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Insulin Resistance and Oligodendrocyte/Microvascular Endothelial Cell Dysfunction as Mediators of White Matter Degeneration in Alzheimer's Disease

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Abstract: In Alzheimer's disease (AD), white matter (WM) degeneration begins early, increases with disease progression, and contributes to cognitive impairment, yet the mechanisms are poorly understood. This article reviews the roles of myelin loss, oligodendrocyte dysfunction, and microvasculopathy in relation to AD WM degeneration. Myelin loss impairs axonal function and its breakdown promotes oxidative stress, inflammation, and lipid peroxidation, further compromising the structure and function of axons. Oligodendrocyte dysfunction impairs homeostatic mechanisms needed to maintain myelin. Microvascular disease with endothelial cell pathology leads to thrombin activation and pro-inflammatory cytokine release, oxidative stress, and increased vascular permeability. Progressive fibrotic

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replacement of smooth muscle cells reduces vaso-responsiveness to metabolic demands. Fibrotic thickening of vessel walls narrows the lumens, rendering them more susceptible to occlusion, endothelial cell injury, and thrombin activation. Since normal physiological functions of oligodendrocytes and microvascular endothelial cells rely on intact insulin/insulin-like growth factor (IGF) signaling through cell survival, metabolic and anti-inflammatory pathways, conceivably, WM degeneration in AD is mediated by insulin and IGF resistance with attendant pathogenic targeting of oligodendroglia and endothelial cells. The apolipoprotein E-ε4 genotype may serve as a co-factor in AD-associated glial-vascular WM degeneration due to its role as a mediator of insulin resistance.

Keywords: Alzheimer's disease; Insulin resistance; oligodendrocytes; thrombin; white matter

INTRODUCTION

Alzheimer's disease (AD) is the 6th leading cause of death and the most prevalent aging-associated dementia, afflicting over 5 million people in the United States. Despite intense and comprehensive research efforts over the past 4–5 decades, we still lack effective disease-modifying therapies, and thus, the annual economic burden of over $170 billion continues to grow (1). Perhaps one of the main obstacles to success has been the failure to appreciate the full spectrum of disease which extends well beyond cerebral accumulations of amyloid-beta (Aβ) and neuronal structural pathologies caused by abnormally phosphorylated tau. AD is mechanistically linked to: (i) insulin resistance; (ii) neuroinflammation; (iii) white matter (WM) atrophy with myelin loss and axonal degeneration; (iv) vasculopathy; (v) leukoaraiosis; (vi) blood–brain barrier disruption; (vii) oxidative stress; (viii) mitochondrial dysfunction; (ix) loss of neuronal plasticity; and (x) synaptic disconnection. Furthermore, consideration should be given to the concept that different mediators of neurodegeneration may emerge at various time points and could be inter-dependent. These points are not addressed by the present-day diagnostic and therapeutic approaches.

In light of the varied and complex nature of AD-associated pathologies, it is not surprising that mono-therapeutic strategies have failed to remediate this disease. The development of a more rational and effective therapeutic design requires that we attain a greater understanding of how various pathogenic processes contribute to the onset and progression of AD. Furthermore, additional information about systemic and central nervous system (CNS) forces that drive the cascade of neurodegeneration could lead to preventive strategies. For example, a better understanding of how co-factors such as vascular disease, head trauma, and lifestyle exposures modify risk and the phenotypic features of AD could ultimately help refine and personalize diagnostics and therapeutics. In this regard, the role of vascular disease in AD has been strongly suggested by the finding that at least 40% of people with clinically diagnosed AD have significant cerebrovascular disease yet neither disease process would be regarded as sufficient to cause dementia (2). Correspondingly, the relatively recent incident decline in AD severity in the United States has been attributed to improved vascular protective care (3).

Now it is time for investigators to re-focus their efforts by capturing a better understanding of the protean pathogenic factors that drive progressive neurodegeneration. Evidence- and mechanism-based approaches are needed to develop multi-pronged therapeutics, utilizing strategies that already have been successful for cancers and other chronic diseases.

AD-ASSOCIATED WM PATHOLOGY

White matter degeneration is a major and consistent but vastly under-studied abnormality in AD (Figure 1). Its occurrence was initially characterized in 1986 by Brun and Englund (4, 5) and subsequently shown to be an early pre-clinical abnormality (6). WM atrophy in AD is most pronounced in the parietal and temporal lobes, followed by the frontal lobes, whereas the occipital lobes tend to be spared (6). Consequently, the severities of WM atrophy correspond with the distribution and degree of cerebral cortical pathology. WM degeneration in AD is associated with loss of myelin and myelinated axons, together with dysfunction or loss of oligodendrocytes, increased activation of astrocytes, that is gliosis, and microvascular disease (7–9). Leukoaraiosis, an extreme form of WM degeneration in which the loss of myelinated axons is extensive and associated with WM hyperintensities by magnetic resonance imaging (MRI) (7–9), is most prominently distributed in periventricular and central compared with subcortical WM (5, 6, 10–15).

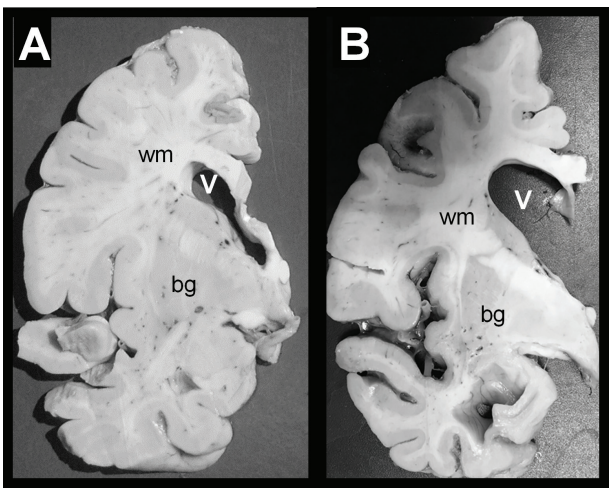

Figure 1 White matter atrophy in AD. Postmortem coronal slices of the left cerebral hemisphere from patients with (A) normal aging or (B) advanced AD. Panels A and B show approximately the same coronal slice levels depicting the cingulate gyrus, corpus callosum, basal ganglia (bg), central and periventricular posterior frontal white matter (wm) and lateral ventricle. Note the markedly atrophic white matter and associated ex vacuo enlargement of ventricles (V) in (B) AD relative to (A) control.

The histopathologic correlates of myelin degeneration in AD have been characterized by assessing relative reductions in Luxol fast blue (LFB) staining intensity and uniformity. LFB reacts with phospholipids and lipoproteins and is suitable for detecting myelin pathology in formalin-fixed, paraffin-embedded tissue. However, additional approaches are required to evaluate axonal degeneration and attrition. Traditional histochemical stains, such as Bielschowsky, utilize silver impregnation to label cytoskeletal proteins in axons, dendrites, and degenerated or dystrophic fibers. Current, more specific approaches employ immunohistochemical staining with antibodies to phosphorylated neuronal cytoskeletal proteins such as neurofilament and tau. In AD, myelin loss and axonal degeneration, respectively, marked by pallor of LFB staining and fragmentation, irregular swelling, and rarefaction of fibers, tend to be more pronounced in central and periventricular compared with subcortical WM, and they increase with severity of AD.

ASTROCYTES, MICROGLIA, AND OLIGODENDROCYTES

Astrocyte activation or gliosis is a conspicuous feature of AD. Gliosis marks responses to cellular and tissue degeneration. One potential outcome of gliosis is tissue repair, but an alternative outcome is the elaboration of pro-inflammatory cytokines that promote oxidative stress and tissue injury, thereby worsening neurodegeneration. WM gliosis is marked by increased glial fibrillary acidic protein (GFAP) immunoreactivity in enlarged (hypertrophic) astrocytes and fibrillary deposits within the extracellular matrix (Figure 2). Dense fibrillary gliosis, which reflects severe degeneration, is most prominently distributed in periventricular and subcortical U-fiber regions. In contrast, central WM gliosis is generally less pronounced and associated with increased reactive hypertrophic astrocytes and variable densities of GFAP-positive fibrillary deposits.

WM gliosis, particularly in the early and intermediate stages of AD, is accompanied by microglial activation. Microglia have rod-shaped, curved, or twisted nuclei and can be detected by immunohistochemical staining with antibodies to common leukocyte antigen (CD45) or ionized calcium-binding adaptor protein-1 (IBA-1) (16). In AD, activated microglia together with reactive astrocytes promote neuroinflammation via increased elaboration of pro-inflammatory cytokines and chemokines and suppression of anti-inflammatory molecules (17). Neuroinflammation causes injury and degeneration of myelin and axons. Although the underlying causes of WM neuroinflammation have yet to be determined, plausible etiologies include insulin/insulin-like growth factor (IGF) resistance and microvascular ischemic injury since both have been demonstrated in AD and are well-documented mediators of inflammation, oxidative stress, and metabolic dysfunction.

Oligodendrocytes synthesize and maintain myelin sheaths needed to support axonal integrity and function. Oligodendrocytes, like neurons, are highly vulnerable to both insulin and IGF-1 resistance and ischemic injury. Loss or dysfunction of oligodendrocytes impairs myelin maintenance, axonal function, and ultimately axonal structure. To better understand the mechanisms of WM degeneration

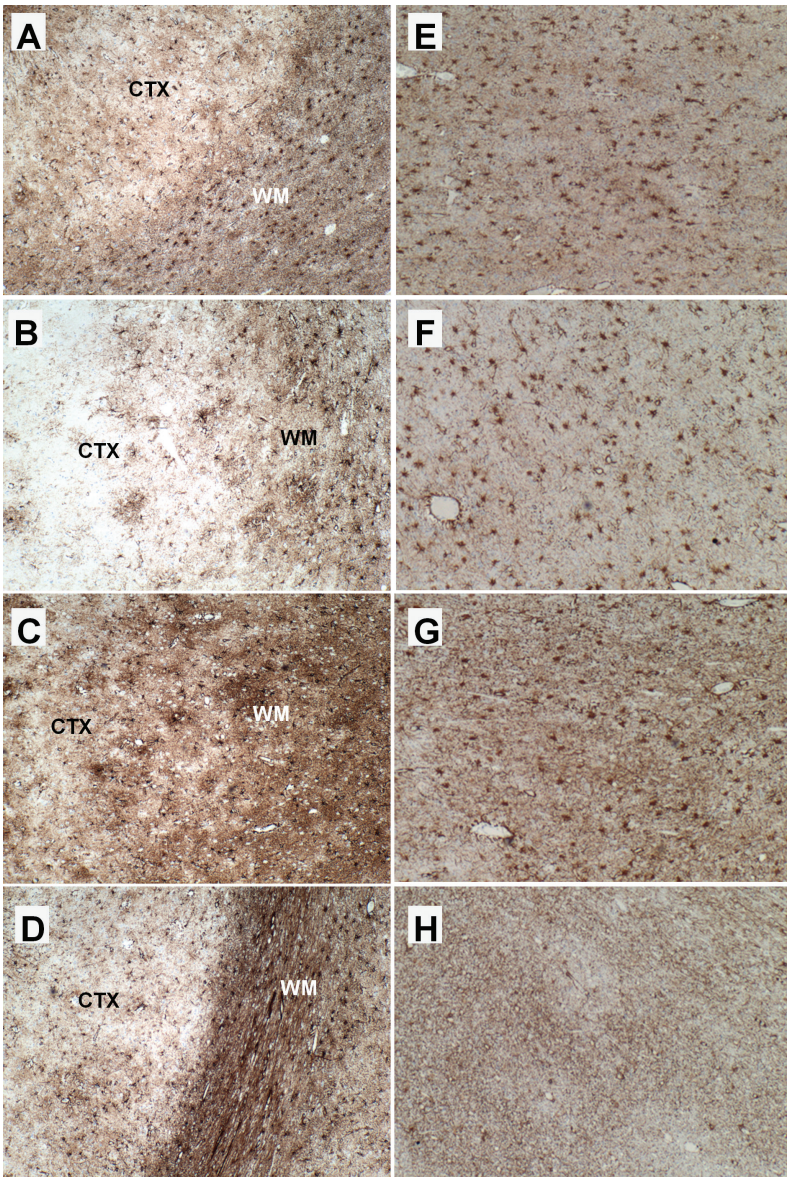

Figure 2 WM gliosis exhibits regional variation in AD. Immunohistochemical staining was used to detect glial fibrillary acidic protein (GFAP) in the(A, E) anterior frontal, (B, F) posterior frontal, (C, G) parietal, and (D, H) occipital cortex (CTX) and underlying white matter (WM) in formalin-fixed paraffin-embedded human postmortem brain tissue. Immunoreactivity was detected with biotinylated secondary antibodies, horseradish peroxidase-conjugated avidin-biotin complexes, and diaminobenzidine (brown precipitant). Panels A–D (100x original magnification) show intense GFAP immunoreactivity in white matter (wm) and variable labeling of the cortex (ctx). Panels E–G (200x original magnification) show abundant GFAP-positive hypertrophic reactive astrocytes (dot-like structures) in a background of diffuse fibrillar labeling, whereas Panel H shows predominantly fibrillar labeling of central occipital WM.

in AD, more information is needed about the biochemical and molecular nature of oligodendrocyte injury and dysfunction leading to myelin and axonal loss. However, recent advances in adult brain WM cell isolation techniques (18), lipidomics mass spectrometry (19, 20), and targeted gene array analysis now provide practical methodologic approaches for characterizing WM oligodendrocyte and myelin lipid pathologies.

BIOCHEMICAL AND CELLULAR BASIS OF WM PATHOLOGY IN AD

White matter is largely composed of myelinated axons. Traditionally, the integrities of myelin and axons are studied by histochemical or immunohistochemical staining. However, overlapping responses to various types of injury and degeneration limit the utility of these approaches for characterizing disease-specific pathologies and responses to treatment. To better understand the nature of AD-associated myelin pathology, biochemical approaches that assess reproducible alterations in lipid composition are needed.

CNS myelin, a specialized membrane synthesized by oligodendrocytes, has a much higher dry mass of lipids (70–85%) compared with proteins (15–30%) and plays a major role in insulating axons to support conductivity. Myelin lipids primarily include cholesterol, glycosphingolipids, sulfatides, gangliosides, phospholipids, and sphingomyelin (21). Many diseases that impair the structural and functional integrity of WM are associated with abnormalities in the expression and metabolism of phospholipids and sulfatides (20, 22–28). Membrane phospholipids have important roles in regulating lipid rafts and receptor functions. Sulfatides, located on extracellular leaflets of plasma membranes (29) and generated via sulfonation of galactocerebroside, regulate neuronal plasticity, memory, myelin maintenance, protein trafficking, adhesion, glial-axonal signaling, insulin secretion, and oligodendrocyte survival (30). Degradation of sulfatide via galactosylceramidase and sulfatidase yields ceramide (29, 31), which promotes neuroinflammation, apoptosis, and production of reactive oxygen species (ROS), and impairs signaling through survival and metabolic pathways (32). Furthermore, deficiencies in membrane sulfatide disrupt myelin's structure, function, and capacity to support neuronal conductivity (32). Thus, imbalances in sphingolipid composition that reduce sulfatide and increase ceramide are potentially important mediators of WM degeneration and attendant cognitive impairment.

Potential role of oligodendrocyte dysfunction as a mediator of WM degeneration

Oligodendrocytes generate and maintain CNS myelin by controlling the expression and activity of enzymes that modulate its biosynthesis, turnover, and degradation (33–36). Loss or damage to myelin impairs neuronal conductivity and

compromises axonal integrity, releasing neurofilament and myelin sulfatides (37). Increases in lipid peroxidation after myelin breakdown exacerbate oxidative damage, neuroinflammation, and astrocyte activation (gliosis). Therefore, the presence of WM atrophy and degeneration early in the course of AD, including in its preclinical stages (6), could be due to pathogenic processes that impair function and survival of mature oligodendrocytes and promote secondary reactive injury via increased oxidative stress, inflammation, and lipid peroxidative. In light of the known importance of insulin and IGF signaling for maintaining a broad array of homeostatic functions in both neurons and oligodendrocytes (38–40), and strong evidence for brain insulin and IGF deficiencies and resistances beginning early in the course of AD (41, 42), it is plausible to hypothesize that impaired signaling through the insulin and IGF receptors also mediates oligodendrocyte dysfunction in AD.

Many critical functions of oligodendrocytes, including cell survival, myelin synthesis, and myelin maintenance, are supported by insulin and IGF-1 signaling (43–46). Consequently, disruption of related networks decreases oligodendrocyte viability, increases oxidative stress, and impairs myelin maintenance and maturation. Likewise, experimental models of chronically impaired brain insulin and IGF signaling exhibit WM atrophy and degeneration (47, 48) together with oligodendrocyte dysfunction (49), all of which can be partly reversed or prevented by early treatment with insulin sensitizers (47, 49). Another consequence of impaired insulin/IGF signaling is dysregulated sphingolipid metabolism resulting in decreased sulfatide and increased ceramide levels (23, 26–28, 50–52). Increases in ceramide can cause WM degeneration via several mechanisms, including inhibition of insulin/IGF signaling through pathways needed for oligodendrocyte survival and metabolic functions, and stimulation of pro-inflammatory and oxidative stress responses (28).

Besides lipids, oligodendrocytes synthesize integral membrane proteins whose expressions are differentially modulated at each stage of myelin maturation as well as in response to injury. As immature oligodendrocyte precursor cells (OPC) pass through phases of differentiation to eventually become mature myelin-producing oligodendrocytes, the proteins needed to support the structure and function of myelin also change. Mature oligodendrocytes express myelin basic protein (MBP), myelin-associated glycoprotein (MAG), myelin oligodendrocyte glycoprotein (MOG), proteolipid protein (PLP) (53), and adenoma polyposis coli (APC) (54), as well as O4 sulfatide (54). PLP is the most abundant protein in CNS myelin (55, 56). Olig 1–3 transcription factors are expressed at various stages of oligodendroglial maturation (54). Injury and degeneration of myelin cause the populations of intact mature functional oligodendrocytes to decline. That effect can lead to the proliferation of immature oligodendrocytes that express different myelin glycoproteins, transcription factors, and myelin glyco- and phospholipids which may not support optimum conductivity and CNS function. The conspicuous abnormalities in oligodendrocyte myelin-associated gene and lipid expression observed in human brains with AD and relevant experimental models including those linked to brain insulin and IGF resistance (19, 22–24, 57), lend strong support the hypothesis that oligodendrocytes are targets of WM atrophy and neurodegeneration in AD.

VASCULOPATHY, VASCULAR DEGENERATION, AND ISCHEMIC INJURY IN AD

Small vessel disease is a recognized component of WM degeneration in AD, but its pathogenesis and contributions to neurodegeneration are poorly understood. The nature of vasculopathy and its progression to vascular degeneration and attendant ischemic injury require mechanistic understanding to guide preventive and therapeutic measures. Important initial steps include drawing distinctions between amyloid and non-amyloid associated vasculopathy and degeneration and characterizing the mediators and consequences of non-amyloid microvasculopathy, which is a feature of WM degeneration in AD.

The well-established AD-associated progressive declines in cerebral blood flow, glucose metabolism, and oxygen utilization suggest that impairments in brain perfusion are important components of AD (58, 59). However, the extent to which vascular disease causes AD or represents an integral component of neurodegeneration remains controversial. Postmortem studies demonstrated cerebral vascular pathology in over 80% of brains with AD (60). In a separate postmortem study, substantial overlap was observed between AD and vascular-mediated injury, but very few cases of dementia could be attributed to vascular disease alone (2). The Gothenburg study reported that mental slowness and deficits in executive function were linked to WM vascular dysfunction and pathology but not cortical vasculopathy (37). Together, these studies suggest that although CNS vasculopathy contributes to AD, it is seldom sufficient to cause dementia on its own (37).

In AD, there are two major types of microvascular pathology: amyloid angiopathy and non-amyloid vasculopathy (Figure 3). Amyloid angiopathy affects vessels in the cerebral cortex and leptomeninges, but not WM (61–63). In AD, non-amyloid vascular degeneration occurs in microvessels, including capillaries, arterioles, and venules in the cerebral cortex, WM, and subcortical nuclei. Non-amyloid microvascular disease is characterized by fibrotic thickening of vessel walls (sclerosis), loss of endothelial cells, thickening of basement membranes, attrition of perivascular tissue (64), reduced vascular density (micro-vasculopenia), and increased vessel coiling (65). Mural sclerosis leads to extreme narrowing of the lumens and reduced vaso-responsiveness, restricting perfusion, particularly in times of increased metabolic demand (66). Chronic hypoperfusion of WM causes ischemic injury ranging from myelin loss to fiber attrition, and in extreme cases, leukoaraiosis and micro-infarcts (5, 13). Another consequence of microvascular pathology is weakening and increased permeability of vessel walls as that occurs in diabetic nephropathy (67). Leakiness of microvessels enables toxins and inflammatory mediators from the peripheral circulation to enter the brain and cause perivascular tissue injury and attrition (58, 68–74).

Potential role of nitric oxide in cerebral microvascular dysfunction and pathology

Nitric oxide (NO) is an important physiological modulator of vascular smooth muscle function and blood flow. However, NO in high concentrations can be cytotoxic due to the activation of stress and inflammatory responses.

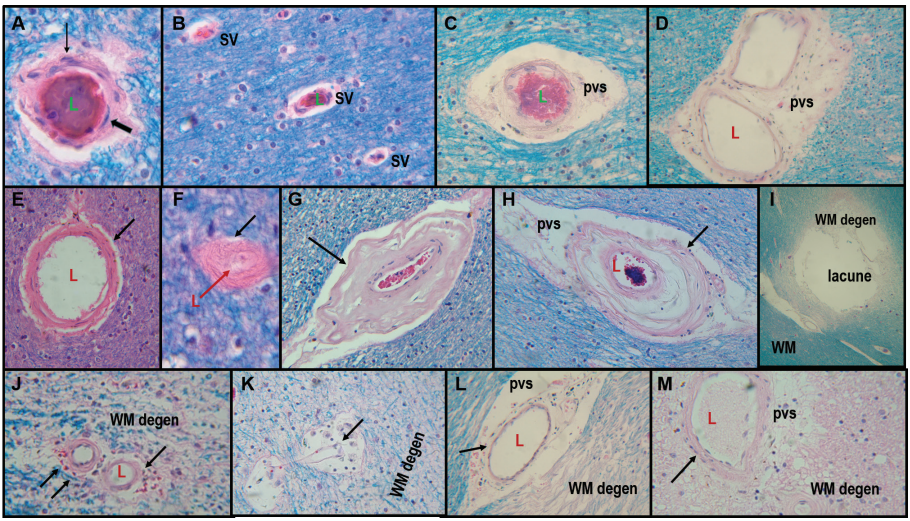

Figure 3 White matter (WM) vascular pathology in AD. Human postmortem parietal lobe samples were fixed in formalin, embedded in paraffin, and stained with Luxol fast blue (LFB), hematoxylin and eosin (LHE). (A, B) Control white matter (A) medium size and (B) small vessels (sv). Fine arrow in A shows normal smooth muscle cells and the broad arrow shows a normal endothelial cell. L = lumen. Panels C–M depict vascular pathology in AD WM. (C) Medium size vessel with reduced smooth muscle and a widened perivascular space (pvs) reflecting to perivascular tissue attrition. (D) Vascular fibrosis with enlarged pvs containing lipid-laden macrophages and hemosiderin deposits. (E) Vascular fibrosis (arrow) and myelin loss (markedly reduced LFB staining compared with A–D, and F–I). (F) Severe microvascular disease with fibrotic thickening (black arrow) and an extremely narrow lumen (red arrow; L). (G, H) Severe arteriosclerosis with degeneration and splitting of vessel walls, narrowing of lumens and in (H), widened pvs. (I) WM lacune (micro-infarct) associated with severe vascular degeneration and circumscribed area of WM degeneration. (J–M) Progressive WM degeneration associated with vasculopathy. WM degeneration is associated with loss of myelin staining, perivascular tissue attrition, and fiber loss. (M) Absent LFB staining and cystic degeneration of WM.

Microvascular degeneration is ultimately mediated by oxidative stress and inflammation. The potential role of NO as a mediator of vascular degeneration in AD was suggested by the findings that high levels of nitric oxide synthase (NOS) activity were co-localized with nuclear p53 in cerebral vessels (75) and cells with increased expression of pro-inflammatory and immune signaling genes (76). A later independent study of postmortem human brains demonstrated increased endothelial NOS (NOS3) immunoreactivity co-localized with nuclear p53 in AD microvascular smooth muscle and endothelial cells, confirming a role for aberrant NOS expression in cerebrovascular cells with increased proneness to apoptosis (77). Together, these findings suggest that non-amyloid vascular degeneration in AD is mediated by increased NO production and activation of inflammatory mechanisms.

However, the simultaneous detection of many sclerotic vessels with increased nuclear p53 but no detectable NOS3 immunoreactivity in either smooth muscle or endothelial cells, that is NOS3 expression was aberrantly down-regulated, suggests additional mechanisms mediate vascular dysfunction in AD (77).

For example, degenerative vascular sclerosis compromises vaso-responsiveness and flow, resulting in chronic ischemic injury that can be exacerbated by repeated and multifocal thrombotic microvascular occlusions. Furthermore, fibrotic degeneration disrupts vessel wall integrity, rendering them more permeable to toxic-inflammatory factors from the peripheral circulation. This phenomenon could account for the perivascular tissue attrition that accompanies microvascular degeneration in AD (Figure 3).

Thrombin activation, inflammation, microvascular occlusion, and ischemic injury in AD

In AD, the cerebral microcirculation is pathophysiologically activated due to endothelial cell overexpression of bioactive, neurotoxic, and inflammatory proteins including thrombin. Endothelial-derived thrombin is a multifunctional protease which in AD, besides promoting vascular occlusion, functions as a stress-activated neurotoxin (78, 79). Mechanistically, thrombin initiates neuronal apoptosis via activation of glial and microglial cells, leading to increased oxidative stress and neuroinflammation (80). Thrombin also stimulates Aβ precursor protein production and cleavage, mediates proteolytic processing of tau, and causes tau hyperphosphorylation and aggregation (80, 81). In essence, vessel-derived thrombin could represent a critical modulator of AD pathology via its regulation of inflammatory and bioactive protein expression. In this regard, thrombin activation of endothelial cells enhances expression and release of many pro-inflammatory proteins including monocyte chemoattractant protein-1 (MCP-1), intercellular adhesion molecule-1 (ICAM-1), IL-1, IL-6, and IL-8 (80, 81). Thus, microvascular disease could initiate and propagate neuroinflammation in AD.

Pro-inflammatory cytokine activation in endothelial cells leads to oxidative stress and thrombin release, with attendant thrombotic luminal occlusion or vessel wall injury causing increased permeability (82). However, the same responses can be mediated by up-regulation of the thrombin receptor protease-activated receptor 1 (PAR-1) or down-regulation of the brain thrombin inhibitor, protease nexin-1 near blood vessels (80). Since brain endothelial cells produce thrombin and also express functionally active PAR-1 and PAR-3 (79, 83), thrombin may initiate autocrine stimulation of a noxious feed-forward cycle. In addition, the intimate proximity of microvascular endothelial cells to microglia, astrocytes, and oligodendrocytes enables secretory products, including thrombin to influence cellular responses via a paracrine-type stimulation. For example, treatment of human microglia with thrombin induces TNF-α/TNR-dependent up-regulation of NF-κB (84). In astrocytes, thrombin activation of PAR-1 leads to increased MMP-9 expression through the regulation of several signaling pathways including PKC, JNK, and MAPK (85).

Microvascular endothelial cells elaborate trophic factors that positively impact oligodendroglia, but under conditions of stress, injury, or inflammation, endothelial cell dysfunction can adversely affect oligodendrocytes. An important role for endothelial-derived factors in oligodendroglial health was suggested by studies showing that endothelial cell-conditioned media enhances survival of OPCs (86, 87). On the other hand, in an experimental animal model of cerebral small vessel disease, early development of endothelial cell dysfunction was found to promote

secretion of heat shock protein 90alpha and subsequently block oligodendroglial differentiation and production of mature myelin (88). Mechanistically, oxidative stress impairs the capacity of endothelial cells to generate trophic factors (89) needed to support critical functions of mature oligodendrocytes, including myelin maintenance.

In AD, WM degeneration could stem from both endothelial and oligodendrocyte dysfunction. Endothelial cell dysfunction leading to excess thrombin release could cause micro-ischemic injury due to thrombosis or perivascular toxic injury mediated by increased vessel wall degeneration and permeability. At the same time, increased thrombin release would compromise the functional integrity of oligodendroglia, impairing survival and maintenance of mature myelin (79, 90–92). The concept that increased thrombin release could impair oligodendrocyte function is supported by the findings that the PAR1 thrombin receptor is a critical extracellular switch that controls myelination and that PAR1 deletion increases myelination (93), survival, maturation, and myelin maintenance. Altogether, the findings suggest that increased thrombin signaling by any one of several mechanisms can lead to micro-vessel-related WM injury in AD. However, the missing link is that we still do not understand the underlying causes of brain microvascular degeneration and endothelial cell dysfunction that lead to thrombin activation. One potential etiopathic candidate is insulin resistance since type 2 diabetes mellitus and other insulin resistance diseases are typically associated with microvascular disease and increased thrombin activation, accompanied by oxidative stress, platelet aggregation, vascular occlusions, and ischemic injury (94, 95). Correspondingly, insulin inhibits thrombin-induced endothelial dysfunction and mitigates microvascular permeability by decreasing thrombin-mediated vascular endothelial-cadherin translocation to the cytoskeleton/nuclear compartment (96).

INSULIN AND INSULIN-IGF SIGNALING IMPAIRMENTS AS MEDIATORS OF WM GLIAL-VASCULAR DEGENERATION IN AD

Considerable research had already demonstrated roles for impaired insulin and IGF-1 signaling in AD cortical and subcortical gray matter structures. However, little information is available regarding alterations of these same signaling pathways in AD WM degeneration, despite evidence that oligodendrocyte survival and function are dependent upon intact insulin and IGF networks. Therefore, additional research on the nature, mechanisms, and effects of impaired insulin and IGF signaling in relation to brain WM degeneration could generate a solid foundation for enhancing a broader understanding of the spectrum of brain pathology in AD.

In AD, deficits in brain energy metabolism, particularly concerning glucose utilization have been recognized for years (97–100). Positron emission tomography (PET) imaging with (18) F-fluoro-deoxyglucose (FDG) is a standard approach for detecting early impairments in brain glucose uptake and utilization (101–103). Insulin and IGF are major regulators of energy metabolism in the

brain, and they have critical roles in maintaining broad neuronal and oligodendrocyte functions (38, 40). Impairments in brain insulin/IGF signaling due to insulin/IGF deficiencies or receptor resistances cause deficits in learning and memory (104).

Postmortem human studies demonstrated that AD is associated with significantly reduced expression of brain insulin and IGF polypeptides and receptors, insulin and IGF receptor tyrosine phosphorylation and receptor binding, activation of downstream pathways that promote cell survival, metabolism, neuronal plasticity, and myelin maintenance, and inhibition of signaling mechanisms that promote oxidative stress, neuroinflammation, cell death, and lipid peroxidation (42, 105, 106). Insulin/IGF deficiencies and resistances increase with Braak stage severity of AD (41, 105) and therefore correlate with accumulations of Aβ and pTau pathologies. The finding that cerebrospinal fluid (CSF) insulin levels decline in the early or intermediate stages of AD (107), and overlap with progressive accumulations of Aβ and advanced glycation end-products (AGEs) (Figure 4A) (52, 107, 108) which cause oxidative stress and neuroinflammation, suggests that insulin deficiency contributes to progressive neurodegeneration in AD.

The human studies linking AD pathogenesis and progression to impairments in brain insulin/IGF signaling are supported by data from experimental models of sporadic AD produced by intracerebral (i.c.) administration of streptozotocin (STZ). STZ, a pro-diabetes toxin, injected into the cerebral hemispheres and ventricles, causes selective insulin deficiency and resistance in the brain with deficits in learning and memory, elevated levels of pTau, Aβ, and ubiquitin, loss of neurons, gliosis, oxidative stress, neuroinflammation, WM atrophy, and microvascular disease (47–49, 104, 109). Importantly, data from these models support the concept that sustained and progressive deficits in brain insulin/IGF signaling cause nearly all of the known structural, functional, biochemical, molecular, and neurobehavioral abnormalities identified in AD. Correspondingly, insulin administration improves working memory and cognition (110–113) and enhances Aβ clearance (114). Moreover, CNS-appropriate insulin sensitizer drugs have been shown to prevent or reduce AD-associated abnormalities in experimental animals (49, 109, 115).

Until now, the adverse effects of brain insulin/IGF deficiencies and resistances have been focused on neurodegeneration and the functional impairments in gray matter structures due to the interest in linking them to standard neuropathological processes. Additional research is needed to determine how insulin/IGF-1 metabolic dysfunction mediates other aspects of AD. In this regard, recent preliminary studies showed that with the increasing severity of AD, WM atrophy and degeneration are associated with corresponding impairments in the expression of Akt pathway proteins and phosphoproteins (de la Monte, S.M. and Tong, M, Unpublished). Compromised signaling along these pathways could lead to loss of structural and functional integrities of oligodendrocytes and myelin. Although the steps leading from brain insulin and IGF deficiencies and resistances to WM degeneration have not yet been delineated, clues may be harnessed from data generated via unrelated experiments. For example, several studies have shown that WM atrophy and degeneration in other models of brain insulin and IGF resistances were associated with significant oligodendrocyte dysfunction. For example, in a rat model of i.c. STZ, WM degeneration was associated with reduced expression of mature myelin-associated genes and increased expression of

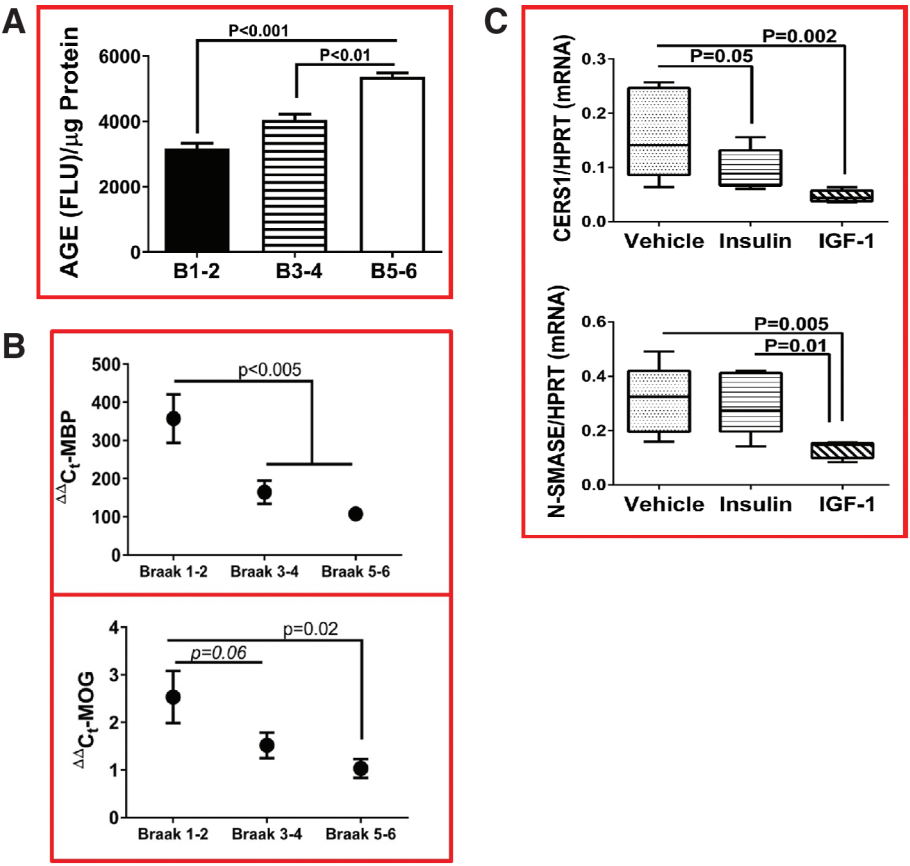

Figure 4 Cellular signaling abnormalities mediating WM degeneration. (A) Advanced glycation end-product (AGE) immunoreactivity was measured in postmortem frontal WM homogenates from humans with normal aging (Braak 0–2; B0–2), moderate AD (B3–4),or severe AD (B5–6) AD using a direct binding ELISA (107). Immunoreactivity was detected with horseradish peroxidase-conjugated secondary antibody and Amplex Red fluorophor. Fluorescence light units (FLU) were measured (Ex 579 nm/Em 595 nm) in a Spectromax M5, and results were normalized to protein content. (B) AD WM atrophy and degeneration are associated with reduced expression of mature MAGs. Quantitative RT-PCR was used to measure mRNA levels of MAG 1 and myelin oligodendroglial glycoprotein (MOG). PCR primer pairs were designed with Primer 3 (http://primer3.sourceforge.net/) software. PCR reactions were performed in a Roche Lightcycler 480 System (116). Gene expression was analyzed using the $^{\Delta\Delta}C_t$ method with results normalized to hypoxanthine-guanine phosphoribosyl transferase, HPRT. (C) Insulin and IGF-1 suppress expression of genes encoding enzymes that produce ceramides or break down sphingomyelin. Frontal lobe WM slice cultures from an i.c. STZ adult Long Evans rat model of sporadic AD (109) were stimulated for 24 h with 10 nM insulin, 10 nM IGF-1, or vehicle (control). Graphs depict the mean ± S.E.M. for each group. Intergroup comparisons were made by one-way ANOVA with the post hoc Tukey's test. Significant P-values and trends are indicated.

immature myelin-associated genes (116), corresponding with deficits in myelin maturation and striking alterations in WM structure (117). Similar observations have been made in human postmortem brains with different severities of AD and WM atrophy (Figure 4B).

An additional feature of experimental brain insulin resistance with WM atrophy is that oligodendrocyte dysfunction is associated with altered expression of sphingolipid metabolizing enzymes such that ceramide accumulation and sulfatide depletion would be favored (Figure 4C) (118, 119). In humans and experimental animals, reductions in brain sulfatide and increases in ceramide correlate with cognitive impairment, oxidative stress, lipid peroxidation, and neuroinflammation (22–24, 120). Ceramides inhibit insulin signaling through PI3K-Akt (25, 121) and increase oxidative stress, Aβ, pTau, and pro-apoptosis activation (122). Furthermore, preliminary studies suggest that insulin and IGF-1 stimulation suppress expression of enzymes that generate ceramides via degradation of sphingomyelin (Figure 4C). Therefore, it is likely that impairments in insulin/IGF-1 signaling in oligodendrocytes are important in the pathogenesis of WM atrophy and degeneration and mediate disease progression in AD.

Proposed role of APOE-ε4

Apolipoprotein E is the major lipid transport protein in the CNS. This 34 kDa protein has three major isoforms (APOE-***ε2***, APOE-***ε3***, and APOE-***ε4***) that differ by single amino acid substitutions at residues 112 and 158 (123). The ***ε4*** allele is the strongest genetic risk factor for late-onset sporadic AD (124–127) in that carriers account for over 50% of all AD cases (128); however, the APOE-***ε4*** risk assessments vary across different countries and ethnicities (129). AD risk is increased by three- or fourfold among APOE-***ε4*** carriers, and 15-fold in APOE-***ε4*** homozygotes. APOE-***ε4*** confers increased risk for AD by reducing brain glucose metabolism in the preclinical stages of disease (130), and ultimately impairing signal transduction through the insulin receptor, reducing Aβ clearance, and increasing Aβ aggregation (131). APOE-***ε4*** may also have a role in mediating AD-associated WM degeneration via insulin resistance (131) and attendant microvascular endothelial cell and oligodendrocyte dysfunction.

Insulin/IGF signaling impairments and glial-vascular WM pathologies in AD

Our overarching hypothesis is that in AD, WM degeneration is mediated by impairments in insulin and IGF signaling that cast a wide net of pathophysiological responses including oxidative stress, inflammation, and dysregulated glucose and lipid metabolism (Figure 5). In WM, the targets of degeneration are oligodendrocytes and microvessels. Reduced signaling through insulin/IGF receptors, IRS and downstream Akt pathways compromises oligodendrocyte survival, myelin maintenance and integrity, and sphingolipid homeostasis, favoring sulfatide depletion and ceramide accumulation. Ceramide-mediated neurotoxicity, inflammation, oxidative stress, lipid peroxidation, and further impairment of insulin signaling reinforce the cascade of WM degeneration.

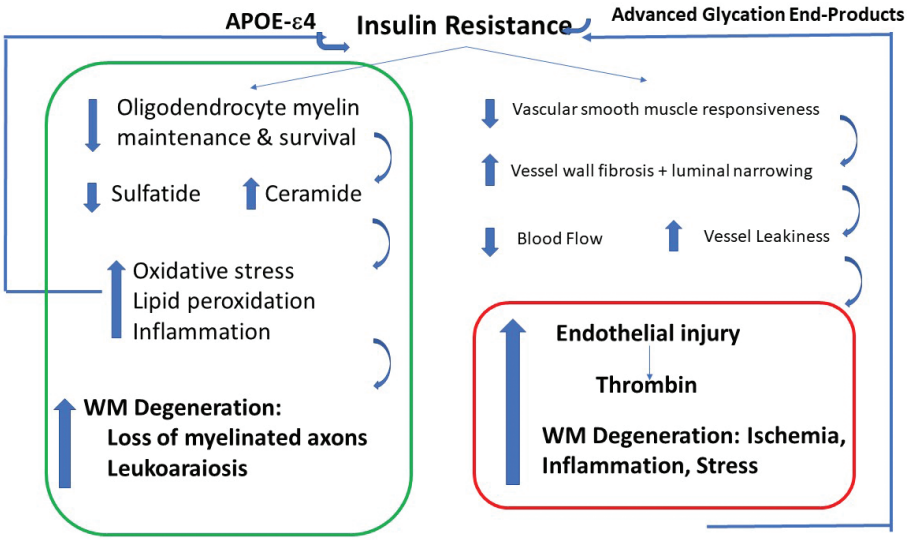

Figure 5 Hypothesis: White matter degeneration in AD is mediated by insulin and IGF resistances that target oligodendrocytes and microvessels. Reduced myelin and vascular integrity promote neuroinflammation, stress, and ischemic injury. APOE-ε4 genotype, obesogenic diets, and poor lifestyle choices have cofactor roles in mediating WM degeneration due to exacerbation of insulin resistance.

Microvascular disease is also driven by insulin deficient and resistant states such as in types 1 and 2 diabetes mellitus. Initially, microvascular disease is mediated by combined effects of hyperglycemia, increased levels of AGE (Figure 4A), up-regulation of receptors for AGE (RAGE), and reduced responsiveness to NOS/NO (67, 132). Therefore, insulin and IGF resistances negatively impact microvascular structural integrity, vaso-responsiveness, and endothelial function. In later stages, microvascular disease is associated with the replacement of smooth muscle by collagen (sclerosis) leading to degenerative mural fibrosis and luminal narrowing, restricted blood flow, reduced vessel wall integrity marked by increased leakiness, and endothelial damage with attendant up-regulation and release of thrombin. Thrombin activation drives inflammation (cytokines), oxidative stress, microvascular occlusions. Microvascular occlusions cause ischemia which can injure oligodendrocytes, myelin, axons, and vessels. Also, vessel wall leakiness exposes perivascular tissue to toxins from the peripheral circulation. Late and probably permanent microvascular-associated WM pathologies in AD include leukoaraiosis with loss of myelin and degeneration of axons, microinfarcts, perivascular tissue attrition, and vasculopenia (vessels can be destroyed by ischemic necrosis). Finally, microvascular disease can drive WM degeneration by worsening insulin resistance, oxidative stress, and inflammation.

Inflammation and oxidative stress are recognized mediators of neurodegeneration in AD (26, 39, 133). Potential sources of stress and inflammation include increased levels of AGE and RAGE expression (107, 108, 132, 134–137), impaired insulin/IGF signaling through Akt pathways, lipid peroxidation linked to myelin

breakdown, and ceramide accumulation. Neuroinflammation in AD is associated with increased pro-inflammatory cytokine expression in astrocytes and microglia (133, 138, 139). Although TNF-α, IFN-γ and IL-1β are key players, preliminary data suggest that neuroinflammatory responses are broader and include activation of pro-inflammatory and inhibition of neuroprotective cytokines/chemokines (17, 140).

CONCLUSION

Combined effects of oligodendrocyte and microvascular dysfunction interact to cause WM degeneration, including leukoaraiosis in AD. Insulin resistance exacerbation by APOE-*ε4* may accelerate AD-associated WM molecular, biochemical, and structural pathologies linked to impaired function of oligodendrocytes and microvascular endothelial cells. Therefore, WM degeneration and cognitive impairment may be preventable or reversible by lifestyle measures that restore insulin responsiveness in the CNS.

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