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Review

Alzheimer ß-amyloid peptides: normal and abnormal localization

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Summary. Alzheimer's disease (AD) neuropathology is characterized by accumulation of "senile" plaques (SPs) and neurofibrillary tangles (NFTs) in vulnerable brain regions. SPs are principally composed of aggregates of up to 42/43 amino acid ß-amyloid (Aß) peptides. The discovery of familial AD (FAD) mutations in the genes for the amyloid precursor protein (APP) and presenilins (PSs), all of which increase Aß42 production, support the view that Aß is centrally involved in the pathogenesis of AD. Aß42 aggregates readily, and is thought to seed the formation of fibrils, which then act as templates for plaque formation. Aß is generated by the sequential intracellular cleavage of APP by ß-secretase to generate the N-terminal end of Aß, and intramembranous cleavage by γ-secretase to generate the C-terminal end. Cell biological studies have demonstrated that Aß is generated in the ER, Golgi, and endosomal/lysosomal system. A central question involving the role of Aß in AD concerns how Aß causes disease and whether it is extracellular Aß deposition and/or intracellular Aß accumulation that initiates the disease process. The most prevalent view is that SPs are composed of extracellular deposits of secreted Aß and that Aß causes toxicity to surrounding neurons as extracellular SP. The recent emphasis on the intracellular biology of APP and Aß has led some investigators to consider the possibility that intraneuronal Aß may directly cause toxicity. In this review we will outline current knowledge of the localization of both intracellular and extracellular Aß.

Key words: Alzheimer's disease, Amyloid precursor protein (APP), Beta-amyloid (Aß), Neuropathology, Cell biology

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Introduction

Alzheimer's disease (AD) is characterized by 1) the accumulation of Aß40/42 (43) peptides in senile plaques (SPs) (Glenner and Wong, 1984; Masters et al., 1985) and 2) by the presence of neurofibrillary tangles (NFTs). While Aß40 is more abundantly generated, it is Aß42 that is considered especially important because it initially deposits as parenchymal SPs in AD and Down syndrome (DS), and specifically increases in all forms of familial AD (FAD) (Selkoe, 2000). The strongest evidence for a pathogenic role for Aß comes from genetic studies of early-onset autosomal dominant forms of FAD. Genetic studies indicate that mutations in the amyloid precursor protein (APP) and presenilins (PSs) are linked to a subset of FAD, and increase Aß production (Hardy, 1997a). Aß is derived by proteolytic cleavage from its precursor APP by ß- and γ-secretase. BACE, a transmembrane aspartyl protease, has been identified as the ß-secretase (Vassar et al., 1999), and studies have suggested that PS may be the γ-secretase (Wolfe et al., 1999). All of the known FAD mutations in the APP gene result in increased production of Aß42. More than 40 FAD mutations in the PS genes have been reported (Selkoe, 1997), all of which also cause an increase in the production of Aß42 (Scheuner et al., 1996). Thus, PSs are thought to either regulate the proteolytic cleavage of APP by γ-secretase or even be the γ-secretases (De Strooper et al., 1998).

Although the molecular mechanism of Aß neurotoxicity remains unclear, Aß deposits found in the brain as SPs are composed of ß-pleated aggregations of Aß peptide. Aß is thought to be especially neurotoxic in its fibrillar form (Small and McLean, 1999). Excess deposition of Aß in the brain is a key pathological hallmark of AD and is more specific for AD than NFTs. Cumulative data support a central role for Aß in the neurodegenerative changes that eventually lead to the most common form of senile dementia, AD.

APP metabolism and Aß production

The interpretation of cellular pathways resulting in Aß production is complicated by the presence of at least three major proteolytic activities (APP secretases) involved in APP metabolism. APP is a ubiquitously expressed type I transmembrane protein, and is a member of a larger gene family including the two amyloid precursor-like proteins (APLP), APLP1 and APLP2. Both APLPs are highly homologous to APP and appear to be proteolytically processed in similar ways. The APP gene is located on chromosome 21 and APP mRNA undergoes alternative splicing to yield 8 possible isoforms. The 695, 751 and 770 amino acid isoforms predominate in the brain. The Aß region of APP is a sequence of up to 42-43 amino acid residues located partially within the ectodomain and partially within the transmembrane domain of APP. APP is cleaved by proteases designated as α-, ß- and γ-secretases (Fig. 1). Cleavages by ß- and γ-secretase at the N- and C-terminal ends of the Aß region respectively, release Aß (Haass et al., 1992; Shoji et al., 1992). α-secretase on the other hand, cleaves within the Aß sequence, thereby precluding the generation of Aß (Mills and Reiner, 1999). Most APP molecules are thought to be cleaved within secretory vesicles and/or close to or at the cell surface by α-secretase. Specifically, α-secretase cleavage occurs within the Aß domain at amino acid 17 of Aß and results in the release of secreted αAPP. Two members of the ADAM (a disintegrin and metalloprotease) family, tumor necrosis factor-α (TNFα)-converting enzyme (TACE or ADAM-17) and ADAM-10, are candidates for α-secretases (Skovronsky et al., 2000).

ß-secretase cleavage at the N-terminus of Aß produces soluble and secreted ßAPP and intracellular ß C-terminal fragments (ßCTF). BACE has been identified as ß-secretase by several groups both by genetic screening and by direct enzyme purification and sequencing (Vassar et al., 1999; Yan et al., 1999; Sinha et al., 1999). Following ß- or α-secretase cleavage, γ-secretase is required for production of Aß and p3, respectively (Haass et al., 1993). Aßx-40/42 refers to truncated Aß peptides, with "x" generally ranging from 1 and 11, but does not refer to p3 (Aß17-42), which is generated following α- and γ-cleavage. Interestingly, Aß11-40/42 was found to be the predominant Aß secreted by primary neurons, and BACE was reported to cleave especially at Aß1 and Aß11 (Gouras et al., 1998; Vassar et al., 1999). It was recently reported that in contrast to Aß1 cleavage, cleavage at Aß GLU11 (Aß11-42) is species specific (Cai et al., 2001). Aß N-terminal heterogeneity is typical for Aß deposited in AD plaques (Roher et al., 1993), but the most abundant N-terminus of plaque associated Aß peptides has yet to be determined (Lemere et al., 1996). Reports indicate that Aßx-42 is preferentially generated in the ER, whereas Aß1-40/42 peptides are predominantly made in the Golgi/TGN (Greenfield et al., 1999). It is thought that the N-terminal truncation extends to a maximum length around amino acid 11 of Aß (Gouras et al., 1998) which renders Aß even more insoluble (Pike et al., 1995). PS is increasingly thought to be γ-secretase (Wolfe et al., 1999). However, the possibility still exists that PS may be a regulatory subunit of γ-secretase, or a protein that is involved in the trafficking of proteins targeted to γ-secretase, rather than γ-secretase itself (Thinakaran, 1999). Only the successive actions of ß- and γ-secretase result in the production of Aß. APP secretases have been under intense investigation due to their role in the production of Aß and are considered to be leading targets for AD therapy. As noted, Aß is produced in a variety of subcellular locations, including the endoplasmic reticulum/intermediate compartment (ER/IC) (Cook et al., 1997; Hartmann et al., 1997; Greenfield et al., 1999), the trans-Golgi network (TGN) (Xu et al., 1997), and the endosomal/lysosomal system (Koo and Squazzo, 1994) (Fig. 2). Cook et al. found that retention of APP in the ER/IC eliminated production of intracellular Aß40, but did not alter intracellular Aß42 synthesis (Cook et al., 1997). This finding suggests that the ER/IC may be an important site for generating this highly amyloidogenic species of Aß. Subsequently, it was reported that Aß40 and 42 are generated predominantly within the trans-Golgi Network (TGN) and packaged into post-TGN secretory vesicles, while Aßx-42 is generated especially within the ER, the latter pool of Aß42 not being secreted (Greenfield et al., 1999). Koo and Squazzo showed that production and release of Aß involve the endocytic pathway, via internalization of cell surface APP by clathrin coated pit-mediated endocytosis (Koo and Squazzo, 1994). A more definitive understanding of the subcellular localization of Aß peptides, especially Aß42, may be important in developing more effective molecular based therapies for AD.

Fig. 1. Schematic diagram of the Aß protein sequence within APP. The sequence within APP that contains Aß partially embedded in the transmembrane domain is expanded and shown by the single-letter amino acid code. The residues in the box show the Aß1-42 peptide. The black circles with two bars show the lipid membrane and the area between these indicates the transmembrane domain. APP is principally cleaved at amino acids 1 and 11 by ß-secretase, 17 by α-secretase, and 40 and 42 by γ-secretase. Stars indicate the secretase cleavage sites. The bold letters above the box indicate the currently known missense mutations in APP identified in familial Alzheimer's disease (FAD) and/or hereditary cerebral amyloid angiopathy. Three-digit numbers refer to the residue number according to the APP770 isoform.

Aß senile plaque pathology

Senile plaques show a topographic distribution that bears some relationship to the stage of disease (Braak and Braak, 1991). The earliest affected regions are neocortical association areas, especially in temporal and parietal lobes. Although neurofibrillary tangles involve medial temporal structures (amygdala and hippocampus) early in the course of AD, these areas usually show relatively little amyloid deposition. It should be noted that although the density of neurofibrillary tangles is considered to parallel the duration of AD fairly well, the same is not true of senile plaques (Arriagada et al., 1992). As the disease progresses, extracellular deposits of amyloid without associated dystrophic neurites (i.e. diffuse plaques) accumulate in the molecular layer of hippocampal dentate gyrus and neostriatum. Primary sensory and motor cortices are especially spared from Aß pathology until later stages of disease. Cases of advanced AD also frequently show amyloid plaques in the molecular layer of cerebellum and even brain stem (Iseki et al., 1989).

In the pathogenesis of AD, accumulation of Aß in the brain, particularly Aß42, is considered to be an important step (Small and McLean, 1999). In 1991, the multiinstitutional Consortium to Establish a Registry for Alzheimer's Disease (CERAD) published diagnostic criteria for AD based on a semi-quantitative assessment of neuritic plaque frequency, correlated with the age of the patients, to arrive at an age-related plaque score. Aß42 is the first species deposited as Aß plaques in both AD and DS, and is the major component of plaque cores in typical late onset AD (Iwatsubo et al., 1994; Lemere et al., 1996) (Fig. 3). Early accumulation of Aß42 is viewed as a common mechanism underlying all forms of AD.

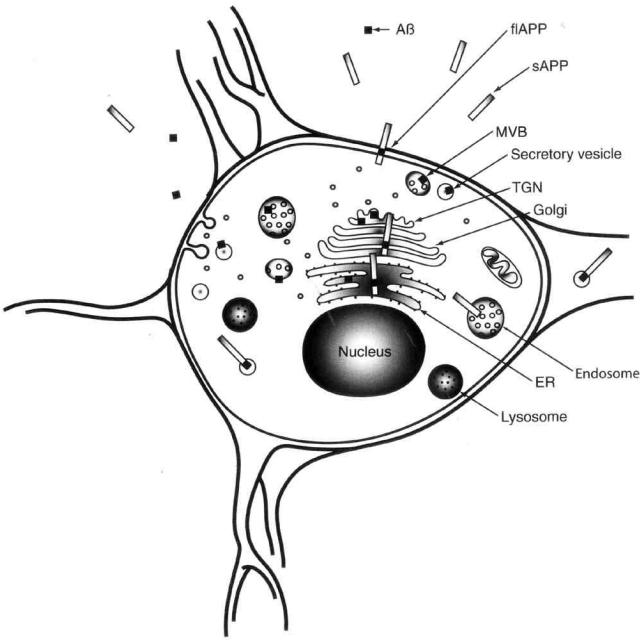

Fig. 2. Intraneuronal pathways of APP metabolism and Aß generation. APP is synthesized in the endoplasmic reticulum (ER) and is transported to the Golgi and trans-Golgi network (TGN) where most APP reside. Eventually it can be trafficked to the cell surface and is secreted or can be reinternalized from the cell surface via endocytosis into the endosomal/lysosomal system. Cleavage of APP to form Aß and other proteolytic products of APP is thought to occur in every organelle where APP resides. APP has also been localized to multivesicular bodies (MVB) where Aß may also be generated.

Substantial genetic, neuropathological, and animal modeling data indicate that Aß plays a central role in initiating a complex cascade that culminates in clinical dementia (Hardy, 1997b). The variability at the carboxyl terminus of Aß appears especially important and affects solubility. The longer Aß42 forms are deposited early as plaques in brain parenchyma while vascular amyloid in AD is composed mainly of Aß40 (Prelli et al., 1988). Aß appears to be secreted by all cells studied, indicating that ß- and γ-secretase cleavages of APP are normal events (Haass et al., 1992). Aß40 is the major form of secreted Aß. However, Aß42, the minor form, aggregates more readily and is thought to seed amyloid fibril polymerization during the early stages of plaque formation (Jarrett and Lansbury, 1993). N-truncated Aß42 was reported to be the first species deposited with AD plaque pathology in DS (Lemere et al., 1996). Several studies show that intracellular Aß42 is produced with N-terminal heterogeneity (Tienari et al., 1997; Wild-Bode et al., 1997; Sudoh et al., 1998; Morishima-Kawashima and Ihara, 1998). However, currently the identity of the plaque associated Aßx-42 is still unresolved, though a recent study suggests Aß11 species to be prominent especially with PS1 mutations (Russo et al., 2000).

Aß aggregation and toxicity

Aß deposition in senile plaques and cerebral vasculature is a pathological hallmark of AD, but whether extracellular amyloid directly contributes to the neurodegenerative process or may just be a by-product of that process remains unknown. Neurotoxicity of Aß is generally thought to be via aggregated extracellular SPs. Aggregation-related toxicity of synthetic Aß was first demonstrated for Aß in rat hippocampal cultures (Yankner et al., 1989; Pike et al., 1991a,b), and subsequently by numerous laboratories in other neuronal systems, including *in vivo* animal models (Lorenzo et al., 2000). It has been generally thought that Aß has to be assembled into highly insoluble extracellular amyloid fibrils to exert its cytotoxic effects on surrounding neurons (Yankner et al., 1989; Pike et al., 1991, 1993; Iversen et al., 1995). Aß toxicity may be mediated by the interaction of fibrillar Aß with neuronal membrane proteins (Lorenzo et al., 2000). Amyloid aggregates form insoluble filaments that are about 7-9 nm in diameter. The fibrillar forms of Aß, ß-pleated amyloid fibrils, consist of antiparallel-pleated sheets, thought to

be especially neurotoxic. Recently, a novel intermediate in the pathway of Aß fibril formation was discovered, which is called amyloid protofibril. The protofibrils are not ß-pleated, but have a secondary structure characteristic of amyloid fibrils and can give rise to mature ß-pleated amyloid fibrils. Some investigators have proposed that it is Aß protofibrils rather than ß-pleated Aß fibrils that are the critical neurotoxic entity (Walsh et al., 1999).

In the normal aging human brain, there are soluble and insoluble forms of Aß present. Although insoluble Aß, which forms amyloid plaques, correlates poorly with the degree of neuropathological damage and cognitive function in AD brain, soluble Aß has a good correlation (McLean et al., 1999; Wang et al., 1999; Naslund et al., 2000). Thus, the soluble pool of Aß is increasingly thought to be the major neurotoxic form.

Understanding how and where Aß aggregation

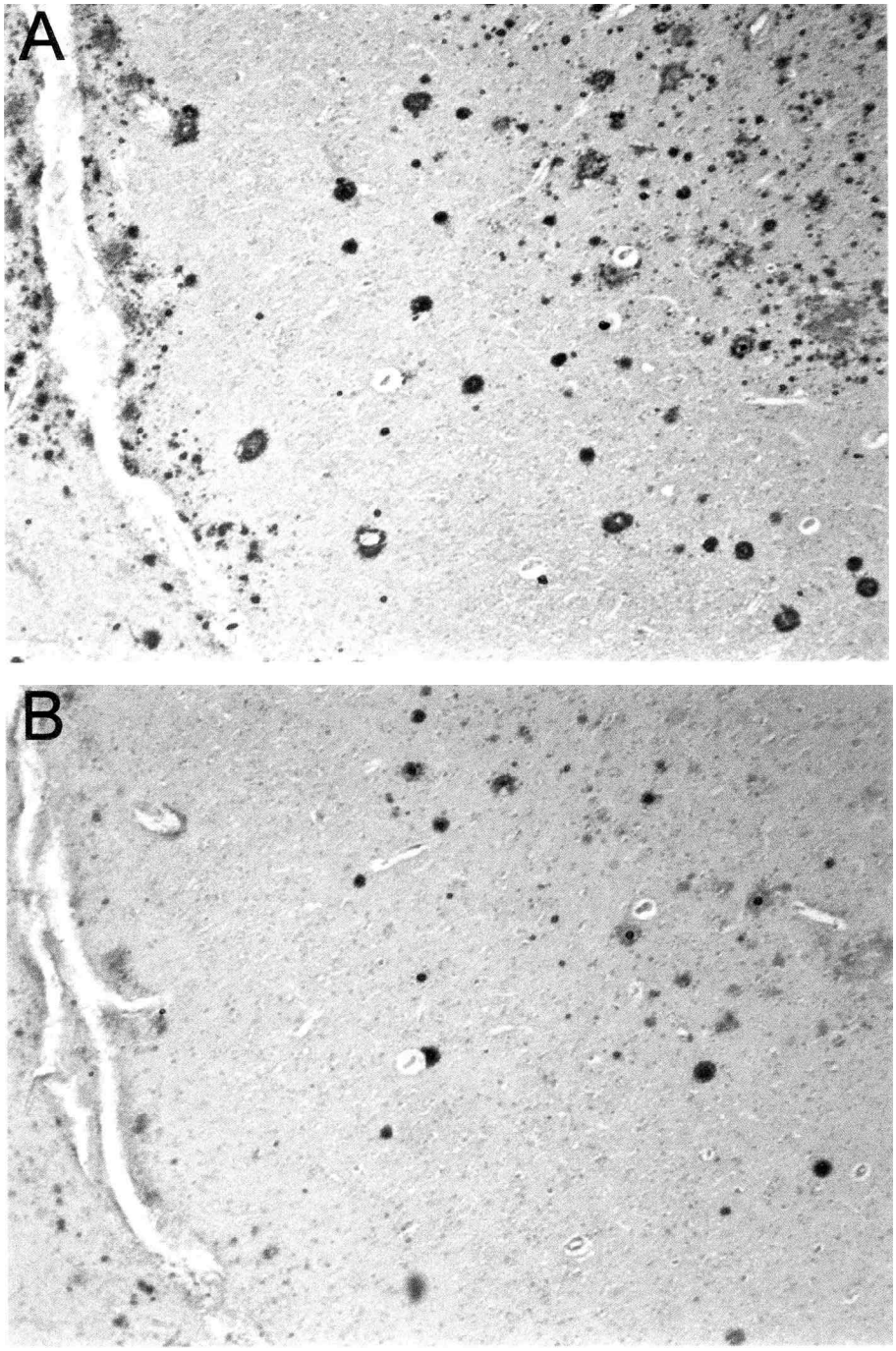

Fig. 3. Comparison between Aß42 antibody **(A)** and Aß40 antibody **(B)** immunoreactivity (IR) using serial sections from postmortem brain tissue of a patient with AD. Aß42 and Abß0 IR differ, with Aß42 being the earliest Aß species in plaques and Aß40 being more prominent in vascular amyloid deposits. x 40

begins may elucidate the mechanism of AD pathogenesis. Recent reports suggest that Aß is generated and accumulates intracellularly (Turner et al., 1996; Skovronsky et al., 1998; Gouras et al., 2000). It has also been reported that intraneuronal accumulation of Aß peptides may precede the detection of extracellular amyloid plaques and NFTs (Gouras et al., 2000), and that this may be associated with neurodegeneration (Chui et al., 1999). Masliah et al. showed by electron microscopy that neuronal processes near plaques can display fine intracellular amyloid fibrils adjacent to rough ER and coated vesicles (Masliah et al., 1996). Recent evidence suggests that neurotoxic effects of Aß may be independent of plaque formation *in vivo* (Hsia et al., 1999; Chui et al., 1999) and independent of ß-pleated Aß formation *in vitro* (Lambert et al., 1998; Hartley et al., 1999; Walsh et al., 1999).

Intracellular Aß is believed to exist within vascular smooth muscle cells (Frackowiak et al., 1994) and microglia *in vivo* (Yamaguchi et al., 2000), but histopathological evidence in neurons is less conclusive. The majority of Aß produced is Aß40; only 5-10% of secretory Aß is the disease linked isoform Aß42 (Gouras et al., 2000). Since Aß42 is the most important Aß isoform for AD and studies indicate that intracellular Aß has a high content of Aß42, up to 1/2 times or more of intracellular Aß is Aß42 (Skovronsky et al., 1998; Gouras et al., 2000), some investigators are suggesting that Aß amyloidogenesis may be initiated within neurons rather than in the extracellular space (Hartmann, 1999; Rosenblum, 1999; Wilson et al., 1999; Gouras et al., 2000). Increasingly, soluble Aß protofibrils, either intra- or extracellularly, are being considered by some to be especially important for Aß toxicity. Aß dimers appear to be preferentially generated intracellularly rather than extracellularly following secretion (Walsh et al., 1999). Recently, in late endosomes of Niemann-Pick type cells (NPC), a novel pool of Aß42 that is regulated by cholesterol has been reported, which appears to be regulated independently of the constitutively secreted Aß pathway (Yamazaki et al., 2000). Intracellular accumulation of Aß42, initially soluble and then increasingly insoluble, might impair cellular functions and directly lead to Aß plaque formation. Accumulation of APP and/or APP CTFs in the ER, Golgi, and/or endosomes, or other Aß42 containing organelles may provide ß- and γ-secretase with additional substrates, resulting in further production of intracellular Aß42. Thus a vicious cycle of intracellular Aß42 production and accumulation may begin.

While the physiological function of APP remains unknown, it was shown that APP is transported by fast axonal transport to synaptic sites (Koo et al., 1990), where APP is preferentially localized, and can be transcytotically transported to dendrites (Schubert et al., 1991). This synaptic and dendritic localization of APP supports the possibility that Aß42 can be generated at synaptic sites where it could induce early impairment of synapses.

Since SPs are observed in the extracellular space in AD brain, SPs have generally been thought to arise from the gradual extracellular aggregation of Aß secreted from neuronal cells. But recent histopathological studies suggest that at least a subset of SPs appear to arise from intracellular Aß aggregation (Gouras et al., 2000; D'Andrea et al., 2001). These studies noted nuclear remnants, seemingly within Aß-burdened neuronal cells found at the center of developing SPs. Moreover, cytoplasmic proteins (i.e. cathepsin D) were found in the extracellular space occupied by SPs, but not outside the boundaries of SPs. Additional evidence for a neuronal origin of SPs comes from the demonstration that SPs contain especially neuron specific mRNAs (Ginsberg et al., 1999). But these studies do not definitively prove that aggregated Aß originates from intracellularly accumulating Aß within neurons or neuronal processes.

Employing hippocampal slice cultures with exogenous Aß42 peptide, Aß42 is demonstrated to be internalized selectively within neurons and as a consequence also to induce a buildup of endogenous neuronal ßCTF (C99), the amyloidogenic precursor to Aß (Bahr et al., 1998). Thus, release of Aß42 could also increase the levels of intracellular Aß42 and thereby induce cell death. Dying neurons might rupture and release the accumulated intracellular Aß42 and C99 into the extracellular space.

Over the past two decades, the small but highly insoluble Aß peptide has taken center stage in AD research. While a multitude of proteins are altered in the brain as a result of the extensive damage caused by the AD pathological process, more than any other peptide, Aß is specifically linked to the disease. The neuropathological hallmark of AD, senile plaques, are composed of aggregated Aß peptides, and mutations in the three known genes causing FAD, including mutations in the Aß precursor protein at or close to the Aß domain, all cause an increase in Aß42. Major challenges for AD research include uncovering the precise biological mechanism by which Aß accumulates and causes disease, and how and whether arresting Aß accumulation can lead to effective treatment for AD. A more precise understanding of the subcellular localization of Aß peptides within neurons may be critical in devising novel molecular based therapies for this most common and ever more prevalent neurodegenerative disease of aging.

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