Road to Alzheimer's Disease: The Pathomechanism Underlying

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Key Words

Alzheimer's disease • Amyloid • Dementia • Neurofibrillary tangles • Oxidative stress • Senile plaques • Tau protein

Abstract

Alzheimer's disease (AD), the most common cause of dementia, results from the interplay of various deregulated mechanisms triggering a complex pathophysiology. The neurons suffer from and slowly succumb to multiple irreversible damages, resulting in cell death and thus memory deficits that characterize AD. In spite of our vast knowledge, it is still unclear as to when the disease process starts and how long the perturbations continue before the disease manifests. Recent studies provide sufficient evidence to prove amyloid β ($A\beta$) as the primary cause initiating secondary events, but $A\beta$ is also known to be produced under normal conditions and to possess physiological roles, hence, the questions that remain are: What are the factors that lead to abnormal $A\beta$ production? When does $A\beta$ turn into a pathological molecule? What is the chain of events that follows $A\beta$? The answers are still under debate, and further insight may help us in creating better diagnostic and therapeutic options in AD. The present article attempts to review the current literature regarding AD pathophysiology and proposes a pathophysiologic cascade in AD.

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Introduction

Alzheimer's disease (AD), a neurodegenerative disorder characterized by slowly progressive dementia and brain atrophy, is peculiar with respect to its subtle onset, insidious course, lack of simple diagnostic tests, effective treatment and last but not least its poor prognosis. AD is the most common type of dementia, accounting for 60–80% of cases [1]. Every 71 s someone in America develops AD; by 2050, it is expected to occur every 33 s [1], and globally there is a new case every 7 s [2]. AD poses an emotional and economic burden to the patient's family as well as society due to the necessity of caregiving and loss of wages. The etiology of AD is multifactorial, involving a host of genetic, environmental and behavioral components. The greatest risk factor is advancing age. The incidence is 1% for those aged ≤ 60 years and roughly doubles every 5 years thereafter [3]. Other risk factors that may be associated with the development of AD include a positive family history, severe head trauma, female gender, previous depression and vascular factors [4]. The disease is divided into two subtypes depending on the age at onset: early-onset AD (EOAD) and late-onset AD (LOAD). EOAD accounts for approximately 1–6% of all cases and manifests roughly between 30 and 60 or 65 years. LOAD, the most common form of AD, accounting for around

Fig. 1. Overview of pathophysiologic mechanisms in AD.

90% of cases, is defined as AD with an age at onset later than 60 or 65 years. Both EOAD and LOAD may occur in people with a positive family history of AD [5]. The clinical phases of AD have been divided into the latent/asymptomatic phase, during which the disease process has started but symptoms do not yet manifest, the prodromal phase/mild cognitive impairment (MCI) and the phase of dementia [6].

Pathophysiology of AD

AD seems to result from a variety of dysregulated mechanisms which incur slowly accumulating damage to the neurons, finally pronouncing their death. The diverse mechanisms hence pose a great difficulty in gaining control over the disease. Various reviews comprehensively discuss the individual mechanisms involved in AD [5–18], but a summarized and integrated pathomechanism has not been described to date and the present review is focused on accomplishing this task.

Our understanding of the pathophysiology of AD is constantly changing. For instance the tangles, a well-known pathological hallmark of AD, earlier thought to be responsible for the disease, now rather seem to reflect the injury which the neurons have endured over a long time. The notion that amyloid β peptide ($A\beta$) and phos-

phorylated tau (P-tau) are pathologic molecules is slowly changing, and their physiological roles as a cellular adaptive strategy to oxidative stress (OXS) are just being realized. Recent evidence indicates that $A\beta$ molecules are produced as cellular defense reaction to OXS and various deranged mechanisms, such as persistent OXS, mitochondrial dysfunction, $A\beta$ production, neurofibrillary tangle (NFT) accumulation, hormone imbalance, inflammation, mitotic dysfunction, impaired calcium homeostasis and genetic components, play a role in the disease process. Though the mechanisms are diverse, neuronal death, the inevitable event, ensues resulting in AD. An overview of the various mechanisms involved and their interaction is shown in figure 1.

OXS – ‘Origin Myth of AD’

Despite all of the evidence that links AD to etiologic components, details on the mechanism which triggers the pathological cascade still remain to be established. The ‘two hit hypothesis’ [9] and the ‘LEARN’ model [19] provide the most comprehensive explanation for the beginning of the cellular insult in AD. OXS, in particular chronic, mild and tolerable levels of OXS, seems to be the major factor initiating AD [9]. Various mechanisms have been reported to cause OXS in AD (table 1).

Abnormal metal homeostasis in the vicinity of neurons is a reason for OXS [7]. Various metals are implicat-

ed in the pathogenesis of AD, including iron, copper and zinc [20]. Iron accumulation was noted in the hippocampus, cerebral cortex, basal nucleus of Meynert and also in AD lesions – senile plaques and NFT. In addition, iron, a transition metal, can form OH[•] via the Fenton reaction [21]. Impaired homeostasis of copper, another important metal component of cytochrome, is also implicated in OXS in AD. Copper has been shown to play a role in reactive oxygen species (ROS) production through its binding to Aβ protein precursor (AβPP). Copper is also highly concentrated within Aβ plaques, creating conditions for Fenton-type reactions through the reduction of Cu(II) by Aβ-H₂O₂ [7]. Aluminum has also received attention in AD [22]; although not convincingly demonstrated, aluminum is highly concentrated in plaques and NFT lesions, suggesting its involvement in AD pathogenesis. Unlike transition metal ions, aluminum cannot initiate redox reactions; however, evidence indicates that it can act synergistically with iron to increase free radical damage [23].

Advanced glycation end products (AGEs) are another source of OXS. In the presence of transition metals, AGEs can either undergo redox cycling [24] or they interact with specific receptors such as the receptor for AGEs (RAGE) [25] and the class A scavenger receptor [26] resulting in ROS production. The proteosomal dysfunction which ensues with advancing age [27] and the lysosomes which accumulate lipofuscin [28] lose their degradative capacity, thus accumulating dysfunctional organelles and contributing to OXS. The hormone dyshomeostasis which ensues with advancing age also contributes to oxidative stress. Studies have shown that estrogen, testosterone and to a lesser extent progesterone have antioxidant, -inflammatory and -amyloid properties, and luteinizing hormone (LH) has opposite effects [29]. With advancing age, the levels of estrogen and testosterone fall, and LH rises, tilting the balance towards a prooxidant state and resulting in OXS. The activated microglia/astrocytes which surround the plaques also have the potential to produce large amounts of ROS by various mechanisms [30, 31].

Though evidence suggests that Aβ fibers are produced as a compensatory response in virtue of their antioxidant properties [32], Aβ, when they aggregate and become aged, are capable of oxidizing various biomolecules. Aβ can induce peroxidation of membrane lipids [33] and lipoproteins [34], generate H₂O₂ [35] and hydroxynonenal [36] in neurons, damages deoxyribonucleic acid (DNA) [37] and inactivate transport enzymes [38]. Aβ can also lead to OXS indirectly by causing mitochondrial dys-

Table 1. Cause of OXS in AD

Metal dyshomeostasis
AGE
Hormone imbalance
Microglial/astrocyte activation
Aβ fibers
Proteosomal/lysosomal dysfunction
Mitochondrial dysfunction

function. The binding of Aβ (Aβ₄₀ and Aβ₄₂) with heme, forming the Aβ-heme complex, has recently been demonstrated [39]. Thus Aβ possibly binds to the newly synthesized heme pool, called the 'regulatory heme' reducing its cellular availability for the synthesis of electron transport chain components [40]. There is also a coexistent defect in several key enzymes of oxidative metabolism, including α-ketoglutarate dehydrogenase and pyruvate dehydrogenase complexes, and cytochrome oxidase in AD [41–43]. The defects in the enzyme complexes could result from the mutations that the mitochondrial DNA sustains as a result of OXS [44, 45].

Studies indicate a decrease in the number of mitochondria per neuron, increased dysmorphic mitochondria and abnormal turnover of mitochondrial components [46]. The decrease in the mitochondrial number and morphological changes occur due to abnormal mitochondrial dynamics. Expression of proteins involved in mitochondrial fusion is significantly reduced, while levels of proteins involved in mitochondrial fission are increased in the hippocampal tissue of AD patients, thus confirming an imbalance between mitochondrial fission and fusion in pyramidal neurons in AD [47]. The ensuing mitochondrial dysfunction not only leads to ROS production but also to a chronic energy crisis in the neurons. The energy depletion may also increase the catabolism of membrane cholesterol [48], and when the reduction in cholesterol is moderate, there is a disorganization of lipid rafts that favors the contact between β-secretase and amyloid precursor protein (APP), which results in increased Aβ production [49].

Thus, OXS, irrespective of its cause in neurons in AD, is sustained, resulting in chronic and persistent OXS. The uniqueness of OXS in AD is that it does not overwhelm oxidative defenses and few neurons (<1/10,000 at any given time) exhibit signs of apoptosis [50], as would be expected under conditions of acute and high-level OXS. Therefore, chronic, tolerable OXS occurs and neurons resort to adaptive responses [51] rather than cell death.

Impaired Antioxidant Defense

Cells normally respond to OXS through an endogenous mechanism regulated at the transcriptional level. A common promoter element, called the antioxidant response element, regulates the genes whose products participate in reducing OXS. Its promoter is bound by a transcription factor, nuclear factor E₂-related factor 2 (Nrf2), which activates transcription in response to OXS [52]. Under basal conditions, interaction with Keap1 (kelch-like ECH-associated protein 1) sequesters Nrf2 in the cytoplasm [53]. Either oxidation of sulfhydryl groups on specific cysteines in Keap1 [54] or phosphorylation of Keap1 and/or Nrf2 induces Keap1 to release Nrf2 [55]. Keap1-released Nrf2 gets stabilized, translocates from the cytoplasm to the nucleus and activates the expression of detoxification enzymes, antioxidant enzymes, reducing molecules and Nrf2 itself [56].

The Nrf2 pathway is important because it has been observed that overexpression of Nrf2 can rescue neurons from mitochondrial complex II inhibition [57] and neurons lacking Nrf2 are more susceptible to OXS through H₂O₂ [58]. Given the presence of OXS in AD, we would expect an upregulation of Nrf2 activity in the nuclei of neurons and astrocytes during disease progression; however, the levels of some antioxidant response element-containing gene products are reduced, suggesting disruption of this pathway [11]. In AD, Nrf2 predominantly occurs in the cytoplasm in hippocampal neurons and not in the nucleus, reflecting impaired nuclear trafficking, suggesting a process blocking Nrf2 nuclear activity in AD, potentially contributing to neuronal dysfunction and/or loss [11].

OXS Signaling

Therefore, in a chronic OXS situation when induction of antioxidant enzymes is insufficient, neurons seem to resort to further structural adaptations like A β production, tau hyperphosphorylation and neuronal hypertrophy by inducing multiple cell signaling pathways. The phosphorylation cascade is modulated by the sequential and collective action of distinct kinases, all of which appear to be related to AD pathophysiology. The 3 members of the MAP kinase family: stress-activated protein kinase (SAPK)/c-Jun amino terminal kinase (JNK), p38 kinase and extracellular signal-regulated protein kinases (ERKs) [59] have received attention owing to their overexpression in AD [60].

The kinases which are known to phosphorylate tau include ERK1 and ERK2 [61], JNK [62], p38 kinase [63], glycogen synthase kinase-3 (GSK3) [64], cyclin-depen-

dent kinase-5 (CDK5) [65], casein kinase I (CK1) [66], cAMP-dependent protein kinase A (PKA) [67] and calcium/calmodulin-dependent protein kinase II (CaM kinase II) [68]. Another interesting molecule playing a role in tau phosphorylation is caspase 2, which cleaves tau at D421 producing Δ tau. Δ Tau is now susceptible to phosphorylation by GSK3 and is more prone to NFT formation [69]. Apart from tau phosphorylation, JNK/SAPK have also been seen to increase amyloid production from neurons [70]. Studies from several groups consistently show that A β induces a two- to threefold activation of JNK/SAPK in different neuronal cell types [71, 72]. A β again may serve to provide a positive feedback to SAPK resulting in their continued activation. It is possible that OXS, as an earlier event, may activate JNK/SAPK; and that elevated levels of A β , as a later event, contribute to the continued and chronic activation of JNK/SAPK [73]. β -Site APP-cleaving enzyme (BACE), an aspartyl protease with β -secretase activity [74], can be induced by OXS [75], which in turn leads to a proportional elevation in the carboxyl-terminal fragments (CTFs) of APP. Since A β is also a source of ROS, OXS production and A β generation may set up a vicious cycle, in which OXS contributes to A β accumulation, and A β in turn induces OXS, thus further enhancing A β production [73].

Hormones and Amyloidogenesis

The association between hormone imbalance and OXS was already briefly mentioned. A large body of evidence indicates that estrogen, progesterone and testosterone are neuroprotective, and, consistent with it, an association between AD and depleted levels of estrogen in women, and testosterone in men, has been reported [76, 77]. Apart from the antioxidant effect, estrogen, testosterone and progesterone to some extent possess (i) anti-inflammatory actions, regulating a wide range of microglial functions, including expression of cytokines, cell surface molecules, apoptotic signaling pathways and free radical generation [78], and (ii) anti-amyloidogenic properties, resulting in an increase in the secretion of α -APPs and a decrease in A β production in cultured human neuroblastoma and cerebrocortical neurons [79]. A recent study has also revealed a potential correlation between apolipoproteins E (ApoE) genotypes and testosterone levels, with low testosterone levels and ApoE ϵ 4 being positively associated with small hippocampal volumes [80].

It has also been shown that in AD patients, serum concentrations of LH are approximately twice as high as in age-matched controls [81], and elevated LH is observed in the AD-vulnerable hippocampus [82]. Unlike estrogen

Table 2. Genes involved and the contribution to AD

Gene	Chromosome	Inheritance	Effect
APP	21q21	autosomal dominant	10–15% of EOAD; alter processing of APP, increasing A β ₄₂ , decreasing A β ₄₀ peptide levels
PSEN1	14q24.2	autosomal dominant	18–80% of EOAD cases; increased A β ₄₂ production, decreased A β ₄₀ production
PSEN2	1q42.13	autosomal dominant	rare; increase the ratio of A β ₄₂ to A β ₄₀ , less effectively than PSEN1
APOE	19q13.32	sporadic	19% of familial LOAD; isoform-specific toxicity, ApoE ϵ 4-mediated amyloid aggregation, and ApoE ϵ 4-mediated tau hyperphosphorylation increased A β transport into the brain, defective A β clearance, immunomodulation

and testosterone, LH is implicated in the promotion of amyloidogenic processing of APP and in the accumulation of A β and amyloidogenic APP-CTFs, whilst decreasing secretion of soluble α -APPs [83]. LH receptor is found to be expressed on a variety of immune cells throughout the body, including macrophages of the brain [84], thus suggesting a role for LH in modulating inflammation. LH dose-dependently increases secretion of reactive oxygen and nitrogen species from human blood leukocytes [85] and upregulates 5'-lipoxygenase, an enzyme important in proinflammatory signaling pathways in hippocampal cell cultures [29].

Thus, with progressive aging and reproductive senescence, there is a gradual fall in neuroprotective hormones, estrogen, progesterone and testosterone with rising LH levels contributing to OXS, inflammation and amyloidogenesis, adding to the pathogenesis of AD.

Role of Genetics in Amyloidogenesis

The early-onset familial forms of AD have an autosomal dominant inheritance linked to 3 genes: APP and presenilins 1 (PSEN1) and 2 (PSEN2), while the most common sporadic form is consistently associated with only 1 gene, the APOE gene [5]. The genes involved and their role in the contribution to AD pathology is summarized in table 2. The genetic abnormalities thus introduce A β relatively early into the game initiating the disease pathology resulting in EOAD. This again correlates well with the proposed pathophysiological cascade in LOAD, where OXS is the early abnormality with A β entry at a relatively later part, and hence late manifestation of the disease.

Amyloid- β Peptide and Hyperphosphorylated Tau Protein – ‘Friends That Turn Foes’

Chronic mild OXS produces compensatory adaptive mechanisms in the neuron which are initially temporary but later become permanent changes. Though A β fibers are one of the pathological hallmarks of AD, it is now believed that the peptide has several physiological roles [18] and is present in the cerebrospinal fluid (CSF) and plasma of healthy individuals throughout life [86]. It has been shown that neurons respond to OXS, both in vitro and in vivo, by increasing A β production. A β is believed to possess antioxidant properties [32] and some authors propose that diffuse amyloid plaques may be a compensatory response aimed at reducing OXS, because a negative correlation has been observed between A β deposition and oxidative damage in patients with Down's syndrome as well as in AD [87–89]. A β also strongly chelates zinc, iron and copper [20], suggesting that one function of A β is also to sequester these metal ions. The role of A β in maintaining neuroplasticity is also appreciated, and APP has been believed to possess a trophic role [90, 91], with increased APP levels during conditions of increased plasticity. Hence, A β production by neurons might be a compensatory response to combat OXS as well maintenance of plasticity.

However, it has been seen that A β can also turn into a nasty foe creating toxicity. The condition under which A β ceases to act as an antioxidant and functions as a pro-oxidant are not clearly understood, but may be dependent on the concentration of the peptide. With time, A β production exceeds the capacity for its clearance, and they begin to accumulate, increasing its concentration towards toxic levels. A β has the capability to induce oxida-

tion of biomolecules provided three important conditions are satisfied: fibrillation, the presence of transition metals and methionine-35. Thus, when conditions turn favorable, the prooxidant effect becomes dominant and A β turns detrimental. There is a dynamic equilibrium between A β fibrils, oligomers and monomers, and oligomeric species are more toxic than monomers or fibrils. Early memory impairment and synaptic dysfunction correlate better with the levels of oligomeric A β but not with fibrillar species [92]. With A β available in abundance, the oligomeric species may be readily available for ensuing damage.

P-tau is another well-known pathological hallmark of AD, which also seems to be an adaptive modification but which results in untoward effects. Tau is essentially present in neurons and mainly functions to stabilize microtubules by interacting via its microtubule-binding domains. By regulating microtubule assembly, tau has a role in modulating the functional organization of the neuron, particularly in axonal morphology, growth and polarity [93]. The tau protein has multiple phosphorylation sites which get phosphorylated in AD. There is evidence that P-tau exerts protective functions. OXS and modification of tau by products of OXS [94] enable neurons to survive for decades [95]. Neurofilament and tau proteins appear adapted to OXS by virtue of their high content of lysine-serine-proline (KSP) domains [96] and may function as a buffer by absorbing lipoxidation- and glycoxidation-derived aldehydes [97]. Phosphorylation also increases the tendency of tau to aggregate readily into paired helical filaments. The microtubule-binding property of tau also seems to be dependent on its state of phosphorylation. P-tau binds with a lesser affinity to microtubules [98], resulting in microtubule instability. In AD, P-tau accumulates in neurons, aggregates into paired helical filaments and loses its microtubule binding and stabilizing function, leading to neuronal degeneration [99].

Thus, both A β as well P-tau may be intended for counteracting OXS and maintaining neurogenesis, but following irreversible changes, they impair cellular function and decrease neuronal function.

A β and Cholesterol Metabolism

The role of cholesterol in the pathogenesis of AD has been given less importance until recently; nonetheless, in neuronal membranes, cholesterol induces large changes in membrane fluidity, regulating the activity of several membrane proteins. A higher input of cholesterol is needed for the formation of new synapses [100], and high-density lipoproteins (HDL) are critical for the maturation of

synapses and maintenance of synaptic plasticity [101]. A high level of low-density lipoprotein and elevated total cholesterol levels are associated with cognitive impairment [102], and high levels of HDL cholesterol were associated with protection against AD [103]. The presence of the ApoE ϵ 4 allele, a cholesterol carrier, another major genetic risk factor for sporadic AD, also suggests that brain cholesterol homeostasis is involved in AD [104]. Cholesterol and A β seem to interact, with one increasing the production of the other and vice versa. Cholesterol is necessary for A β production [105], and hypercholesterolemia is associated with increased intraneuronal and extracellular accumulation of A β [106]. A β , on the other hand, modifies cellular cholesterol distribution and the rate of cholesterol esterification altering cholesterol homeostasis [107]. A β also increases hippocampal lipid synthesis and cholesterol uptake [108], and promotes the accumulation of cholesterol and ceramides [109].

Apolipoproteins play a role in A β metabolism and transport, although they do not cross the blood-brain barrier (BBB) and may regulate the flux of A β between the central nervous system (CNS) and the periphery. ApoE ϵ 4 is thought to increase translocation of A β from blood to brain [110] and results in increased A β deposition in the brain. Mouse models that overproduce and deposit A β combined with transgenic animals lacking ApoE tend to have reduced A β deposition [111], suggesting a role for ApoE in A β transport. Apart from its role in A β trafficking, ApoE also performs immunomodulatory functions. In the brain, the principal cells secreting ApoE are astrocytes and microglia [112], and ApoE ϵ 4 is associated with increased production of proinflammatory cytokines compared with ApoE ϵ 3 [113]. Even a single amino acid change that differentiates ApoE ϵ 4 from ApoE ϵ 3 and ApoE ϵ 2 in mice results in protein misfolding and endoplasmic reticulum stress in astrocytes, leading to severe cognitive deficits [114]. A β and APP also stimulate the formation of the proapoptotic molecule 7 α -hydroxycholesterol from cholesterol [115] and neuronal death is again the inevitable result.

Excitotoxicity and AD

Excitotoxicity is another mechanism known to be involved in AD pathophysiology. Excessive stimulation of the N-methyl-D-aspartate (NMDA) receptor and the subsequent massive influx of extracellular Ca²⁺ can be highly toxic to neurons [12]. A β and tau seem to enhance the activation of NMDA receptors [12], resulting in increased A β and tau levels. Various studies indicate an imbalance in glutamate homeostasis with impaired clearing of glu-

tamate from the synaptic cleft, leading to extracellular glutamate accumulation [116]. Quinolinic acid, the precursor of nicotinamide adenine dinucleotide in the kynurenine pathway, has also been found to be a direct activator of the NMDA receptor contributing to excitotoxicity [13]. The peculiarity with hippocampal neurons in AD is that, when compared to cholinergic and glutamatergic neurons, the γ -aminobutyric acid (GABA)ergic neurons are relatively spared, with the precise reason being unknown [117]. Another feature is that chronic growth factor deprivation, which occurs with aging, changes GABA transmission from an inhibitory to an excitatory stimulus, contributing to excitotoxicity [118].

Pathological excitation leads to prolonged, tonic activation, sustained local depolarization and the influx of cations that trigger further glutamate release [119]. This vicious circle could lead primarily to swelling of the neurons because of the increased cation concentration and consequent water influx, and secondly to delayed Ca^{2+} -dependent neuronal degeneration [120].

Perturbed Ca^{2+} Homeostasis

Calcium, a vital element for the functioning of neurons, plays multiple physiological roles in the form of controlling membrane excitability, triggering neurotransmitter release, gene expression, neuronal growth, differentiation and transition to apoptosis [121]. Various studies have indicated age-related changes that occur in Ca^{2+} homeostasis in the form of elevated intracellular Ca^{2+} levels/enhanced Ca^{2+} influx through voltage-dependent Ca^{2+} channels secondary to excitotoxicity; impaired ability of mitochondria to buffer Ca^{2+} , and disturbed Ca^{2+} regulation in ryanodine and $\text{Ins}(1,4,5)\text{P}_3$ -sensitive Ca^{2+} stores [122]. Similar abnormalities in Ca^{2+} regulation were also observed in neurons exposed to OXS and in patient studies [123]. The various mechanisms due to which Ca^{2+} metabolism is disturbed have already been reviewed [10, 124]. Thus Ca^{2+} metabolism is impaired in sporadic as well as familial AD, implying a vital role in the pathogenesis of AD. The increase in intracellular Ca^{2+} can thus activate (i) CaM kinase II: producing P-tau; (ii) calpain: producing an active p25/CDK5 contributing to P-tau; calpain I activation also brings about cytoskeletal breakdown [125]; (iii) calcineurin and calpains: enabling long-term depression and leading to the inhibition of long-term potentiation, modifications in the neuronal cytoskeleton, synaptic loss and neuritic atrophy, and (iv) excessive Ca^{2+} , taken up by mitochondria through mitochondrial Ca^{2+} uniporter, opening mitochondrial permeability transition pores and leading to apoptosis [124].

Autophagy and AD

Autophagy is a double-edged sword playing protective as well as pathological roles. Insufficient protective autophagy could cause damaged cellular components to accumulate over many years affecting normal cell function. Alternatively, cell death could be the result of overactive, pathological autophagy [126]. The role of autophagy was indicated in studies with mice lacking Atg5 [127] or Atg7 [128] who showed severe neurodegeneration. In neurons, along with the steps involved in formation, autophagosomes and endosomes from the distal axon must be retrogradely transported to the soma in order to fuse with lysosomes [129], so disturbances in any of the steps may deregulate autophagy leading to unwanted consequences. The tau phosphorylation could result in impaired axonal transport of autophagosomes with accumulation in the distal axon. Autophagosomes and other prelysosomal autophagic vacuoles have been noted in AD brains particularly within neuritic processes [130], suggesting impaired axonal transport.

Deregulation can also occur with the intrinsic mechanism for autophagy. Most of the recent evidence points towards proteolytic failure as the major dysregulated step in autophagy [131]. In AD, autophagy may help to protect the cell in the initial stages degrading the dysfunctional mitochondria and the oxidatively damaged components. Damaged mitochondria are subjected to cellular digestion by mitophagy, a specialized kind of protective autophagy. According to the mitochondrial-lysosomal axis theory of aging [132], lysosomes slowly lose their inherent ability to completely remove oxidatively damaged structures [133] and mitochondrial turnover progressively declines with age, resulting in accumulation of damaged organelles, decreased ATP production, release of apoptotic factors and, eventually, cell death. Even internalized $\text{A}\beta_{1-42}$ is slowly degraded and therefore accumulates in lysosomes, followed by leakage of lysosomal enzymes into the cytosol and cellular toxicity [134]. In advanced disease, it is possible that the activation of pathological autophagy might also contribute to neuronal death.

Hence, impaired autophagy due to blocked transport of autophagosomes in axons, proteolytic failure in protective autophagy and activation of pathologic autophagy could be involved in the AD pathophysiology.

Mitotic Dysfunction

Neurons of the brain have long been considered to reside in a terminally differentiated G_0 state, but around 14 years ago, susceptible cortical neurons were shown to display an ectopic presence of various cyclins, cyclin-depen-

dent kinases and cyclin-dependent kinase inhibitors in AD, indicating the possibility of cell cycle activation [135, 136]. Thus susceptible, postmitotic, neuronal populations actually exit G₀ and reenter the G₁ phase. This reentry is probably supported by activation of selective signal transduction pathways, transcriptional activation that results in cytoskeletal changes such as tau phosphorylation, alterations in mitochondrial activity and DNA replication [137–139]. Although they may enter the cell cycle they do not progress further, resulting in cell cycle arrest and death. The significance of such a reentrant phenotype is not clear, but a dysregulated cell cycle in these neurons may contribute to their eventual death in AD [9].

Mechanisms of Neuronal Death

Though the perturbed mechanisms are diverse in AD, they all lead to the ultimate event, neuronal death. The detection of cleaved caspases and the accumulation of cleaved caspase substrates in post-mortem AD brain tissue have suggested that apoptosis may play a role in the subsequent neuronal loss in AD [140]. Multiple caspases have been implicated in the progression of AD. mRNA expression of caspases 1–3 and 5–9 were elevated in the brain of AD patients compared with controls [141]. Also, the cytoplasmic tail of APP can be cleaved by caspases at Asp₆₆₄ (APP₆₉₅), generating a new CTF of 31 residues named C31 [142], and the expression of C31 alone is cytotoxic to the cell, thus potentiating the susceptibility to apoptosis. Caspases also cleave tau and enhance NFT formation [69] contributing to synaptic dysfunction and neuronal death. In vitro studies have shown that A β induces upregulation of proapoptotic molecules, such as Bax and Bim, and downregulates anti-apoptotic proteins, such as Bcl-2, Bcl-X_L or Bcl-w, thus again contributing to apoptosis [143, 144]. As discussed earlier, excessive Ca²⁺ when taken up by mitochondria through mitochondrial Ca²⁺ uniporter leads to opening of mitochondrial permeability transition pores and apoptosis [124]. A β and APP also can stimulate the formation of 7 α -hydroxycholesterol, a proapoptotic molecule, from cholesterol [115].

Accounting for A β Overproduction

Although much has been said and done, we are, however, still unable to explain why a person should develop AD sporadically. We are aware of the key role played by

A β in the disease process and in the process of understanding its physiological role, but there are still missing links which need addressing. Comparing the different types of AD, it would not be a surprise that the disease mechanisms may be similar once A β assumes the lead role, and what would vary are the different circumstances that result in A β accumulation.

A β is produced through the proteolysis of APP, a large type 1 transmembrane protein, and initial cleavage of APP by β -secretase followed by γ -secretase [145] is essential to release A β peptides. BACE1, the authentic β -secretase in the brain, enables site-specific cleavage precisely at Asp+1 and Glu+11 of A β [146], generating the N terminus of A β and a membrane-bound C-terminal fragment called C99. γ -Secretase processing of C99 produces several mature A β peptides with heterogeneous C termini ranging from 38 to 43 residues in length [146]. The two main toxic species are A β ₄₀ and A β ₄₂, with A β ₄₂ assuming only 10% of A β production but more prone to aggregates due to its hydrophobicity [147]. Although the activity of both β - and γ -secretases is needed, BACE activity is thought to be the rate-limiting factor for A β production [148], and hence continuous BACE1 activity is essential for sustaining A β levels in AD.

With EOAD, the mechanism is quite straightforward since it is either due to mutations in APP or presenilin, rendering APP to be processed more towards the A β pathway; however, in case of LOAD, the precise reason for A β production still remains unclear. Having said that BACE activity is the rate-limiting factor for A β production, logically, in case of sporadic AD, either there is an increase in BACE activity or levels, or a combination of both; alternatively there could be defective clearance of A β . Evidence indicating that familial AD caused by the APP Swedish mutation that only enhances APP cleavage by BACE1 implies elevated BACE1 activity alone can lead to AD. Various studies have shown that levels of BACE1 protein and activity are increased in AD brain [149–151]. BACE is an acute-phase protein, and studies have shown increased enzyme levels in conditions such as OXS [75], hypoxia [152], ischemia [153], traumatic brain injury [154] and impaired glucose metabolism [155]. The above-said conditions, being well-known risk factors associated with AD, can result in sustained and up to twofold increased BACE activity [149].

Multiple endogenous pathways exist for A β degradation [156] and include neprilysin, insulin-degrading enzyme, endothelin-converting enzyme, angiotensin-converting enzyme and metalloproteinase 9 [156], but their levels are reduced in AD [157]. Apart from the increased

enzyme activity, defects in Aβ clearance also play an important part in AD pathogenesis. Aβ is transported across the BBB in both the blood-to-brain and brain-to-blood directions by RAGE [158] and a low-density lipoprotein receptor-related protein (LRP-1) [159], respectively. RAGE expression increases with age at the BBB [160], resulting in more influx, suggesting another mechanism for Aβ accumulation in the CNS. The expression of LRP-1, the transporter responsible for Aβ efflux, is also decreased [161], thus blocking Aβ clearance from the CNS. ApoEε4, when present, also contributes to the transfer of Aβ from blood to CNS [110].

However, it is not clear as to which mechanism is impaired early in each case or how many mechanisms are perturbed in AD, and the precise reason for Aβ accumulation is yet to be defined. The mechanisms resulting in Aβ accumulation are summarized in table 3.

Pathoanatomy in AD

Apart from the peculiarity of the disease process, anatomical involvement as well as progression are also strikingly similar in most cases. It has been well known for a long time that temporoparietal regions are the most affected in AD. Many studies have definitively demonstrated that NFT distribution is initially limited to entorhinal cortices, spreading to temporoparietal association cortices later, followed by frontal and, ultimately, primary sensory and visual areas [162, 163]. In a study by Thompson et al. [164], the sequence of gray matter involvement in AD was mapped, and, along with the disparity in cortical involvement, there was asymmetric hemispherical involvement. The left hemisphere is involved first, followed by the right in a similar sequence but after a time lag. Disease progression is also not continuous, and specific regions are relatively spared, for instance primary sensory and motor cortices, until later in disease. Surprisingly, healthy controls also lose gray matter volume at rate of 1% per year [164], but the loss is limited to the frontal cortex, CA1 and subicular regions of the hippocampus. The loss in gray matter is secondary to cell shrinkage and reduced dendritic length in healthy controls [165] whereas in AD, a combination of these processes as well as substantial neuronal loss is noted [166].

Astonishingly, again there appears to be a reason behind the site-specific involvement in AD. The fact that hippocampi and limbic cortices bear the brunt of injury is not surprising due to their high baseline plasticity and active involvement in memory formation. Increasing ev-

Table 3. Causes of Aβ accumulation in AD

Increased β-secretase expression and activity
Decrease in Aβ-degrading enzymes
Increased RAGE expression/Aβ influx into the CNS
Decreased LRP-1 expression/Aβ efflux

idence highlights the fact that axonal sprouting, dendritic remodeling and perhaps reactive synaptogenesis are higher in the limbic system than in other parts of the cerebral cortex [167, 168]. Further, the markers of axonal sprouting [169], neurotrophic factor expression [170] and dynamics in dendritic growth [171] have been found to be highest in the transentorhinal, entorhinal and hippocampal regions, gradually decreasing in the posterior parietal and prefrontal areas, being almost negligible in the primary motor and sensory areas. This pattern accurately mirrors the sequence and pattern of mapped anatomic loss in AD, which was discussed earlier [164].

The high dynamicity of the neurons in these regions, which demands active metabolism, exposes these neurons to OXS at an early stage, probably explaining their vulnerability in AD. The risk factors of AD, viz aging, the ApoEε4 allele [172] and hormone imbalance with reproductive senescence [173], are all known to cause neuronal dysfunction and negatively affect plasticity. To begin with, slowly increasing OXS, secondary to metal accumulation, AGEs or inflammation, might cause subnormal neuronal function, resulting in additional work burden on the neurons to maintain hippocampal function. The neurons now adopt a compensated state in which organ function remains uncompromised, but neurons become now more susceptible to additional blows. Over the years, such compensatory processes would lead to chronically high and eventually unsustainable levels of plasticity-related cellular activity. Recalling the neurotrophic properties of APP [91], the role of P-tau in regulating neuroplasticity [93] and antioxidant properties of Aβ and tau [32], a chronically high demand for plasticity-related activity upregulates the level of tau and Aβ. Experimental evidence has already shown that high levels of plasticity are associated with increased expression and phosphorylation of tau [174] and APP levels [90]. The resultant P-tau polymerizes to NFT leading to cytoskeletal dysfunction and neuronal degeneration while toxic Aβ species themselves can produce synaptic damage and cell death directly [175]. The adjacent neurons would now be facing the plasticity demand and the process continues and

gradually extends to tightly linked adjacent areas of the limbic-paralimbic cortex, resulting in horizontal spread of NFT [176], reasoning the early and prominent involvement of the limbic system out. The loss of limbic neurons now places the plasticity burden on the reciprocally connected association areas, resulting in accumulation of NFTs which spread vertically as well as centrifugally [176], thus gradually involving adjacent regions and accounting for the anatomical cascade in AD.

Interindividual Variation

Along with the pathogenesis, even the clinical manifestations remain an enigma. Age at presentation varies broadly in case of AD, with EOAD manifesting roughly between 30–60 years and LOAD after 60 years. Even patients with extensive pathological lesions in the brain have remained cognitively normal [177], and about 20–40% of cognitively normal elderly people have evidence of significant brain A β plaque deposition [178]. The clue stems from the fact that even in patients with established AD mutations, onset age varies. Although convincing proof is yet to be established, the concept of a ‘cognitive reserve (CR)’ could provide an explanation. According to the CR model, the brain actively attempts to cope with brain damage using preexisting cognitive processing approaches or utilizing compensatory approaches [179] altering neuroplasticity. Thus, the same amount of brain damage or pathology may have different effects on different people, even if brain size remains constant. As a result, individuals with more CR would be more successful at coping with the same amount of brain damage [180]. Apart from the CR, 4 alternate splice variants are found for BACE [181], hence the differential expression of the variant secretase can differ in APP processing and modulate the rate of A β production and contribute to the interindividual variation [6]. It is not known whether individual differences in the antioxidant reserve exist and if they do, whether they contribute to interpatient differences.

Integrated Pathomechanism

Although the individual mechanisms are well established, an integrated cascade remains to be proven. Jack et al. [6] observed biomarkers in AD and found that (1) the biomarkers become abnormal in a temporally ordered manner with progressing disease, (2) A β -plaque biomarkers are dynamic early in the disease, before the

appearance of clinical symptoms (3) biomarkers of neuronal injury, dysfunction and degeneration present later in disease and correlate with clinical symptom severity, (4) NFT accumulation and cerebral atrophy, spread from medial temporal limbic areas to adjacent limbic and paralimbic areas, and later to the isocortical association cortex, and (5) a lag phase of unknown duration is present between A β plaque formation and the neurodegenerative cascade. Considering the above findings in mind, some assumptions can be derived, which might give orientation for future proceedings although hypothetical (fig. 2).

The finding that markers of A β formation present before clinical features [6] indicates that A β is crucial in the pathogenesis of AD and the time taken for A β accumulation decides the age of disease onset. In case of sporadic AD, the process of A β production is rather complex and involves multiple deregulated mechanisms; however, it is difficult to say convincingly as to which process is the earliest to be perturbed. The A β accumulation occurs over years, probably decades, accounting for the late manifestation of sporadic AD. This is substantiated by the fact that EOAD occurs in patients with mutations in APP or PSEN, for example, resulting in increased A β accumulation. In ApoE ϵ 4 carriers, there is decreased A β clearance and neuroinflammation [112] accounting for early A β accumulation. The left shift in the A β accumulation curve [6] accounts for the early manifestation of the disease.

Preclinical AD corresponds to the asymptomatic period during which the disease process continues subtly. During this phase, disease process remains confined to the hippocampus/limbic cortex, rather than the CNS in general. In response to the perturbations, neurons adopt a compensated state maintaining plasticity, during which there is A β production. The individual stressor in this case is rather weak, but with more and more abnormal mechanisms stepping in, there is amplified A β production. Neuropathological studies in preclinical AD have shown that there is A β accumulation in the brain although in the absence of significant global OXS, probably due to the lack of A β oligomerization [182]. In addition, there is no significant increase in the protein carbonyl content but rather neuronal hypertrophy, along with nuclei and nucleolar hypertrophy [183]. The exact nature of this adaptation and its resilience over time is uncertain, but indicates the compensatory response of neurons, representing the resistance displayed by the neurons to survive the stressors, which could last a definite but variable period before the onset of cognitive decline.

The stage of neurodegeneration encompasses MCI and dementia, and occurs due to synaptic loss and neuronal

Fig. 2. Hypothetical pathophysiological cascade in AD. * = Addition of a new mechanism into the pathway; ETC = electron transport chain; - - - = the period of decline in neuronal function.

death gradually leading to atrophy. The findings observed during this stage were again in temporal order with decreased metabolism shown in fluorodeoxy glucose positron emission tomography occurring first, followed by increased CSF-tau and brain atrophy in MRI [6]. This part of the pathophysiology is probably common to both EOAD and LOAD. MCI, a state between normality and full-blown AD, represents a stage of transition from normality and heralds a rapid decline in neuronal function. Many patients with the clinical syndrome of MCI presented the 'AD pattern' of hypometabolism in temporoparietal regions [184], which predicts progression to clinical AD.

Only few longitudinal neuropathological studies on clinically diagnosed MCI are available, and some common features are reported. Between preclinical AD and MCI, a significant increase in the level of protein carbonyls, indicative of significantly elevated oxidative stress in MCI has been observed despite equal levels of pathology [185]. Specifically, carbonylation of heat shock protein (HSP90) was significantly increased and levels of ubiquitin carboxyl-terminal esterase L1 (UCLH1) were decreased in MCI compared to preclinical AD and controls [185], indicating the onset of proteosomal dysfunction that was normal in preclinical AD. Patients with MCI showed significantly increased neuritic plaques in all neocortical regions and the amygdala compared with controls. NFTs were significantly increased in the amygdala, entorhinal cortex, CA1, subiculum and inferior parietal lobule in MCI compared with normal controls [186]. Neuritic plaques were increased in the amygdala and subiculum, and NFTs were significantly increased in middle frontal and middle temporal gyri, amygdala and subiculum in EOAD compared with MCI [186]. In a longitudinal study by Scheff et al. [187], synapse numbers were decreased by 18% in the stratum radiatum of hippocampal CA1 and 13% in the outer molecular layer of the dentate gyrus in MCI patients compared with normal controls but did not reach statistical significance. However, comparing MCI to AD, synapse number was significantly decreased in AD by 44% in the outer molecular layer and 55% in the stratum radiatum. Thus, AD pathology is much greater in MCI patients than in controls, but it is intermediate between controls and AD.

Pathophysiologically, it is during this phase that the disease process begins to dominate compensatory mechanisms. As discussed earlier, oligomerized A β is probably the major culprit in this stage and worsens neuronal function via multiple mechanisms. The failure of antioxidant defense mechanisms reflected by improper Nrf2 localization [11] in the nucleus also plays a role in AD

pathogenesis. The levels of A β degrading enzymes are decreased in AD, and A β begins to accumulate with the A β production exceeding its clearance. In fact, A β can directly induce synaptic damage and neuronal apoptosis, resulting in accelerated neuronal death placing additional plasticity demand on the surviving neurons that fail to cope with. Tau phosphorylation probably reflects the neuronal attempt to maintain plasticity. However, P-tau, due to its tendency to aggregate, develops into NFTs, further impairing neurogenesis. Subsequently, both A β and P-tau individually as well as synergistically produce neuronal degeneration and death. Various other mechanisms, such as perturbed cholesterol metabolism, mitotic dysfunction or caspase activation, also contribute to the neuronal decline and death.

This process propagates to nearby and associated regions; initially, it is limited to entorhinal cortices, spreading to temporoparietal association cortices, and then to frontal and, ultimately, to primary sensory and visual areas [162, 163]. This sequential involvement suggests that at a particular time period, different sites of the brain might be in different phases of the disease. The patient might be asymptomatic for a variable period during this phase depending on the individual's CR. Once a particular threshold is reached beyond CR, it manifests as memory deficits and MCI, and later progresses to dementia.

Conclusion and Future Directions

Thus, the pathophysiology of AD is enigmatic and puzzling, and further studies are needed to bring the complete picture. Our failure to understand the temporal sequence of events is probably the reason for our failure in various treatment trials. For instance, inflammation plays an important role in the early phases of the disease; hence and rightfully, various treatment trials with non-steroidal anti-inflammatory drugs have failed. Considering age-related neuronal loss and plasticity changes, it is possible that every individual might develop AD with advancing age, but the interperson variation in antioxidant reserve, APP processing, neuronal adaptations and CR affect the age of AD onset, and at least theoretically every individual might develop AD in due course if they survive. If proven that OXS is the main trigger of AD, prevention of AD might have to be taken to the primordial prevention level beginning with diet and exercise.

The precise reason for early hippocampal involvement and sparing of other regions is still not clear. Autopsy studies may be planned in different age groups, studying

the integrity of individual mechanisms throughout the course of AD in order to define the disease cascade. Sorting out the disease cascade will only help us in devising specific screening or diagnostic tests for AD and in guiding specific management. Proteomic studies elucidating the differential expression of proteins at various sites in the brain could explain the site-specific involvement in AD. Although preventive trials with neuroprotective

strategies in the general public might raise ethical concerns, they can be planned and might be done in ApoE ϵ 4 carriers to demonstrate possible benefits.

Disclosure Statement

The authors have no conflict of interest to declare.

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