Advances in Alzheimer's disease

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ABSTRACT **The problem of the etiology of Alzheimer's disease has not been solved. But in the past several years there have been significant extensions of our knowledge of the disease and advances in determining the molecular changes underlying the disorder. There is now convincing evidence that the dementia per se is caused by loss of neurons and synapses, particularly in neocortex and hippocampus. The molecular aspects of amyloid and its precursor protein have been defined. The nature of intracellular changes leading to accumulation of the paired helical filament is beginning to be understood. For the first time, putative risk factors can be described in terms of pathogenetic mechanisms. Thus, it may become possible in the not-too-distant future to discover interventions that will slow the progress of this devastating disease.—Katzman, R.; Saitoh, T. Advances in Alzheimer's disease. *FASEB J.* 5: 278–286; 1991.**

Key Words: Alzheimer's disease • epidemiology • positron emission tomography • genetics • neuritic plaque • neurofibrillary tangle • paired helical filament • amyloid • amyloid β-protein precursor • protein kinases

THE 1980S WAS A DECADE OF EXTRAORDINARY events with regard to Alzheimer's disease (AD)[1] research. In 1980, the year that the Alzheimer's Association was formed, the *New York Times* had only a single article on the disease. Today, descriptions of recent advances, drug trials, and articles about caregivers or new management techniques appear several times a month in many major U.S. newspapers; Alzheimer's disease has indeed become a household word. To a great extent this increased interest in AD is a result of the recognition of the public health importance of AD and the activities of lay organizations, such as the Alzheimer's Association; but it also reflects the recognition of the scientific advances now being made in understanding AD (1).

DEMOGRAPHY OF ALZHEIMER'S DISEASE

AD is the epitome of an age-dependent disorder. It accounts for about two-thirds of the cases of dementia in the United States and its age-specific prevalence increases logarithmically with age (**Fig. 1**). The incidence of AD increases from about 0.1% at age 60–65 to as high as 47% of the population over age 85 (2). In studies of mortality, the age-specific death rate of AD victims is two to four times that of individuals in the general population. A particular consequence of this in regard to research is that the control individuals are ordinarily free of other terminal diseases, and hence the number of autopsy specimens available for research study of control individuals whose normal cognition has been documented during life is very limited. The importance of control brains in AD research becomes apparent when it is recognized that much of the progress in AD research has depended on clinical-pathological correlations.

ALZHEIMER'S DISEASE AS A CLINICAL-PATHOLOGICAL ENTITY

Alzheimer's first report of this disease described the clinical features of a woman who died at age 55 of a disorder manifesting memory, language, and behavioral changes progressive over a 5-year period, and in whom the application of new silver staining techniques revealed the presence of the abnormal structures now referred to as neuritic plaques (NP) and neurofibrillary tangles (NFT) within neocortex and hippocampus, features that are the hallmarks of this disorder. AD was immediately accepted as an important entity in terms of its applicability to individuals under the age of 65. For many years the relationship of AD to the dementia symptoms that occur late in life was a matter of controversy, largely because some cognitively normal older individuals had pathologic changes in their brain reminiscent of AD, whereas other individuals who were demented showed the presence of multiple infarcts or strokes or other pathology and did not have NP or NFT in their brain. It was not until the last 30 years that it was recognized that a number of disorders could produce the symptoms of dementia. The classic study by Blessed and colleagues (3) in Newcastle clearly identified a subset (but a major subset) of those in their 70s and 80s with dementia, who not only showed AD changes but in whom the number of NP in neocortex correlated well with mental status tests and functional evaluations carried out during the year before death.

WHAT CAUSES DEMENTIA?

The AD brain shows marked atrophy. This loss is widespread but not uniform. The association neocortex is markedly involved (4, 5) as is the hippocampus and parahippocampal structures including the entorhinal cortex, olfactory cortex and olfactory bulb, the cholinergic forebrain basal nucleus (of Meynert), the dorsal tegmental serotonergic nuclei, and the locus ceruleus noradrenergic nuclei. Within neocortical association areas, Terry and associates (6) have shown there is a marked loss of large (>90 μm in cross-sectional area) neurons, the pyramidal neurons; neurons that provide cross connectivity within association cortices. On the other hand, the primary somatosensory, visual, and auditory cortices, the motor cortex, cerebellum, basal ganglia, brain stem, and much of the thalamus are relatively spared.

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[1]Abbreviations: AD, Alzheimer's disease; APP, amyloid β/A4-protein precursor; Ca/CaM-kinase II, calcium/calmodulin-dependent kinase II; CK I and CK II, casein kinase I and II; HSPG, heparan sulfate proteoglycan; IL 1, interleukin 1; kb, kilobase; KPI, protease inhibitor of the Kunitz type; MID, multi-infarct dementia; NFT, neurofibrillary tangle; NP, neuritic plaque; PET, positron emission tomography; PHF, paired helical filament; PKC, protein kinase C; PN 1 and 2, protease nexins 1 and 2; SPECT, single photon emission computed tomography; τ, tau protein.

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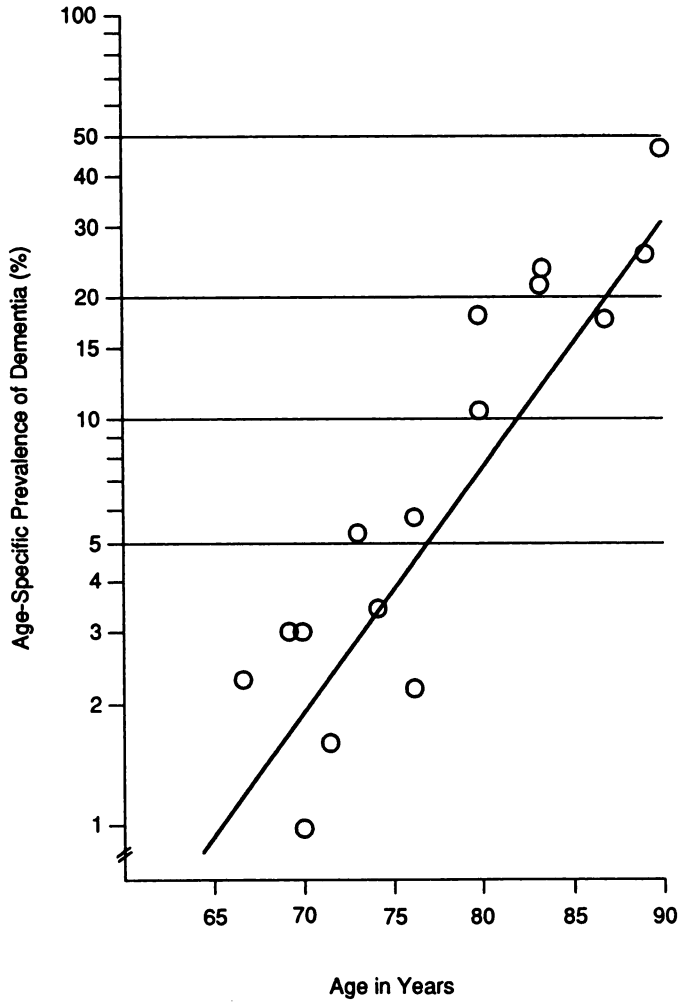

Figure 1. Age-specific prevalence of dementia. Adapted from Cross and Gurland (66), with additional data from Zhang et al. (62) and Evans et al. (2).

The finding in the Newcastle prospective study that the number of NP averaged over several neocortical association areas correlated well with functional dementia scores and mental status scores (r between 0.6 and 0.7) was impressive (3). This suggested that perhaps within the affected brain regions the NP was to a large extent responsible for the dementia. However, further analysis of their data indicates that the correlation coefficient depends in part on the presence in their sample (as would be expected) of individuals who did not have NP and were not demented. Although the finding that individuals without NP for the most part were not demented in the absence of strokes was important in associating dementia and pathological markers of AD, removal of these individuals from the correlation matrix reduced the correlation coefficient to <0.4. Recently, independent studies from three groups (7–9) have found a marked loss of synapses in the association neocortex in AD. Terry and colleagues (8), using an antibody to synaptophysin, an integral hexameric channel membrane protein of small presynaptic neurotransmitter-containing vesicles (10), have found that the density of this synaptophysin marker is not only markedly reduced in the AD brain compared with control brains, but that within the brains of AD patients the correlation coefficient between the mental status score given in the Newcastle series by Blessed and colleagues and synaptophysin density exceeded 0.7 ($P < 0.001$).

Using quantitative ultrastructural techniques, DeKosky and Scheff (9) found a similar correlation between mini-mental status scores and synpatic density measures in cortical lamina III of frontal cortex biopsies. Thus, loss of synapses and neurons no doubt leads to the loss of cognitive functions and the development of dementia. This finding, in regard to synaptic density, although only recently quantitated, has been widely accepted. The question then arises as to what causes the loss of neurons and synapses.

MOLECULAR NEUROPATHOLOGY OF ALZHEIMER'S DISEASE

The neuritic plaque and the amyloid and its precursor

Based on histochemical and ultrastructural studies, it has been established that the NP in its mature form consists of a central core of an extracellular Congo red-staining fibrous protein, meeting ultrastructural as well as histochemical criteria for amyloid; surrounding this amyloid core are degenerating nerve endings, both dendritic and axonal, which contain numerous lysosomes, abnormal mitochondria, as well as a few intact-looking synaptic endings with presynaptic vesicles. Immunohistochemical methods have confirmed that the individual neurites contain neurotransmitter markers so that in a given NP one cluster of degenerating endings may stain with an antibody to somatostatin, another cluster with antibodies to choline acetyltransferase, another cluster to antibodies to dopamine β-hydroxylase, etc., indicating that neurons representing many different neurotransmitter systems contribute processes for the formation of the NP. In the mature NP, the neurites also contain PHF as will be described below.

In addition to the amyloid present in the NP, there is a variable degree of amyloid in blood vessels in AD neocortex. Chemically identifying amyloid in the NP had been difficult because of amyloid's insolubility. In 1984, Glenner and Wong (11) selected AD brains heavily laden with vascular amyloid in both intracerebral and meningial blood vessels and were able to isolate this amyloid and obtain a partial sequence of this ~4.2-kDa peptide, a peptide now referred to as amyloid β/A4 protein. This sequence information made it possible to obtain antibodies to the amyloid and to demonstrate that the core amyloid was similar or identical to vascular amyloid. In addition, oligonucleotides could then be synthesized to be used as probes to obtain the cDNA for the parent molecule of the amyloid peptide. Subsequently, the gene for the precursor protein, now termed amyloid β/A4-protein precursor (APP), was identified on chromosome 21, sequenced, and the promoter region was obtained. The promoter of the APP gene has been shown to have the typical structure of a housekeeping gene having consensus sequences for SP1, AP-1, Hox proteins, and heat-shock element-dependent regulation (12). In fact, synthesis and processing of APP seem to be regulated by many growth factors including nerve growth factor, fibroblast growth factor, epidermal growth factor, and interleukin 1 (IL 1). There is some uncertainty as to whether the heat-shock regulatory region is actually functional, a matter of considerable importance. Among the risk factors for AD are such events as head trauma and episodes of ischemia secondary to coronary artery disease. One can make the assumption then that this type of stress would lead to an up-regulation of amyloid production, if indeed the heat-shock regulatory site were functional.

Molecular cloning of the cDNA encoding amyloid β/A4-protein revealed that the protein is synthesized as a large precursor molecule, which is then processed into β/A4-protein in a way that is not yet fully understood (reviewed in refs 13 and 14). There are at least four different forms of APP, three of which contain a domain showing a strong homology with protease inhibitors of the Kunitz type (KPI) (15). These proteins contain 714, 751, and 770 amino acids whereas the form without the protease inhibitor domain contains only 695 amino acids. The four types of APP known to date are encoded by four distinct mRNA molecules of ~3.4–3.9 kilobases (kb). The APP gene, present in a single copy per haploid genome, consists of 18 exons and 17 introns, totaling more than 50 kb (16). The mRNAs for the four types of APP are all derived from the alternative splicing of these exons. The regulation of this splicing might have important biological effects especially with regard to the proportion of APP-695 that is the only form without a KPI. Most peripheral cells produce APP-751 or APP-771, but in the brain as much APP-695 is produced as a form with the KPI insert. It is reported that the proportion of the KPI-containing form of APP relative to the APP-695 form is elevated in AD cortex and hippocampus and has a positive correlation with the number of plaques in the region of the brain (17). The selective reduction in the concentration of APP-695 relative to KPI-containing forms of APP is observed in behaviorally impaired old rats but not in behaviorally intact old rats (18).

Based on the analysis of the predicted amino acid sequence, APP was initially proposed to be a cell surface receptor or a transmembrane glycosylated protein (reviewed in ref 14; see **Fig. 2**). The 42 amino acid domain of the molecule

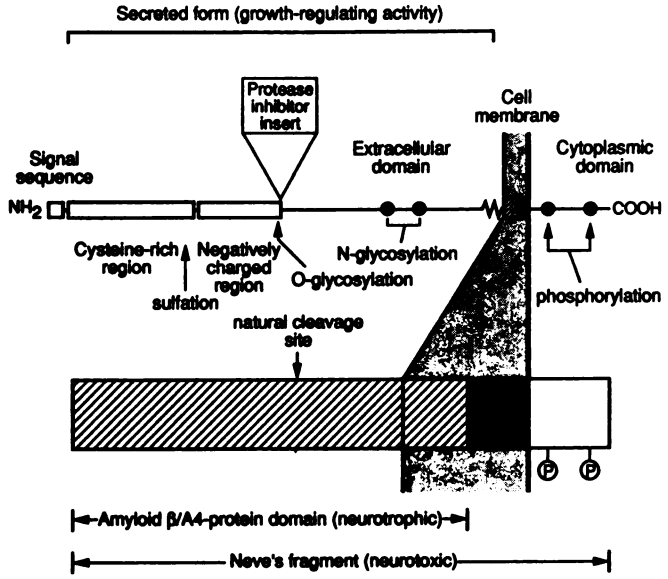

Figure 2. Schematic representation of amyloid β/A4-protein precursor. APP is synthesized as a membrane protein, cleaved near the membrane, and secreted. The biological significance of post-translational modification (sulfation, O-glycosylation, N-glycosylation, and phosphorylation) is unknown. There are at least six isoforms of APP, with and without a protease inhibitor domain. APP may have several biological activities: growth regulating (NH_2 terminal), neurotrophic (amyloid domain), and neurotoxic (COOH terminal). The aberrant processing of APP which produces amyloid is to be elucidated, although the first step seems to be a defect in the natural cleavage of APP within the amyloid β/A4-protein sequence.

corresponding to β/A4-protein is partly within the membrane and partly outside the cell. Amino acids 1 to 28 of β/A4-protein are located outside whereas amino acids 29 to 42 are part of the membrane-spanning fragment. However, evidence has accumulated that suggests that APP products may be secreted, perhaps into extracellular matrix, perhaps into synaptic clefts. APP can be detected in the medium conditioned by cells in culture such as PC12 and fibroblasts (19), and a soluble form of APP is found in plasma, possibly being secreted from platelets. The secreted form of APP is smaller than the form detected in the particulate (membrane) fraction in brain, PC12, and fibroblasts, suggesting that cleavage of APP is necessary for its secretion and that a small COOH-terminal portion of the molecule (including the transmembrane fragment and the intracellular domain) remains in the membrane whereas the large NH_2-terminal portion is released. Recent evidence indicates that the cleavage occurs between amino acids glutamine 15 and leucine 17 of β/A4-protein, thus very close to the membrane and within the amyloidogenic domain itself (20). Another line of investigation demonstrated that amino acids 18 to 28 of β/A4-protein are required for the cleavage (21). In fact, the mutation from glutamic acid to glutamine at amino acid 22 of β/A4-protein seems to be responsible for the amyloidogenesis in Hereditary Cerebral Hemorrhage, Dutch type (22). However, the protease that catalyzes this cleavage has not yet been identified. Under normal physiological conditions, APP is cleaved within the β/A4-protein domain, and thus no amyloid production is possible. In other words, formation of β/A4-protein might imply that, under pathological conditions, the normal cleavage of APP is impaired. This possibility has caught the attention of many investigators who are now seeking to determine the exact degradative process of this protein in AD. Potentially this is one of the primary events taking place in the development of the disease.

Recently it was shown that the secreted form of APP (major NH_2-terminal portion of molecule) has a growth-regulating activity, probably through an autocrine mechanism (23). Another line of study demonstrated that the secreted form of APP with a KPI domain is identical to a previously described protease inhibitor, protease nexin 2 (PN 2) (24, 25). PN 2 is secreted from fibroblasts and regulates their growth through protease inhibition. The molecular mechanisms by which APP without a KPI domain regulates cell growth are not known.

In the NP of AD, abnormal neuritic processes cluster around a core of amyloid. A simplified explanation of this is that a constituent of amyloid causes the neuritic sprouting around plaques. Cotman and his colleagues (26) tested the possibility by treating hippocampal cultures with β/A4(1-28) and β/A4(1-42). It seems that β/A4(1-28), a peptide homologous to the first 28 amino acid residues of amyloid β/A4-protein, enhanced neuronal survival without affecting neuritic extension or branching. On the other hand, β/A4(1-42), a peptide homologous to the full 42 amino acid residues of amyloid β/A4-protein, enhanced neuronal survival as well as a number of neurites, neuritic extension, and branching. Therefore, it is possible that plaque amyloid is not the tombstone of a nonfunctional protein but has a significant role in the pathogenesis of NP.

In addition to the NH_2-terminal and β/A4-protein regions of APP, the COOH-terminal region also has biological activity. Neve and her colleagues expressed a COOH-terminal 105-amino acid portion of APP in PC12 cells and fibroblasts and detected this peptide in the conditioned medium (27). Surprisingly, this COOH-terminal APP peptide was neurotoxic in terminally differentiated PC12 cells or hippocampal

neurons, whereas the same peptide induces mitosis in non-neuronal cells. Although this peptide was artificially manufactured in this experiment, it is not unreasonable to speculate that this peptide is generated under pathological conditions where the NH_2-terminus of β/A4-protein is cleaved but the COOH-terminus is not. Detection of this COOH-terminal portion of APP in vivo seems to be crucial for this hypothesis to be valid.

There is also the possibility that the abnormality in AD may represent a local overproduction or accumulation of APP, which is not completely degraded by a normal but minor pathway. This possibility is based on the fact that individuals with Down's syndrome who have three sets of chromosome 21 and produce 50% more APP than do individuals who do not have Down's syndrome, develop AD changes—including NP—in the brain, by the time they reach 35 to 50 years of age. Moreover, in brains of Down's individuals in their late teens and early twenties, accumulations of amyloid wisps scattered in a small region, patterns observed with appropriate antibodies to amyloid β/A4-protein and termed diffuse plaques, are present (28). These foci are also found in other situations that may be precursors to AD. However, the diffuse plaques that are present in neocortex and hippocampus, as would be expected if they were precursors of NP, are also present in cerebellum which does not show the development of the mature NP. Thus, the deposition of these precursor proteins alone cannot be responsible for eliciting the development of the neurite degeneration seen in the mature NP.

In addition to β/A4-protein, α_1-antichymotrypsin has been shown by Abraham and colleagues (29) to be an amyloid component. α_1-Antichymotrypsin was reported to be synthesized by reactive astrocytes near the NP. It is not known if α_1-antichymotrypsin is actively involved in amyloidogenesis. A recent study demonstrated that α_1-antichymotrypsin levels in plasma are elevated in AD patients. It is possible that, being systemically elevated, this enzyme is adsorbed to the amyloid. One other molecule extrinsic to amyloid, yet important, is heparan sulfate proteoglycan (HSPG) (30). HSPG may be deposited in the developing plaque by adjacent and infiltrating astrocytes and neurons. Because HSPG can activate and promote neuritic outgrowth, it may serve as a trophic factor in attracting neurites to the plaque area.

In classical mature plaques, immunoglobulin and complement factors are also reported to be components of amyloid, suggesting the involvement of immune systems in amyloidogenesis. A recent finding that activated microglial cells, believed to be brain homologs of the macrophage, are closely associated with amyloid fibrils (31) strongly supports this contention. Furthermore, it has been demonstrated that a cytokine, IL 1, is excessively synthesized in the activated microglia in AD cortex (32). IL 1 has been shown to stimulate APP synthesis (33). Although a net increase in APP concentration is not found in AD, there might be an increase in the local concentration of APP surrounding activated microglia.

In summary, APP is an important protein involved in the regulation of cell growth and neuronal development. This protein must be processed and secreted to be biologically fully active. Abnormal processing of APP is thus likely to produce a nonfunctional protein or even a cytotoxic protein. In AD patients, the processing of APP is altered, as demonstrated by the presence of β/A4-protein as amyloid which is not supposed to be produced if the precursor molecule is normally processed. It is possible that abnormal APP processing in AD leads to an important alteration in the regulation of cell growth. This, in turn, could lead to the massive neuronal loss observed in AD brains.

Neurofibrillary tangles, paired helical filaments, and tau

The NFT in the neuronal cell body is composed of paired helical filaments (PHF) which are also found in the neuritic component of NP and neuropil thread. The PHF are relatively insoluble to detergent and resistant to proteases. The PHF, 28 to 36 nm in width, are two twisted filaments 14–18 nm in diameter which coil counter-clockwise around each other with a periodicity of 70–90 nm. A recent study using the quick-freeze, deep-etch, replica method revealed that the PHF often form parallel bundles which are cross-linked to each other with thin filaments, ~6 nm in diameter, at relatively regular intervals (34).

One step in understanding the significance of NFT in AD is to determine PHF composition. Immunochemical and biochemical techniques have revealed many molecules in PHF. Tau (τ) has been shown to be a major PHF protein constituent in NFT (35). Ubiquitin is also a constituent of PHF. Neurofilament proteins (160 and 200 kDa) and microtubule-associated protein 2 (MAP 2) may also be a part of PHF. A recent report further identified phosphorylated MAP 5 as a component of PHF (36). Because MAP 5 is involved in neurite outgrowth and is expressed in differentiating neurons as a phosphorylated form, it is likely that the PHF is not a mere product of neurodegeneration but may result from an aborted effort of somatodendritic sprouting of pyramidal cells. Along with these cytoskeletal proteins, casein kinase II (CK II) was found to colocalize with the NFT (37). This is the first protein kinase colocalized with PHF and two constituents of PHF, τ and MAP 5, seem to be overly phosphorylated. Therefore, it is possible that enzymes important for PHF formation are also found colocalized with PHF. A recent report suggests that kinase is not the only regulatory protein associated with PHF. Apparently a protease inhibitor, protease nexin-1 (PN 1), is associated with PHF and may also be found in the neuritic component of plaques (38).

Alz-50, a monoclonal antibody raised against brain homogenate from AD patients (39), intensely stains NFT. This antibody detects a 68-kDa protein (A68) which is enriched in AD brain. Alz-50 recognizes τ on the Western blot, and anti-τ antibodies recognize A68. Therefore, it is likely that A68 is related to τ, although it is not a classical τ protein because it has a higher molecular weight than τ. A recent study demonstrated that a phosphorylation event is involved in the generation of the Alz-50 epitope. Alz-50 stains neurons which do not form NFT but are vulnerable to NFT formation (40). Furthermore, overly phosphorylated τ is found in neurons vulnerable to NFT formation (41). Thus, it is likely that protein phosphorylation is involved in the pathogenesis of AD lesions.

Protein phosphorylation

Protein phosphorylation and dephosphorylation are important in maintaining cellular homeostasis and gene expression. Many growth factors and hormones maintain cellular functions through the regulation of protein kinases and phosphoprotein phosphatases which catalyze phosphorylation and dephosphorylation. Therefore, a change in the normal balance of these reactions can lead to cellular dysfunction and eventual death. In AD, the process of cell death might be exacerbated by the formation of NFT and NP which might also be brought about by a disruption in the

balance of normal protein phosphorylation and dephosphorylation. Several brain proteins associated with AD have been shown to be abnormally phosphorylated in vivo by immunohistochemical technique or through in vitro phosphorylation assays (**Table 1**). These abnormally phosphorylated proteins include τ, neurofilament proteins, MAP 5 (36, 42), P60, and P86 (37). The increase in P60 phosphorylation in AD cortex has a positive correlation with the number of NFT but not with that of plaques or reactive gliosis. Thus, P60 or P60 kinase might be involved in the formation of NFT. Also, several protein kinases have been shown to have abnormal activity and/or abnormal neuronal localization in AD (37, 43) (see Table 1). These include CK II, protein kinase C (PKC), and Ca^{2+}/calmodulin-dependent kinase II (Ca/CaM-kinase II). CK I and cAMP-dependent kinase, however, are relatively preserved.

Protein kinase C (PKC) is a key enzyme in mediating the action of neurotrophic factors to maintain neuronal survival. Total PKC concentration measured by phorbol ester binding is decreased, its compartmentalization altered, and the in vitro phosphorylation of major PKC substrates is reduced in AD cortex. These changes are fairly specific in AD and are not observed in other neurodegenerative diseases such as multi-infarct dementia (MID) and Pick's disease. The most affected PKC isozyme is the βII form (PKC(βII)); PKC(γ) isozyme is least affected in AD (44). Anti-PKC(βII) stains the periamyloid region of NP, and anti-PKC(βI) stains the neuritic component of plaques. The finding that APP is phosphorylated by PKC at its cytoplasmic domain (45) and the possible regulatory function of this phosphorylation regarding lysosomal processing of APP (19) might link the aberrant PKC and APP metabolism and their colocalization in AD plaques. Lysosomal enzymes cathespin B and D are also found closely associated with amyloid in the plaques (46). As described previously, dilated neurites in NP contain many lysosomes which might participate in APP processing and possibly in amyloidogenesis.

Abnormally phosphorylated τ, neurofilament, and MAP 5 are associated with NFT (36, 47). However, τ is overphosphorylated before NFT formation, and therefore may be one of the early steps in NFT formation (41). It would be important to determine the protein kinase and/or phosphatase that is responsible for the aberrant phosphorylation of τ, MAP 5, and neurofilament proteins in AD. Despite extensive studies by many investigators, the effort to identify these enzymes has not been successful. Because CK II was found to be localized to NFT, it is attractive to speculate that CK II is involved in the generation of AD-associated overphosphorylation of NFT constituents. Alternatively, it is possible that new kinases such as tubulin-dependent τ kinase (47) or the P60 kinase, as described above, might be involved in aberrant NFT phosphorylation.

PERIPHERAL ALZHEIMER'S DISEASE MARKERS

If AD were a hereditary disorder, and if an aberrant gene is expressed outside the brain tissue, it is likely that some biochemical abnormalities are found in peripheral tissues. Numerous studies demonstrate this to be the case (48). Many abnormalities have been reported so far in AD fibroblasts, including altered energy metabolism, increased X-ray and alkylating reagent sensitivity, Ca^{2+} binding to cell surface, intracellular free Ca^{2+} concentration, reduced adhesiveness and spreading, reduced secretion of cholinergic differentiation factor and APP, altered responsiveness to β-adrenergic agonists, and reduced PKC immunoreactivity. Abnormalities are not limited to fibroblasts but are found in many cell types including platelets, lymphocytes, and erythrocytes obtained from AD patients. A recent study

TABLE 1. *Protein kinases and phosphoproteins altered and/or associated with AD pathologies*[a]

Kinases and phosphoproteins	AD pathology	Alteration	Kinase(s) that phosphorylate(s) substrate
Tau (τ)	NFT	Excessively phosphorylated	PKC, CK II, Ca/CaM-kinase II, PKA, and PTK
Neurofilament[b]	NFT	Phosphorylated in NFT	PKC, Ca/CaM-kinase II, and PKA
MAP 5	NFT	Phosphorylated in NFT	PKC, Ca/CaM-kinase II, and PKA
P60	NFT	Correlated with NFT	Not identified
P86 (MARCKS?)	–	Decreased in AD	PKC
Spectrin	NP	Associated with neuritic components of plaques	CK II
APP	NP	Present in the neuritic components of plaques	PKC and Ca/CaM-kinase II
CK II	NFT	Reduced in AD; associated with NFT	
PKC(βI)	NP	Found in the neuritic components of plaques	
PKC(βII)	NP	Diminished and translocated from membrane to cytosol; found at the center portion of plaques, apposed to amyloid	
Ca/CaM-kinase II	NFT	Coincides with NFT localization	

[a] Protein kinases and phosphoproteins affected in AD are tabulated as well as their suggested association with AD lesions. See text for details and references. Note that distinct sets of protein kinases and phosphoproteins are involved in the AD pathologies, NP and NFT. Components found to be associated with NFT are also found as PHF in the neuritic portion of NP.
[b] 160- and 200-kDa proteins.

shows the presence of a diffuse plaque-like structure in peripheral tissues such as skin and intestine, further substantiating the above contention (49). More severe abnormalities are detected from cells obtained from familial AD patients than from sporadic AD patients. It is possible that because of the strong deviation from normal biochemistry, familial AD has an earlier onset than sporadic AD.

Certain manipulation of normal fibroblasts or cultured neurons may induce AD-associated molecular changes. For example, intoxication of cells with mitochondrial uncoupler CCCP and glutamate induces Alz-50 and PHF antigenicity, whereas PKC down-regulation induces Alz-50 antigenicity and in vitro P60 phosphorylation (37, 48, 50), which suggests that some extrinsic environmental factors might induce AD-associated alterations, whereas in AD cells, some genetic deficiency or alteration causes the expression of biochemical markers of AD. Thus, peripheral tissue might be a system of choice for the identification of genetic alterations which make individuals more susceptible to AD.

GENETICS OF ALZHEIMER'S DISEASE

One form of AD has been described as an autosomal-dominant hereditary disorder (51). It is not distinguishable from sporadic AD neuropathologically or clinically, although the onset of the symptoms tends to be at a younger age. Moreover, the presence of AD-like pathology in all patients with Down's syndrome (chromosome 21 trisomy) after 35 years of age suggests the involvement of a gene on chromosome 21 in the development of AD-like pathology. However, the FAD (putative defective gene in familial AD) locus on the long arm of chromosome 21 identified for a group of families with early-onset AD (52) is distinct from and closer to the centromere than the APP gene. This locus has been excluded as a site of an AD gene in another group of families, the so-called Volga German families, as well as in studies of families with late-onset AD. Recently, a site on chromosome 19 has been identified for some late-onset families (53). In addition there is the earlier report by Weitkamp of a familial AD gene on chromosome 14 (54). Because of the presence of an α_1-antichymotrypsin gene on chromosome 14, this report deserves further follow-up.

In spite of widely accepted homology between the pathology of elderly Down's syndrome patients and AD patients, there are important differences. The concentration of serum and brain APP is increased 1.5-fold in patients with Down's syndrome compared with that in controls, whereas the APP levels in AD patients are not different from controls (55). Therefore, although it is possible that increased levels of APP might account for the amyloid deposition in Down's syndrome, additional factors must operate in AD. The other important difference between Down's syndrome and AD is found in lipid metabolism. An increase in the levels of phosphomonoesters and phosphodiesters is found in AD. Phosphodiesters are considered to be the markers of membrane breakdown, whereas phosphomonoesters are the markers of fast lipid turnover. The increased levels of phosphomonoesters are found in patients in an early stage of AD, although this change is not found in patients with Down's syndrome (56). Ultrastructural analysis of diffuse plaques demonstrates the involvement of membranes in the early stage of amyloid deposition. Altered lipid metabolism found in AD by Pettegrew and colleagues (56) might be an additional factor (beside APP overdose) operating for the amyloid deposition.

POSITRON EMISSION TOMOGRAPHY

Positron emission tomography (PET) can be used to measure regional metabolism in the brain using ^{18}F deoxyglucose, and blood flow using ^{15}O (57, 58). Another technique for measuring blood flow or the uptake of chemicals by the brain is single photon emission computed tomography (SPECT), a technique somewhat more readily available than PET although not as precise. In cases that meet the clinical criteria for probable AD, PET studies often show a typical biparietal and temporal pattern of apparent reduced metabolism sometimes greater on one side than on the other, and both techniques show a similar pattern of reduced blood flow. The extent to which the reduced glucose metabolism represents a reduction of metabolism in surviving neurons rather than an anatomical loss of the most actively metabolizing structures, that is the synapses, is uncertain. In these studies, greater reduction of cerebral metabolism on one side of the brain is often associated with corresponding cognitive asymmetry, showing how variable the onset of the disease may be when one begins to look at specific regions of the brain, even in patients who meet the clinical criteria for *probable* AD.

The ability of these imaging techniques to identify atypical cases of AD or to identify AD in the presence of other disorders such as Parkinson's disease, normal pressure hydrocephalus, or MID has not been demonstrated yet. However, typical cases of AD and MID are readily distinguished on PET; there is no change in oxygen extraction by cerebral cortex in AD, as measured by ^{15}O, whereas similar PET measurements show change in oxygen extraction in a multifocal pattern in vascular dementia. This suggests that the decrease in cerebral blood flow in AD is secondary to the decrease in neuronal activity, and is not a consequence of ischemia. Moreover, PET studies using tracers that do not readily cross the blood-brain barrier do not show any alteration in this barrier in AD. Thus, it is unlikely that there is a primary vascular component to AD.

RISK FACTORS

The two most important risk factors for AD are age and family history. The prevalence of AD is a semi-log function of age (Fig. 1) rising from less than 1% at ages 60 to 65, to more than 30% (reported to be as high as 47% in the East Boston study) beyond the age of 85. There is certainly an important genetic factor in AD with some families showing an apparent autosomal dominant inheritance as described in the Genetics section above. An increase in risk for AD among first-degree relatives of AD probands who were under the age of 75 at onset of illness is well established both in studies utilizing clinically diagnosed probands and in the studies based on autopsy-confirmed probands (59). On the other hand, based on clinical observations, there are many sporadic cases with no family history of the disease despite reasonable longevity in parents. In two well-conducted case control studies, 60% of AD patients had no evidence of family aggregation. Perhaps the most important fact is that there is only a 50% concordance in identical twins (60). The finding of autopsy-proven AD in two identical twins with an onset of almost 15 years apart (61) suggests that even in genetic cases, nongenetic factors play a crucial role. Factors other than age and genetic makeup must be important in determining who will develop AD.

Recent epidemiological studies, including case control studies, population-based surveys, and longitudinal studies

of volunteer cohorts, have begun to reveal new and at times unexpected risk factors, including lack of education (62), a history of serious head trauma (63, 64), and in the very elderly, a history of myocardial infarct (65). These risk factors may be considered in terms of two different mechanisms. In regard to the effect of the lack of formal education, it can be postulated that such lack might lead to a decrease in synaptic reserve, resulting in an earlier onset of symptoms. As we noted previously, the degree of dementia in AD is a function of synaptic loss. On the other hand, other risk factors such as head trauma and brief periods of ischemia associated with coronary artery disease might lead to the deposition of diffuse plaques which could hypothetically lead to the later development of AD. This dual set of mechanisms to explain risk factors is speculative but soon should be testable.

CONCLUSION

Decades of combined efforts of epidemiology, neuropathology, biochemistry, molecular biology, cell biology, genetics, and biophysics now converge toward a fuller understanding of AD. This disease is an age-associated neurodegenerative disorder the process of which is deeply affected by the genetic makeup of individuals and many epigenetic factors that are yet to be elucidated. The clinical expression of AD, the deterioration of intellectual faculty, is likely brought about by reduced synaptic connectivity partially caused by neuronal loss. Although the biochemical and cellular mechanisms for the neuronal loss are not fully elucidated, it is probable that altered intracellular signal transduction mechanisms, exemplified by aberrant protein phosphorylation and protein degradation, are involved. These altered intracellular biochemical reactions are also likely to be involved in the formation of two pathological hallmarks of AD, the NFT and NP. FJ

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