AWARD LECTURE (REVIEW)

Neuropathologic Changes Provide Insights into Key Mechanisms Related to Alzheimer Disease and Related Dementia

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Alzheimer disease (AD) is a chronic disease characterized by a progressive decline in memory and cognition. AD progression is closely correlated with neuropathologic changes and accumulation of the two main hallmark lesions, senile plaques and neurofibrillary tangles. Nevertheless, deciphering the complex biological aspects of AD requires not only looking for the neuropathologic changes as the cause but rather as the collective responses to a disease process that are essential to maintain life during aging but ultimately generate a nonfunctional brain. Chronic conditions, such as AD, represent a new homeostatic balance or disease state, where the organism responds or adapts to maintain life. The pathologic diagnosis of AD still remains the gold standard for precise diagnosis of dementia, commonly in conjunction with cognitive-memory tests and brain image scans. Herein, we present a general overview of the main neuropathologic hallmarks and features of AD and related dementia, revealing the key biological and functional changes as potential drivers of age-dependent brain failure related to AD. The present work reflects some of the main ideas presented during the American Society for Investigative Pathology Rous-Whipple Award Lecture 2021. (Am J Pathol. 2022, 188:1–7; https://doi.org/10.1016/j.amjpath.2022.07.002)

Alzheimer disease (AD) is a progressive neurodegenerative disorder characterized by memory loss, diminishing mental functions, and cognitive impairment. 1 Around 6.5 million Americans, aged ≥65 years, have a diagnosis of AD and related dementia, and this number is expected to reach 13.8 million by 2060. 2 Today, AD is the sixth-leading cause of death in the United States, with 121,499 official death certificates in 2019. AD is by far the most common cause of dementia, comprising 60% to 80% of all cases.

In the original 1907 article, “On an Unusual Illness of the Cerebral Cortex,” by Alois Alzheimer, he described the neuroanatomy of a demented 51-year-old woman for the first time. 3 The article describes an unknown illness characterized by a rapid loss of memory, disorientation, altered behavior, marked difficulties in reading, misspelement, and disorientation, that progressed in severity over 4.5 years of observation. A post-mortem examination showed an evenly atrophic brain and arteriosclerotic changes. Brain tissue revealed striking changes in the neurofibrils, and the presence of thick fibrils in the neurons. He observed that “the change of the fibrils seems to be a parallel process of deposition of a pathological metabolic substance in the neurons whose closer examination is still pending.” Additional interesting comments from Alois Alzheimer included the observation that around one-fourth to one-third of the neurons whose closer examination is still pending. 4

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The Rous-Whipple Award is given by the American Society for Investigative Pathology (ASIP) to a senior pathologist with a distinguished career in experimental pathology research and continued productivity at the time of the award. George Perry, Ph.D., recipient of the 2021 ASIP Rous-Whipple Award, delivered a lecture entitled “Pathology in Alzheimer Disease: A Protective Response?” on April 27, 2021, at the 2021 ASIP Annual Meeting at Experimental Biology (held virtually).
concluded that “we are dealing with a special illness” and that “a histological examination will enable us to determine the characteristics of some of these cases.” It is now recognized that elderly patients with AD and related dementia have an abnormal aggregation of misfolded proteins forming neurotoxic bundles within the cerebral cortex.

**Neuropathologic Hallmarks of Alzheimer Disease**

The hallmark pathologies of AD are the accumulation of amyloid-β (Aβ) peptide into senile plaques (SPs) outside neurons and of twisted strands of hyperphosphorylated tau protein into neurofibrillary tangles (NFTs) inside neurons in the brain. Figure 1 shows immunohistochemistry location of SPs and NFTs in AD brain tissue. The deposition of these protein aggregates is accompanied by complex molecular and cellular responses that lead to synaptic failure, neuroinflammation, and eventually progressive neuronal death. Over the last decades, one of the main focuses in AD has been to decipher the mechanisms of formation of SPs and NFTs, with the objective of identifying potential therapeutic targets. This knowledge could be used to reduce the development of AD and for the development of a possible cure or effective treatments of AD and related dementias.

The causes of AD are not completely understood but probably include a combination of many factors, including aging, genetics, environmental conditions, and lifestyle. Although aging is the most important risk factor for neurodegeneration, it is not a direct cause of AD; only one-third of all people aged ≥85 years may have AD, and many elderly people never develop dementia (https://www.nia.nih.gov/health/what-causes-alzheimers-disease, last accessed June 7, 2022). Findings of SPs and NFTs in normal aging and in patients with traumatic brain injury are common, although neither fully correlated with cognitive loss, but they were seldom questioned as to the unique cause of AD.

AD is classified into two types, early-onset familial AD and late-onset or sporadic AD. Remarkably, late-onset or sporadic AD is by far the most common type of AD, whereas only ≤1% of all cases correspond to early-onset familial AD. Genetic factors are implicated in both types of AD. In particular, the apolipoprotein E-ε4 (APOE-ε4) gene on chromosome 19 has shown a higher risk of AD, but inheriting an APOE-ε4 allele does not definitively correlate with the development of AD because many carriers simply do not develop dementia. Moreover, APOE-ε3 gene is the most common type, with no evidence suggesting it may decrease or increase the risk of AD. In contrast, APOE-ε2 is a rare form of this gene that may provide some protection or lower risk against AD. Subjects who inherit genes with specific mutations associated with early-onset familial AD usually develop symptoms as early as their late 30s and mid-60s. In particular, genetic studies of AD subjects have identified several mutations in three genes: amyloid-β precursor protein (AβPP) on chromosome 21, presenilin 1 (PSEN1) on chromosome 14, and presenilin 2 (PSEN2) on chromosome 1. The function of AβPP is not completely clear, but probably implicated as a neuronal receptor, in synaptic formation, and on hormone-metabolite regulation. AβPP is the precursor of Aβ peptide, which is produced by its proteolytic processing by PSEN1, PSEN2, and other enzymes. Identification of mutations in AβPP, PSEN1, and PSEN2 genes was associated with early-onset familial AD and an overproduction of Aβ peptide that forms neurotoxic fibrils and SPs. Nevertheless, the association of these genes with AD has been misinterpreted or placed as directly causal. Causality requires demonstrating that Aβ removal either prevents or reverses the development of AD.

Unfortunately, there are no effective treatments for AD. Current treatments only focus on the management of symptoms and maintenance of mental function, with limited effectiveness. Over the last years, the main approach for the development of therapeutic compounds against AD has been focused mainly on Aβ. In particular, there has been the development of multiple monoclonal therapeutic antibodies intended for the removal of neurotoxic forms of Aβ, but
surprisingly, in over a dozen clinical trials, the AD patients have not benefited significantly, as expected or as causality would dictate. The cumulative research indicates that Aβ is not the only driving force of AD, but still raises the question of what Aβ is doing that genetically and biologically associates it to the development of AD pathology causing progressive neuronal failure. In the same direction, therapeutic antibodies targeting aggregated tau into NFTs are being evaluated for removing tau deposits from the brain, but initial studies focused on tau removal have also not reversed disease progression. The failure of multiple therapeutic compounds (small drugs, inhibitors, and antibodies) targeting Aβ and tau opens the question of whether the biological roles of Aβ and tau in AD are still complete, wrong, or even completely reversed. There is a striking mismatch between lesions of SPs/NFTs and symptoms of AD; a significant number of subjects show neuropathologic changes with widespread deposition of SPs/NFTs (observed at autopsy) but with relatively normal cognitive conditions and without manifesting dementia.9

Roles of Oxidative Stress in Alzheimer Disease

As discussed previously, the etiology and pathogenesis of AD are not completely described, but oxidative stress is a key component. Oxidative stress refers to a state of cellular imbalance between the production of reactive oxygen species and reactive nitrogen species with the antioxidant defense systems.10 Proteins, lipids, and genetic material may experience oxidative damage during oxidative stress, altering their functions-structure and promoting mutations. In the AD brain, the amount of oxidative damage is increased in comparison with healthy elderly individuals. This damage includes elevated levels of intracellular reactive oxygen species/reactive nitrogen species, protein carbonyls, and 3-nitrotyrosine, increased lipid peroxidation, significant DNA damage as 8-hydroxy-deoxyguanosine produced by reactive oxygen species/reactive nitrogen species, and 8-hydroxyguanine in RNA.10

Almost three decades ago, we performed pioneering studies of oxidative damage in AD, particularly to understand the chemical and biological properties of SPs and NFTs. In brain tissue from AD, we identified oxidative modification of neuronal proteins through nitration, and these changes were also located within NFTs (Figure 2, A and B).11 Conversely, the levels of nitrotyrosine in age-matched controls were undetectable through immunocytochemistry in the cerebral cortex. Furthermore, oxidative damage in AD is also present in lipid membranes, as demonstrated by immunocytochemical detection of lipid peroxidation (4-hydroxynonenal) in brain tissue from AD patients (Figure 2, C and D).12 Surprisingly, the lipid peroxidation collocated with NFT lesions but not with SPs. The oxidative damage in AD is extended to the genetic material, as demonstrated by identifying 8-hydroxy-deoxyguanosine and 8-hydroxyguanine in neurons within the hippocampus, subiculum, entorhinal cortex, as well as frontal, temporal, and occipital neocortex (Figure 2, E and F).13 The subcellular localization of the oxidative damage mainly in the cytoplasm allowed us to hypothesize that mitochondrial components may be the source of free radicals promoting oxidative stress in AD. The signs of oxidative damage are more prominent early in the disease and reduce with disease progression; this relationship is more significant in APOE-e4 carriers.14 With these studies, we conclude that the damage of oxidative stress conditions is widely extended in AD to all biomolecules, including lipids, proteins, sugars, and nucleic acids in brain cells. Moreover, the oxidative...
damage to each type of biomolecule specifically increased in vulnerable populations of neurons during AD. The range of oxidative damage types in AD suggested the involvement of Fenton reactions as the source of free radicals that promote oxidative damage, particularly focusing on abnormal levels of oxidation-reduction (redox)—active metal ions, such as copper and iron, which are the source of reactive oxygen species and other redox-generated free radicals. Figure 3, A and B, shows the histochemical detection of iron in AD, the deposition of iron collocated mainly with SPs and NFTs.

The oxidative stress conditions correlate with a transition from normal aging to the onset of cognitive impairment, and later on to prodromal AD. This occurs mainly by affecting the hippocampus and temporal cortex with oxidative damage, and is mostly restricted to the neuronal components in the cytoplasm, not APC and NFTs. In fact, affected neurons with NFTs had reduced oxidative damage, and Aβ levels negatively correlated with oxidative damage in AD and Down syndrome. Overall, these observations suggested that the overexpression and aggregation of Aβ and tau could be part of complex neuronal molecular responses that are responsive to oxidative stress conditions in the aging brain. To gain further insight, we performed experiments to understand the brain responses to oxidative stress. First, we determined that NFTs are not formed by the aggregation of pure tau protein but also are intimately associated with other proteins, such as heme oxygenase-1, an enzyme that converts heme to biliverdin/bilirubin—transforming an oxidant to an antioxidant. Second, the levels of tau phosphorylation are correlated with heme oxygenase-1 expression in neuronal cells, and tau is regulated through signal transduction pathways that are modulated by oxidative stress to play a role in the cytoprotection of vulnerable neurons.

Finally, Aβ peptide binds different redox-active metals, including Cu(II), Fe(II/III), and Zn(II), to stabilize them.

Figure 3  Histochemical location of oxidation-reduction—active iron in Alzheimer disease (AD). A: Tissue section from AD brain (arrowheads indicate the presence of neurofibrillary tangles, and arrows indicate amyloid senile plaques). B: Control (non-AD) brain. C: X-ray spectromicroscopy (STXM) image showing the overall plaque morphology. D: Composite STXM image showing plaque morphology (blue), Cu²⁺ (green), Cu³⁺/Cu⁰ (red), and iron (gray) content. E: Iron oxidation state difference map of the region highlighted in B. Stronly absorbing oxidized iron (Fe³⁺) is shown as light contrast, and chemically reduced iron (Fe²⁺ and/or Fe⁰) is shown as dark contrast. F: High-resolution (HR) composite image. G: Copper oxidation state difference map of the region highlighted in B. In the oxidation state difference map, oxidized copper (Cu³⁺) is shown as light contrast, and chemically reduced copper (Cu²⁺ and/or Cu⁰) is shown as dark contrast. Reproduced with permission from Proc Natl Acad Sci U S A, 1997, 94:8666 (A and B) and Sci Adv, 2021, 7:eabf6707 (C–G).
from redox cycling reactions in the AD brain.\textsuperscript{21,22} The interactions between A\textsubscript{β} peptide and redox-active metal ions promoted the oxidation of His residues of A\textsubscript{β}, and favored their aggregation into APC.

In recent studies, we demonstrated APC from AD contain metallic elements within them, including copper and iron in multiple valence states (Cu\textsuperscript{2+}, Cu\textsuperscript{2+}, Fe\textsuperscript{3+}, and Fe\textsuperscript{3+}), as observed with synchrotron-based X-ray spectromicroscopy (Figure 3, C–G).\textsuperscript{16} The advanced imaging and quantitative spectroscopic techniques allowed us to directly demonstrate the involvement of redox-active metal ions in redox cycling metabolism. Furthermore, advanced X-ray spectromicroscopy imaging revealed the presence of nanometer size metallic aggregates of copper (Cu) and iron (Fe) within purified amyloid plaque cores, and in correlation with deposition of iron in AD pathology. Remarkably, APC from advanced AD also contained iron aggregates formed by redox-active species, Fe\textsuperscript{3+}, Fe\textsuperscript{2+}, and metallic iron (Fe), and forming nanostructured aggregates.\textsuperscript{1,3} Some of these metallic species should not be stable to air, demonstrating A\textsubscript{β} peptide has unique properties responsible for its antioxidant activity, this through binging of redox-active ions and promoting their aggregation within APC.

Overall, it was observed not only do SPs and NFTs play a role in oxidative stress responses in brain cells, but that several signal transduction pathways are induced during the development of neurodegenerative processes of AD, such as mitogen-activated protein kinase/extracellular receptor kinase,\textsuperscript{25} neuroinflammation, and cell cycle reentry (https://www.alzforum.org/news/researchnews/targeting-glial-cells-towards-selective-cns-specific-antiinflammatory-drugs, posted June 2, 2002, last accessed May 5, 2022), and what we think may be the most fundamental: dysfunctional glucose metabolism by induction of the pentose phosphate pathway and increased neuronal reduction to produce the cellular antioxidant compound glutathione.\textsuperscript{26}

Mitochondrial Dysfunction in the Pathogenesis of Alzheimer Disease

Another neuropathologic feature related to the neurodegenerative processes of AD is mitochondrial dysfunction. Specifically, this includes mitochondrial structural and functional integrity alterations, mitochondrial biogenesis and dynamics, axonal transport, mitophagy, and mitochondrial proteostasis.\textsuperscript{27} These biological changes raise the question of where and how they occur in vulnerable cell populations in the AD brain. We focused our efforts on elucidating the mitochondrial responses during AD, because mitochondria contain abundant levels of metalloproteins and are the main source of free radicals and highly oxidant compounds, such as superoxide (O\textsubscript{2}'). Just as most oxidative damage is dependent on redox-active ions Cu/Fe, the mitochondria oxidative metabolism is almost completely dependent on these metals as cofactors and acceptors.

Mitochondria from AD brain samples were examined with probes to mitochondrial DNA, enzyme proteins, and enzyme-prosthetic groups, such as cytochrome oxidase 1. We found that the same population of neurons displaying a high degree of oxidative damage also contained abundant mitochondrial debris, mostly located within autophagosomes.\textsuperscript{28} These subcellular structures also contained ferrooxidase-ferroreductase, the activity to change iron redox state to catalyze both redox silencing and oxidative damage.\textsuperscript{15}

Close examination of an aging brain series revealed those after the age of 40 years showed similar—although reduced—mitochondria autophagy to that observed in patients with AD. These observations suggested a new molecular mechanism related to AD pathogenesis, one where the activation of mitochondria autophagy is at the center of neuronal damage. Figure 4 illustrates the neuronal responses to oxidative stress and their impact on mitochondrial dysfunction. Our studies of mitochondria transport, fusion-fission, and turnover all have consistently shown morphologic and functional abnormalities related to the progression of AD. Activation of mitochondria autophagy poses several challenges for affected neurons. Specifically, the alterations in metal ion transport/homeostasis are key components, because redox-active Cu/Fe ions that are liberated during the process of mitochondria turnover can cause massive oxidative damage to all compartments of neurons and even the entire brain if not properly counterbalanced by the antioxidant systems. From the cumulative evidence, we hypothesize that A\textsubscript{β} and tau may play this role as a neuroprotectant by binding free metal ions to reduce their reactivity. This molecular function of A\textsubscript{β} and tau is essential during neurodegeneration, and mutations in A\textsubscript{β}PP may lead to improper functionality in neuronal cells and, consequently, lead to early-onset AD.

Mitochondria are at the center of aerobic energy production, producing >95% of all cellular ATP, also controlling programmed cell death processes, and a master neuronal signaling, calcium (Ca\textsuperscript{2+}). Calcium controls microtubule assembly, and these microtubules are decreased by up to 90% in neurons affected by AD, as well as diminished in the brain during normal aging.\textsuperscript{29} Proteolysis is also controlled by calcium, as is synaptic vesicle fusion. Loss of mitochondrial functions seems to be at the center of oxidative stress responses, promoting autophagy, altered redox-active biometal dynamics, and activation of neuronal survival mechanisms in neurodegeneration. During aging, diminished mitochondrial functions are met with critical compensations by A\textsubscript{β}/tau/HO-1/autophagy/survival responses that are all essential to maintaining brain function. As aging progresses, the compensation mechanisms deployed in the brain grow and may ultimately reveal themselves as pathology. With continued aging, some of the compensation mechanisms could fail to maintain normal brain function and rather redirect cellular metabolism to survival rather than function. This choice is evolutionarily
selected as a response to temporary brain stress insults rather than the ever-present effects of normal aging. This interpretation is consistent with the lack of correlation between neuronal loss and cognition: simply, in AD, the neurons are there but not functioning. Although these affected neurons might correspond to senescent cells, all neurons of the same population display pre-apoptotic changes without death, and their therapeutic removal will only advance disease progression. Moreover, mutations in AβPP lead to the improper molecular processing of Aβ rather than primary causation. Finally, and most significantly, the removal of Aβ, tau, or even redox-active metals will not benefit AD patients by reversal of the primary driver because each is an important neuroprotective response, explaining why many therapies focused on Aβ removal have had no benefit in cognition, memory, and brain function.

Mitochondria are highly responsive to changes in cell homeostasis, cellular stress, inflammation responses, environment, nutrition, and exercise status, all of which play major factors in the potential development of AD. All these factors are directly or indirectly linked to the dysfunctions in energy metabolism as key drivers to the development of AD. Potential therapies maintaining mitochondrial functions will delay AD, as observed with some anti-diabetes drugs (metformin and insulin, among others). Similarly, promoting more efficient autophagy or restoring calcium levels could lower neuronal oxidative stress. Focusing on the complex biology of AD through pathology will continue to reveal potential or alternative therapeutic insights that will benefit AD patients and their families.

Conclusions

Alzheimer disease is still a complex chronic neurologic disease with no effective treatments. The molecular and structural changes observed in AD brain have helped to establish the key mechanisms involved in the progression of the disease, and to differentiate them from normal aging processes. The main neuropathologic features are synaptic failure, neuronal loss, and mainly the widespread presence of amyloid plaque cores and neurofibrillary tangles, but these abnormal protein aggregates are more complex than we thought. Over the last years, with the advancement of novel quantitative and advanced imaging techniques, the ultrastructural and chemical signatures of SPs and NFTs revealed the presence of inorganic aggregates formed by biometals, such as Fe, Cu, Zn, and Ca. Furthermore, additional pathologic features not frequently analyzed include alterations in mitochondria, lipids, and inflammation responses, and altered neuronal phenotype. Remarkably, clinical decisions are often based on histopathologic/microscopic results, such as in cancer, but in the case of AD this is not possible, and the confirmation of neuropathology is performed postmortem. We still face significant challenges to fight AD, expecting that soon we will have effective therapeutic approaches and sensitive/effective molecular diagnostic tools.

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References


