Research into the molecular mechanisms of Alzheimer disease (AD) continues to clarify important issues in aberrant protein processing while seeking to identify therapeutic targets. Mutations of genes on chromosomes 1, 14 (presenilins 1 and 2), and 21 (the amyloid-β [Aβ] amyloid precursor protein [APP]) cause the familial forms of AD that often begin before age 65. An allelic polymorphism on chromosome 19 (apolipoprotein E) affects the age of onset of the more common forms of sporadic AD. Multiple studies in transgenic mice provide strong evidence to support the view that Aβ amyloid formation is an early and critical pathogenic event: mice expressing pathogenic human APP mutations develop Aβ deposits; coexpression of mutant presenilin genes accelerates the rate of Aβ deposition; and apolipoprotein E plays a role in this process. Thus, the 3 established genetic causes or risk factors for AD affect Aβ deposition. The fact that elevation of the Aβ42/Aβ40 ratio (differing only in 2 amino acids in length) is also linked to amyloid deposition in the APP mice and is temporally linked to cognitive impairment suggests that Aβ42 may be a principal inducing factor of AD. The exact sequence of events is still unknown, but the transgenic models generated so far have shown their usefulness in clarifying this complex part of the pathology. The continuing progress in elucidation of the molecular pathogenesis of AD suggests a range of rational pharmacological interventions for this disorder. The most promising strategy involves the development of approaches to retard, halt, or prevent Aβ-mediated disease progression, and these can now be tested in transgenic animals.

More elderly people are being affected by Alzheimer disease (AD), pathologically marked by extracellular plaques with fibrils of amyloid-β (Aβ) peptide and intraneuronal tangles of polymerized tau. The prevalence of AD is 3% for individuals aged 65 to 74 years; 18.7% for those aged 75 to 84 years, and 47.2% for those 85 years and older. Although it most frequently occurs in the elderly, this disorder also afflicts younger patients. Most AD cases are late in onset and are sporadic, whereas a small percentage are early in onset and often segregate within families (FAD), suggesting a genetic cause.

The amyloidocentric theories of AD propose that Aβ plaque depositions or partially aggregated and/or soluble Aβ trigger a neurotoxic cascade causing neurodegeneration and AD. The arguments are based on in vitro studies suggesting that Aβ is toxic to neurons, and on the measurements of increased release of Aβ by cells expressing FAD mutant genes. The concurrent development of intracellular neurofibrillary lesions in AD also follows a stereotyped pattern. The principal consequence of these lesions is a loss of synaptic function in the affected regions of the brain. However, lines of evidence suggest that amyloid plaques per se might not be the primary mechanism underlying AD neurodegeneration. Soluble oligomeric forms of Aβ, probably complexed with other factors such as apolipoprotein E (ApoE), may be more relevant.

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Early onset
(younger than 65 y, 
about 1%-3% of all AD)
21q21.2
APP
Increased total Ap production; increased ratio of Aβ42/Aβ40
Aggressive, rapidly advancing disease (duration < 7 y), often with abnormal movements

Late onset
(most common form)
19q13.2
ApoE
The e2, e3, and e4 alleles may interact differentially with Aβ, and thereby affect toxicity or clearance from the brain
More indolent progression of disease (duration > 10 y)

12p
α2M
May interact with extracellular clearance of Aβ through LRP

12
LRP

* AD indicates Alzheimer disease; APP, amyloid precursor protein; PS, presenilin; ApoE, apolipoprotein E; LRP, low-density lipoprotein receptor–related protein; and Aβ, amyloid-β.

Amyloid Aβ and the Amyloid Precursor Protein (APP)

The APP is a type I transmembrane glycoprotein containing a large extracytoplasmic region, a transmembrane domain, and a small cytoplasmic tail. There are several isoforms composed of 672 to 714 amino acids derived by alternative splicing of a single gene on chromosome 21. APP695, APP696, and APP770 are the most frequent, and of these, APP695 is the most common isoform in the brain. Amyloid-β and associated pathogenic peptides are released from APP through the proteolytic actions of α-, β-, and γ-secretases.

Mutations in the APP gene account for 5% to 20% of the cases of early-onset FAD. The first FAD mutation to be identified was APP692, causing a variant form of AD with thus a link between amyloid burden and cholinergic impairment in AD. The potency of Aβ as a cholinergic neuromodulator suggests that it is a potent inhibitor of acetylcholine release. A greater understanding of the mechanism by which Aβ-related peptides and the cholinergic system interact may lead to greater understanding of AD symptomatology. Amyloid-β might also modulate other neurotransmitters. In addition, Aβ has been shown to disrupt neuronal [Ca2+] homeostasis. The role of neuronal [Ca2+] overload in initiating [Ca2+] dependent neurotoxicity and death is well documented, and thus a link between [Ca2+] and morphological or functional losses in AD has been proposed. The mechanism of Aβ toxicity supposedly involves disruption of neuronal [Ca2+] homeostasis which may be implicated in the formation of neurofibrillary tangles (largely consisting of tau).

Presenilins 1 and 2

Since the discovery of linkage of some early-onset AD families to chromosome 21, molecular biological research in AD suggests that a locus on chromosome 14 causes about 70% of early-onset AD. However, the dis-
covery that neither the chromosome 21 nor the 14 loci was linked to AD in the Volga German families suggested the presence of another locus. This linkage led to the identification of chromosome 14 gene (presenilin [PS] 1) and the Volga German locus on chromosome 1 (PS2).

Mutations in PS1 are the most common cause of FAD; mutations in the PS2 gene are a rare cause. More than 50 missense mutations have been identified in PS1, and these mutations account for 25% to 30% of early cases of FAD. All mutations represent a toxic gain-of-function. Many of these mutations occur within transmembrane domains or immediately adjacent to the predicted cytoplasmic loop domain. The most provocative insights pertaining to the mechanisms by which mutant PS1 causes AD emerged from studies of the conditioned medium from fibroblasts or the plasma from affected members of pedigrees with PS1/PS2-linked mutations. Surprisingly, FAD-linked PS1/PS2 variants influence processing at the γ-secretase site and may cause AD by increasing the extracellular concentration of highly amyloidogenic Aβ42 species, thus fostering Aβ deposition in the brain. The age of onset of AD in families harboring PS1 mutations is young, ranging from 30 to 60 years, whereas the age of onset in cases with PS2 mutations is older (55-70 years). Thus mutations in APP, PS1, and PS2 cause AD and may, therefore, illuminate a pathway for the development of therapeutic targets.

**Apolipoprotein E**

The apolipoprotein E (ApoE) gene at 19q13.2 has been shown to be a late-onset AD risk factor. Apolipoprotein E is a polymorphic lipoprotein involved in the transmembrane transport of cholesterol and is thought to play an important role in neuronal growth and in the central nervous system response to injury, particularly in the hippocampal region.

The relevance of ApoE to late-onset AD was examined by genotyping patients from late-onset FAD kindreds for the ApoE polymorphisms. A strong allelic association between AD and the ε4 allele of ApoE was reported by using late-onset FAD families, the frequency of ε4 in patients with AD being 0.52 compared with 0.14 to 0.16 in controls. The effect of ApoE on susceptibility has been confirmed in multiple racial groups and in numerous studies. Subsequent analyses showed that the ε4 allele acts in a dose-related manner to increase risk and decrease age of onset both in late-onset familial and in sporadic AD, and in early-onset sporadic AD, so that those with 2 ε4 alleles are at greatest risk. In contrast, the ε2 allele affords a protective effect in late-onset AD in most populations studied. Apolipoprotein E ε4 promotes the early appearance of Aβ and neurofibrillary tangles in the elderly; it also forms a tight complex with Aβ in the extracellular space. The dissociation of Aβ from ApoE may prove to be the basis of its association with AD. However, it must be emphasized that ApoE ε4 is neither necessary nor sufficient to cause late-onset AD, but this locus may account for about 50% of the genetic etiology of late-onset AD.

Discovery of the ApoE gene as a major risk factor for AD has opened doors for many new lines of research. It has led to epidemiologic studies showing that age of onset can vary by as much as 20 years, depending on which form of the gene one carries. Finding the relationship between ApoE and AD provides a crucial biological marker for epidemiologic studies. Another important challenge is to use this genetic information to devise new and more effective interventions.

**Tau Immunoreactivities**

In recent years, it has become apparent that in a variety of neurodegenerative, infectious, and developmental disorders, intracellular tau can aggregate and form fibrils, often in the absence of extracellular amyloid deposits. The gene for tau is located at 17q21-22. Abnormally phosphorylated tau aggregates to form paired helical filaments and straight filaments within neurofibrillary tangles and in dystrophic neurites. In contrast to AD, frontotemporal dementia with parkinsonism chromosome 17 type (FTDP-17), a recently defined disease entity, is clinically characterized by disturbed executive function, personality changes, bradykinesia, and rigidity. Several mutations in the tau gene have now been linked to FTDP-17. The effect of these mutations is to lower the affinity of tau for tubulin and to allow the 4-repeat isoform to accumulate. In AD, however, only hyperphosphorylated forms of tau are found, and presumably are a consequence of the toxic effect of Aβ.

**Metal-Mediated Oxyradical and Peroxide Formation**

A novel hypothesis that involves oxyradicals in AD suggests that Aβ itself generates free radicals in a metal-dependent mechanism. There is an association between increased levels of the oxidative stress–related enzymes, glucose 6-phosphate dehydrogenase, superoxide dismutase (SOD), and heme oxygenase-1 and AD. Reactive free radicals and peroxides can be very toxic to cells. Reactive oxygen species have been proposed to cause neuronal injury in several neurological disorders and ischemic brain injury. Vitamin E, a free-radical scavenger, prevents neuronal death induced by Aβ and has been shown to have some beneficial effects in AD.

**Aβ and Endoplasmic Reticulum–Associated Binding Protein**

Recent evidence suggests that Aβ binds an intracellular polypeptide known as endoplasmic-reticulum–associated binding protein (ERAB), which is overexpressed in neurons affected in AD. The ERAB sequence indicates that it might be an enzyme such as a hydroxysteroid dehydrogenase or an acetoacetyl-CoA reductase. The ERAB normally functions in cellular metabolism and biosynthesis, and these functions may be perturbed by Aβ. By interacting with intracellular Aβ, ERAB may contribute to the neuronal dysfunction associated with AD. The ERAB may contribute to the pathogenesis of AD by being an intracellular target for Aβ, mediating cellular stress and, ultimately, apoptosis due to increased amounts of Aβ.
ANIMAL MODELS

Despite intensive efforts in both academia and industry, a fully authentic transgenic mouse model of AD has not yet been created. Nevertheless, overexpression of Aβ in various mouse lines has produced a variety of phenotypes that, as a first approximation, are remarkably similar to the human AD condition (Table 2). These first-generation models will undoubtedly be crafted into successive generations in which all features of AD will be present. Even at this stage, the first-generation models are providing an assay system in which selected details of pathogenesis and therapeutic intervention can be evaluated.

Transgenic Models of Mutant Human APP With Aβ Amyloid Deposition

To generate animal models of Aβ amyloidogenesis and the associated lesions of AD, many researchers have created transgenic mice that overexpress wild-type APP, FAD-linked APP variants, or C-terminal fragments of APP (see Table 3).

These efforts have resulted in 3 lines that recapitulate some of the key neuropathological features of human AD. In the “Games mouse,” the platelet-derived growth factor β-promoter was used to drive expression of a human APP minigene that encodes the FAD-linked APP (V717F) mutation in an outbred strain. The construct contained portions of APP introns 6 through 8, which presumptively enhanced alternative splicing of exons 7 and 8. Levels of human APP messenger RNA and protein substantially exceeded levels of endogenous APP. At about age 6 to 9 months, transgenic animals began to exhibit deposits of human Aβ in the hippocampus, corpus callosum, and cerebral cortex. As the animals aged (≥9 months), the density of the plaques increased until the Aβ staining pattern approached that of AD. Most plaques were intimately surrounded by glial fibrillary-
Acidic protein–positive reactive astrocytes, and also compressed and distorted the surrounding neuropil as seen in the AD brain. There was also some dystrophic neuritic components and loss of synaptic density with regional specificity resembling that of AD. Unfortunately, no behavioral and cognitive assessments have yet been published.

In a second line of “Hsiao mice,” the hamster prion protein promoter was used to overexpress human APP with Lys-Met to Asn-Leu (Swedish) mutations (human APP695sw). The brains of one of these lines (Tg2576) showed elevated levels of Aβ40 (5-fold increase) and Aβ42 (14-fold increase); there were some dystrophic neurites around moderate numbers of Aβ deposits as plagues and around vessels in amygdala, hippocampus, and cortex. The Tg2576 mice showed impairments at a young age on several memory tests, including the Morris water maze, a spatial reference memory task, and the Y-maze alternation task. At age 3 months, these mice showed normal learning and memory in spatial reference and alternation tasks, but by age 9 to 10 months, they were impaired. In the third model, the “Novartis mouse,” a combination of human APP mutations (Swedish KM670/671 NL and the V717I) was driven by the Thy-1 promoter. Abundant amyloid plaques with notable neuritic changes occurred, but in the absence of typical tau-positive neurofibrillary tangles.

Each of the above 3 principal lines of transgenic models have been restricted in distribution to the wider research community because of commercial considerations. Hopefully this will change in the near future as better models emerge. Nevertheless, the present 3 lines should be sufficient to convince all but the most resolute skeptic that the APP/Aβ pathway is at the center of AD. Some features of the disease, most noticeably the formation of tau that contains paired helical filaments, activation of the complement pathway, and a robust clinical phenotype, have yet to be achieved. It also seems that the impairment of cognitive functions observed in some transgenic mice is not necessarily due to the generation of Aβ but might result from either overexpression of wild-type APP or the accumulation of Aβ precursors, or both.

The CT100 Mice

Mice engineered with the C-terminal 100 residues of APP (CT100) are designed to be used for the study of γ-secretase and its inhibitors. Several lines are now available (Table 3), with considerable variation in phenotypes. None yet exhibits the extent of extracellular Aβ deposition seen with the full-length APP constructs, and indeed there may be an accentuation of intracellular Aβ accumulation, suggesting that the CT100 construct is aberrantly targeted in the intracellular pathway.

Mutant PS Mice

The preliminary analysis of mice expressing PS constructs shows that mutant PS1 selectively increases brain Aβ42, and suggests that the PS mutations probably cause AD through a gain of deleterious function that increases the amount of Aβ42 in the brain. However, these mice do not show AD pathologic lesions. This is intriguing, and suggests that overexpression of the rodent Aβ sequence alone is insufficient for Aβ amyloid aggregation and plaque/perivascular deposition. This interpretation is consistent with the findings from 2 studies that double transgenics (mutated PS × human APP) result in an acceleration of cerebral Aβ deposition. In the first study, transgenic mice were generated that expressed either wild-type or mutant PS1 (A246E), and these were crossed with mice that expressed a murine APP transgene with a humanized Aβ domain (mouse/human-APPsw). In the second, the doubly transgenic progeny were derived from a cross between a Tg2576 line and a mutant PS1M146L transgenic line. Both studies demonstrated that mice co-expressing mutant PS1 with mutant APP develop Aβ deposits much earlier than age-matched controls.

Human APP Overexpression on the ApoE Null Background

The effects of ApoE on amyloid deposition have been tested by mating apoE−/− mice with APP-V717F transgenic mice. At age 6 months, APP-V717F × ApoE−/− mice showed robust amyloid deposition, whereas APP-V717F × ApoE exhibited only sparse, diffuse Aβ deposits. These studies suggest that ApoE either directly or indirectly may influence the aggregation or influence the clearance of Aβ peptides. A full description of the kinetics of Aβ biogenesis, aggregation, and degradation in this model is awaited with interest.

Human APP Overexpression Combined With Oxidative Stress

In an attempt to investigate the role of oxidative stress responses in the pathogenesis of AD, it is relevant that transgenic animals show the same type of oxidative stress responses that are found in AD and that these directly correlate with the presence of Aβ deposits. Several groups are now engaged in programs in which the human APP transgenic lines are being modulated through the superoxide dismutase-1 and glutathione peroxidase pathways.

APP Knockout Mice

Mice with functionally inactivated alleles of APP were generated by deleting its promoter and first exon. The mutant animals weighed 15% to 20% less than age-matched wild-type controls. The mutant mice also showed an impaired neurological and muscular function (reactive gliosis and decreased locomotor activity and forelimb grip strength). However, since neuronal cell damage or loss in the brains of APP-deficient mice did not occur, the mechanisms responsible for the reactive gliosis in these mice could not be determined. It was postulated that the absence of substantial phenotypes in APP knockout mice may be related to functional redundancy provided by the homologous amyloid precursor-like protein molecules 1 and 2 that are expressed at high levels and have developmental and cellular distributions similar to APP. This has now been confirmed through the analysis of double knockouts in which some combinations (eg, APP × amyloid precursor-like protein molecule 2) are not viable.
PS1 Knockout Mice

To examine the in vivo role of PS1 in mammalian development, mice with a targeted disruption of the PS1 gene were generated. Homozygous mutant mice failed to survive beyond the first 10 minutes after birth. The most striking phenotype observed in PS1 knockout (KO) embryos was a severe abnormality in the development of the axial skeleton and ribs. Fibroblast cultures established from these embryos have revealed a remarkable phenomenon: these cells accumulate the C-terminal fragments of APP, suggesting that the PS molecules play a direct or indirect role in the activity of γ-secretase. It may yet transpire that PS forms a direct complex linking γ-secretase directly to APP, and thereby provides an elegant solution to the effect of PS mutations on Aβ40/42 processing.

CONCLUSIONS

The relationship between amyloid deposits and neurofibrillary lesions remains an important unresolved issue in our understanding of the pathogenesis of AD. The lack of consensus regarding the mechanisms by which Aβ causes neurodegeneration also highlights one of the key weaknesses with the hypothesis that this molecule causes AD. The concentration of Aβ used in almost all in vitro neurotoxic studies is between 1 and 100 μg/mL, which is as much as 10,000 times the concentration of Aβ in human cerebrospinal fluid. Further problems concern the specificity of the synthetic peptides used; most toxicity studies have used synthetic peptides that might behave differently from the peptides generated in vivo. Abundant Aβ deposits can be present in cognitively normal individuals, and it is the presence of intracellular neurofibrillary lesions that correlates better with the presence of cognitive changes. More recent studies from our laboratory, however, show that it is the soluble pool of Aβ that may prove to be the major determinant of neurodegeneration.

Further understanding of the critical age-dependent factors that confer vulnerability to Aβ neurotoxicity may provide better insight into the neurodegenerative mechanisms involved in AD, with potentially significant therapeutic innovations. It is widely believed that mutations and polymorphisms in other genes, as yet unknown, will modulate susceptibility to AD and will therefore be found to be additional trait-dependent markers. Neuroprotective growth factors such as the neurotrophins, basic fibroblast growth factor, and insulin-like growth factors may play such a role.

Whether therapeutic agents that affect the concentration, deposition, aggregation, degradation, clearance, or toxicity of Aβ will influence the clinical and pathological features of AD is still unclear. Nevertheless, it seems likely that approaches that reduce the concentration of Aβ or the rate of amyloid aggregation and deposition in proximity to synapses and neuronal cell bodies will be beneficial for patients with AD. Even with existing transgenic mouse models, a start for screening and testing therapeutic efficacy of lead compounds has commenced. The results of initial endeavors will be known shortly.

References