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Pathophysiology of Alzheimer's Disease

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Alzheimer's disease (AD) is the most common form of senile dementia, affecting 10% of individuals older than 65 and nearly 50% of those older than 85. The prevalence of AD in the United States is estimated to be approximately 4.5 million and is predicted to increase to up to 13.2 million in the next 50 years. Although basic research in AD has made remarkable progress in the past 2 decades, currently available drugs are able only to improve cognitive symptoms temporarily, and no treatment can reverse, stop, or even slow the inexorable neurodegenerative process. The pathophysiology of AD is associated with a variety of factors, including the extracellular deposition of β-amyloid (Aβ) plaques, accumulation of intracellular neurofibrillary tangles, oxidative neuronal damage, and inflammatory cascades. It is widely believed, however, that an increase in the production of the Aβ peptide, the main component of amyloid plaques, is central to the pathogenesis of the disease. Since the first description of the neurotoxic properties of the Aβ peptide, a vast number of studies have investigated the cellular and molecular pathology triggered by Aβ. This article reviews the basic knowledge of the pathophysiology of AD. Progress in understanding the cellular and molecular alterations that are responsible for the neuron's death may help significantly in developing more effective medications to slow the progression of this devastating disease.

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AD is a neurodegenerative disorder characterized clinically by progressive deterioration of cognition, behavior, and functionality that impairs activities of daily living significantly. Morphologically, the disease is characterized by brain atrophy and by enlarged cerebral ventricles. From a biochemical point of view, the most clear-cut and consistent finding is a deficit of the cholinergic system, decreased levels of choline acetyltransferase, and other cholinergic markers. Histologically, AD is characterized by extracellular deposits, called cerebral plaques, composed of a dense proteinaceous core containing the Aβ peptide surrounded by dead and damaged neurons. The other typical histopathologic hallmarks of AD are the neurofibrillar tangles within neurons, formed mainly by a filamentous, hyperphosphorylated form of the microtubule-associated protein τ. Whereas most cases of AD occur sporadically, approximately 5% of patients develop the disease early as a result of fully penetrant autosomal dominant gene mutations.

Epidemiology of Alzheimer's disease

The incidence and prevalence of AD rise with increasing age and are higher in women in part because of their increased longevity. The incidence of AD ranges from 1% at ages 65 to 70 to approximately 4% over age 85. In the United States, the number of new cases per year is expected to triple from approximately 420,000 in 2000 to more than 1.3 million in 2050 [1]. Estimates of prevalence of AD range from the lowest figure of 3% of the population at 65 years to the highest reported estimate of 47% of

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people over age 85. The prevalence of AD in the United States in 2000 was estimated to be 4.5 million [2]. By 2050, this number will increase by almost threefold, to 13.2 million. In the United States, AD currently is the eighth leading cause of death, with approximately 63,000 deaths per year and a death rate of 21.8 deaths per 100,000 population [3]. The death rate of AD is increasing by approximately 6% per year. The median survival from initial diagnosis recently was estimated to be 4.2 years for men and 5.7 years for women [4].

Genetics of Alzheimer's disease

The presence in some families of AD individuals who have an autosomal dominant inheritance pattern has allowed for the discovery of disease genes. Mutations on three genes, known as causative genes, are fully penetrant and cause aggressive forms of early-onset AD. The causative genes are those encoding amyloid precursor protein on chromosome 21q21 (*APP*), presenilin 1 on chromosome 14q24 (*PSEN1*), and presenilin 2 on chromosome 1q42 (*PSEN2*). Mutations in these genes account for approximately 5% of the total number of AD cases [5]. There also are other genes, known as susceptibility genes, believed to be involved in the pathogenesis of AD through complex interactions with environmental factors. Allele polymorphism of one of these susceptibility genes, that encoding apolipoprotein E on chromosome 19q13 (*APOE*), is associated definitely with increased risk for late-onset AD in many studies. It is suspected that several other AD-susceptibility genes exist, and their identification is the subject of ongoing research [5]. Ultimately, a variety of genes probably will be determined to confer risk, each one responsible for only a small fraction of all cases. Overall, the four genes linked to AD (*APP*, *PSEN1*, *PSEN2,* and *APOE*) account for approximately half of the total genetic risk [6].

Causative genes

Historically, the first identified gene causing AD was that encoding the substrate (*APP*), from which the Aβ peptide is generated [7]. The other two AD causative genes discovered encode the enzymes (presenilins) that produce Aβ from *APP* [8,9]. Mutations in genes encoding *APP* or presenilins cause dysregulation of *APP* processing with an increase of the amount of Aβ produced (Fig. 1). The accumulation of Aβ seems to be an early and initiating event that triggers a series of downstream processes, including misprocessing of the τ protein. This cascade ultimately causes neuronal dysfunction and death and leads to the clinical and pathologic features of AD.

Amyloid precursor protein

APP is a type I transmembrane glycoprotein produced in many cells and processed through the secretory or endosomal-lysosomal pathways. Alternative splicing of APP provides a total of 10 isoforms, with lengths of 304, 639, 677, 695, 696, 714, 733, 751, 752, and 770 amino acids [10]. The major *APP* isoforms are those with 695, 751, and 770 amino acids. The 695 amino-acid form is expressed preferentially in neuronal tissue. Although the physiologic functions of *APP* remain unclear, its ubiquitous expression during development and in several adult tissues suggests a fundamental role in cellular physiology. *APP* seems to be involved in the static cell-substrate adhesion or neurite outgrowth synaptogenesis, synaptic plasticity, and promotion of neuronal cell survival [11]. *APP* also is involved in the regulation of cell movement [12]. Exposure of cortical neurons to *APP* monoclonal antibodies leads to neurite degeneration followed by caspase-dependent apoptosis [13]. The soluble secreted form of *APP* (sAPPα) shows similarities with growth factors and increases the proliferation of embryonic neural stem cells [14].

Mutations of *APP* are connected to a limited number of early-onset familial AD cases [15]. To date, 22 single–amino-acid and 1 two–amino-acid (the Swedish mutation K670N/M671L) mutations of *APP* are identified (see Fig. 1). Four of these mutations are not pathogenic. Most of the mutations are located adjacent or in proximity to the cleavage site of γ-secretase. The most frequent *APP* mutation is V717I (the London mutation) [16]. Carriers of the so-called "Arctic" mutation (E693G), found in a Swedish family, show decreased Aβ42 and Aβ40 levels in plasma but increased protofibril formation [17]. This *APP* mutation is highly amyloidogenic in vivo [18]. The most recently discovered *APP* mutations are one found in a Japanese family within the Aβ sequence (D678N) [19] and a novel mutation at the β-secretase (BACE1) cleavage site [20].

Presenilins

Presenilin 1 (PS-1) and presenilin 2 (PS-2) are two similar 8-domain transmembrane proteins that, within the γ-secretase complex, catalyze the last step of the *APP* cleavage generating the Aβ40 and Aβ42 peptides. Six alternative splicing forms of PS-1 are known with 184, 374, 378, 409, 463, and

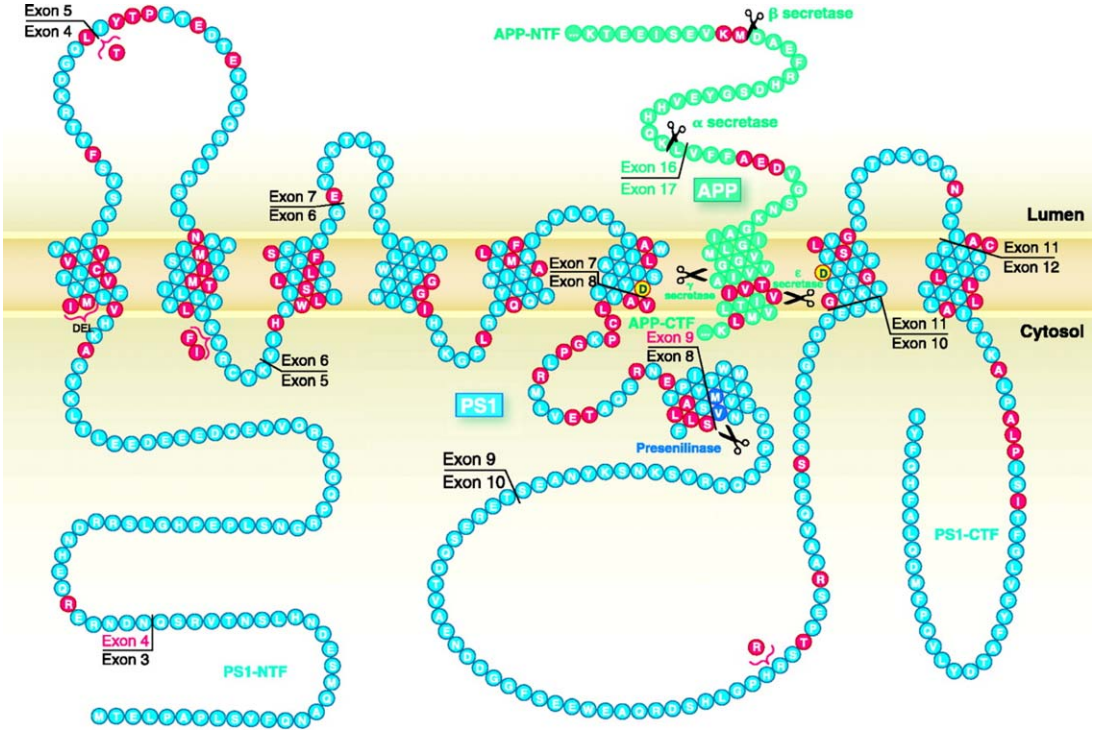

Fig. 1. Amino-acid sequence of PS-1 (*blue circles*) and of the C-terminal portion of APP (*green circles*) generating Aβ. Mutations in PS-1 and APP known to cause familial AD are depicted in red. The β, α, γ, and ε cleavage sites are indicated by small scissors. The two amino acids involved in the endoproteolytic cleavage of PS-1 are shown in dark blue. The two aspartate residues of the catalytic site of PS-1 are highlighted in yellow. Exon junctions also are shown. CTF, C-terminal fragment of PS-1; NTF, N-terminal fragment of PS-1. (*From* Hardy J, Selkoe DJ. The amyloid hypothesis of Alzherimer's disease: progress and problems on the road to therapeutics. Science 2002;297:355; with permission.)

467 amino acids. Two isoforms of PS-2 are sequenced with 414 and 448 amino acids. To date, 144 missense mutations of PS-1 are identified, two of which are not pathogenic (see Fig. 1). Eleven mutations of PS-2 are known, one of which is not pathogenic. PS-1 mutations are the cause of the most severe form of dominant familial AD, with complete penetrance and an onset occurring as early as 30 years of age [5]. Studies in cell lines expressing human mutated presenilins, transgenic mice bearing mutated human presenilins, and plasma samples and brain sections of patients who have presenilin mutations show that PS-1 and PS-2 missense mutations increase the production of Aβ42, the highly selfaggregating and neurotoxic form of Aβ [21]. These mutations alter the conformation of presenilins, apparently enhancing coordination between the two catalytic aspartates of presenilins (respectively, in position 257 and 385) and the peptide bond between amino acids alanine and threonine of *APP* [22]. Certain PS-1 mutations are associated with atypical phenotypes, including spastic paraparesis, language deficits and frontal behavioral disturbances [23]. A PS-1 mutation (G183V) was found to cause clinically frontotemporal dementia (FTD) and histologically neurofibrillary tangle pathology without amyloidosis [24]. Cultured cells transfected with this mutated presenilin produced normal amounts of Aβ42, an observation that raised questions as to the presumption that all autosomal dominant forms of AD could be linked to the accumulation of Aβ in the brain.

The last described presenilin mutations include one (L174R) in two members of a Bavarian family [25] and one (V97L) in members of a Chinese family [26].

Susceptibility genes

Although mutations in the APP, PS-1, and PS-2 genes cause less than 5% of cases of AD, 25% of persons who have late-onset forms of the disease

have had a close relative who has had dementia [5]. Indeed, there are many families that have a heavy load of late-onset AD but do not have mutations in APP, PS-1, or PS-2 genes. Thus, intense research efforts are dedicated to identifying additional genetic factors in the more common late-onset form of the disease. Genetic linkage studies involving hundreds of families have produced evidence for several chromosomal regions suspected of harboring genes related to AD. These include regions on chromosomes 6, 9, 10, 12, and 19 [27]. So far, careful scrutiny of these regions has resulted in the identification of *APOE* as an unequivocal gene associated with late-onset AD. More than 100 additional genes have been investigated as susceptibility genes through genetic-association studies, but none of them are confirmed fully [28]. It is estimated that there are at least six additional susceptibility genes influencing the age at onset and the risk for sporadic AD [29]. The hunt continues and additional loci are expected to be discovered as the result of genomewide screening [16].

Apolipoprotein E

Apoliprotein E (apoE) is a 299–amino-acid protein involved in transport and metabolism of lipids [30]. Ninety percent of circulating apoE derives from the liver. apoE also is synthesized in other organs (lung, ovary, muscle, spleen, and kidneys), including the central nervous system, where it is made by glia, macrophages, and neurons. Three major isoforms of apoE are known (apoE2, apoE3, and apoE4) and these are encoded by three natural allelic apoE variants (ε2 [*APOE*E2*], ε3 [*APOE*E3*], and ε4 [*APOE*E4*]). The structural differences of different apoE isoforms are small. apoE3 has cysteine in position 112 and arginine in positions 158. In apoE4, arginine replaces the cysteine in position 112. In apoE2, the arginine in position 158 is replaced by cysteine. The most common *APOE* allele is *APOE*E3*, present in 40% to 90% of the population. The *APOE*E3* is present in 10% to 15% of the general population with frequency higher in northern than in southern European regions [31]. *APOE*E2* is the least frequent allele (approximately 10%).

The polymorphism of the *APOE* gene is considered the most important, best-documented, genetic susceptibility risk factor for late-onset AD [5]. Individuals who carry the *APOE*E4* have an increased risk for developing AD compared with noncarriers, with a threefold to fourfold higher risk for heterozygotes and a ninefold to tenfold higher risk for homozygotes [32]. In the population of patients who have AD, approximately 45% of *APOE* alleles are of the ε4 type [33]. Conversely, the *APOE*E2* seems to protect from AD [32]. Interestingly, there are few individuals who have mutated APP who also carry the *APOE*E2* who fail to develop dementia [34]. Nevertheless, the putative protective effect of *APOE*E2* remains debated [35]. Many genetic epidemiologic studies robustly confirm an enhanced risk for developing AD in subjects who have one or two *APOE*E4*s who are in their sixth or seventh decade of life. The effect is modified by gender, however, and is age specific, with its peak effect observed at approximately age 70 [36]. There are octogenarians carrying two *APOE*E4*s who seem to have escaped the *APOE*E4* effect. The purified apoE4 isoform is reported to have higher affinity for Aβ than apoE3 [37], suggesting it may act as a pathologic chaperone stabilizing the β-sheet structure of Aβ fibrils [38] and impairing Aβ clearance [39]. Elegant experiments in *APOE* knockout and transgenic mouse models show that the presence of apoE4 increases the amount of fibrillar Aβ deposits markedly in the brain compared with the effects of apoE3 [40]. The *APOE*E3* genotype, however, is neither necessary nor sufficient for the occurrence of AD. Thus, there are other genetic or environmental factors that alone or in conjunction with *APOE*E4* can modify the risk for AD [27].

Susceptibility loci on chromosome 10

Genome screenings aimed at identifying genetic susceptibility risk factors for late-onset AD find a locus on chromosome 10 that modifies risk for AD by elevating Aβ42 plasma levels independently from the *APOE* genotype [41,42]. Several strong candidate genes (VR22, PLAU, and IDE) support the linkage.

The VR22 gene encodes α-T catenin, which is a binding partner of β catenin, known to interact with PS-1. VR22 has variants that influence high plasma Aβ42 levels [43].

The IDE gene encodes insulin-degrading enzyme, which has a central role in the degradation and clearance of Aβ by microglial cells and neurons [44]. Recently, the existence of pathologic variants in the region harboring IDE that influences late-onset AD with high plasma Aβ42 levels has been suggested [45].

The PLAU gene encodes urokinase-type plasminogen activator, which converts plasminogen to plasmin. Aβ aggregates induce PLAU expression, thereby increasing plasmin, which degrades aggregated and nonaggregated forms of Aβ. Single missense mutation (P141L) in urokinase-type plasminogen activator recently was suggested to increase Aβ42 in late-onset AD [46].

Susceptibility loci on chromosome 12

A genomic screen of families who have late-onset AD reveals a susceptibility locus on chromosome 12 not linked to the presence of the *APOE*E4* [47].

The genes encoding α2-macroglobulin (α2M) and low-density lipoprotein receptor-related protein (LRP) present on this chromosome initially were considered suspects. Later, it was proposed that a genetic variation in a transcriptional factor, LBP-1c/CP2/LSF, is the susceptibility factor for late-onset AD [48]. α2M is a serum protease inhibitor, also expressed in the brain implicated in AD on the basis of its ability to mediate the clearance and degradation of Aβ [49]. α2M polymorphisms resulting from a deletion allele or an amino-acid substitution (V1000I) are hypothesized to explain its association with AD. More recently, new population-based studies are published in which α2M deletion/insertion polymorphisms are not associated with risk for developing AD [50,51] . Thus, it may be possible that the locus on chromosome 12 responsible for AD susceptibility does not correspond to α2M but to an adjacent gene.

The low-density LRP is a multifunctional receptor that is highly expressed in the brain. LRP is the main apoE receptor expressed in neurons, mediates neurite outgrowth, is responsible for the endocytosis of secreted APP, and is detected in senile plaques, dystrophic neuritis, and reactive astrocytes in AD brain [52]. Recently, it was shown that LRP interacts with β-secretase on the cell surface and acts as a β-secretase substrate [53]. Genetic association between two different LRP polymorphisms (a TTTC repeat and a cytosine to thymine silent substitution) and AD is reported. These associations, however, are not confirmed in other studies and the link remains controversial [54].

Several members of the glyceraldehyde-3-phosphate dehydrogenase (GAPD) gene family are found, including one within the 12p candidate region associated with late-onset AD [55]. GAPD is a key enzyme in cellular energy production and also plays an important role in several other cellular processes, including neuronal apoptosis and neurodegenerative diseases, including AD. It is known to bind APP [56] and Aβ [57].

Microtubule-associated protein τ

Microtubule-associated protein τ promotes microtubule assembly and stability and is involved in the establishment and maintenance of neuronal polarity. Eight isoforms of protein τ are known: PNS-τ (757 amino acids), fetal-τ (315 amino acids), τ-A (351 amino acids), τ-B (380 amino acid), τ-C (409 amino acids), τ-D (382 amino acids), τ-E (411 amino acids), and τ-F (440 amino acid). Isoform PNS-τ is expressed in the peripheral nervous system (PNS), whereas the others are expressed in the central nervous system. Protein τ is a major component of the intracellular filamentous deposits (neurofibrillar tangles) that histologically define not only AD but also other neurodegenerative diseases, including progressive supranuclear palsy, corticobasal degeneration, Pick's disease, and argyrophilic grain disease. Neurofibrillar tangles consist mostly of paired helical filaments of protein τ in the hyperphosphorylated, insoluble form [58]. For a long time, it was unclear if the dysfunction of protein τ follows disease or if disease follows τ dysfunction. This was resolved when mutations in gene encoding τ on chromosome 17q21 (*MAPT*) were found to cause the inherited FTD and parkinsonism (FTDP-17), historically also termed Pick's disease [59]. Currently, 32 different mutations are identified in more than 100 families [60]. Approximately half of the known mutations reduce the ability of protein τ to interact with microtubules and increase its propensity to assemble into abnormal filaments. The other mutations perturb the normal ratio of protein τ isoforms [61]. Although no mutations in protein τ are yet associated with AD, the abundance of neurofibrillar tangles correlates with the degree of neurodegeneration. The presence of neurofibrillar tangles in AD may manifest a "downstream" response to the pathologic events initiated by this disease [6]. In a triple transgenic mouse model of AD harboring mutant genes for APP (K670N/M671L), PS-1 (M146V), and protein τ (P301L), Aβ deposition develops before the tangle pathology [62]. Similarly, studies in double transgenic mouse expressing the Swedish mutation of APP and the τ protein containing three of the mutations present in FTDP-17 show that Aβ facilitates the phosphorylation of τ and its aberrant aggregation [63].

Other susceptibility genes

Many other genes emerging from studies on cohorts of sporadic AD cases and age- and sex-matched controls are hypothesized as associated to sporadic AD.

Neprilysin is a zinc metalloproteinase reported as a major Aβ-degrading enzyme expressed in the brain [64]. The decreased expression and activity of neprilysin may contribute to the development of AD by promoting the accumulation of Aβ. The gene encoding neprilysin (NEP) is located on chromosome 3q25. At least 10 single nucleotide polymorphisms in the NEP are found to be associated to sporadic

AD, suggesting that genetic variations within or extremely close to the NEP might influence the susceptibility to AD [65,66].

Ubiquilin 1 (UBQLN1) is a highly expressed protein that promotes the accumulation of uncleaved PS-1 and PS-2 by stimulating their biosynthesis. The gene encoding UBQLN1 is one of several candidate genes for AD located near a well-established linkage peak on chromosome 9q22. Variants in UBQLN1 substantially increase the risk for AD, possibly by influencing alternative splicing of this gene in the brain [67].

β-secretase is the enzyme the starts the first cleavage of APP to generate Aβ. The gene encoding β-secretase (BACE1) is located on chromosome 11q23 near a region with increased lod scores for AD. The biologic functional and genetic association studies indicate that the BACE1 gene may be a genetic risk factor for late-onset AD. BACE1 gene polymorphism C786G may act as an *APOE*E4*-dependent risk factor for developing late-onset AD in Chinese populations [68].

Tumor necrosis factor-α (TNF-α) is a potent immunomodulator that may mediate neurotoxicity [69], which makes it an appropriate AD candidate gene. The gene encoding TNF-α is located near a chromosomal region, 6p21, which shows genetic linkage association with AD in several of the full-genome screens in AD [28]. Recent studies, however, suggest that TNF-α may act as a risk factor only for vascular dementia [70] and that 308 adenine/guanine (A/G) polymorphism is not associated with late-onset AD [51].

α1-antichymotrypsin (ACT) is a serine protease inhibitor and an acute phase protein. ACT is synthesized predominantly in the liver and other tissues, including the brain. ACT is a major component of the senile plaques and may act as a pathologic chaperone, promoting Aβ assembly into neurotoxic fibrils. Increased levels of ACT are found in the brain and peripheral blood of patients who have AD [71]. ACT gene is located on chromosome 14q32. It contains common bi-allelic polymorphisms, adenine/thymine (A/T) transversion, resulting in either an alanine or a threonine at codon 15 in the signal peptide sequence. The alanine allele is associated with an increased risk for AD only in *APOE*E4* carriers [72]. Other studies, however, do not confirm these findings [73]. More recently, reports suggest that the T allele of a guanine/thymine (G/T) polymorphism at position 51 of ACT is associated with an increased risk for early-onset of AD independent of the presence of the *APOE*E4*. After manifestation of the disease the ACT TT genotype also is associated with faster cognitive decline in patients who have the *APOE*E4* [74].

Nongenetic risk factors for Alzheimer's disease

AD is caused by a combination of genetic and environmental factors, and the role of environment cannot be ignored. A lot of effort has been devoted to identifying those specific genetic and environmental factors, determining their relative importance, understanding their interactions, and capitalizing on this knowledge to treat and prevent the disease.

Traumatic head injury

Several studies suggest a link between risk for developing AD and traumatic head injury, but the association is controversial [75]. Human postmortem and experimental studies show Aβ deposition and τ pathology after head injury. A pig model of brain injury shows that even a mild trauma with full recovery leads to the accumulation of Aβ and formation of neurofilament inclusions [76]. An overexpression of APP is observed in the brains of gunshot survivors [77] and in head-injured sheep [78]. Aβ levels in the cerebrospinal fluid also are increased in patients who have traumatic head injury and in animal models of brain trauma. Studies involving large groups of participants, however, such as the Multi-Institutional Research in Alzheimer Genetic Epidemiology (MIRAGE) study [79] and Rotterdam [80] studies, provide contradictory results. A recent analysis of 15 case-control studies supports an association between a history of previous head injury and the risk for developing AD, but this association was restricted to male patients [81]. The presence of *APOE*E4* is associated with worse neurologic impairment in head injury [82] but the influence of the *APOE* genotype on the prognosis of traumatic brain injury is under discussion [75].

Hypercholesterolemia and high-density lipoprotein cholesterol

The relationship between total serum cholesterol, its high- and low-density lipoprotein fractions, and AD is the subject of several epidemiologic studies. The results of these studies sometimes are contrasting. A small Finnish study of 444 elderly men suggests that regardless of genetic status, high serum cholesterol represents an independent risk factor [83]. Similarly, another small French study involving 334 elderly subjects suggests that regardless of *APOE* status and other potential confounding variables, elevated high-density lipoprotein cholesterol reduces the risk for AD significantly [84]. Alternatively, other epidemiologic studies show that lipid levels

are lower in persons who develop AD, not higher. For example, a study of 987 elderly subjects, most of them of Caribbean Hispanic origin, concludes that total cholesterol has a weak but significant inverse association with incident AD, regardless of *APOE* genotype [85]. A recent study of 392 subjects shows that high cholesterol in late life is associated with decreased dementia risk [86]. The conflicting results may be explained by the timing of the cholesterol measurements in relationship to age and clinical onset of dementia. Studies in patients taking cholesterol-lowering drugs, such as 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins), also are contradictory. Some studies conclude that statin use lowers the risk for dementia [87], whereas others conclude that this association is artifactual [88].

Results of epidemiologic studies prompted many laboratory studies. In vitro, APP751-transfected cells with elevated cholesterol levels show a dramatic reduction in secretion of sAPPα, the nonamyloidogenic metabolite of APP [89]. In cultured neurons, cholesterol depletion obtained by combination of lovastatin treatment and methyl-β-cyclodextrin extraction, reduces production of Aβ below detectable levels [90] by shifting the APP processing to α-secretase [91]. At least some of the actions of statins on α-secretase seem to occur via the rho/ROCK pathway, which can modulate sAPPα generation [92]. In contrast, recently it was shown that statins may determine an accumulation of intracellular amyloidogenic metabolite of APP (sAPPβ) and Aβ via an isoprenoid-dependent mechanism [93]. Adding complexity to the picture, recently it was shown that in human neuroblastoma cells, treatment with statins decreased the association of the γ-secretase complex significantly with lipid rafts [94]. In vivo, rabbits on a 2% cholesterol diet developed increased Aβ levels within the neurons of their brains [95]. Studies in transgenic animal models of AD show that fat feeding increases brain plaque load and that hypocholesterolemic agents, such as statins, lower plaque burden [96,97].

Definitive answers on the role of cholesterol in AD can be obtained only from clinical studies. Indeed, quite a few prospective clinical trials of cholesterol-lowering statins in AD patients are available. Most of these studies are negative. In addition, two large randomized, placebo-controlled clinical trials fail to show any effect of statins (simvastatin and pravastatin) on cognitive performance or on dementia development [98,99]. Perhaps the only encouraging evidence, albeit preliminary, is the apparent ability of atorvastatin to slow progression of dementia in AD [100].

Homocysteine, folate, and vitamin B_{12}

Elevated serum homocysteine levels are considered a risk factor for cognitive decline and AD, but data from prospective studies are controversial. Serum concentration of homocysteine increases with age. In healthy elderly subjects, plasma total homocysteine concentrations are correlated negatively with cognitive performance [101,102] and high plasma homocysteine levels are associated with brain atrophy [103]. High homocysteine levels are associated with low vitamin B_{12} and folate levels but it is unknown if it is homocysteine toxicity or vitamin insufficiency that is responsible for the observed cognitive dysfunction. In healthy elderly subjects, low folate levels appear to be a risk factor for cognitive decline [104]. Vitamin B_{12} and folic acid supplementation normalize plasma total homocysteine concentrations but do not affect cognitive performance [105]. In patients who have AD, plasma homocysteine levels are described as significantly higher than in controls [106] but this observation is not confirmed in other studies [107,108]. No correlations between homocysteine levels and cognitive functioning are found [109] and high homocysteine levels are not found associated with a decrease in memory scores over time [107]. Hence, there is little evidence to justify treating cognitive impairment with vitamin B_{12} or folate supplementation. This is consistent with the findings from recent systematic reviews of randomized double-blind trials, which have not found any evidence of potential benefit of vitamin supplementation. A systematic review of the literature establishes that folic acid plus vitamin B_{12} is effective in reducing the serum homocysteine concentrations, but there is no beneficial effect of folic acid on cognition in older healthy women and in patients who have mild to moderate cognitive decline and different forms of dementia [109]. A recent study shows that homocysteic acid, an oxidized metabolite of homocysteine, induces intraneuronal accumulation of Aβ42 that is associated with cytotoxicity [110]. But further research is required to establish if raised serum total homocysteine is a cause or consequence of disease [111].

Alcohol

Light to moderate alcohol, in particular wine, intake may be related to a low risk for AD [112]. This is apparent in two major studies. A French population-based prospective study involving 3777 subjects finds a protective effect of wine consumption on AD with adjusted odds ratios of 0.55 and 0.28 for subjects consuming 1 to 2 and 3 to 4 glasses of wine per

day, respectively [113]. These results are confirmed from the analysis of the Rotterdam Study involving 5395 subjects. Light to moderate drinking (one to three drinks per day) is associated significantly with a lower risk for any dementia, with a hazard ratio of 0.58 [114]. A third, smaller study involving 402 subjects again finds that light to moderate drinking is associated significantly with a decreased risk for AD compared with nondrinking, with an adjusted relative risk 0.50 [115]. A further study involving 980 subjects reveals that the association between wine consumption and lower risk for AD is confined to individuals who do not have the *APOE*E4* [116]. Intake of liquor, beer, and total alcohol is not associated with a lower risk for AD. Other studies find a modest, although significant, protective effect of alcohol intake, with an odds ratio of 0.83 [117].

Other dietary risk factors

Several other dietary elements and foods are reported to be either risk or protective factors for the development of AD [118]. Antioxidant vitamins E and C have received particular attention, because animal and in vitro studies suggest that oxidative stress has a role in the pathogenesis of AD. Analysis of the Rotterdam Study finds that high intake of vitamin C and vitamin E is associated with lower risk for AD with rate ratios of 0.82 and 0.82, respectively [119]. Another prospective study conducted in 815 individuals suggests that vitamin E from food, but not other antioxidants, may be associated with a reduced risk for AD [120]. This association was observed only among individuals who did not have the *APOE*E4*. A major 2-year, double-blind, placebo-controlled study of 341 patients who had moderate AD detected a significant delay in clinical worsening for patients treated with vitamin E (2000 IU/day), although statistical analysis applied to this study is questioned [121]. A recent 3-year, double-blind, placebo-controlled trial in 769 subjects who had mild cognitive impairment shows that vitamin E supplementation (2000 IU/day) does not reduce the progression to AD [122]. Thus, a recent review concludes that there are no clear-cut answers as to whether or not vitamin E is worth prescribing for AD [123].

Reports on other dietary factors are inconsistent and there are few large epidemiologic studies exploring the associations between nutrients and AD. Available data do not permit definitive conclusions regarding diet and AD or specific recommendations for diet modification for the prevention of AD [124].

Prevalent pathophysiologic hypothesis: the β-amyloid cascade

Neuritic plaques, the characteristic lesions found in the brain of patients who have AD, are composed mainly of aggregates of a peptide with 40 or 42 amino-acid residues, known as Aβ. The Aβ peptide is the result of the metabolic processing of a complex transmembrane glycoprotein, known as APP. APP may be processed metabolically according to two pathways (Fig. 2). In the so-called "nonamyloidogenic pathway," the α-secretase enzyme cleaves APP within the Aβ sequence and releases its transmembrane fragment, sAPPα, which seems to exert neuroprotective activity. In the amyloidogenic pathway, the β-secretase enzyme releases sAPPβ plus a 12-kd protein fragment (C99), which in turn is cleaved by the γ-secretase complex giving way to Aβ. The γ-secretase complex consists in a catalytic component, presenilin, and three cofactors, nicastrin, anterior pharynx defective 1 (Aph-1), and presenilin enhancer 2 (Pen-2) (Fig. 3). Presenilin is activated biologically by endoproteolysis that generates two heterodimers that each present the key aspartate residue.

The correlation among Aβ histopathologic lesions, brain cell death, and cognitive deficiency in AD represents the so-called "Aβ hypothesis" of the disease. This hypothesis was conceptualized in 1991 by Hardy and Allsop [125] and updated in 2002 by Hardy and Selkoe [126] (Fig. 4).

The generation of the β-amyloid hypothesis

The Aβ peptide first was sequenced from the meningeal blood vessels of patients who had AD in 1984 [127]. A year later, Aβ was recognized as the primary component of the senile plaques of AD brain [128]. In 1987, it was realized that the gene encoding APP is located on chromosome 21 [129], the same gene found in Down syndrome (trisomy 21) that develops the same Aβ neuropathology as AD. In 1990, it was found that a mutation in the APP gene causes Aβ deposition in the cerebral vessel walls of Dutch patients who had hereditary cerebral hemorrhage and amyloidosis [130]. These findings provided a genetic framework for the Aβ hypothesis (see Fig. 4) [125].

The development of the β-amyloid hypothesis

Soon after the generation of the Aβ hypothesis, a series of studies supported it. The 28 residue synthetic peptide homologous to the Dutch APP variant was found to accelerate fibril formation in vitro [131].

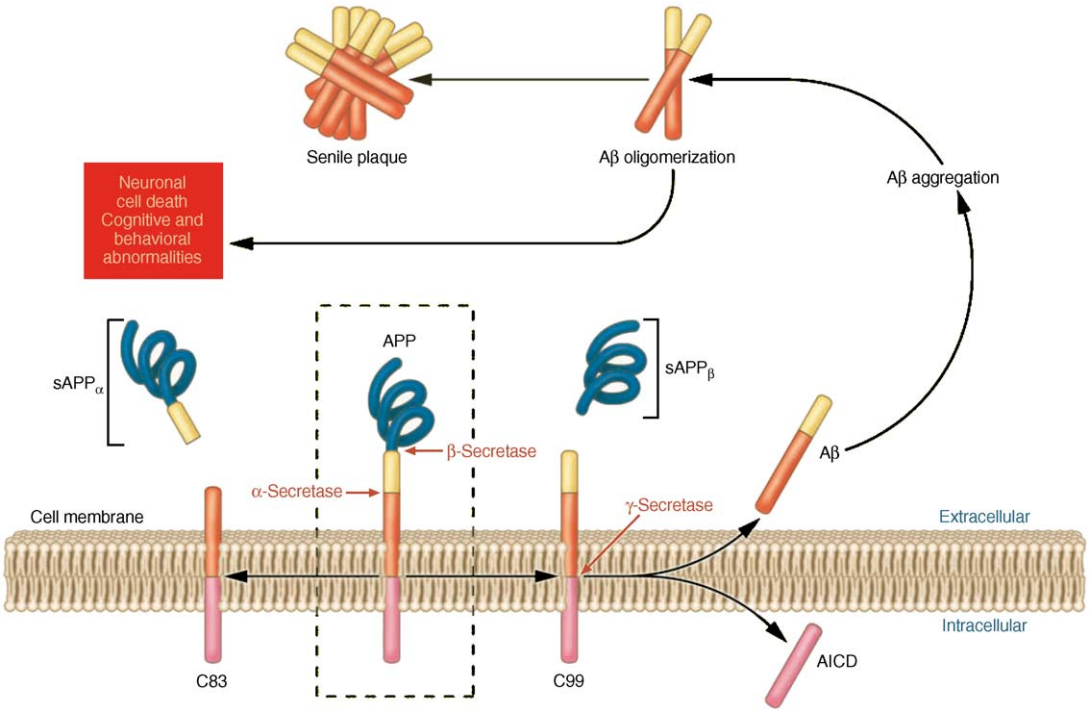

Fig. 2. Schematic representation of APP processing and Aβ accumulation. Mature APP (*center, inside dashed box*) is metabolized by two competing pathways, the α-secretase pathway that generates sAPPα and C83 (also known as CTFα) (*left*) and the β-secretase pathway that generates sAPPβ and C99 (*right*). The carboxyterminal fragment C99 is cleaved by γ-secretase to generate Aβ and the APP intracellular domain (AICD). The carboxyterminal fragment C83 also is cleaved by γ-secretase for the secreted peptides p3 (not shown). Aβ aggregates into small multimers (dimers, trimers, and so forth), known as oligomers. Oligomers seem to be the most potent neurotoxins, whereas the end-stage senile plaque is relatively inert. (*From* Gandy S. The role of cerebral amyloid β accumulation in common forms of Alzheimer disease. J Clin Invest 2005;115:1122; with permission.)

Several APP mutations causing familial AD were discovered, most of them concentrated at the cleavage sites of APP [132–134]. These mutations promote generation of Aβ by favoring proteolytic processing of APP by β- or γ-secretase. The subsequent demonstration that mutations in PS-1 and PS-2 (the major components of the γ-secretase complex) causing familial AD also enhance the processing of APP to form amyloidogenic Aβ strongly reinforced the hypothesis [21]. The finding that APP transgenic mice with *APOE* knocked out show a marked reduction in cerebral Aβ deposition suggested a role

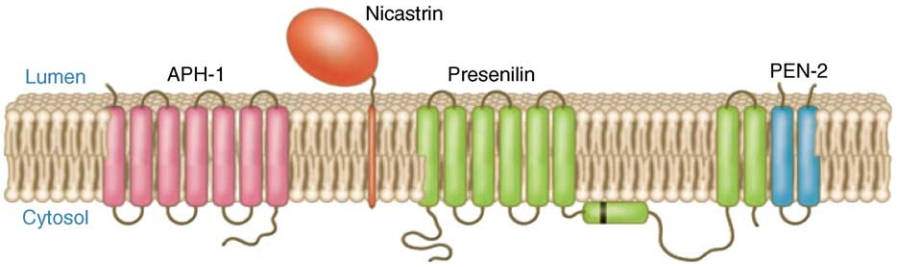

Fig. 3. Schematic representation of the four components of the γ-secretase complex. The γ-secretase complex consists of a catalytic component, presenilin, and three cofactors, nicastrin, Aph-1, and PEN-2. Presenilin is activated biologically by endoproteolysis that generates two heterodimers that each present the key aspartate residue. (*From* Gandy S. The role of cerebral amyloid β accumulation in common forms of Alzheimer disease. J Clin Invest 2005;115:1124; with permission.)

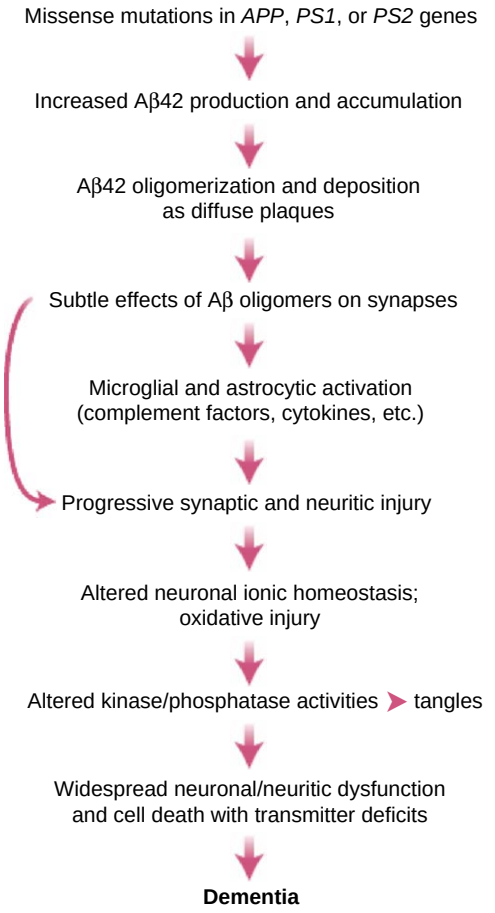

Fig. 4. The sequence of pathogenic events leading to AD proposed by the amyloid cascade hypothesis. The curved violet arrow indicates that Aβ oligomers may injure the synapses and neurites of brain neurons directly, in addition to activating microglia and astrocytes. (*From* Hardy J, Selkoe DJ. The amyloid hypothesis of Alzherimer's disease: progress and problems on the road to therapeutics. Science 2002;297:354; with permission.)

for Aβ in *APOE* polymorphism, a risk factor for sporadic AD [135]. In 1998, mutations in the gene encoding the τ protein were identified as responsible for FTD, suggesting that the neurofibrillary tangles in AD brains likely are deposited after changes in Aβ metabolism and initial plaque formation [136]. In addition, transgenic mice overexpressing mutant human APP and mutant human τ undergo increased formation of τ-positive tangles, again suggesting that either APP or Aβ influences the formation of neurofibrillary tangles [137]. The discovery of a locus on chromosome 10 that determines an increase in Aβ in patients who have late-onset AD [41] and the allele-specific association between this chromosome 10 locus and the gene encoding insulin-degrading enzyme, the enzyme responsible for Aβ degradation in neurons [138], suggests that impaired Aβ catabolism and clearance may contribute to the risk for late-onset AD. Recently, it was found that Aβ deposition develops before the tangle pathology in triple transgenic mice expressing simultaneous mutant genes for human APP, PS-1 and τ protein [139].

In addition to this genetic evidence, there are several biochemical studies that reinforce the Aβ hypothesis. Brain deposition of Aβ42 plaques precedes the formation of neurofibrillary tangles or overt neuronal loss and the appearance of clinical symptoms in familial AD with PS-1 mutation [140]. Brain levels of Aβ40 and Aβ42 are elevated early in patients who have AD, and levels of both peptides, especially Aβ42, were correlated strongly with cognitive decline. In the frontal cortex, Aβ was elevated before the occurrence of significant τ pathology [141]. Cerebrospinal fluid concentrations of Aβ42 were significantly lower in patients who had AD compared with controls, suggesting a diminished clearance of the toxic peptide from central nervous system [142]. Patient who have incipient AD [143] and patients who have mild cognitive impairment [144] have higher plasma levels of Aβ42 than matched controls. Finally, expression and activity of β-secretase, the enzyme that initiates the amyloidogenic metabolic pathway, seem to be increased in the brain of sporadic AD [145,146]. Taken together, these findings are consistent with the notion that cerebral Aβ accumulation is the primary influence in AD and that the rest of the disease process, including τ tangle formation, results from an imbalance between Aβ production and Aβ clearance.

The role of β-amyloid oligomers

The specific forms of Aβ that cause injury to neurons in vivo are not established definitively but soluble oligomeric species of Aβ are considered the "bad guys." These soluble species include small globular structures 2.7 to 6.0 nm in diameter, called Aβ-derived diffusible ligands [147], and curvilinear structures, known as protofibrils [148]. Aβ oligomers are elevated strikingly in AD brain tissue [149] and in the brain tissues of transgenic mouse models of AD [150]. In neuronal cell cultures, Aβ oligomers are found strongly neurotoxic [151]. In vivo, Aβ oligomers rapidly blocked long-term potentiation, a classic experimental paradigm for synaptic plasticity [152]. Fibrillar aggregates of Aβ cause local

structural disruption of synapses and neurite breakage in transgenic mouse models of AD [153].

The role of cholesterol

Cholesterol is shown to alter APP processing and Aβ deposition in vitro and in vivo. The addition of exogenous cholesterol to APP-transfected cells leads to an increase of Aβ40 and Aβ42 secretion [154]. Introduction of high cholesterol diet induces Aβ deposition in rabbits [94] and mice [95]. Other studies find, however, that increased dietary cholesterol reduces brain APP metabolism [155]. High cholesterol may affect Aβ production through the stabilization of APP, β- secretase, or γ-secretase within the lipid rafts on the cell membrane of neurons. Increased cholesterol also may change the fluidity of the membrane in a way that maintains APP in close proximity with β- and γ-secretase (Fig. 5).

Intraneuronal β-amyloid accumulation

In addition to the deposition of Aβ into extracellular plaques, there is increasing evidence that Aβ also accumulates intracellularly as monomeric and oligomeric species and that this process may be an early event in AD pathophysiology [156]. Studies in cell cultures, postmortem brain tissue of patients who have AD, and transgenic AD mouse models suggest that intracellular Aβ may be neurotoxic by stimulating oxidative stress and apoptotic cell death.

First evidenceof intracellular generation of Aβ was reported in human neurons derived from a teratocarcinoma cell line [157]. Aβ peptides seem to be generated at different subcellular sites with Aβ40 solely in the trans-Golgi network [158] and Aβ42 in the endoplasmic reticulum and the Golgi compartments [159]. Intracellular Aβ seems to be mainly in oligomeric form, mainly dimeric [160].

Autoptic studies on brain tissue of patients who have AD show that Aβ42 accumulates in pyramidal neurons of hippocampus and entorhinal cortex [161]. With increasing cognitive dysfunction and plaque deposition, intraneuronal Aβ42 immunoreactivity seems to be attenuated [161]. Intracellular Aβ deposition is evident before the appearance of paired helical filaments-positive structures, indicating that it is one of the first neurodegenerative alterations in the AD brain [162].

Human neuroblastoma cells stably expressing the Swedish mutation of APP show increased intracellular Aβ levels [163]. Several PS-2 and few PS-1 mutations lead to enhanced intracellular Aβ42-levels with a concomitant decrease in intracellular Aβ40 [164]. The ratio of intracellular Aβ42:Aβ40 also increases markedly in cells coexpressing APP and either mutant PS-1 or PS-2 [163].

Studies in transgenic mouse models of AD also support the pathophysiologic role of intraneuronal Aβ. Transgenic mice expressing mutant human APP in combination with mutant PS-1 show intracellular Aβ accumulation in hippocampal and cortical neurons before extracellular plaque deposition [165,166]. As observed in postmortem brain tissue of patients who have AD, the intraneuronal Aβ immunoreactivity declines with increased extracellular plaque

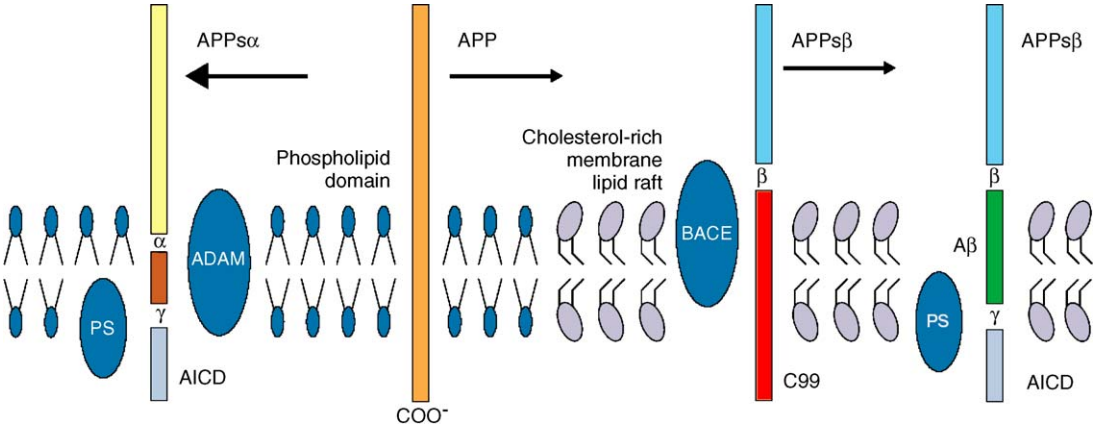

Fig. 5. Schematic representation describing the possible role of cholesterol in the APP processing. Nonamyloidogenic cleavage of APP by α-secretases (ADAM) requires a membrane domain that is poor in cholesterol and rich in phospholipids. Amyloidogenic cleavage of APP by the β-secretase (BACE) and γ-secretases (PS) requires a membrane domain that is cholesterol rich, such as a lipid raft. (*From* Casserly I, Topol E. Convergence of atherosclerosis and Alzheimer's disease: inflammation, cholesterol, and misfolded proteins. Lancet 2004;363:1140; with permission.)

accumulation [167]. The intraneuronal Aβ deposition in hippocampus seems to be increased fourfold by high fat and cholesterol diet in transgenic mice expressing APP with the Swedish mutation [168]. Triple-transgenic mice expressing mutant APP in combination with mutant PS-1 and mutant protein τ show early intraneuronal Aβ immunoreactivity together with synaptic dysfunction before plaque or tangle deposition was evident [139].

Besides intraneuronal Aβ production, cellular uptake of Aβ from the environment is a second mechanism that contributes to intracellular Aβ accumulation. Selective intracellular accumulation of Aβ42 is reported in cells treated with synthetic Aβ-peptides and occurs via endocytosis [169]. This accumulation seemd specific for Aβ42 that seems resistant to degradation. Aβ40 and shorter peptides are eliminated with a half-life of approximately 1 hour [170].

The mechanisms with which intracellular Aβ may be neurotoxic are not understood completely. It is reported that intracellular Aβ42 correlates with apoptotic cell death in brains of patients who have AD [171]. Transgenic mice expressing Aβ42 in neurons show extensive neurodegeneration with morphologic and biochemical evidence that neuronal death occurred via apoptosis [172]. Rat cortical neurons expressing human APP underwent apoptosis as soon as they accumulated intracellularly Aβ42 [173]. Treatment of cell cultures with apoptosis-inducing substances, such as etoposide or melphalan, led to an increase in cellular Aβ42 in damaged neurons [174]. In addition to apoptotic mechanisms, it seems that stimulation of oxidative stress may mediate toxicity of intracellular Aβ. Human neuroblastoma cells treated with hydrogen peroxide shows a significant increase of intracellular Aβ levels [175].

There is accumulating evidence suggesting that intraneuronal Aβ42 is a major risk factor for neuron loss and a trigger for the Aβ cascade of pathologic events. Thus, Aβ pathology may be seen in AD as a continuous process from an initial abnormal Aβ intracellular accumulation to the well-established extracellular Aβ aggregation, culminating in the formation of amyloid plaques and dystrophic neuritis.

Physiologic functions of β-amyloid

It is still unclear if Aβ has any normal physiologic role in the brain [176]. Aβ peptide is present in the cerebrospinal fluid and in plasma of healthy individuals throughout life [177]. Studies in cultured rat cortical neurons incubated with exogenously added Aβ peptides indicate that unaggregated Aβ40 has no neurotoxic effect and stimulates an increase in voltage-dependent C^{2+} channel current activity, suggesting that Aβ may act as a physiologic regulator of ion channel function in neurons [178]. In vitro studies show that spontaneous neuronal activity stimulates β-secretase, leading to an enhanced secretion of the Aβ peptide in normal neurons and in neurons overexpressing the Swedish mutated APP [179]. In turn, secreted Aβ depresses neuronal activity via inhibition of the NMDA excitatory pathway. This negative feedback loop, in which neuronal activity promotes Aβ production and Aβ secretion decreases synaptic activity, could maintain the physiologic homeostasis of neurons. In AD, neurons might fail to be depressed by Aβ, leading to a gradual build-up of neuronal activity and further Aβ secretion. Alternatively, the production of Aβ might become independent from neuronal activity [176]. Other in vitro studies suggest that physiologic production of Aβ is important for neuronal viability [180]. Incubation with β- or γ-secretase inhibitors induces death in neuronal cells but not in non-neuronal cells. The addition of Aβ40 prevents the toxicity of secretase inhibitors in neuronal cells. The protective effect of Aβ40 is concentration dependent with significant effects at concentrations as low as 10 ppm. More recently, it is proposed that Aβ, at physiologic concentrations, may stimulate postsynaptically the expression of proteins related to synaptic plasticity via cyclic adenosine monophosphate–responsive, element-regulated gene expression [181].

Evidence against the β-amyloid hypothesis

The Aβ hypothesis of AD also is criticized strongly. The most frequent objection is that the number of amyloid deposits in the brain does not correlate well with the degree of cognitive impairment experienced by patients in life [182]. In addition, the histopathologic work of Braak and Braak shows that the distribution pattern and packing density of amyloid deposits are of limited significance for differentiation of neuropathologic stages [183]. The disease severity correlates much better with Aβ assayed biochemically than with histologically determined plaque counts, and the concentration of soluble Aβ species seems to correlate with cognitive impairment [184–186]. Recently, it was shown that soluble oligomers of Aβ, but not monomers or insoluble amyloid fibrils, may be responsible for synaptic dysfunction in the brains of patients who have AD and in AD animal models. The so-called ‘‘AD diffusible ligands’’ [147] or protofibrils [148] cause subtle injury to cultured neurons. Injection in

rats of Aβ oligomers can inhibit long-term potentiation in the hippocampus, which is required for memory formation [152]. Thus, it is hypothesized that large polymeric aggregates (such as the amyloid plaques) represent inactive reservoirs of species that are in equilibrium with smaller, putatively neurotoxic assemblies (Aβ oligomers).

Another concern arises from the fact that some presenilin mutations that increase Aβ production strongly seem to be associated with atypical symptoms, such as spastic paraparesis (weakness affecting the lower extremities), rather than cognitive symptoms typical of AD [187].

Transportation and degradation of β-amyloid

Enormous progress has been achieved in understanding how Aβ is generated from APP and huge pharmacologic efforts have been made in trying to interfere with Aβ production. In the past few years, interest also has been directed to the fate of Aβ once it is generated from APP and the pharmacologic approaches to boost the processes involved in the physiologic clearance of Aβ from the brain. Soluble Aβ can be removed from the brain via two basic mechanisms: favoring its transportation across the blood-brain barrier and augmenting its proteolytic degradation [188].

Transportation of Aβ across the blood-brain barrier

Aβ can be transported across the blood-brain barrier and exported out of the brain into the blood stream either by direct binding to LRP or by first binding apoE and α2M and then transported by LRP. Once Aβ enters the blood stream, it can re-enter the brain via the receptor for advanced glycation end products (RAGE) or be delivered via chaperone molecules, such as apoE or α2M, to peripheral sites of degradation, such as the liver or kidney [189]. In addition, evidence also exists suggesting that 10% to 15% Aβ can enter into the cerebral spinal fluid from the brain interstitial fluid and onward into the bloodstream [190].

Low-density lipoprotein receptor-related protein

LRP is an approximately 600-kd multifunctional receptor that is highly expressed in the brain. LRP is found in plaque deposits in brains of patients who have AD. A key role for LRP in exporting Aβ from brain is proven in several animal studies. Initially, Aβ forms a complex with α2M or apoE on the luminal side of endothelium. This Aβ complex binds LRP and then is internalized to be delivered to lysosomes for subsequent degradation or transported for transcytosis across the blood-brain barrier into plasma [191]. Recently it was shown that Aβ can be transported across the blood-brain barrier and be cleared from the brain after directly binding LRP [192]. LRP favors clearance of Aβ40 over Aβ42. Specific anti-LRP antibodies are shown to reduce efflux of Aβ40 from mice brain by up to 90% [190]. Cerebral amyloid load is doubled in human APP transgenic mice engineered to possess low levels of LRP at the blood-brain barrier and no expression of the critical LRP chaperone, the receptor-associated protein [193].

Receptor for advanced glycation end products

RAGE is a cell surface multifunctional receptor that binds a large array of different ligands [194]. Immunocytochemical studies demonstrate that in AD the expression of advanced glycation end products is elevated in neurons close to Aβ deposits and in the cells of Aβ containing vessels. Glycation of Aβ markedly enhances its aggregation in vitro, and the glycation of protein τ, in addition to hyperphosphorylation, seems to enhance the formation of paired helical filaments. Further, RAGE is found to be a specific cell-surface receptor for Aβ with affinity in the nanomolar range [195]. The binding of Aβ to RAGE is shown to stimulate expression of proinflammatory cytokines and to decrease cerebral blood flow [189]. The active participation of RAGE in the pathogenesis of AD also is confirmed in RAGE-engineered transgenic mice. Down-regulation of RAGE decreases the influx of Aβ from the periphery into the central nervous system [196]. Double transgenic mice overexpressing mutant human APP and RAGE display exaggerated neuropathologic findings and early abnormalities in spatial learning and memory [197]. Conversely, transgenic mice bearing a dominant-negative RAGE construct targeted to neurons crossed with animals with mutant human APP display preservation of spatial learning and memory and diminished neuropathologic changes [197].

Metabolic degradation of Aβ

Current evidence in vivo and in vitro suggests that there are three major players in amyloid turnover: insulin-degrading enzyme, neprilysin-converting enzyme, and endothelin-converting enzyme (ECE), all of which are zinc metallopeptidases. Other proteases also are implicated in amyloid metabolism, including angiotensin-converting enzyme, urokinase-type plasminogen activator, tissue plasminogen activator, and plasmin, but for these the evidence is less

compelling [198]. Aβ also can be internalized and degraded by activated microglia in the brain.

Neprilysin-converting enzyme and ECE are homologous membrane proteins of the M13 peptidase family, which normally play roles in the biosynthesis or metabolism of regulatory peptides. Insulin-degrading enzyme is distinct structurally and mechanistically. The regional, cellular, and subcellular localizations of these enzymes differ, providing an efficient and diverse mechanism for protecting the brain against the normal accumulation of Aβ. Reduction in expression levels of some of these proteases after insults (eg, hypoxia and ischemia) or aging might predispose to the development of AD. Conversely, enhancement of their levels by gene delivery or pharmacologic means could be neuroprotective. Even a small enhancement of Aβ metabolism could slow the progression of the disease.

Insulin-degrading enzyme

Insulin-degrading enzyme (IDE) is a 110-kd protein that, in addition to Aβ, hydrolyzes several regulatory peptides, including insulin, glucagons, atrial natriuretic factor, transforming growth factor-α, β-endorphin, amylin, and APP intracellular domain [199]. Several studies support the role of IDE in Aβ degradation [200], the most convincing being those in transgenic animals. IDE knockout mice show increased cerebral accumulation of endogenous Aβ [201]. Double transgenic mice overexpressing IDE and human mutated APP show a 50% decrease in cerebral Aβ40 and Aβ42 levels and Aβ load [202]. Further support for the role of IDE in AD pathogenesis derives from recent reports of its genetic association with late-onset AD [45]. Hippocampal IDE mRNA levels are found lower in subjects who have an *APOE*E4*, suggesting that the genetic risk conferred by *APOE*E4* may be mediated in part by its effect on IDE expression [203].

Neprilysin

Neprilysin is a type II membrane protein that hydrolyzes circulating biologically active peptides, including enkephalin, cholecystokinin, neuropeptide Y, and substance P [204]. The catalytic site of neprilysin is exposed extracellularly. Expression of neprilysin in primary cortical neurons led to significant decreases in Aβ40 and Aβ42 levels in culture media in a dose-dependent manner [205]. The proof that neprilysin is the major Aβ-degrading enzyme in vivo derived from studies in knockout mice, in which neprilysin deficiency resulted in defects in the degradation of exogenously administered Aβ and in the metabolic suppression of the endogenous Aβ levels in a gene dose-dependent manner [206]. Conversely, transgenic overexpression of neprilysin in neurons significantly reduced brain Aβ and amyloid plaque formation [202]. Neuronal upregulation of neprilysin in young transgenic mice expressing the Swedish mutated form of APP led to reduction of brain Aβ levels and delayed Aβ plaque deposition in young but not in aged animals with pre-existing plaque pathology [207]. Unilateral intracerebral injection of a lentiviral vector expressing human neprilysin reduced Aβ deposits by half with respect to the untreated side in transgenic mouse models of amyloidosis [208]. Finally, the recent findings that neprilysin immunoreactivity is decreased in AD brains [209] and that single nucleotide polymorphisms of the gene encoding neprilysin is associated with patients who have sporadic AD [66] reinforce the role of neprilysin in the pathogenesis of AD.

Endothelin-converting enzyme

ECE, a member of the M13 family, is a key component in the regulation of blood pressure and electrolyte balance. ECE-1 also is proposed as Aβ-degrading enzyme. It is shown that treatment of endogenous ECE-expressing cell lines with the metalloprotease inhibitor phosphoramidon causes a twofold to threefold elevation in extracellular Aβ concentration that is believed the result of inhibition of intracellular Aβ degradation [210]. Mice deficient for ECE-1 and a closely related enzyme, ECE-2, show increased Aβ40 and Aβ42 brain levels [211]. Further support of a potential role of ECE in the pathophysiology of AD derives from a large case-control study that finds that a functional genetic variant (A allele) of the gene encoding ECE reduces risk for late-onset AD [212].

Mechanisms of β-amyloid toxicity

Various mechanisms are proposed to explain the pathway by which Aβ induces neuronal cell death, including intracellular calcium accumulation, reactive oxygen species (ROS) and nitric oxide production, inflammatory processes, and increased sensitivity to apoptosis.

Oxidative stress

Oxidative stress is a term indicating processes that lead to uncontrolled production of highly ROS. Increased oxidative stress is an early event in AD [213]. Cells in the brains of AD patients exhibit

abnormally high amounts of oxidatively modified proteins, lipids, and DNA, especially in the areas in which Aβ is abundant [214]. Aβ aggregation is triggered by the presence of the redox-active ions, Fe^{2+} and Cu^{+}, and this interaction generates hydrogen peroxide. The formation of hydrogen peroxide leads to lipid peroxidation with the consequent production of 4-hydroxynonenal, a neurotoxic aldehyde that covalently modifies proteins on cysteine, lysine, and histidine residues (Fig. 6). Some of the proteins modified oxidatively by this Aβ-induced oxidative stress include membrane transporters, guanosine triphosphate (GTP)-binding proteins, and ion channels. Oxidative modifications of τ by 4-hydroxynonenal and other ROS can promote its aggregation and thereby may induce the formation of neurofibrillar tangles. Aβ also can cause mitochondrial oxidative stress and dysregulation of calcium ion (Ca^{2+}) homeostasis, resulting in impairment of the electron transport chain, increased production of superoxide anion radical (O_2^-), and decreased production of ATP. O_2^- is converted to hydrogen peroxide by the activity of superoxide dismutase (SOD). O_2^- also can interact with nitric oxide (NO) via NO synthase (NOS) to produce peroxynitrite (ONOO*). Interaction of hydrogen peroxide with Fe^{2+} or Cu^{+} generates the hydroxyl radical (OH*), a highly reactive oxyradical and potent inducer of membrane-associated oxidative stress that contributes to the dysfunction of the endoplasmatic reticulum (see Fig. 6) [215].

Attack of DNA by ROS, particularly OH*, leads to the formation of approximately 20 major products. Of the possible base adducts resulting from oxidative stress, one of the most prominent appears to be the result of the hydroxylation of guanine at the C8 position, leading to the formation of 8-oxoguanine and the corresponding formamido-pyridine [216]. These markers of oxidative stress are localized to neurofibrillary tangles and senile plaques in AD brains [217]. DNA damage is a feature of neuron cell death that has been detected in AD and in experimental models of AD. Studies demonstrate an increased oxidative DNA damage in AD brain. DNA damage induced by free radicals or enzymatic modifications can be a trigger that initiates the cell death program.

The role of nitric oxide

NO is synthesized from L-arginine by NOS. In tissues, NOS occurs in three isoforms: endothelial

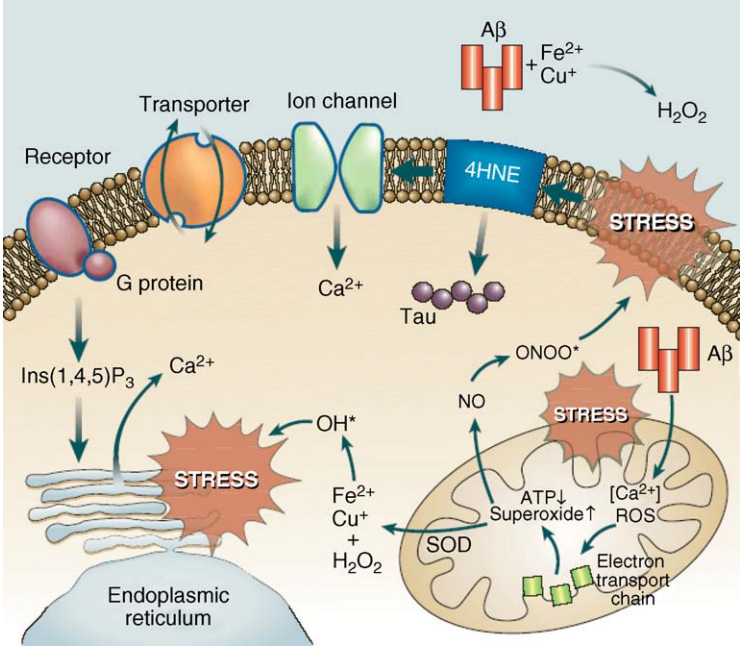

Fig. 6. Schematic overview of the neuronal oxidative stress induced by Aβ. The neurotoxic action of Aβ involves generation of ROS and disruption of cellular calcium homeostasis. (*From* Mattson MP. Pathways towards and away from Alzheimer's disease. Nature 2004;430:633; with permission).

(eNOS), neuronal (nNOS), and inducible (iNOS). In the brain, all three isoforms of NOS are present and iNOS and eNOS are expressed aberrantly in AD, especially in association with Aβ deposits [218]. Dysregulation of vascular NO production can occur from chronic cerebral hypoperfusion during aging [219]. Increased deposition of Aβ results in increased mRNA and protein expression of iNOS and generation of NO [220]. NO can interact rapidly with superoxide anion O_2^- forming more reactive peroxynitrite ($ONOO^-$). $ONOO^-$ can induce lipid peroxidation and functional alterations in proteins and DNA, eventually leading to neuronal death [216].

The role of inflammatory mediators

Several studies indicate that Aβ deposition activates a series of inflammatory reactions that may mediate neuronal death. In the AD brain there are elevated levels of a diverse range of proinflammatory molecules. These inflammatory molecules are produced principally by activated microglia and astrocytes, which are found clustered within and adjacent to the senile plaque.

The inflammatory products released by Aβ-activated microglia include cytokines, such as interleukin 1 (IL-1), interleukin 6 (IL-6), TNF-α, and transforming growth factor-β (TGF-β, [221]. Although their expression is induced by the presence of Aβ, these cytokines also are able to promote the accumulation of Aβ in a vicious circle that fuels the progression of the disease [222]. Activated microglia also secrete chemokines, a diverse group of small proteins that controls the recruitment of cytotoxic and helper T lymphocyte to the sites of inflammation. These chemokines include interleukin 8 (IL-8), interferon-γ–inducible protein, macrophage inflammatory protein-1α, macrophage inflammatory protein-1β, and monocyte chemoattractant protein-1 (MCP-1) [223]. Additionally, studies in glial cultures show that Aβ activated nuclear factor-κB, a transcription factor for several inflammatory mediators, including IL-1β and IL-6 [224].

In AD, the number of reactive astrocytes is increased and the expression of phospholipase A2 in these cells is upregulated, leading to increased arachidonic acid/prostaglandin inflammatory pathway activity. It is shown that Aβ deposition is accompanied by activation of astrocyte to secrete chemokines, in particular MCP-1 and regulated on activation, normal T-cell expressed and secreted (RANTES), which serve as potent macrophage chemoattractants [225].

Finally, there also is evidence that Aβ can bind and activate the classical complement cytolytic pathway [226].

The role of intracellular calcium

Ca^{2+} plays a fundamental role in learning and memory and also is involved in neuron survival and death. The inability of neurons to regulate calcium homeostasis is an aspect of AD pathogenesis that seems intimately involved in the dysfunction and death of neurons [227]. Several studies in cultured neurons from patients who have AD and from transgenic mice bearing AD-causing mutations of PS-1, PS-2, or APP show alterations of cellular calcium homeostasis [228]. These alterations include increased amount of calcium released by the endoplasmic reticulum, defect in capacitative calcium entry, and calcium influx pathway activated by depletion of intracellular stores [229]. These effects seems linked to the increased production of Aβ42 and the decreased levels of sAPPα [215]. Aβ may perturb calcium regulation by inducing oxidative stress, which impairs membrane calcium pumps and enhances calcium influx through voltage-dependent channels and ionotropic glutamate receptors (see Fig. 6). Aβ can promote calcium influx by forming channels in membranes or by activating cell surface receptors coupled to calcium influx. Presenilin and APP mutations are a rare cause of AD, however, and there is no evidence that a calcium-regulating action of presenilin is involved in sporadic forms of AD. Recent studies suggest, however, that wild-type PS-1 normally may serve a calcium-regulating function in the endoplasmic reticulum [230].

Disturbances of calcium regulation in AD may not be limited to neurons. Studies in lymphocytes from patients who have familial or sporadic AD demonstrate abnormalities in calcium signaling similar to those in neurons [231]. Thus, perturbed cellular calcium homeostasis seems to be a widespread abnormality in familial and sporadic forms of AD that may contribute to the disease process.

Disturbed energy metabolism

Neurons are highly dependent on glucose for ATP generation necessary for ATP's various functions, including synaptic transmission. ATP production depends on a well-preserved mitochondrial structure and function. When mitochondria fail to provide adequate energy, partial neuronal depolarization and loss of calcium homeostasis occur and may lead to neuronal apoptosis. Biochemical studies show that the

mitochondrial electron transport chain is defective in sporadic AD [232]. Mitochondrial dysfunction is linked to oxidatively damaged mitochondrial DNA and eventually may lead to cell death. How exactly this translates into loss of specific neuronal populations, including cholinergic forebrain, hippocampal pyramidal and cortical neurons, is unclear.

Brain imaging studies demonstrate deficits in glucose use in living patients who have AD, an abnormality that may occur before the onset of clinical symptoms [233]. The activities of key enzymes involved in energy metabolism (cytochrome c oxidase, pyruvate dehydrogenase complex, and α-ketoglutarate dehydrogenase complex) are decreased in brain cells of patients who have AD. It is proven that altered proteolytic processing of APP may contribute to impaired energy metabolism. Transgenic human APP mice show age-dependent decrease in glucose metabolism in brain regions involved in cognitive processes [234]. Moreover, hypoxic tolerance is decreased significantly in young human APP mutant mice, suggesting an early role for perturbed energy metabolism [235]. Insulin resistance, a sign of systemic abnormality in glucose regulation, is considered a risk factor for AD [236]. Caloric restriction, which enhances insulin sensitivity, attenuates Aβ deposition in transgenic mouse models of AD [237]. All these findings support a role for perturbed glucose metabolism in AD pathogenesis, although this remains to be established in humans.

Cerebral hypoperfusion

Cerebral blood flow is impaired abnormally in AD. Neuroimaging detection of cerebral hypoperfusion in selected brain regions seems to predict AD at the mild cognitive impairment stage, and possibly even earlier, with consistent accuracy. Brain hypoperfusion initiates oxidative stress and cognitive decline and neurodegeneration is reinforced further [238].

Rodent models of ischemic brain show that brain hypoperfusion enhances APP mRNA expression [239] and increased APP accumulation in surviving neurons [240]. In vitro experiments show that freshly solubilized Aβ enhances constriction of cerebral and peripheral vessels. In vivo, intra-arterially infused freshly solubilized Aβ40 in rats results in decreased blood flow and increased vascular resistance specifically in cerebral cortex [241]. A decreased cerebral hemodynamic response to electrical stimulation is documented in a human APP transgenic mouse model of AD [242]. These data suggest that Aβ deposition may contribute to the cerebral hypoperfusion observed early in the AD process.

Dysfunction of the axonal transport

Many of the neurons affected in AD are relatively large and have long axons. Damage to axons of such neurons with alterations in axonal transport is observed in the early stages of patients who have AD and also in young AD transgenic mice [243]. Axonal defects consist of swellings that accumulated abnormal amounts of microtubule-associated and molecular motor proteins, organelles, and vesicles. APP and its proteolytic enzymes, PS-1 and β-secretase, are transported axonally to synaptic terminals in brain regions affected in AD [244]. Reductions in microtubule-dependent transport of APP may stimulate its proteolytic processing with the consequent accumulation of Aβ [245]. Aβ can have many adverse effects on the functions and integrity of pre- and postsynaptic terminals, including inducing oxidative stress, impairing calcium homeostasis and perturbing the functions of mitochondria and the endoplasmic reticulum.

Activation of apoptotic death

Apoptosis is a form of programmed cell death that involves changes in the cytoplasm, endoplasmic reticulum, mitochondria, and nucleus. Typically it includes the production or activation of proteins, such as p53, Bax, Bad, and Par-4. These proteins increase the membrane permeability of mitochondria with the release in cytoplasm of cytochrome c and apoptosis-inducing factor. The membrane permeability of endoplasmic reticulum also is increased with the consequent release of calcium. The latter events then activate cysteine aspartyl proteases, called caspases, which cleave various protein substrates. Caspase-mediated cleavage of cytoskeletal proteins and ion channel proteins causes cell shrinkage and may prevent necrosis, whereas cleavage of DNA degrades chromosomes.

Presenilin mutations causing familial AD render neurons vulnerable to apoptosis induced by Aβ and other stimuli, apparently by altering calcium regulation of endoplasmic reticulum [246]. APP mutations also are sufficient to trigger apoptosis in cultured cells [247]. Different studies show that extracellular amyloid deposits and intracellular Aβ may activate caspases, leading to cleavage of cytoskeletal proteins, including protein τ [248]. In addition to Aβ, C-terminal proteolytic products of APP

are implicated in neuronal apoptosis [247]. Increased expression of p53, a DNA damage indicator, is observed in neurons exposed to Aβ [249]. Indeed, neurons treated with inhibitors of p53, agents that stabilize mitochondrial and endoplasmic reticulum membranes or caspase inhibitors, are resistant to being killed by Aβ [250]. Other studies suggest that Aβ increases the expression of the death effector Bax and simultaneously downregulates the antiapoptotic bcl2 [251].

Minor pathophysiologic hypothesis: the τ-protein cascade

Neurofibrillary tangles consist of paired helical filaments found in the cytoplasm of neurons primarily in the hippocampus [252]. The formation of neurofibrillary tangles in AD is related directly to abnormal function of protein τ. τ is a microtubule-associated phosphoprotein that plays a critical role in supporting axonal transport and promotion of cell stability. The major polypeptides of paired helical filaments are microtubule-associated protein τ. The level of τ in AD neocortex is sevenfold higher than in aged control brain and this increase is in the form of abnormally phosphylated protein [253]. The hyperphosphorylation of τ results in reduced binding of τ to microtubules and subsequent disruption of axonal transport [254]. It is proposed that abnormal hyperphosphorylation of τ results in neurodegenerate effects via inhibition of microtubule function and impairments of neuronal axonal transportand a toxic gain of function in which hyperphosphorylated τ forms inert polymers, which become neurofibrillary tangles.

Several protein kinases are implicated in the pathologic hyperphosphylation of τ in AD, including glycogen synthase kinase-3 (GSK-3) and cyclin-dependent kinase. GSK-3 is demonstrated to phosphorylate endogenous τ [255] and plays a role in the brain by regulating cytoskeletal processes, synaptic plasticity, and modulation of microtubule dynamics. Neurons exhibit strong GSK-3 immunoreactivity in AD compared with normal controls and is found to colocalize in areas of granulovaculear degeneration [256].

Specific protein phosphatases, including phosphatase 2A (PP-2A), are shown to have reduced activity in AD brains. PP-2A is demonstrated in in-vitro studies to dephosphorylate several phosphoserine residues in τ [257]. Furthermore, a recent study demonstrates reduced PP-2A expression in the hippocampus of AD brains, suggesting that reduction of this enzyme may be a critical factor in the hyperphosphylation of τ in AD [258].

Several studies correlate the severity of AD with the accumulation of neurofibrillary tangles rather than Aβ [259]. Coexistence of τ and Aβ in the majority of AD brains, however, suggests a pathologic interaction between these two proteins. This hypothesis is supported by a transgenic mouse model in which animals are crossbred to express mutant τ and APP. In these mice, neurofibrillary tangle pathology is enhanced substantially in the limbic cortex [137], suggesting a synergistic neurodegenerative effect between these two proteins.

Conversely, there are several neurodegenerative disorders in which τ is regarded as the primary pathologic hallmark feature. These taupathies include frontotemporal dementia (FTD), Pick's disease, corticobasal degeneration, and progressive supranuclear palsy. FTD, although less common than AD, accounts for a significant percentage of patients who have dementia, especially those who present with earlier onset and primary psychiatric symptoms. FTD is linked to mutations in the τ gene with subsequent aggregation of τ and neurodegeneration. In comparison, mutations in τ are not implicated in AD.

Summary

In the past 2 decades, huge progress has been made in understanding the cellular and molecular processes that initiate and feed the AD cascade. The enhanced knowledge of the pathophysiology of AD lays the groundwork for significant improvements in the diagnosis and pharmacologic treatment of the disease. Fundamental advancement has been made in understanding the genetic basis of the disease with the identification of causative genes (APP, PS-1, and PS-2) for early-onset familial AD. In addition, several susceptibility genes are proposed as associated with the late-onset form of the disease, but only the polymorphism of the *APOE* gene consistently is confirmed to play a role in the most frequent form of the disease. The recognition that causative genes of familial AD encode the substrate and the enzymes that generate the Aβ peptide and that mutations of these genes cause brain accumulation of Aβ, the most prominent histopathologic hallmark of the disease, represents the most convincing argument for the generation of the Aβ hypothesis of AD. Although other pathophysiolgic pathways are proposed for AD (protein τ hyperphosphorylation, oxidative

stress, inflammatory cascade, and so forth) the most prominent one is the Aβ hypothesis. Detailed understanding of cerebral degeneration and accumulation of Aβ has generated the most promising hopes for the discovery of disease-modifying treatments. This goal has been facilitated greatly by the development of well-characterized and reproducible transgenic animal models of the disease. Recent progress in the refinement of the Aβ hypothesis includes the identification of the potential toxicologic role of intracellular Aβ. The recent elucidation of the physiologic pathways of Aβ clearance and degradation may generate new biologic targets for drug discovery. Progress needs to be made, however, in achieving a clear understanding of the mechanisms that link Aβ accumulation and neuronal death. Ultimately, the only way to validate the Aβ hypothesis fully is to identify Aβ interfering drugs that are effective in inhibiting disease progress. We predict that the next 5 years will be crucial in this respect.

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