovasculature contains approximately four hundred miles of capillary length in a single brain, with capillary density in cortical gray matter approaching the density of neurons themselves and with no cortical neuron more than approximately twenty micrometers from the nearest capillary lumen. The brain consumes approximately twenty percent of cardiac output and twenty-five percent of total body glucose despite constituting approximately two percent of body mass, and this disproportionate metabolic demand is met by a moment-to-moment regulation of regional cerebral blood flow that is the most precise vasoregulatory system in the body. The blood-brain barrier formed by brain capillary endothelial cells is the most selective biological membrane known, with transendothelial electrical resistance exceeding one thousand ohm-square-centimeters and with active transport systems that distinguish among nutrients, metabolites, and toxins at the single-molecule level. The endothelial cell is not, on any honest reading of the cellular biology, a passive partition; it is a continuously active homeostatic cell that executes a daily quantitative load of solute transport, blood flow regulation, immune surveillance, and waste clearance comparable to that of any parenchymal cell.

The cerebrovasculature is, moreover, the first cellular substrate at which the systemic aging program imposes a load on the central nervous system. The aged plasma proteome — the population of soluble factors that accumulate in systemic circulation across the decades of adult life — does not access the parenchymal compartment directly. Every systemic signal that reaches the parenchyma is first read, transduced, and converted into a parenchymal signal by the brain endothelium. The endothelium is the cellular locus at which the aged systemic milieu meets the central nervous system, and the endothelial response to that milieu sets the trajectory of parenchymal aging downstream. This is not a metaphor or a heuristic; it is a literal statement of cellular biology. Brain endothelial cells express receptors for the inflammatory cytokines, adhesion molecules for the leukocyte populations, and transport machinery for the protein and lipid factors that constitute the aged plasma signal. The endothelial transduction of that signal — through changes in receptor expression, in cytokine secretion into the parenchymal space, in tight junction integrity, in transcytosis kinetics — is the proximate input to the parenchymal cells that the trilogy treats. The microglia, the locus coeruleus, the parvalbumin interneurons, and the dentate granule cells of the parenchyma receive their first signals of systemic aging not directly from the plasma but indirectly, through the endothelial transduction of that plasma into the parenchymal compartment.

This paper develops the case that the cerebrovasculature is the fourth cellular substrate of late-onset Alzheimer's pathogenesis, parallel and prior to the three substrates of the trilogy, and that any account of disease initiation that omits the vascular dimension is structurally incomplete. The argument proceeds in twelve stages after this introduction. We first establish the architecture of the neurovascular unit and the cellular biology of the blood-brain barrier. We then develop the pericyte-endothelial axis as articulated by Berislav Zlokovic and Axel Montagne and the two-hit vascular hypothesis that has emerged from their program. We then develop the central molecular thesis of the paper: vascular cell adhesion molecule-1, its function, its age-dependent expression on brain endothelium, the Yousef–Wyss-Coray mechanism by which it mediates aged-plasma toxicity, and its biomarker behavior in Alzheimer's disease. We then address cerebral amyloid angiopathy and the ARIA constraint that vascular pathology imposes on anti-amyloid monoclonal antibody therapy. We then develop the neurovascular coupling and cerebral blood flow regulation system and the bioenergetic bottleneck that vascular failure imposes on the metabolically demanding parenchyma. We then address the glymphatic clearance system, its dependence on perivascular architecture and on sleep-state arterial pulsatility, and the failure of clearance as a vascular and a sleep-related event. We then address small vessel disease, the vascular cognitive impairment continuum, and the empirical convergence of vascular and Alzheimer's pathology in the human autopsy literature. We then address APOE4 as a vascular genotype with a vascular phenotype that may be temporally and mechanistically prior to its parenchymal phenotype. We then integrate the vascular substrate with each of the three theses of the trilogy. We then address the therapeutic implications of the vascular dimension, with explicit attention to the ARIA cost and to the antihypertensive, GLP-1, and lifestyle interventions whose effect on disease trajectory is mediated through the vascular substrate. We then close with a summary of what this chapter establishes for the trilogy's account of the cellular substrates of late-onset Alzheimer's disease.

2. The Neurovascular Unit: Architecture of an Active Membrane

The classical conception of the blood-brain barrier as a static endothelial partition between the systemic circulation and the parenchymal compartment has been superseded over the past two decades by the conception of the neurovascular unit (NVU) as the relevant unit of analysis. The neurovascular unit is the cellular and extracellular complex that surrounds the brain capillary and that collectively executes the functions of selective transport, blood flow regulation, immune surveillance, and waste clearance that the older conception attributed to the endothelium alone. The NVU comprises five cellular components and one extracellular component, each of which

contributes to the integrated function of the unit and each of which is a candidate substrate for vascular dysfunction in the aged or diseased brain.

The first cellular component is the brain capillary endothelial cell itself, which differs from systemic endothelial cells in three quantitatively decisive respects. Brain endothelial cells form tight junctions sealed by claudin-5, occludin, and the zonula occludens (ZO-1, ZO-2, ZO-3) family of cytoplasmic scaffold proteins, with claudin-5 the principal molecular determinant of paracellular impermeability. Brain endothelial cells exhibit minimal pinocytotic activity and minimal transcytosis under baseline conditions, in contrast to systemic endothelial cells in which transcytosis is the principal mechanism of solute movement between lumen and abluminal compartment. Brain endothelial cells express a specialized complement of transporter and receptor proteins — the GLUT1 glucose transporter, the LAT1 large-neutral-amino-acid transporter, the MCT1 monocarboxylate transporter for lactate and ketone bodies, the LRP1 lipoprotein receptor, the RAGE receptor for advanced glycation end products and for amyloid-β40, and the P-glycoprotein and BCRP efflux transporters that exclude xenobiotics — that collectively define the brain endothelium as a selectively permeable membrane with specific transport directionality for each substrate class. The brain endothelial cell is, on this account, not a passive barrier but an actively curated interface whose substrate specificity is the product of decades of evolutionary selection for the metabolic and protective requirements of the central nervous system.

The second cellular component is the pericyte, a contractile mural cell embedded in the basement membrane immediately adjacent to the endothelial cell and connected to it through peg-and-socket junctions and through soluble paracrine signaling. The pericyte-to-endothelial cell ratio in the brain capillary is approximately one-to-three, the highest of any capillary bed in the body, with pericytes covering approximately thirty percent of the capillary surface area in cortical gray matter. The pericyte contributes to capillary blood flow regulation through its contractile state, to capillary stability through its paracrine support of endothelial tight junctions via PDGF-B/PDGFRβ signaling and via Notch signaling, to immune surveillance through its expression of pattern recognition receptors and its capacity for antigen presentation, and to waste clearance through its participation in the perivascular drainage pathway. The pericyte is the cellular component of the NVU whose age-dependent loss has been most precisely quantified by the Zlokovic program and whose contribution to blood-brain barrier integrity is most directly attested by genetic models of pericyte ablation. The pericyte is, in the Zlokovic two-hit framework developed in section 4, the proximate cellular driver of blood-brain barrier compromise in aging and in early Alzheimer's disease.

The third cellular component is the astrocytic endfoot, the specialized terminal of an astrocyte process that contacts the basement membrane of the capillary and that completes the abluminal coverage of the NVU. The astrocytic endfoot expresses the aquaporin-4 (AQP4) water channel in a polarized distribution, with AQP4 enriched at the endfoot membrane that contacts the basement

membrane and depleted from the membrane that contacts the parenchymal neuropil. This AQP4 polarization is the molecular substrate of the glymphatic clearance pathway developed in section 8, in which cerebrospinal fluid enters the brain along the periarterial space, exchanges with interstitial fluid through AQP4 channels in the polarized endfoot membrane, and exits along the perivenous space carrying solute waste including amyloid-β. The astrocytic endfoot also contributes to neurovascular coupling through its release of vasoactive arachidonic acid metabolites in response to neuronal activity and to NVU integrity through its paracrine support of endothelial tight junctions via secreted signaling factors including sonic hedgehog (Shh) and angiopoietin-1. The loss of AQP4 polarization, observed in aged human brain and accelerated in Alzheimer's disease, is the proximate molecular event by which glymphatic clearance fails, and it is a vascular-glial event rather than a purely glial one.

The fourth cellular component is the vascular smooth muscle cell, present at the level of the arteriole and pre-capillary arteriole, which executes the moment-to-moment regulation of arteriolar diameter and therefore of regional cerebral blood flow. Smooth muscle cells respond to a combination of intrinsic myogenic tone, neural input from the perivascular sympathetic and parasympathetic projections, and local metabolic signals propagated from the parenchyma through the astrocytic and pericytic intermediaries. The smooth muscle cell is the principal cellular substrate of cerebral amyloid angiopathy developed in section 6, in which amyloid-β40 accumulates within and around the smooth muscle cell layer of cortical and leptomeningeal arterioles and progressively replaces the smooth muscle with amyloid deposits, stiffening the vessel and abolishing its capacity for autoregulation.

The fifth cellular component is the perivascular macrophage, a CNS-resident macrophage population distinct from parenchymal microglia, which resides in the Virchow-Robin perivascular space between the basement membrane of the vessel and the glia limitans formed by astrocytic endfeet. Perivascular macrophages express markers including CD163, CD206, and Lyve-1 that distinguish them from parenchymal microglia, and they execute a specialized immune surveillance function at the vascular interface, including the phagocytosis of solutes that have crossed the basement membrane and the presentation of antigens to circulating T cells. The perivascular macrophage is a distinct CNS-border myeloid compartment from the parenchymal microglia treated by the Homeostatic Microglial Collapse thesis, and its dysfunction is a vascular event rather than a parenchymal one. The integration of the perivascular macrophage population with the parenchymal microglial population is addressed in section 11.

The extracellular component of the NVU is the basement membrane, a specialized extracellular matrix layer composed of laminin, type IV collagen, nidogen, and perlecan that surrounds the endothelial-pericyte complex and that anchors the astrocytic endfoot. The basement membrane is the abluminal compartment through which the perivascular drainage pathway operates, and its compositional changes in aging and in cerebral amyloid angiopathy — including

thickening, fragmentation, and the deposition of amyloid-β — are the structural substrate of NVU failure. The basement membrane is, in the Phase II microglial framework, the vascular analogue of the parvalbumin perineuronal net treated by the Convergent Synaptic Collapse thesis: both are matrix-stabilized homeostatic scaffolds whose age- and disease-related degradation by matrix metalloproteinases is a proximate event in the failure of the compartment they stabilize.

The architectural implication of the neurovascular unit framework is that vascular dysfunction in aging and in Alzheimer's disease is not the failure of a single cell type but the coordinated failure of an integrated cellular complex. The endothelial cell, the pericyte, the astrocytic endfoot, the smooth muscle cell, the perivascular macrophage, and the basement membrane fail together in a stereotyped sequence whose mechanistic logic the subsequent sections of this chapter develop.

3. The Blood-Brain Barrier: Tight Junctions, Transporters, and the Cost of Selectivity

The blood-brain barrier, as a property of the brain capillary endothelial cell, is the product of three molecular subsystems whose combined action produces the high selectivity of the brain microvasculature. The first subsystem is the tight junction complex; the second is the polarized expression of solute transporters and efflux pumps; the third is the suppression of vesicular transcytosis. The dysfunction of any of these three subsystems is sufficient to compromise barrier integrity, and the age- and disease-related dysfunction of all three has been documented in the human Alzheimer's brain.

The tight junction complex of the brain endothelial cell comprises three classes of transmembrane proteins — the claudin family (principally claudin-5 with smaller contributions from claudin-3 and claudin-12), the occludin protein, and the junctional adhesion molecules (JAM-A, JAM-B, JAM-C) — anchored to the actin cytoskeleton through the cytoplasmic scaffold proteins of the ZO family. Claudin-5 is the principal molecular determinant of paracellular impermeability to small molecules. Genetic deletion of claudin-5 in mice produces a size-selective opening of the barrier to molecules below approximately 800 daltons within the first day of life and is incompatible with postnatal survival. The age-dependent reduction in claudin-5 expression at the human brain capillary, documented by Montagne, Zlokovic, and colleagues in multiple cohorts spanning the adult lifespan, is the proximate molecular event by which paracellular permeability increases with age. The reduction is gradual through the fourth and fifth decades, accelerates in the sixth decade, and is detectable by dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) as an age-dependent increase in barrier permeability that begins in the hippocampus

and the medial temporal cortex before generalizing to other regions. The hippocampal-temporal regionalization of early BBB compromise is precisely the regionalization of the Phase II hippocampal bridgehead identified by the trilogy, and the temporal coincidence of these events is, on the present argument, the foundational observation that brings the vascular dimension into the trilogy's framework.

The polarized transporter expression of the brain endothelial cell is the subsystem that converts the selective barrier into a curated interface. The GLUT1 glucose transporter, expressed at the luminal and abluminal membranes in approximately equal density, executes the bidirectional facilitated diffusion of glucose between blood and brain across a concentration gradient established by the high parenchymal glucose consumption. GLUT1 expression at the brain endothelium declines with age in human autopsy studies, and the GLUT1 reduction is accelerated in Alzheimer's disease. The reduction is mechanistically coupled to the hypometabolism observed on fluorodeoxyglucose positron emission tomography (FDG-PET) in early Alzheimer's disease, in which the temporoparietal hypometabolism that is one of the most reliable imaging biomarkers of the disease reflects, at the vascular level, the reduced glucose transport capacity of the brain endothelium and not (or not only) the reduced glucose consumption of the parenchyma. The conflation of these two interpretations in the older imaging literature has been resolved by the demonstration that GLUT1 reduction precedes parenchymal hypometabolism, that GLUT1-heterozygous mice exhibit Alzheimer-like cognitive deficits in the absence of parenchymal amyloid or tau pathology, and that the relationship between endothelial glucose transport and parenchymal glucose consumption is, in the aging and diseased brain, a vascular-to-parenchymal coupling whose failure is initiated at the vascular end.

The LRP1 receptor at the abluminal membrane of the brain endothelial cell executes the principal route of amyloid-β efflux from brain to systemic circulation. LRP1 binds free amyloid-β with submicromolar affinity, transcytoses the peptide across the endothelial cell to the luminal membrane, and releases it into the blood, where it is cleared by hepatic LRP1 and by systemic proteases. The RAGE receptor at the luminal membrane executes the reverse process, binding circulating amyloid-β and transcytosing it back into the parenchyma. The balance between LRP1-mediated efflux and RAGE-mediated influx is, in the healthy adult brain, weighted heavily toward efflux, with a net amyloid-β clearance rate sufficient to maintain steady-state parenchymal amyloid concentrations in the picomolar range. The balance shifts with age and with the onset of Alzheimer's disease: LRP1 expression at the abluminal membrane declines, RAGE expression at the luminal membrane increases, and the net clearance reverses to a net influx. The vascular component of amyloid accumulation in Alzheimer's disease is therefore a transporter-level imbalance at the brain endothelium, not (or not only) an overproduction event at the neuronal source.

The P-glycoprotein (P-gp, ABCB1) and breast cancer resistance protein (BCRP, ABCG2) efflux transporters at the luminal membrane execute the active efflux of xenobiotics and of a subset of endogenous metabolites against their concentration gradient. P-gp also contributes to amyloid-β efflux as a secondary substrate, and the age-dependent reduction in P-gp expression at the human brain endothelium has been documented as a parallel contributor to vascular amyloid accumulation. The therapeutic implication of P-gp reduction is that the pharmacokinetics of central nervous system-active drugs in the elderly are systematically different from those in younger subjects, with implications for both efficacy and toxicity of treatments administered in late life.

The suppression of vesicular transcytosis at the brain endothelial cell is the third subsystem, and it is the subsystem whose dysregulation has the most direct relevance to the inflammatory hypothesis of brain aging developed in section 5. The brain endothelial cell suppresses transcytosis at baseline through the action of MFSD2A, a membrane lipid transporter that imports docosahexaenoic acid (DHA) lysophosphatidylcholine into the endothelial cell and that maintains a lipid composition incompatible with caveolar formation. Loss of MFSD2A produces a transcytosis-permissive endothelial phenotype in which previously excluded large molecules — including immunoglobulins, plasma proteins, and inflammatory factors — cross the barrier through caveolar transcytosis. The age-dependent reduction in MFSD2A expression at the human brain endothelium is the molecular substrate of the increased transcytotic activity observed in the aged brain, and it is the substrate on which the VCAM-1 mechanism developed in section 5 operates.

The cost of selectivity at the blood-brain barrier is the metabolic and structural burden it imposes on the endothelial cell. The brain endothelial cell maintains its tight junction complexes, its polarized transporter expression, and its suppressed transcytosis through a continuous metabolic expenditure that is comparable per unit mass to that of a neuron. The brain endothelial cell is, on this account, not merely a structural element of the vasculature but an active homeostatic cell whose failure under metabolic or inflammatory stress is the proximate event by which the barrier compromises. The downstream consequences of barrier compromise — fibrinogen extravasation, IgG infiltration into the parenchyma, leukocyte transmigration, water and solute imbalance — are the cellular events through which the trilogy's parenchymal substrates are exposed to signals from the systemic compartment, and they are the cellular events that section 5 of this chapter traces to the VCAM-1 gateway.

4. Pericyte Loss and the Zlokovic Two-Hit Vascular Hypothesis

The systematic analysis of pericyte dysfunction in Alzheimer's disease and in vascular aging is the principal contribution of Berislav Zlokovic's program at the University of Southern California, extending across more than two decades and integrating cellular biology, imaging, and human genetics. The Zlokovic two-hit vascular hypothesis, articulated in its mature form in a series of papers between 2011 and 2020 and developed quantitatively by Axel Montagne, Daniel Nation, and colleagues within the Zlokovic group, holds that pericyte loss is the proximate driver of blood-brain barrier breakdown in aging and in early Alzheimer's disease, and that BBB breakdown is itself the proximate driver of parenchymal injury through fibrinogen extravasation, leukocyte infiltration, and the secondary cytokine cascade that follows. The two hits in the framework are vascular and amyloid: vascular injury initiates the cascade, and amyloid deposition follows and amplifies it, but the vascular event is causally and temporally prior to the parenchymal one.

The cellular biology of pericyte support for the brain endothelial cell is mediated principally by the platelet-derived growth factor B / PDGFRβ signaling axis. Brain endothelial cells secrete PDGF-B as a paracrine factor; pericytes express PDGFRβ as the cognate receptor and depend on its signaling for their survival, recruitment, and maintenance at the capillary. Genetic ablation of either Pdgfb in endothelial cells or Pdgfrb in pericytes produces a phenotype of pericyte loss, BBB breakdown, microvascular degeneration, and cognitive impairment in mice, with the severity scaling with the degree of pericyte loss. Pericyte-deficient mice exhibit accelerated amyloid-β accumulation in the parenchyma, secondary to LRP1 reduction at the brain endothelium that is itself a downstream consequence of the loss of pericyte support. The PDGF-B / PDGFRβ axis is therefore the molecular substrate through which the pericyte maintains the endothelial cell, and its disruption produces a coordinated failure of the entire vascular unit.

The Montagne dynamic contrast-enhanced MRI program established the first in vivo evidence of BBB breakdown as an early event in the human aging trajectory and as an early event in cognitive impairment. In a 2015 paper in *Neuron*, Montagne and colleagues reported a regional BBB permeability map across the adult human lifespan in cognitively healthy volunteers, with the principal finding that BBB permeability increases with age in a regionally specific pattern, beginning in the hippocampus and the medial temporal cortex in the sixth decade and extending to the parietal and prefrontal cortex in the seventh and eighth decades. The hippocampal regionalization of early BBB breakdown was confirmed in subsequent papers spanning multiple cohorts and was extended to subjects with mild cognitive impairment, in whom hippocampal BBB permeability was elevated relative to age-matched controls in the absence of detectable amyloid or tau pathology by cerebrospinal fluid biomarkers. The implication of these findings is that BBB breakdown in the human aging brain is a hippocampal-first event whose temporal precedence over amyloid and tau accumulation has now been documented in multiple independent cohorts, and that it is detectable by imaging in vivo in the same temporal window in which the Phase II hippocampal bridgehead of the trilogy is consolidating.

The biomarker correlate of pericyte loss in human cerebrospinal fluid is soluble PDGFRβ (sPDGFRβ), the shed extracellular domain of the pericyte receptor that is released into the CSF as pericytes are damaged or eliminated. The Montagne group established CSF sPDGFRβ as a quantitative biomarker of pericyte injury, with elevations correlating with hippocampal BBB permeability on DCE-MRI, with cognitive decline in longitudinal follow-up, and with subsequent conversion from mild cognitive impairment to clinical dementia. The CSF sPDGFRβ elevation is, in the Zlokovic-Montagne data, present at the preclinical stage and is independent of CSF amyloid-β and tau measurements, establishing pericyte injury as a vascular event with a measurable trajectory distinct from the parenchymal events captured by the AT(N) biomarker framework.

The APOE4 vascular phenotype, developed in detail in section 10, is mechanistically linked to the pericyte axis through the cyclophilin A / matrix metalloproteinase-9 pathway. APOE3 and APOE2 isoforms suppress the cyclophilin A / NF-κB / MMP-9 signaling axis in pericytes through a normal interaction with the LRP1 receptor, with the suppression maintaining pericyte function and BBB integrity. APOE4 fails to suppress this pathway, with the consequence that cyclophilin A is constitutively elevated, NF-κB is activated, MMP-9 is secreted, and the tight junction proteins of the adjacent endothelial cell — claudin-5, occludin, ZO-1 — are degraded by the MMP-9 protease. The APOE4 vascular phenotype is, on this molecular account, a pericyte-mediated event whose proximate effector is matrix metalloproteinase-9 and whose proximate target is the endothelial tight junction complex. The Montagne group has extended this molecular framework to a clinical population, demonstrating in 2020 that APOE4 carriers exhibit hippocampal BBB breakdown years before they exhibit amyloid accumulation or cognitive symptoms, and that the BBB breakdown is the earliest detectable biomarker change in the APOE4 trajectory.

The two-hit vascular hypothesis in its mature form holds, then, that pericyte loss is the proximate driver of BBB breakdown, that BBB breakdown is the proximate driver of parenchymal injury through fibrinogen extravasation and inflammatory infiltration, and that the cascade is amplified by but does not require amyloid deposition. The framework predicts, and the imaging data confirm, that the temporal sequence of biomarker changes in Alzheimer's disease begins with pericyte injury (CSF sPDGFRβ elevation) and BBB breakdown (DCE-MRI permeability), proceeds through neuroinflammation (CSF cytokine elevation, microglial activation on PET), and only later includes the parenchymal markers of amyloid and tau accumulation that have dominated the AT(N) biomarker literature. The implication for the trilogy is that the vascular event is causally upstream of the parenchymal events that the trilogy treats and that the Phase 0 stage of the disease — the stage that precedes the bioenergetic ignition of the locus coeruleus and the microglial transition of the parenchyma — is a vascular stage whose cellular substrate is the pericyte-endothelial axis.

5. Endothelial VCAM-1 and the Aged Systemic Milieu

The central molecular thesis of this chapter is that vascular cell adhesion molecule-1 (VCAM-1, CD106) is the proximate molecular gateway through which the aged systemic milieu accesses the parenchymal compartment of the brain. The thesis emerges from the convergence of three bodies of work: the immunological characterization of VCAM-1 as a leukocyte adhesion molecule in the systemic vasculature, the Wyss-Coray program at Stanford on the soluble plasma proteome of aging and the effects of young versus aged plasma on brain biology, and the Yousef–Wyss-Coray identification of brain endothelial VCAM-1 as the necessary mediator of aged-plasma toxicity to the hippocampal neurogenic niche. This section develops each of these bodies of work in turn and then integrates them into the framework of the present chapter.

VCAM-1 is an immunoglobulin-superfamily transmembrane glycoprotein expressed on activated endothelial cells. The molecule comprises seven immunoglobulin-like extracellular domains, a single transmembrane domain, and a short cytoplasmic tail. VCAM-1 is not expressed on resting vascular endothelium; its expression is induced in response to pro-inflammatory cytokines, principally tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), through an NF-κB-dependent transcriptional program. The induction kinetics are rapid: VCAM-1 mRNA is detectable within thirty minutes of TNF-α exposure, and surface VCAM-1 protein is detectable within two to four hours, with peak expression at twelve to twenty-four hours. The cognate ligand of VCAM-1 is the α4β1 integrin (very late antigen-4, VLA-4) expressed on lymphocytes, monocytes, eosinophils, and basophils. VCAM-1 binding to α4β1 mediates the firm adhesion of leukocytes to activated endothelium that precedes their transendothelial migration into the underlying tissue. The systemic immunological function of VCAM-1 is, therefore, to mark a vascular bed as an inflammatory site and to recruit the leukocyte populations that respond to that signal.

The Wyss-Coray program at Stanford established, beginning in the late 2000s, that the soluble plasma proteome of young and aged mice differs in a quantitatively substantial fashion and that the differential effects of young and aged plasma on brain biology are mediated by these soluble factors. Saul Villeda, then a graduate student in the Wyss-Coray laboratory, reported in 2011 in *Nature* that heterochronic parabiosis — the surgical joining of the systemic circulation of a young and an aged mouse — produced an aging effect on the brain of the young mouse and a rejuvenating effect on the brain of the aged mouse, with the principal cellular substrate of the rejuvenation being the hippocampal neurogenic niche and the cognitive substrate being hippocampal-dependent learning and memory. The Villeda finding was extended in 2014 to demonstrate that the rejuvenating effects of young blood on aged brain were attributable to soluble factors in young plasma and could be reproduced by systemic administration of young plasma to aged mice, and that the toxic effects of aged blood on young brain were attributable to soluble factors in aged plasma. The Wyss-Coray program subsequently identified candidate factors mediating both the rejuvenating and the toxic

effects, with eotaxin, β2-microglobulin, and a small number of other proteins emerging as candidate mediators on the toxic side.

The principal finding of the Yousef–Wyss-Coray work, published in *Nature Medicine* in 2019 with Hanadie Yousef as lead author, is that vascular cell adhesion molecule-1 — specifically, soluble VCAM-1 (sVCAM-1) released from the surface of activated endothelium by metalloproteinase shedding — is the single most age-upregulated protein in mammalian plasma, with plasma sVCAM-1 concentrations rising by approximately tenfold across the adult mouse lifespan and by approximately fivefold across the adult human lifespan. The Yousef paper went further and established the mechanistic chain by which plasma VCAM-1 mediates aged-plasma toxicity to the brain. Aged plasma was shown to induce VCAM-1 expression on brain endothelial cells in young recipient mice, with the induction detectable at the protein level by immunohistochemistry and at the surface level by flow cytometry of isolated brain endothelial cells. The VCAM-1 induction on brain endothelium was shown to be necessary for the downstream effects of aged plasma on the brain: pharmacological antibody blockade of VCAM-1, and tissue-specific conditional deletion of Vcam1 in brain endothelial cells, both abolished the aged-plasma effects on hippocampal microglia, on hippocampal neurogenesis, and on hippocampal-dependent cognitive performance. The brain endothelial VCAM-1 was thus established as the necessary mediator of the aged-plasma signal to the brain, and the molecule was established as a candidate therapeutic target for the prevention of brain aging.

The mechanistic chain through which brain endothelial VCAM-1 transduces the aged-plasma signal into a parenchymal effect comprises three steps that the Yousef paper and subsequent work have progressively elucidated. The first step is the engagement of brain endothelial VCAM-1 by α4β1-expressing leukocytes — principally aged monocytes whose surface α4β1 is itself elevated in the aged state — with the engagement producing firm leukocyte adhesion at the brain microvasculature. The leukocyte adhesion is not necessarily followed by transmigration into the parenchyma in the aged brain; the firm adhesion may be sustained without transmigration for hours or days, during which the adherent leukocyte secretes inflammatory cytokines that diffuse across the endothelium into the parenchymal compartment. The second step is the endothelial signaling that VCAM-1 engagement initiates within the brain endothelial cell. VCAM-1 has a short cytoplasmic tail but transduces signals through interactions with the actin cytoskeleton and with the ezrin-radixin-moesin family of scaffold proteins. VCAM-1 engagement on the endothelium activates NADPH oxidase and produces a local reactive oxygen species pulse, which in turn activates endothelial NF-κB and induces the secretion of inflammatory cytokines including interleukin-6 (IL-6) and CCL2 (MCP-1) into the abluminal compartment. The third step is the parenchymal reception of these endothelial-secreted cytokines by the perivascular macrophages and the parenchymal microglia, with the consequence that the homeostatic microglial signature treated by the Homeostatic Microglial Collapse thesis is destabilized by a signal whose origin is at the vascular interface and whose mediator is brain endothelial VCAM-1.

The Yousef–Wyss-Coray mechanism establishes, therefore, a direct molecular chain from the aged systemic milieu, through the brain endothelium, to the parenchymal microglia and the hippocampal neurogenic niche. The chain is: aged plasma soluble VCAM-1 and aged monocytes in systemic circulation induction of VCAM-1 on brain endothelium engagement of α4β1 monocytes at brain endothelium endothelial NADPH oxidase / NF-κB activation endothelial secretion of IL-6 and CCL2 into the abluminal compartment microglial activation in the parenchymal compartment loss of homeostatic microglial signature impaired hippocampal neurogenesis and cognitive decline. Each step in the chain is supported by direct experimental evidence in mice, and the human correlates of each step are documented in independent biomarker and imaging studies.

The clinical and biomarker correlates of the VCAM-1 mechanism in human Alzheimer's disease are substantial and convergent. Cerebrospinal fluid VCAM-1 is elevated in the preclinical, prodromal, and dementia stages of Alzheimer's disease relative to age-matched cognitively normal controls. The CSF VCAM-1 elevation correlates with CSF total tau and phosphorylated tau, with cortical thinning on structural MRI, and with subsequent cognitive deterioration in longitudinal follow-up of cognitively unimpaired and mildly impaired subjects. Plasma sVCAM-1 has been validated as a peripheral biomarker of brain endothelial activation and tracks with hippocampal BBB permeability on DCE-MRI in cohorts in which the two measurements have been combined. The biomarker behavior of VCAM-1 is therefore not the behavior of a downstream marker of advanced disease but the behavior of an early-stage marker whose elevation precedes the parenchymal events of amyloid and tau accumulation in the natural history of the disease.

A further connection between VCAM-1 and Alzheimer-relevant amyloid metabolism was established by recent proteomic work demonstrating that VCAM-1 is the principal physiological substrate of BACE2, the homolog of BACE1 (β-secretase) whose function had remained partially obscure prior to this identification. BACE2 cleaves VCAM-1 in a manner analogous to BACE1's cleavage of amyloid precursor protein, releasing soluble VCAM-1 ectodomain into the extracellular space and leaving a membrane-bound C-terminal fragment. The CSF concentration of the BACE2-specific cleavage product of VCAM-1 has been proposed as a pharmacodynamic biomarker for BACE1-selective inhibitors, in which the maintenance of BACE2 activity at a near-normal level is desirable while BACE1 is suppressed. The VCAM-1 / BACE2 connection is, on its own, a remarkable convergence: the principal molecular gateway of aged-plasma toxicity to the brain is regulated by a member of the same enzyme family that produces amyloid-β, and the cleavage of VCAM-1 by BACE2 is the principal known physiological function of an enzyme whose pharmacological inhibition is a therapeutic target for Alzheimer's disease.

The implication of the VCAM-1 mechanism for the trilogy is that the molecular gateway between the aged systemic milieu and the parenchymal compartment is now identified, mechanistically characterized, and biomarker-validated. The endothelial VCAM-1 gate is the

proximate molecular event by which systemic aging is converted into brain aging, and it is the proximate molecular event upstream of the Butovsky-defined homeostatic microglial signature whose loss the Homeostatic Microglial Collapse thesis treats as the central mechanism of Phase II. The vascular substrate is therefore not parallel to but causally prior to the microglial substrate, and the trilogy's account of Phase II is structurally incomplete without an explicit treatment of the VCAM-1 gateway as the upstream input.

6. Cerebral Amyloid Angiopathy and the ARIA Constraint

Cerebral amyloid angiopathy (CAA) is the deposition of amyloid-β within and around the smooth muscle cells of cortical and leptomeningeal arterioles and small arteries, and it is the vascular pathology that intersects most directly with the parenchymal amyloid pathology of Alzheimer's disease. CAA is present at autopsy in approximately ninety percent of subjects with clinical Alzheimer's disease and is the principal vascular comorbidity of the parenchymal disease. The amyloid that accumulates in the vessel wall is principally amyloid-β40, in contrast to the predominantly amyloid-β42 species that accumulates in parenchymal plaques, with the differential distribution reflecting the higher solubility and the slower aggregation kinetics of amyloid-β40 and the perivascular drainage pathway through which soluble amyloid is cleared from the parenchyma along the basement membranes of cortical arterioles. The CAA deposition is therefore a clearance pathway event: amyloid-β40 produced in the parenchyma is cleared along the perivascular drainage pathway and accumulates in the vessel wall when the perivascular drainage capacity is exceeded.

The cellular biology of CAA progression follows a stereotyped sequence in which amyloid-β40 first deposits in the basement membrane of the vessel, then accumulates in the smooth muscle cell layer, then progressively replaces the smooth muscle cells, and finally compromises the structural integrity of the vessel wall. The advanced stages of CAA include vessel-in-vessel deposition, fibrinoid necrosis of the affected vessel wall, and microaneurysm formation. The clinical consequences of advanced CAA include lobar intracerebral hemorrhage, cortical superficial siderosis, convexity subarachnoid hemorrhage, and the cerebral microbleeds that are detected on gradient-echo and susceptibility-weighted MRI as small foci of hemosiderin deposition in the cortical and subcortical territory.

The intersection of CAA with anti-amyloid monoclonal antibody therapy is the principal therapeutic constraint that the vascular dimension imposes on the current treatment landscape for Alzheimer's disease. The amyloid-related imaging abnormalities (ARIA) that emerge as adverse events of lecanemab, donanemab, and the earlier aducanumab are vascular events: ARIA-E is the

magnetic resonance imaging signature of vasogenic edema attributable to leak of plasma constituents across the compromised vessel wall, and ARIA-H is the imaging signature of microhemorrhage attributable to rupture of CAA-affected vessels. The mechanism is interpretable within the framework of the present chapter as follows: the monoclonal antibodies bind amyloid-β within the parenchyma and within the vessel wall, recruit microglia to the antibody-amyloid complexes, and stimulate amyloid clearance through Fc-receptor-mediated phagocytosis. The clearance of amyloid from the vessel wall is, however, a destabilizing event for a vessel whose structural integrity has already been compromised by amyloid replacement of the smooth muscle layer, and the consequence is the leak or rupture of the affected vessel. The ARIA events are therefore not idiosyncratic toxicities of the antibodies but predictable consequences of the interaction of the antibody mechanism with the underlying CAA pathology. The clinical decision to administer or withhold anti-amyloid monoclonal antibodies in a given patient is, in current practice, substantially constrained by the patient's burden of cerebral microbleeds on baseline MRI and by the patient's APOE genotype, with APOE4 homozygotes exhibiting the highest CAA burden and the highest ARIA risk.

The implication of CAA and ARIA for the trilogy's account of therapeutic intervention is that the vascular substrate imposes constraints on parenchymal-directed therapy that are not visible from a purely parenchymal analysis. The decision to clear parenchymal amyloid by monoclonal antibody must be evaluated against the vascular cost of that clearance, and the patient stratification that minimizes the vascular cost is itself a function of the vascular biomarkers — APOE genotype, baseline microbleed count, CAA imaging score — that the present chapter develops. The trilogy's account of disease modification is, therefore, materially affected by the inclusion of the vascular substrate, and the appropriate stratification of intervention by vascular biomarker is a contribution that the vascular chapter makes to the trilogy's therapeutic framework.

7. Neurovascular Coupling and the Bioenergetic Bottleneck

The cerebral blood flow regulation system executes the moment-to-moment matching of regional blood flow to regional metabolic demand, with the matching tight enough that the relationship between regional neural activity and regional blood oxygenation is the basis of functional magnetic resonance imaging. The system is sometimes referred to as neurovascular coupling, and it operates through a combination of fast neural input from perivascular projections, intermediate-timescale signaling from astrocytes through arachidonic acid metabolites and through potassium siphoning, and slow-timescale autoregulation by smooth muscle myogenic tone. The system fails progressively in aging and in Alzheimer's disease, with the consequence that regional cerebral blood flow becomes

increasingly dissociated from regional metabolic demand and that local hypoperfusion intervals occur during periods of high neural activity.

The cellular biology of the failure has been characterized principally by Costantino Iadecola and his colleagues at Cornell, whose program has demonstrated that the neurovascular coupling response is reduced in magnitude and prolonged in latency in aged mice, in APOE4 carriers, and in subjects with prodromal Alzheimer's disease. The reduction is detectable on functional MRI as a reduced BOLD response amplitude to a given sensory or cognitive stimulus, and it correlates with cognitive performance across cohorts. The underlying cellular events include the loss of pericyte responsiveness to the metabolic signals propagated from the parenchyma, the impairment of smooth muscle relaxation in response to nitric oxide and to arachidonic acid metabolites, and the disruption of the astrocyte-mediated signaling through which the parenchymal demand signal is transmitted to the vascular wall.

The bioenergetic implication of impaired neurovascular coupling is the recurrent local hypoperfusion of high-demand parenchymal regions during periods of high activity. The hypoperfusion is sub-ischemic — it does not produce frank infarction — but it is sufficient to drive transient hypoxia, reduced ATP availability, and the consequent dysregulation of the energy-dependent cellular processes that the Bioenergetic Collapse thesis treats. The recurrent hypoperfusion is, on the present account, an upstream driver of bioenergetic stress in the parenchyma, with the implication that the brainstem locus coeruleus ignition treated by the Bioenergetic Collapse thesis may be vascularly conditioned. The locus coeruleus is unusually vulnerable to hypoperfusion: its neurons are highly metabolically active, are densely packed in a small brainstem nucleus, and depend on a single arterial supply from the superior cerebellar artery whose dysregulation in aging is more pronounced than that of cortical arterial supply. The bioenergetic vulnerability of the locus coeruleus may therefore be a vascular vulnerability in the first instance, with the parenchymal NAD depletion treated by the Bioenergetic Collapse thesis a downstream consequence of vascularly conditioned hypoxic stress.

The clinical correlate of impaired neurovascular coupling is the reduced cerebral blood flow detectable on arterial spin labeling MRI in cognitively normal APOE4 carriers and in subjects with prodromal Alzheimer's disease, with the regional pattern of hypoperfusion preceding the regional pattern of hypometabolism on FDG-PET and the regional pattern of cortical thinning on structural MRI. The temporal sequence — hypoperfusion first, then hypometabolism, then atrophy — is consistent with the present chapter's framework in which vascular dysfunction is causally upstream of parenchymal failure.

8. Glymphatic Clearance Failure and the Sleep-Vascular Interaction

The glymphatic clearance pathway, characterized principally by Maiken Nedergaard and Jeffrey Iliff and their colleagues, is the cerebrospinal-fluid-to-interstitial-fluid exchange system through which solute waste — including amyloid-β, tau, and metabolic byproducts — is cleared from the parenchymal compartment of the brain. The pathway operates as follows: cerebrospinal fluid produced by the choroid plexus enters the periarterial Virchow-Robin space along cortical and leptomeningeal arteries, exchanges with interstitial fluid through aquaporin-4 channels in the polarized astrocytic endfoot membrane that contacts the basement membrane, percolates through the parenchymal interstitium carrying solute waste, and exits along the perivenous space to be drained through the meningeal lymphatic vessels into the deep cervical lymph nodes. The pathway is driven by arterial pulsatility, with the pulse pressure of cortical arteries pumping the periarterial CSF into the parenchymal compartment in a wave-like manner that propagates along the vascular tree.

The glymphatic clearance system has two principal features that link it to the vascular substrate and to the sleep-state biology of the brain. The first feature is its dependence on aquaporin-4 polarization at the astrocytic endfoot: AQP4 must be enriched at the endfoot membrane that contacts the basement membrane and depleted from the membrane that contacts the parenchymal neuropil for the glymphatic flow to be directional and effective. The loss of AQP4 polarization, observed in aged human brain and accelerated in Alzheimer's disease, abolishes the directionality of the flow and reduces the clearance efficiency by an order of magnitude. The second feature is its sleep-state dependence: the glymphatic flow is approximately ten times greater during slow-wave sleep than during waking, with the difference attributable to the expansion of the interstitial space during sleep and to the altered vasomotor pattern that accompanies slow-wave activity. The implication is that the glymphatic clearance of amyloid-β occurs primarily during sleep and that sleep disruption, common in aging and in early Alzheimer's disease, is a proximate cause of impaired amyloid clearance.

The vascular and sleep-related contributions to glymphatic failure intersect at the arterial pulsatility that drives the flow. Arterial stiffening, common in aging and accelerated in hypertension, reduces the pulse pressure available to drive the periarterial CSF flow and impairs glymphatic clearance independently of the AQP4 polarization changes. The combination of arterial stiffening, AQP4 depolarization, and sleep disruption produces an additive impairment of glymphatic clearance in the aged and the early Alzheimer's brain, with the consequence that the steady-state parenchymal concentrations of amyloid-β and tau are elevated by a factor of two to five relative to the young adult baseline even in the absence of changes in production rate.

The implication of glymphatic failure for the trilogy is that the parenchymal amyloid accumulation treated by the Convergent Synaptic Collapse thesis is, in significant measure, a vascular-glial clearance event rather than a parenchymal production event. The intraneuronal Aβ42 accumulation at the synaptic endosome that the inside-out paradigm treats is itself conditioned by the steady-state parenchymal Aβ concentration, which is in turn conditioned by the glymphatic clearance capacity. The vascular and sleep-related contributions to glymphatic capacity are therefore upstream of the parenchymal amyloid trajectory, and the trilogy's account of Phase I and Phase II amyloid dynamics is structurally incomplete without an explicit treatment of the glymphatic substrate.

9. Small Vessel Disease, White Matter, and the Vascular-Alzheimer's Continuum

The cerebral small vessel disease (cSVD) family of pathologies — including arteriolosclerosis, lipohyalinosis, microbleeds, lacunar infarcts, white matter hyperintensities, and the broader spectrum of cerebrovascular changes that accumulate with age and with vascular risk factor exposure — is the substrate of vascular cognitive impairment and of mixed dementia, the syndromes in which vascular pathology contributes substantially to the cognitive presentation that is clinically indistinguishable from Alzheimer's dementia. The autopsy literature on the prevalence of mixed pathology has converged on the finding that the majority of subjects with clinical Alzheimer's dementia at autopsy exhibit substantial cerebrovascular pathology in addition to the parenchymal amyloid and tau pathology that defines the diagnosis, with the consequence that the "pure Alzheimer's" pathology that has dominated the clinical research framework is, in fact, the exception rather than the rule in the elderly population that bears the bulk of the disease burden.

The white matter hyperintensities that accumulate with age, with hypertension, with diabetes, and with the broader spectrum of vascular risk factors are the principal imaging correlate of cSVD, and they are quantitatively associated with cognitive impairment in cross-sectional and longitudinal cohorts. The cellular biology of the white matter lesion is the consequence of chronic small vessel hypoperfusion, with oligodendrocyte vulnerability to hypoxic stress driving the demyelination and axonal compromise that produces the imaging finding. The white matter lesion is therefore a vascular lesion expressed through a glial substrate, and its impact on cognitive function is the disconnection of cortical regions whose communication depends on the affected white matter tracts. The cognitive impact of white matter lesions is dose-dependent and is additive to the cognitive impact of cortical atrophy and of Alzheimer's pathology in mixed dementia.

The Vascular Contributions to Cognitive Impairment and Dementia (VCID) framework, developed under the auspices of the National Institute of Neurological Disorders and Stroke and articulated principally by Steven Greenberg, Marilyn Albert, and colleagues, treats the vascular and Alzheimer's contributions to cognitive impairment as additive substrates with overlapping risk factors and overlapping clinical presentations. The framework emphasizes the practical impossibility of distinguishing the two contributions on clinical grounds in the elderly population and the importance of treating the vascular substrate as a target of intervention even in subjects whose presentation has been characterized as "Alzheimer's dementia" by the standard clinical criteria.

The implication of the vascular-Alzheimer's continuum for the trilogy is that the disease the trilogy treats is, in the elderly population at the bulk of disease burden, almost always a mixed pathology in which the vascular and parenchymal substrates contribute jointly to the cognitive presentation. The trilogy's account of the three parenchymal substrates is correct as far as it goes, but it is structurally incomplete in its omission of the fourth vascular substrate whose contribution to the mixed presentation is, in many subjects, comparable to or greater than the contribution of any individual parenchymal substrate.

10. APOE4 as a Vascular Genotype

The apolipoprotein E ε4 allele is the dominant genetic risk factor for late-onset Alzheimer's disease, with heterozygotes carrying approximately threefold elevated risk and homozygotes carrying approximately twelve-fold elevated risk relative to APOE3/APOE3 controls. The molecular biology of APOE4 has been treated principally as a parenchymal phenomenon — APOE4-mediated impairment of amyloid-β clearance from the parenchyma, APOE4-mediated dysregulation of intracellular lipid trafficking, APOE4-mediated impairment of synaptic plasticity — and the trilogy's treatment of APOE4 has been similarly parenchymal. The present chapter develops the case that APOE4 is, in significant measure, a vascular genotype whose vascular phenotype may be temporally and mechanistically prior to its parenchymal phenotype.

The vascular phenotype of APOE4 was developed in cellular detail by Berislav Zlokovic and his colleagues in a series of papers beginning in the early 2010s and culminating in a 2020 *Nature* paper from the Montagne and Zlokovic groups in which the human in vivo phenotype was articulated. The cellular biology, as developed in section 4, is the failure of APOE4 to suppress the cyclophilin A / NF-κB / MMP-9 signaling axis in pericytes through the LRP1 receptor, with the consequence that MMP-9 is constitutively elevated and the tight junction proteins of the adjacent endothelial cell are progressively degraded. The Montagne 2020 paper extended the cellular framework to a human

in vivo cohort and demonstrated that APOE4 carriers exhibit hippocampal BBB breakdown years before they exhibit detectable amyloid accumulation or cognitive symptoms, with the BBB breakdown the earliest detectable biomarker change in the APOE4 trajectory. The implication is that APOE4 affects the brain first vascularly, before it affects the brain parenchymally, and that the vascular phenotype is the leading edge of the APOE4 trajectory.

The independent confirmation of this finding has come from multiple imaging cohorts in which the regional pattern of hippocampal BBB breakdown in young and middle-aged APOE4 carriers — detected by DCE-MRI and corroborated by CSF sPDGFRβ elevation — has been documented before the appearance of detectable amyloid PET signal or cognitive symptoms. The BBB breakdown is regionally consistent with the hippocampal bridgehead identified by the trilogy as the Phase II target and temporally precedes the parenchymal events of the trilogy by an estimated decade or more.

The integration of APOE4-as-vascular-genotype with the trilogy is straightforward but materially modifies the trilogy's account of risk stratification and of intervention timing. The trilogy's treatment of APOE4 has been principally as a modifier of the parenchymal substrates — APOE4-mediated impairment of microglial homeostatic maintenance, APOE4-mediated dysregulation of bioenergetic metabolism, APOE4-mediated potentiation of synaptic toxicity. The vascular phenotype is upstream of all of these and provides a mechanistic explanation for the temporal precedence of APOE4-related brain changes over the cognitive symptoms of disease. The implication for intervention is that the appropriate temporal window for APOE4-directed therapy is the vascular window — the third through fifth decades — at which the vascular phenotype is established but the parenchymal substrates have not yet been engaged. The therapeutic candidates appropriate to this window are vascular: control of hypertension, treatment of hyperlipidemia, suppression of the cyclophilin A / MMP-9 axis through emerging pharmacology, and the lifestyle interventions whose vascular benefits are documented.

11. Integration with the Three Theses of the Collapse Trilogy

The vascular dimension as developed in this chapter integrates with each of the three theses of the trilogy in a distinct manner, with the integration in each case extending and qualifying the trilogy's account without contradicting it. The present section develops the integration thesis by thesis.

The integration of the vascular dimension with the Bioenergetic Collapse thesis is the most direct of the three. The locus coeruleus, identified by the Bioenergetic Collapse thesis as the disease's

earliest parenchymal substrate, is anatomically dependent on a single vascular supply from the superior cerebellar artery and is metabolically demanding at a level that places it among the most vascularly vulnerable parenchymal substrates in the brain. The neurovascular coupling impairment developed in section 7, the glymphatic clearance failure developed in section 8, and the pericyte-endothelial dysfunction developed in section 4 collectively converge on the locus coeruleus as a target of vascularly conditioned hypoxic stress whose downstream consequence is the NAD depletion that the Bioenergetic Collapse thesis treats as the proximate driver of brainstem aminergic failure. The vascular substrate is, on this integration, the conditioning substrate of the bioenergetic substrate, with the temporal sequence vascular hypoperfusion bioenergetic stress PARP-1 hyperactivation NAD depletion locus coeruleus failure.

The integration of the vascular dimension with the Homeostatic Microglial Collapse thesis is, in this chapter's argument, the central integration and the principal contribution of the vascular dimension to the trilogy. The Butovsky-defined homeostatic microglial signature, identified by the Homeostatic Microglial Collapse thesis as the cellular substrate whose loss is the proximate driver of Phase II, is destabilized by signals whose origin is at the vascular interface. The VCAM-1 mechanism developed in section 5 establishes the molecular chain by which the aged systemic milieu is converted into a parenchymal signal that destabilizes the microglial homeostatic signature: aged plasma brain endothelial VCAM-1 α4β1 monocyte adhesion endothelial NF-κB endothelial cytokine secretion microglial signature loss. The vascular substrate is, on this integration, the upstream driver of the microglial transition, with the implication that the Phase II microglial event treated by the trilogy is, in mechanistic terms, a vascularly initiated event whose proximate driver is brain endothelial VCAM-1.

The integration of the vascular dimension with the Convergent Synaptic Collapse thesis is the most indirect of the three but is mediated through the glymphatic clearance pathway developed in section 8 and through the cerebral amyloid angiopathy axis developed in section 6. The intraneuronal Aβ42 accumulation at the synaptic endosome that the inside-out paradigm of the Gouras framework treats is conditioned by the steady-state parenchymal Aβ concentration, which is in turn conditioned by the glymphatic clearance capacity. The vascular and sleep-related contributions to glymphatic capacity are therefore upstream of the parenchymal amyloid trajectory that drives the synaptic substrate. The parvalbumin perineuronal net, the central matrix-stabilized scaffold of the Convergent Synaptic Collapse thesis, has its vascular analogue in the basement membrane treated in section 2, and the MMP-mediated digestion of both scaffolds is executed by overlapping molecular mediators whose vascular and parenchymal expression is co-regulated. The vascular substrate is, on this integration, the conditioning substrate of the synaptic substrate, with the implication that the Phase III synaptic event treated by the trilogy is, in mechanistic terms, vascularly conditioned in its earliest stages.

The cross-thesis implication of these three integrations is that the vascular dimension is the fourth substrate of the trilogy and is causally and temporally prior to each of the three parenchymal substrates. The Phase 0 stage of disease — the stage that precedes the bioenergetic ignition, the microglial transition, and the synaptic disintegration — is the vascular stage, and its cellular substrate is the neurovascular unit treated in section 2 and the molecular substrate of brain endothelial VCAM-1 developed in section 5.

12. Therapeutic Implications: The Vascular Window and the ARIA Cost

The vascular dimension as developed in this chapter has substantial implications for the therapeutic landscape of Alzheimer's disease and for the appropriate stratification and timing of intervention. The principal implications are three.

The first implication is the identification of a vascular intervention window in the third through fifth decades of life, during which the vascular substrate is the active site of pathological progression and during which intervention directed at the vascular substrate would be expected to produce the largest effect on subsequent disease trajectory. The interventions appropriate to this window are not novel pharmacology in most cases but established cardiovascular medicine: control of hypertension to standards now supported by the SPRINT-MIND trial data, treatment of hyperlipidemia, control of diabetes and of the broader metabolic syndrome, smoking cessation, and the lifestyle interventions — physical activity, dietary modification, sleep hygiene — whose vascular benefits are extensively documented. The translation of cardiovascular standards of care to the prevention of Alzheimer's disease is the principal therapeutic implication of the vascular dimension, and the implication is that the population for whom this translation is most relevant is precisely the middle-aged APOE4 carrier population whose vascular phenotype is in its leading-edge phase. The middle-aged population is, however, the population least often engaged by current Alzheimer's clinical trial frameworks, which focus principally on the late-prodromal and early-symptomatic stages in which parenchymal pathology has already accumulated.

The second implication is the ARIA constraint on anti-amyloid monoclonal antibody therapy developed in section 6. The decision to administer or withhold lecanemab, donanemab, or other antibodies in a given patient is materially affected by the vascular substrate — APOE genotype, CAA burden, baseline microbleed count — and the stratification of intervention by these variables is now a standard component of clinical practice for the agents that have been approved. The vascular dimension as developed in this chapter provides the mechanistic framework for

understanding why the stratification matters and why the ARIA events are predictable consequences of the antibody mechanism in subjects with substantial vascular amyloid burden.

The third implication is the identification of brain endothelial VCAM-1 as a candidate molecular target for intervention. The Yousef–Wyss-Coray demonstration that antibody blockade of VCAM-1 rescues the aged-plasma phenotype in mice establishes a proof-of-concept for VCAM-1-directed intervention in aging and, by extension, in early Alzheimer's disease. The development of clinical-grade VCAM-1 antibodies, the validation of VCAM-1 inhibitors in early-stage human trials, and the establishment of biomarker frameworks for monitoring VCAM-1-directed therapy are the appropriate next steps for the translation of the Wyss-Coray finding from mice to humans. The molecule itself is well-characterized, the antibodies are technically straightforward to produce, and the population for whom the intervention is most relevant is identifiable by the combination of age, APOE genotype, plasma sVCAM-1 elevation, and DCE-MRI evidence of hippocampal BBB permeability that the present chapter has developed.

The vascular dimension as developed in this chapter does not displace the parenchymal substrates of the trilogy as targets of intervention; it adds to them. The integrated therapeutic framework that emerges from the trilogy's four-substrate analysis is a phased framework in which vascular intervention in the third through fifth decades is followed by bioenergetic intervention in the fifth decade, by microglial intervention in the sixth decade, and by synaptic intervention in the seventh decade and beyond. The framework is, in effect, a developmental pharmacology of late-onset Alzheimer's disease in which the four substrates are addressed in the temporal sequence in which they engage and in which the cumulative effect of intervention across the four substrates is the disease-modifying effect that has eluded the single-target approach of the past two decades.

13. Conclusion: The Vascular Dimension as the Fourth Substrate

This chapter has developed the case that the cerebrovasculature is the fourth cellular substrate of late-onset Alzheimer's pathogenesis, parallel and prior to the three parenchymal substrates treated by the Collapse Trilogy, and that the molecular gateway between the aged systemic milieu and the parenchymal compartment is brain endothelial vascular cell adhesion molecule-1. The case rests on five principal observations developed across the preceding twelve sections. The first observation is that the cerebrovasculature is anatomically and physiologically an active homeostatic system whose component cells execute a daily load comparable to that of any parenchymal compartment. The second observation is that pericyte loss and blood-brain barrier breakdown are the earliest

detectable biomarker changes in the human aging brain and in the APOE4 trajectory, preceding the parenchymal events of amyloid and tau accumulation by an estimated decade or more. The third observation is that brain endothelial VCAM-1 is the most age-upregulated protein in mammalian plasma and is the necessary mediator of aged-plasma toxicity to the hippocampal neurogenic niche, with antibody blockade of VCAM-1 sufficient to rescue the aged-plasma phenotype in mice. The fourth observation is that the cerebral amyloid angiopathy and ARIA constraint, the neurovascular coupling impairment, the glymphatic clearance failure, the small vessel disease continuum, and the APOE4 vascular phenotype collectively constitute a coherent vascular substrate whose engagement is upstream of and conditioning to the engagement of the three parenchymal substrates of the trilogy. The fifth observation is that the therapeutic implications of the vascular dimension — the vascular intervention window in midlife, the ARIA stratification of anti-amyloid therapy, the VCAM-1 candidate target — extend the trilogy's therapeutic framework substantially and identify a population for whom intervention is currently underutilized.

The trilogy's account of late-onset Alzheimer's pathogenesis is, with the addition of the vascular dimension, a four-substrate account: vascular, bioenergetic, microglial, synaptic. The four substrates engage in temporal sequence across the third through eighth decades of adult life and converge on the cognitive presentation that is clinically recognized as Alzheimer's dementia. The first substrate — the vascular — is the substrate at which the aged systemic milieu meets the central nervous system, and it is the substrate whose Phase 0 engagement sets the trajectory of the three subsequent substrates. The cellular biology of the first substrate is the cellular biology of the neurovascular unit; the molecular biology of the first substrate is the molecular biology of brain endothelial VCAM-1; the temporal biology of the first substrate is the biology of the third through fifth decades of adult life. The trilogy is, with the addition of this chapter, no longer a three-thesis account but a four-thesis account, and the vascular thesis takes its place as the first of the four in the temporal sequence of disease engagement.

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