ABSTRACT

The pathophysiology of Alzheimer’s disease (AD) is complex, involving many neurotransmitter systems and pathophysiologic processes. Although a great deal remains to be understood, the number and diversity of aberrant mechanisms increases the hope of finding effective and diverse therapeutic interventions for the prevention and treatment of AD. The long-held hallmarks of AD—β-amyloid plaques, neurofibrillary tangles (NFTs), and neuronal cell death—are well-known and central factors in the neurodegenerative process. However, plaques and NFTs are not unique to AD. These same structural changes occur with normal aging and in many other neurodegenerative disorders. In AD, they are uniquely distributed to particular regions within the brain. This article reviews the pathophysiology and course of neurological damage seen during AD progression, from preclinical to severe stages, and describes currently held hypotheses on the causes and mechanisms leading to neuronal cell death. Recent studies and debate are expanding our understanding beyond the definitions and details of AD pathology to explore how the sequence and the timing of events within the life cycle influence the development of symptoms and illness progression. A fascinating (and somewhat alarming) finding is that the initial changes in neuronal function and neurotransmitter availability may actually begin years, even decades, before there is any evidence of clinical symptoms. Grasping the details of AD pathophysiology is fundamental to understanding illness progression and assessing the potential merits and side effects of therapeutic interventions. (Adv Stud Pharm. 2005;2(4):126-135)

Alzheimer’s disease (AD) has 3 consistent neuropathologic hallmarks: plaques of β-amyloid protein (amyloid plaques), neurofibrillary tangles (NFTs), and neuronal degeneration. Plaques and NFTs were first discovered by Alois Alzheimer in a 1906 autopsy of a demented patient. Figures 1 and 2 show examples of plaques and NFTs after histologic staining. However, plaques and NFTs are not unique to AD, as these same structural changes occur with normal aging and in many other neurodegenerative disorders. A distinguishing feature of AD is that the plaques and NFTs are localized to areas in the brain corresponding to the clinical symptoms. Although the development of plaques and NFTs eventually leads to a noticeable clinical condition, the process is thought to start years before the initial onset of symptoms (preclinical AD). To understand these relationships, it is useful to have a general knowledge of the brain landscape.

Structural Changes in the Alzheimer’s Disease Brain

The brain areas involved in memory include the cortex, entorhinal cortex, and hippocampus (Figure 3). A defining symptom of AD is memory loss, particularly in short-term recall. Preclinical AD begins in the entorhinal cortex, which connects the hippocampus, the structure responsible for memory formation (short- and long-term memory; Figure 4), to the cerebral cortex. Magnetic resonance imaging studies indi-
cate that neuronal loss (measured by atrophy in these regions) may start years before signs of memory loss emerge. As the brain atrophies, cerebrospinal fluid fills in the space previously occupied by brain tissue.

Mild to moderate AD is characterized by prominent memory loss and a decline in the ability to process complex thoughts. In the brain, atrophy continues within the cerebral cortex (Figure 5). These changes result in the common symptoms of short-term memory loss, difficulty with balancing the checkbook or other complex activities, difficulty recalling well-known names, confusion about familiar places, and even mood and personality changes.

By the severe stage of AD, cortical atrophy has become prominent in the areas that control speech, reasoning, sensory processing, and conscious thought. As larger areas of the cortex and hippocampus atrophy, the size of the lateral and third ventricles increases (Figure 6). As expected with this degree of brain atrophy, the symptoms of severe AD increase in severity (ie, impaired long-term memory, seizures, incontinence, weight loss, no recognition of loved ones, unable to sit up, and groaning/moaning/grunting). Interestingly, the cortical motor strip and brain stem are left relatively intact. The progression of atrophy is shown in Figure 7.

LEARNING

The human brain is estimated to have 100 billion neurons, which form 100 trillion synapses. Connections between neurons are established during brain development, primarily through the formation of synapses between the presynaptic neurons and postsynaptic neurons. Experience and learning strengthen neuronal connections through repeated excitatory and inhibitory inputs to postsynaptic neurons. In early AD, the strengthening of new inputs is diminished because of the depletion of specific neurotransmitters (acetylcholine, serotonin, and norepinephrine; Figure 8) and neuronal cell death. As AD progresses, these established neuronal connections become increasingly disrupted throughout much of the cerebral cortex, slowly destroying a lifetime of experiences while new learning becomes more difficult, then impossible.

DEGENERATIVE PROCESSES IN ALZHEIMER’S DISEASE

Recent studies and debate are expanding our understanding beyond the definitions and details of AD pathology to explore how the sequence and timing
of events within the life cycle influence the development of symptoms and illness progression. Central to the degenerative process is the development of plaques and tangles, although many other abnormalities are also occurring during the progression of AD. First, we discuss information known about the hallmark pathologic features (plaques and tangles) and then review other processes thought to be involved during the course of AD.

**Amyloid Hypothesis and the Development of Plaques**

The central role of β-amyloid plaques amongst the many diverse pathophysiologic processes of AD neuronal degeneration is now well established. Plaques are seen in all persons as they age, but in AD, the density is higher and the plaque distribution correlates with the areas of neuronal degeneration and clinical symptoms. β-amyloid plaques are clumps of insoluble peptides that result from the aberrant cleavage of amyloid precursor protein (APP), a transmembrane protein. The exact function of APP is uncertain, although several roles have been suggested, including that of an adhesion protein for cell-to-cell contact and structure, a factor involved in promoting neurite growth and perhaps differentiation, and as a modulator of gene transcriptional activity. APP is cleaved by 3 enzymes—β-secretase, γ-secretase, and α-secretase. Normally, cleavage by β-secretase, followed by γ-secretase, yields a soluble 40 amino acid peptide (Figure 9). In AD, a variant form of the γ-secretase cleaves APP at an incorrect place, creating a 42 amino acid peptide called Aβ42 or Aβ, which is not soluble and aggregates into identifiable clumps termed β-amyloid plaques (Figure 10).

α-secretase serves a protective function as it cleaves APP at a site that prevents Aβ formation. Potentially, α-secretase agonists and γ-secretase inhibitors may provide alternate mechanisms useful in developing new therapeutic interventions for the prevention and treatment of AD.

There are 3 genes identified in familial AD (APP, PS1 [presenilin 1], and PS2 [presenilin 2]), and all are known to be involved with the formation of Aβ. APP (identified as a gene rather than the protein by the use of italics) is the gene that codes for APP (the protein). It is located on chromosome 21, which may explain why people with Down's syndrome (trisomy 21—3 copies of chromosome 21) ultimately develop AD and do so at a younger age than the general population. 5
There are at least 20 known mutations of APP, more than 140 known mutations in PS1, and approximately 10 in PS2. PS1 and PS2 code for presenilin, the catalytic subunit of γ-secretase.\textsuperscript{5,6}

In mice bred specifically for mutant APP, increases in Aβ correlate with neuropathologic and behavioral changes similar to AD.\textsuperscript{7} When these findings are considered together, the amyloid hypothesis is very strongly supported, especially in familial AD. Immunization against Aβ is under investigation in animals and humans.\textsuperscript{7,8} Although promising results have been achieved, there have been problems with encephalitis. Achieving better tolerability is the focus of intense research at this time.

Recent studies also show that Aβ can aggregate in several ways—as oligomers (eg, aggregates of 2 or 3 peptides), polymers (larger aggregates or plaques), and fibrils. Oligomers are soluble, yet appear to induce damage to the neuron and synapse as their presence in the synapse strongly correlates with the severity of cognitive impairment. Therefore, it appears that neurotoxicity from the Aβ peptide likely occurs through several mechanisms.\textsuperscript{7,9}

**TANGLES**

Neurofibrillary tangles are seen as dying or dead neurons when viewed after histologic staining (Figure 2). NFTs result from the destruction of neuronal microtubules caused by the modification of their supporting protein, tau (Figure 11).\textsuperscript{7} Microtubules are essential components of neuronal cell structure; they deliver nutrients and assist in synaptic transmission along the length of the neuronal axon. During AD pathogenesis, tau proteins become hyperphosphorylated, disrupting their bonds to microtubules, thus collapsing microtubule structure and destroying the neuron’s transport and communication system. Neuronal cell death ensues. As with β-amyloid plaques, hyperphosphorylation of tau with resulting development of NFTs occurs in several other neurodegenerative disorders, in addition to normal aging.\textsuperscript{10} It is the distribution within the brain and their co-occurrence with plaques that make NFTs a distinctive AD hallmark. Although the causal relationship is unclear, hyperphosphorylation of tau is thought to occur after, thus secondary to, plaque formation.\textsuperscript{5,11}

**BEYOND PLAQUES AND TANGLES**

In addition to plaques and NFTs, several other pathophysiologic processes are associated with AD.
progression. However, it is unclear if they occur concurrently or sequentially with regard to plaques and NFT formation. If they occur sequentially, are these other processes the cause or result of plaques and NFTs? Although the AD pathophysiologic cascade is complicated, each discovery of a neurotransmitter or pathologic process leads to the potential discovery of new therapeutic targets through research and drug development.

**Cholinergic Hypothesis**

Acetylcholine is an important neurotransmitter in brain regions involving memory. As expected, loss of cholinergic activity correlates with cognitive impairment (Figure 8). In AD, cholinergic abnormalities are the most prominent of neurotransmitter changes, primarily because of the reduced activity of choline acetyltransferase (an enzyme involved in acetylcholine synthesis). By late-stage AD, the number of cholinergic neurons is markedly reduced, particularly in the basal forebrain (ie, more than 75% loss of cholinergic neurons).

Acetylcholine binds to 2 postsynaptic receptor types: muscarinic and nicotinic. Presynaptic nicotinic receptors influence the release of neurotransmitters important for memory and mood (ie, acetylcholine, glutamate, serotonin, and norepinephrine). It is known that blocking nicotinic receptors impairs cognition (seen in humans and animals), whereas nicotinic agonists may improve memory (based on studies in rodents and nonhuman primates).

Loss of nicotinic receptor subtypes in the hippocampus and cortex has been observed in AD. Muscarinic receptors are not involved in the development of dementia, but blocking these receptors (as seen with anticholinergic agents) can cause confusion. Four of 5 approved medications for AD increase acetylcholine levels in the synapse. This medication class, the cholinesterase inhibitors, acts by blocking the enzyme acetylcholinesterase, which is responsible for acetylcholine degradation. It is thought that by maintaining or increasing acetylcholine levels in the synapse, memory loss and cognitive function through cholinergic neurons could be restored or maintained, even during neuronal degeneration.

**Glutamatergic and Excitotoxic Hypothesis**

Glutamate, the primary excitatory neurotransmitter, is virtually ubiquitous in the central nervous system (CNS) and involved in essentially all CNS functions. It is estimated to be involved in roughly 66% of all brain synapses. Glutamatergic neurotransmission is involved in learning, memory, and the shaping of neuronal architecture (plasticity). Importantly, most glutamatergic neurons are projection neurons that provide information from one brain area to another. As projection neurons, they influence cognition through connections to cholinergic neurons in the basal forebrain and cerebral cortex. A significant finding is that AD brains have fewer NMDA receptors (1 of 3 types of glutamate receptors, including AMPA...
and kainate) than normal. There also appears to be excessive or unregulated glutamate signaling, which eventually leads to neurotoxicity. This is not caused by excess glutamate production or release, but it is caused by postsynaptic receptor defects that result in sustained low-level activation. The role of NMDA receptors in learning and the effect of sustained low-level activation are shown in Figure 12. Based on animal studies, dysregulation at the glutamate NMDA receptor is thought to perpetuate a vicious cycle of neuronal damage. Continuous activation of the glutamate NMDA receptor leads to chronic calcium influx that interferes with normal signal transduction and, over time, increases production of APP. Increases in APP are associated with higher rates of plaque development and, as noted earlier in this article, hyperphosphorylation of tau protein (thus NFT formation) followed by neuronal toxicity. In Part 2 of this series, we will discuss the role of memantine as a treatment for the calcium dysregulation that occurs at the NMDA receptor because of excessive glutamate signaling.

The second type of receptor defect occurs during glutamate reuptake. During normal transmission, glutamate is cleared from the synapse by reuptake into the nerve terminal and surrounding glial cells. Studies in brain tissue of patients with dementia are now showing reduced numbers of glutamate reuptake sites, and an association between aberrant (increased) expression of one type of glutamate transporter and NFTs. This particular transporter is normally expressed in glia, but in patients with AD, it is found in high numbers in neurons. Although a correlation between increased expression of a glutamate transporter and the development of AD pathology is counterintuitive (ie, increased transporter expression would be expected to cause an increase in glutamate reuptake), the investigators suggest that the aberrant expression may lead to NFTs, may be caused by NFTs, or may be a mechanism to control or prevent excitotoxic damage.

**OXIDATIVE STRESS**

Oxidation can include the combination of a substance with oxygen or free radical damage. The oxidative process of adding an oxygen molecule to a protein can be a normal part of cellular function or may be aberrant, with a resulting change in the protein’s form and ability to function properly. Free radical damage occurs when an oxygen or nitrogen molecule containing an unpaired extra electron (termed species) reacts with other molecules to achieve a stable configuration. During this process a high-energy electron is thrown off (termed a free radical) that can cause cellular and molecular damage. Either of these oxidative processes can cause oxidative stress with resulting cellular damage. The brain is especially vulnerable to damage from oxidative stress because of its high oxygen consumption rate, abundant lipid content, and relative paucity of antioxidant enzymes compared to other organs.

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**Figure 12. NMDA Receptor Transmission in Healthy and Degenerating Neurons**

At rest, the normal NMDA receptor is blocked by magnesium. When a large release of glutamate (such as with an action potential) occurs, glutamate binds. Magnesium can then leave the receptor, allowing calcium influx, causing depolarization (the cell becomes less negatively charged), and signal propagation ensues. Learning requires a large change in voltage at the postsynaptic receptor. When the receptor is at rest, the level of noise is kept low by the magnesium block. When the receptor is activated, the voltage change of the membrane from the large calcium influx allows a signal to be detected. A large absolute difference between signal and noise is required for learning to occur. When the NMDA receptor is undergoing sustained activation, the signal-to-noise ratio is virtually one, thus learning is prevented. The sustained release of calcium also causes neuronal damage.

fact, Aβ induces lipid peroxidation and generates reactive oxygen and nitrogen species. Oxidative damage has been demonstrated in virtually all types of neuronal macromolecules (eg, lipids, carbohydrates, proteins, and nucleic acids). Oxidation can impaire neuronal function. Oxidized lipids change the structure and function of synaptic membranes resulting in neuronal death or synapse loss. Modifications to sugars lead to advanced glycation end-products (the attachment of sugars to proteins, lipids, or nucleic acids, usually a sign of disease or age), which cause irreversible protein cross-linking and the development of protein aggregates. For example, oxidation is known to damage the principle glutamate transporter. Advanced glycation end-products themselves can create free radicals and reactive species, in addition to up-regulating proinflammatory cytokines (discussed later in this article). In addition, because neurons are post-mitotic cells (ie, they have exited the cell cycle and are fully differentiated), oxidative damage is cumulative and usually not repaired. In AD, neuronal DNA and RNA also become oxidized. DNA breaks, nicking, and fragmentation are seen, which suggest deficient DNA repair mechanisms. Oxidative stress is thought to be important early in the progression of AD and is temporally linked to the development of plaques and NFTs. Antioxidants, including vitamin E, have been investigated as a possible treatment for AD. However, no satisfactory interventions have been developed yet in this category. This may be an example where timing is a critical aspect of a drug’s efficacy. For example, perhaps antioxidants may be more efficacious very early in the AD process, but later, after the oxidative damage is done, are unable to impact the pathologic course or clinical presentation.

**Chronic Inflammation**

Neurons are not the only brain cells affected in AD. Microglia, 1 of 3 glial cell types (along with astrocytes and oligodendrocytes) in the CNS, are involved in immune and inflammatory responses to injury or infection within the brain. During the AD process, microglia are activated, releasing potentially cytotoxic molecules, such as proinflammatory cytokines, reactive oxygen species, proteinases, and complement proteins. Within the CNS, cytokines stimulate inflammatory processes that may promote apoptosis (programmed cell death) of neurons and oligodendrocytes and induce myelin damage. Another measure of inflammation is the finding of increased prostaglandins, which are produced by the cyclo-oxygenases COX-1 and COX-2, in the AD brain. β-amyloid deposits, NFTs, and damaged neurons may stimulate inflammation as a natural response to cell damage, as occurs outside the CNS, but inflammatory processes can backfire, causing more damage than protection. As noted by Akiyama et al, because these stimuli are discrete, microlocalized, and present from early pre-clinical stages of AD, inflammation in AD is also microlocalized, discrete, and chronic. In Part 2 of this series, we will review the use of anti-inflammatory drugs under investigation for AD prevention and treatment.

**Other Neurotransmitter Deficiencies**

During the AD process, brain regions where acetylcholine, serotonin, and norepinephrine are prominent neurotransmitters become altered (Figure 8). These neuronal pathways originate in the basal forebrain and brain stem and innervate widespread areas of the cerebral cortex. Research is also focusing on neurotransmitters that emerge from the midbrain, notably norepinephrine and serotonin (5-hydroxytryptamine). Many studies have underscored the importance of serotonin in affective illness, and depression is a common comorbidity with AD. It has been shown that the number of specific serotonin receptors (5-HT2A and possibly the serotonin transporter, which is responsible for serotonin reuptake) are altered in AD brains. In the temporal cortex of patients with AD, the number of 5-HT2A receptors is reduced and the extent of the reduction correlates with the decline in Mini-Mental State Examination score, but it is independent of the presence of behavioral symptoms. Patients with late-onset AD who are homozygous for selected polymorphisms of the 5-HT2A and 5-HT2C receptor are 5 times more likely to have major depressive illness than heterozygotes. The number of serotonin transporters in the temporal cortex of patients with AD with significant anxiety before death appears to be preserved, but it is reduced in nonanxious patients with AD. Patients with both copies of a “high-activity allele” of the serotonin transporter have higher rates of anxiety before death. Currently, dual inhibitors of serotonin transporter and acetylcholinesterase are under investigation in animal studies and appear to affect levels of these neurotransmitters.

Norepinephrine levels are reduced and norepinephrine neurons are lost in AD. Evidence suggests that norepinephrine may play an important role in some of the behavioral and psychological symptoms of
dementia (agression, agitation, and psychosis), in addition to memory loss. Animal data also suggest that loss of norepinephrine neurons in the midbrain and norepinephrine depletion potentiate the inflammatory responses to Aβ, suggesting that the loss of these neurons exacerbates neuronal cell death and inflammation in AD.

CHOLESTEROL

The brain contains the highest amount of cholesterol of any human organ. Although the CNS accounts for only 2% of body mass, it contains almost 25% of the body's unesterified cholesterol. Cholesterol is now also implicated in AD pathogenesis. In vivo and in vitro data suggest that elevated cholesterol levels increase Aβ production, whereas reduced cholesterol synthesis (eg, as a result of statin drugs) reduces Aβ levels. Cholesterol reduction may also reduce the risk or severity of dementia through protection from vascular risk factors, which often coexist in patients with AD. The APOE (apolipoprotein E)-ε4 allele is associated with higher cholesterol levels, is a dose-dependent risk factor for AD, and correlates with an increase in Aβ pathology. In a study of 218 Japanese-American men, as part of the Honolulu-Asia Aging Study, cholesterol levels were measured at midlife and late-life and compared to the number of plaques and NFTs. On autopsy, the results showed a strong linear association between mid-life and late-life high-density lipoprotein cholesterol and the number of these AD hallmarks in the cortex. In Part 2 of this series, we will look at the role of statins for the prevention and treatment of AD.

TIMING AND SEQUENCE OF NEURONAL DAMAGE

As we develop a clearer picture of the brain cell components damaged during AD, it is clear that the pathophysiologic processes are complex. An important remaining issue is to determine whether there is a single initiating event that prompts a cascade of events or if multiple events must be present simultaneously. Traditionally, Aβ generation has been considered to be the first step of an "amyloid cascade." This process, along with oxidation, excitotoxicity, inflammation, and the hyperphosphorylation of tau, contributes to the neurotransmitter abnormalities and neuronal cell death, which are then manifested as cognitive and behavioral abnormalities (Figure 13). Other hypotheses have also been proposed, as described later in this article.

Two-Hit Hypothesis

Zhu et al have recently proposed a “2-hit” hypothesis for AD pathology—neurons may encounter aberrant mitotic signaling, causing them to re-enter the cell cycle, or be subjected to oxidative stress. It is unclear why neurons would encounter aberrant mitotic signaling, causing them to re-enter the cell cycle, but some components of the cell-cycle mechanism are activated in vulnerable neurons in AD. Re-entering the cell cycle may ultimately cause death because the mitotic structures needed for cell division are not observed in neurons. The cell would then undergo a “mitotic catastrophe,” contributing to its eventual death. Either of these stresses alone could be survived through compensatory mechanisms. However, if both insults occur (oxidative stress and aberrant mitotic signaling), a threshold is reached from which the neuron cannot recover and the AD phenotype of neuronal loss and clinical symptoms ensue.

Figure 13. Diagram of the Amyloid Cascade

**SYNAPTIC FAILURE**

Selkoe describes the clinical presentation of AD symptoms as a synaptic failure, rather than a disorder of neurodegeneration.11 Synapses appear to be the initial target in AD pathogenesis, with subtle reductions in synaptic efficacy before neuronal cell death. “Synapse loss matters; loss of whole neurons comes later and matters less.” He points out that the earliest symptom of AD (memory loss) is “remarkably pure” beginning with the loss of short-term memory (usually of trivial details) and progressing onto longer-term memory, with more serious ramifications. These changes occur while motor and sensory functions remain intact.11 Histologic analysis reveals substantially decreased (approximately 30%) total numbers of synapses and synapses per cortical neuron in AD brains 2 to 4 years after clinical onset.44 There may also be a closer correlation between synapse loss and cognitive deficits than with the number of plaques or tangles, although studies measuring Aβ peptide levels and cognitive decline also showed a strong correlation.49,50

**CONCLUSIONS**

As we learn more of the details involved in the pathophysiology of AD, we gain insight into potential therapeutic targets. The long-held hallmarks of AD—β-amyloid plaques and NFTs—are certainly major factors amongst the myriad neurodegenerative processes. Perhaps more interesting is that these changes in neuronal function and neurotransmitter availability start years before clinical symptoms, and they appear to correlate, to greater or lesser extents, to the hallmark symptoms of AD. Optimal treatment approaches will need to focus on the pathophysiologic changes in AD and address multiple mechanisms because of the complex nature of this devastating disease.

**REFERENCES**

24. Scott HL, Tannenberg AG, Dodd PR. Variant forms of neuronal glutamate transporter sites in Alzheimer disease cere-