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Commentary

Amyloidosis in Aging and Alzheimer's Disease

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Alzheimer's disease (AD), the most common cause of senile dementia,[1,2] is characterized by the presence of senile plaques, neurofibrillary tangles, neuropil threads, and congophilic angiopathy in a variety of brain regions including amygdala, hippocampus, and neocortex.[3,4] In cases of AD, amyloid is present in diffuse deposits, plaques, and congophilic vessels.[5,6] One of the characteristic features of cellular pathology of AD is the presence of senile plaques, which are complex structures, the principal components of which are neurites and amyloid. Amyloid is a 4-kilodalton (kd) peptide (β/A4, Aβ) comprised of 11-15 amino acids of the transmembrane domain and 28 amino acids of the extracellular domain of the amyloid precursor proteins (APP).[7–11] Plaque and vascular amyloid are peptides of 42 or 39 residues, respectively.[5]

Beginning with the seminal work of Dr. George Glenner,[8] the central role of amyloid in Alzheimer's disease (AD) has become increasingly well documented. This review briefly discusses several recent advances in the understanding of amyloidogenesis.

Amyloid Precursor Protein

Encoded by a gene located on chromosome 21,[11] APP pre-mRNA are alternatively spliced to produce at least four mRNA that encode Aβ-containing proteins (i.e., APP-695, -714, -751, and -770).[11–14] APP-751 and -770 contain a domain homologous to the Kunitz family of serine protease inhibitors.[12,13] Maturing through a constitutive secretory pathway,[15] APP are posttranslationally modified by the addition of N- and O-linked carbohydrates and by phosphorylation and tyrosine sulfation.[15,16] *In vitro*, APP are cleaved at the plasma membrane[17,18] between positions 16-17 within the Aβ domain, releasing the ectodomain of APP.[19–22] This pathway is presumably utilized *in vivo*, because secreted APP are present in cerebrospinal fluid.[15,23] An NPXY sequence in the intracellular domain determines the ability of APP to be retrieved via a clathrin-coated pit pathway[24] (Sisodia, personal observation). APP can be internalized and degraded in endosomal/lysosomal compartments,[17,25–28] where proteases generate C-terminal fragments containing the entire Aβ domain.[17,27] In cultured cells, manipulations that increase protein kinase C activity increase the level of APP secretion, and the relationships between phosphorylation of residues in the APP cytoplasmic domain and secretion are an active area of investigation.[29–31]

APP mRNA isoforms are present in neurons of the peripheral and central nervous systems.[32,33] In rat sciatic nerve, APP (predominantly full-length APP-695) are delivered by fast anterograde transport to axons and terminals.[34,35] In the central nervous system, APP are also transported as full-length forms, and processed N- and C-terminal species are present at terminals, where it has been speculated that APP play a role in synaptic interactions. In terminal fields of the rat perforant pathway, APP appear to be cleaved by secretion-related mechanisms (i.e., within Aβ) as well as by mechanisms that generate potentially amyloidogenic fragments (Sisodia, Koliatsos, and Price, personal observations).

APP: A Source of Parenchymal Amyloid Deposits

Neuronal APP are considered to be one source of Aβ deposits in the brains of aged primates,[7,36–39] cases of Down's syndrome,[10,40] and individuals with AD.[7,9,28,41,42] In each of these settings, neuronal APP accumulate in neurites located in proximity to Aβ deposits[28,38,39,42,43]; the codistribution of neurites and Aβ sug-

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gests that these two abnormalities are linked.[28,41,42,44] Alterations of the normal processing of neuronal APP at synaptic sites may generate amyloidogenic fragments that, upon subsequent proteolysis, form Aβ deposits in the brain parenchyma.[7,36–38,40,42] The suggestion of a neuronal source of Aβ does not exclude contributions to amyloidogenesis by other cells, particularly microglia and astrocytes. Moreover, it is likely that vascular amyloid arises from local processing by cells associated with blood vessels.[6] Mechanisms that lead to the formation of amyloid *in vivo* and the consequences of amyloid deposition are not fully defined but could include: alterations in the efficiencies of secretory versus endocytic maturation of APP; changes in the stoichiometry of APP-695 versus -751/770 expression in affected cells[32]; overexpression of APP, as occurs in trisomy 21; and inherited mutations of the APP gene, as has been documented in some cases of familial AD[45–49] and in patients with hereditary cerebral hemorrhage with amyloidosis, Dutch type (HCHWA-D).[50,51] Recent experimental studies using model systems have begun to define some of the issues raised by investigations of amyloidogenesis in AD.

Transgenic Animals

Although initial studies designed to examine age- or disease-associated alterations in ratios of different APP transcripts/isoforms did not disclose consistent patterns in these measures,[32,33,52] one *in situ* hybridization study demonstrated that levels of APP-751 mRNA (relative to APP-695 mRNA) were increased in subsets of neurons affected in AD.[32] If APP-751 overexpression influences the maturation/cleavage of APP or alters the interactions of APP with proteases, then changes in these ratios could facilitate the formation of Aβ. To test this hypothesis, a human APP-751 cDNA under the control of a neuron-specific enolase promotor was used to produce transgenic mice.[53] A polymerase chain reaction (PCR)-based approach showed the presence of transgene-derived mRNA; by immunoblotting, levels of APP appeared to be increased relative to nontransgenic littermates. A relative increase in APP occurred in neurons, and some extracellular Aβ deposits were present in the hippocampus and cortex. These results suggest that changes in ratios of neuronal APP isoforms, with a relative increase in APP-751, may promote amyloidogenesis. However, before animals that overexpress APP-751 are accepted as a model of early AD, investigators must provide more complete documentation concerning levels of transgene mRNA, absolute levels of identifiable transgene products, and the character of putative amyloid deposits, particularly in older animals.

Other investigators have taken different approaches. One group introduced a transgene that encoded the 4-kd β-peptide under the transcriptional control of 1.8 kb of the human APP promoter.[54] In these transgenic animals, levels of transgene mRNA were a fraction of the levels of endogenous mouse APP mRNA. Small clusters of Aβ-like immunoreactivity were visualized in the hippocampus, but it became apparent that C57BL/6J mice, a contributor to the Aβ transgenic mouse line, normally develop age-related clusters of intracytoplasmic inclusions within astrocytic processes. These lesions, possibly a murine corpora amylacea-like structure, exhibit nonspecific immunostaining patterns with a variety of polyclonal antibodies,[55] and it is likely that these lesions represent age-associated astrocytic inclusions that, for unknown reasons, bind a variety of antibodies. Several additional research groups have made mice using constructs coding for the C-terminal 100 amino acids of APP, a possible neurotoxic fragment.[56] Using a Thy-1 promotor, Kawabata et al[57] showed high levels of transgene-derived mRNA specifically expressed in brain and enriched in neurons. Eight-month-old animals were reported to show amyloid deposits, neuritic plaques, neurofibrillary tangles, and neuronal degeneration in hippocampal formation, amygdala, and cortex. Patterns of Aβ and A68 immunoreactivities were said to be similar to those seen in cases of AD. However, thioflavin S preparations did not demonstrate amyloid or tangles convincingly. Significant discrepancies were noted between thioflavin images and those generated by silver stains and immunocytochemistry (Price and Walker, personal observations). Subsequent studies of additional animals derived from these lines did not replicate the initial observations; moreover, biochemical studies showed no evidence of the transgene product (Sisodia and Price, personal observation), and the paper has been retracted. More recently, two other groups have made transgenic animals expressing the C-terminal 100 amino acids under the influence of either the brain dystrophin promoter[58] or the cytomegalovirus (CMV) promoter (K Fukuchi and GM Martin, personal communication). The former animals show accumulations of Aβ-immunoreactive deposits in cell neuronal bodies and neuropil, and aggregates of C-terminal epitopes were present in abnormal-appearing neurites in hippocampus.[58] By PCR methods, transgene-derived mRNA were present, but immunoblotting revealed that the encoded polypeptide product was expressed at low levels. Transgenes encoding the APP C-terminal 100 amino acids and driven by a CMV promoter were expressed at substantial levels, but animals of up to 6 months of age did not show clinical signs or histopathologic abnormalities in any tissues (K Fukuchi and GM Martin, personal communications).

Aged Nonhuman Primates

At present, the most satisfactory model of Aβ amyloidogenesis is *Macaca mulatta,* which develop age-

associated impairments in performance on memory tasks[59] and brain abnormalities, including deposits of amyloid[36–39,60] early in the third decade of life. These monkeys exhibit enlarged axons, nerve terminals, and dendrites (neurites) filled with abnormal membranes, dense bodies, lysosomes, cytoskeletal elements, and abnormal-appearing mitochondria.[7,36] Neurites are derived from several populations of neurons.[61–63] Old animals also show diffuse deposits of Aβ, senile plaques, and amyloid angiopathy.[7,36–38,63,64] Aβ is readily demonstrable in proximity to swollen APP-enriched neurites filled with lysosomes and abnormal membranes,[39] observations consistent with a neuronal origin for some of the parenchymal deposits. Other cells, including astrocytes and microglia, could also participate in the formation of these lesions. For example, in aged monkeys a_1-antichymotrypsin (ACT) appears to be, in part, synthesized in astrocytes.[33,60] Moreover, these aged monkeys, particularly squirrel monkeys, also show deposition of Aβ and ACT around blood vessels,[37,38,60,65] and these deposits may arise from the local vasculature or other cells. We have speculated that APP may play an important role in synaptic interactions, perhaps functioning to maintain synaptic stability. Thus, a defect in APP processing could lead to altered normal functions of APP at synapses, including synaptic disjunction, a potentially reversible process, followed by irreversible synaptic disconnection. In the oldest animals, retrograde abnormalities, similar to those occurring after axotomy, may appear in cell bodies. Eventually, cytoskeletal elements in perikarya become severely perturbed, leading to the formation of neurofibrillary tangle-like structures (L.C. Cork, Walker and Price, personal observations). In the brains of old animals, modest reductions in cholinergic, monoaminergic, and certain peptidergic markers have been detected,[66,67] but levels of these markers never reach the severities documented in the brains of cases of AD. Our investigations of aged primates are compatible with the elegant studies of Terry, Masliah, and colleagues[68–70] showing synaptic abnormalities in the brains of cases of AD.

In Vivo *Injections of Aβ*

Recent research suggests that Aβ can be toxic to neurons *in vivo*.[56,71–73] Consistent with the concept that Aβ may be neurotoxic is the observation that neuronal degeneration occurs when amyloid cores, isolated from the brains of individuals with AD, are injected into rat hippocampus and cortex.[74] After the *in vivo* injection of Aβ, some neurons in proximity to the site of injection develop neurofibrillary-like pathology, and, in some animals, there is evidence of neuronal loss.[72] The most dramatic neuropathologic changes are reported to occur when the Aβ peptide is injected into the brains of aged nonhuman primates.[75] These investigations raise important issues concerning the pathogenetic role of amyloid in neurotoxicity. Some groups have been able to confirm the neurotoxicity of Aβ *in vivo*, whereas others have not. The severity of Aβ toxicity seems, in part, to depend on the peptide and may be related to the aggregation state of the peptide, observations consistent with the studies of Cole and colleagues[74] in which AD plaque cores were shown to be toxic when injected *in vivo*. An upcoming volume of *Neurobiology of Aging* is devoted to this area of research and is reviewed in our Commentary in that volume.[76] At present, it is difficult to draw conclusions concerning the neurotoxicity of Aβ peptides, given the variability of the results among laboratories. If neurotoxicity cannot be produced reliably and unequivocally, then it seems unlikely that this model will be useful for analyzing pathogenetic mechanisms of AD or for testing therapeutic strategies.

Future Directions

During the past 5 years, significant advances have been made in the understanding of Aβ amyloidogenesis. Future research will focus on the trafficking and processing of APP in specific cells (i.e., neurons, astrocytes, glia, and vascular cells) and in identifying the *in vivo* processing events in control animals and in models in which the biology of APP is perturbed. The proteases leading to the generation of amyloidogenic fragments and Aβ must be defined, and mechanisms controlling these processes need to be clarified. Experimental manipulations will provide important information concerning the ways to influence the processing of APP. A major line of research will take advantage of exciting new findings in molecular genetics. Hypotheses concerning mechanisms of amyloidogenesis may be tested best by strategies in which APP mutations linked to the human disease[46] are introduced to the gene coding for APP. The effects of these mutations on the processing and formation of Aβ-containing fragments can be examined in transfected cells *in vitro* by grafting cells transfected *in vitro* into the brain and by examining lines of transgenic mice. Transgenic animals can then be studied using strategies that have proved useful in the analysis of behavioral and brain abnormalities in aged nonhuman primates. If transgenic mice reproduce the neuropathologic abnormalities that occur in aged primates, cases of AD, and individuals with Down's syndrome,[8,10,36,38–40] the role of Aβ, which began with the work of Dr. George Glenner, will have been established as central in the pathogenesis of AD.

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