The Presence, Origin, and Significance of Aβ Peptide in the Cell Bodies of Neurons

WILLIAM I. ROSENBLUM, MD

Abstract. Interest in the Aβ amyloid in Alzheimer disease (AD) has largely focused on the Aβ in the neuropil, an extracellular site. Here much attention has been given to the possibility that Aβ acts as a neurotoxin. However, increasing emphasis is now being given to the relationship between neurofibrillary tangles (NFT) and the degree of cognitive decline, as opposed to the relationship between decline and senile plaques, the sites of extracellular Aβ deposition. This review focuses attention on the existence and significance of Aβ in the cell body of the neuron. The review brings together diverse strands of literature indicating: (1) the tau-positive, paired helical filaments that are the main component of NFT are not themselves the source of the amyloid-like staining (Congo red birefringence) of PHF, and are not, in fact, an “amyloid”; (2) there is Aβ within the cytoplasm of neurons affected by AD and in other conditions characterized by tau-positive neurofibrillary tangles; (3) peptides derived from portions of the Aβ precursor can bind to PHF; (4) the affected neurons are the source of extracellular Aβ in their vicinity and are unable to maintain the synaptic structures that depend upon the integrity of the neuronal cell body; and (5) debates about whether the intracellular Aβ is an amyloid depend upon beliefs about its tertiary structure and assumptions concerning the relationship between the size of self-aggregating Aβ molecules, their tertiary structure, and their ability to generate Congo red birefringence without necessarily being detected as ultrastructural filaments 5–10 nm wide.

Based upon this literature, it is suggested that the Congo red birefringence generated by NFT is caused by Aβ, intimately bound to the NFT. Moreover, whether defined as an amyloid or not, the Aβ in the neuronal cell body indicates an abnormal processing of the precursor molecule on the way to its ultimate transmembrane domain. Deranged neuronal functioning, which leads to this abnormal processing and/or the intracellular Aβ itself, may be the cause of subsequent functional and morphologic abnormalities in the brain.

Key Words: Aβ amyloid; Alzheimer disease; Neurofibrillary tangles; Perikaryon.

INTRODUCTION

Neurofibrillary tangles (NFT) and senile plaques (SP) have long been the features used by neuropathologists to make the diagnosis or to suggest the presence of Alzheimer disease (AD) (1). Recent reports suggest that the numbers and location of NFTs have more importance than SP for both the diagnosis and an understanding of the pathogenesis of AD (2, 3). However, the vast majority of studies on intraneuronal pathology in AD focus on NFT formation and its relationship to the presence of the abnormally phosphorylated cytoskeletal protein called tau (1). Less attention has been given to the presence and importance of the Aβ amyloid peptide (Aβ) within the perikaryon.

The presence of Aβ within the perikaryon is important for 2 reasons. First, it reflects the fact that the neuron can be an important source of extracellular Aβ. This is located in SP within the neuropil (1). Many investigators have suggested that this amyloid can act as a neurotoxin (1). Recent emphasis on NFT does not negate the potential importance of extracellular amyloid acting as a neurotoxin within the SP, nor does this emphasis discount the large and growing body of evidence linking this amyloid burden to structural damage (1, 4–10). Secondly, the presence of Aβ within the perikaryon serves as a marker for a pathological cellular biochemistry which may be the cause of, or the initial step in, a cascade of pathological alterations in neuronal form and/or function. In addition, intraneuronal Aβ provides a putative toxin that might act within the cell rather than only in the extracellular space of the neuropil.

This review focuses on the existence and possible role of the intraneuronal Aβ in the pathogenesis of Alzheimer disease (AD). In so doing, it is inevitable that there be some reference to the literature concerning SP, extracellular Aβ, and its suggested neurotoxicity. However, because these subjects have been extensively reviewed by diverse authors, and because the main focus of the present review is Aβ within the neuronal cell body, references to the other important aspects of Aβ will be limited.

DOES AN AMYLOID EXIST WITHIN THE PERIKARYON?

The presence of intraneuronal Aβ peptide may not have received the attention that extracellular Aβ has received because the latter frequently develops post translational changes that bestow the characteristics of an amyloid (11). These changes involve beta pleated sheet formation and self-aggregation of the pleated sheet molecules to form filaments 5 to 10 nm wide. The beta pleated sheets, thought by some workers to arrange themselves in an antiparallel array (12), but recently shown to have a parallel array (13), trap the molecules of certain dyes to produce characteristic staining reactions (11, 12, 14–16). Chief among the latter is the staining by Congo red and the green birefringence produced when the stained structures are viewed microscopically with polarized light. In fact, contemporary definitions of amyloid

From the Division of Neuropathology, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia.

Correspondence to: William I. Rosenblum, MD, 305 Tarrytown Drive, Richmond, VA 23229.

575
rely on all 3 of the aforementioned features: beta pleated sheet formation, green birefringence, and the presence of characteristic filamentous when viewed by electron microscopy (11). The beta pleat configuration is assumed when Congo red birefringence is found, but can only be proven by demonstrating a characteristic X-ray diffraction pattern in samples extracted from brain. In many of the extracellular deposits of Aβ found in Alzheimer disease, usually in association with local degeneration of dendrites and axon terminals, the Aβ displays all 3 characteristics of an amyloid. In contrast, there has usually been failure to find characteristic amyloid filaments within the neurons. This is so even though both Congo red birefringence (17–19) and the appropriate x-ray diffraction pattern (20) have been demonstrated in neurons containing neurofibrillary tangles or in extracts of such neurons, and when Aβ can be demonstrated in such neurons by immunohistochemistry (for examples see ref 17, 18, 21).

One must assume that the failure to consider the potential importance of the intraneuronal Aβ is related to the belief that in the absence of characteristic filamentous, no such amyloid can be said to exist within the perikaryon. In fact, those who first demonstrated Aβ within tangential bearing neurons ascribed this to an epitope actually residing within the paired helical filaments (PHF) that form the tangle (17). This implied that, by coincidence, the PHF were a different amyloid than that contained in extracellular SP, and that by chance this different amyloid shared epitopes with the extracellular Aβ. This conclusion was apparently based on the belief that PHF could simultaneously take on a beta pleated sheet configuration, which would account for the Congo red staining and birefringence of such neurons. This belief was supported by a single X-ray diffraction study (20) that reported that PHF extracted from tangle bearing neurons gave the diffraction pattern of amyloid.

Very recently, using atomic force microscopy, the PHF have been reinterpreted as twisted ribbons (22). The relevance of the newly interpreted tertiary structure to the selective binding of dyes and to Congo red birefringence remains to be determined. In any case, one may still read (e.g. ref 18, 19) that the amyloid "signal" generated by Congo red staining of tangle bearing neurons is produced by the PHF. However, earlier statements (17) calling NFT an amyloid were made prior to our knowledge that the NFT consist largely of a hyperphosphorylated form of the cytoskeletal protein known as tau (1). Therefore, to believe now that PHF are an amyloid that binds Congo red, one must assume that hyperphosphorylated tau forms not only paired helical filaments or twisted ribbons, but also that these are simultaneously beta pleated sheets. Alternatively, it may be that some other kind of molecular array is binding the Congo red and other dyes to give the NFT the tinctorial properties of an amyloid (14). However, in 1995 a thorough reinvestigation of the supposed amyloid-like properties of PHF was carried out. It was shown that PHF have none of the properties of an amyloid and do not show the X-ray diffraction pattern of beta pleated sheets (23). The authors of this study concluded that in the previous (20), and to my knowledge the sole, study showing this property of PHF, the latter were actually contaminated by bona fide Aβ from the affected brains.

Because of the significance of this conclusion it is important to discuss the differences between the methods used to isolate PHF in the 2 conflicting studies (20, 23). The first (20) used a technique said to recover "neurofibrillar tangles" (24). The second (23) isolated and further purified the "soluble PHF fraction." However the electron microscopic pictures illustrated in each study (20, 23) show apparently identical paired helical filaments. If the filaments in the earlier study contained chemical elements that were stripped from the filaments in the later study, this would not prove that such elements (e.g. beta pleated sheet molecules) were an intrinsic part of tau, now known to be the essential element of the PHF. Rather, as asserted by Schwers et al (23), it would simply show that the "purified" PHF isolated by Kirschner et al (20) contained elements, not discernible on the electron microphotographs, that were bound to but not an integral part of what we now know is tau. In other words, the morphologic structure called a neurofibrillary tangle is, on both light and electron microscopy, a conglomerate of 2 or more molecular species. Only one of these, tau, is the essential element of the PHF and is not an amyloid. Another, Aβ, is bound to the tau and gives the neurofibrillary tangle the tinctorial properties of an amyloid.

If the latter conclusion is correct, then the Congo red signal and similar dye-binding signals from dyes that selectively stain amyloid must be coming from molecules other than those in the PHF themselves. If so, then why are the narrow straight filaments that characterize the ultrastructure of most amyloids not seen in neurons? The question assumes that the Aβ peptide forms such filaments as soon as it forms aggregates sufficient to bind the Congo red. However, there are no studies that prove this assumption and the existence of dye binding multimers without identifiable filamentous form remains an open possibility (25, 26). In fact, microaggregates of Aβ bind Congo red in extremely low concentrations (300–500 ng/ml) without displaying the light scattering properties of fibrils (15, 26). Higher concentrations of Aβ are required for fibril formation (26).

In addition to raising the possibility that small multimers of Aβ are responsible for the Congo red signal in PHF bearing neurons, one should also point out that other portions of the Aβ precursor molecule (BPP) have been shown to "decorate" PHF, and the PHF bind the βPP fragment (27, 28). The PHF may also bind Aβ (29). One may then ask whether such binding assists the Aβ in assuming an
ordered beta pleated sheet form sufficient to bind Congo red without forming filaments of the Aβ molecule.

Aβ PEPTIDE IS PRESENT IN THE NEURONAL CELL BODY

The preceding section presents diverse reasons for abandoning the idea that the PHF are an amyloid that shares epitopes with extracellular Aβ. This idea originated before the tau-dependent structure of PHF was known. The preceding section asserts instead that the amyloid-like staining of the tangle bearing neurons is more likely due to Aβ aggregates in the perikaryon. If one demands ultrastructural filaments of less than 10-nanometer width to define amyloid, then the characteristic dye staining is insufficient grounds for saying that the perikaryon contains amyloid. However, such a narrow definition is arbitrary and grew out of the development of electron microscopic technology. Moreover, those who called NFT an amyloid (e.g., 17, 19) did so in the absence of the 5–10-nm-wide straight filaments typical of amyloids in general. Therefore, if one demands the presence of such filaments to prove that Aβ exists in the cell body as an amyloid apart from the NFT, then one is inconsistent in insisting that the PHF, which do not have such an ultrastructure, are an amyloid. However, whether one agrees or disagrees with the idea that Aβ in neurons is often in the form of an amyloid, one must acknowledge the numerous studies that show that the Aβ peptide is present in tangle bearing neurons.

Over the last twenty years there have been numerous reports from diverse laboratories that antibodies directed against Aβ can stain the neuronal cell body. Apparently this staining is sometimes localized to the sites of lipofuscin deposits in the perikaryon (30, 31). But often the immunopositive material is most notable in neurons containing NFT. This staining is sometimes diffuse, but often delimits structures with forms identical to that of NFT stained either with silver stains or with antibodies directed against tau (17, 21, 32–34). The latter suggests that the Aβ is in intimate contact with the PHF that make up the tangles.

One of the reasons for doubting the presence of Aβ within the cell has been the often cited fact that amyloid fibrils are insoluble (1, 11). This observation leads naturally to the question of how the amyloid could have entered the neuron; a question that frequently arises during informal discussions of the importance of Aβ in AD. However, if we focus on the importance of the Aβ peptide rather than on the insoluble tertiary structure which it may finally take on, the question of passage of insoluble material need not arise. Moreover, extracellular Aβ in so-called “diffuse” senile plaques can exist in Congo red negative, and largely, but perhaps not entirely (35), non-filamentous form, which one might imagine could enter the perikaryon. An alternative view suggests that the portion of the membrane spanning βPP, which contains the Aβ fragment, is cleaved from that precursor and internalized for processing into Aβ within the neuron (1).

However, neither of these suggestions are needed to explain the intracytoplasmic location of Aβ. There is now well-established evidence that the Aβ is an intracellular product of an abnormal, alternative processing of the βPP prior to the arrival of the βPP in its normal transmembrane domain (36–43). The normal processing of βPP with some cleavage into peptides more soluble than the Aβ peptide, occurs in the trans–Golgi network (38), from which clathrin-coated vesicles may arise which transport the βPP to it transmembrane location. The abnormal processing occurs in a different acidic compartment. The relative strengths of the 2 paths may be controlled by phosphorylations under the control of kinases at sites still to be identified (42, 43). Thus, the accumulation of significant amounts of Aβ within the cytoplasm does not require internalization from an external source.

THE POTENTIAL IMPORTANCE OF GLYCOSAMINOGLYCANS IN THE NEURON

A wide variety of glycoproteins are found in concert with diverse amyloids whenever amyloid is deposited in any part of the body (44). These glycoproteins appear to precede the formation of the insoluble fibrils of the amyloidogenic peptide. The precise role played by the glycoproteins in guiding or aiding self-aggregation and/or fibril deposition of the peptide has not been determined. However, the fact that deposition of the glycoproteins precedes formation of the mature amyloid suggests that the former may be an essential determinant of the latter. Thus it is important to note that in Down syndrome babies, neurons with cytoplasm full of glycosaminoglycans have been demonstrated as early as 1 day of life (45). Many of these neurons are destined to become tangle bearing in subsequent years. The tangles have the same properties as those of AD patients without Down syndrome (1, 45); and Down patients with and without AD have significant deposits of Aβ in their brains. Thus our attention is called once again to a pathologic process within the perikaryon that leads to both Aβ production and the production of PHF. Since deposition of glycosaminoglycans within the neuronal cell body is distinctly abnormal particularly at an early age (45), it might even be that this, rather than the later abnormal processing of βPP, is the trigger for events that lead to abnormal form and function of neurons.

IS INTRANEURONAL Aβ HARMFUL AND/OR A MARKER FOR IMPORTANT INTRACELLULAR DAMAGE?

Again the attention paid to the question of amyloid toxicity has centered on the role of Aβ in the extracellular space of the neuropil rather than the cell body (1). There are 2 forms of Aβ; one is 40 amino acids long and the
other is 42 amino acids long. The latter is more insoluble and neurotoxicity is more often ascribed to the 1–42 fragment (1, 4, 18). Thus toxicity is most often ascribed to the fibrillar, “insoluble” form of Aβ. This assertion raises a serious and unsolved question: how can insoluble material effect adjacent structures? Recently, diffusible, non-fibrillar ligands of the 1–42 fragment have been found to be extraordinarily toxic to neurons in tissue culture, and to have immediate inhibitory effects on neural function in the hippocampus (46). Since the initial deposition of Aβ[1–42] in the neuropil (1), and certainly in the neuron, is in the prefibrillar structure, the stage is set for toxic events produced by this molecule whether or not, at that point, we call it an “amyloid.”

The SP that characterize AD are of various types. This may explain the wide variation between reports that test the link between plaque concentration and degree of cognitive decline. The ability to detect such a relationship may depend upon the type of plaque that is counted and this in turn depends upon the staining technique that is used (47, 48). It has been known for many years, but is frequently overlooked, that the best correlations between mental function and plaque count appear when only tau positive (49) neuritic plaques are used in the analysis (50). In this regard, it is of interest that such plaques have been reported to congregate where there are neurofibrillary tangles (49). This implied that the paired helical filaments and tau are produced in both the cell body and the processes of the same set of neurons. Two reasonable and not mutually exclusive hypotheses explain this: 1) either the cell body and processes suffer from an identical biochemical aberration; or 2) some product of the cell body is responsible for inducing the formation of tau and PHF. Finding a neurocentric gradient of Aβ and of βPP around the cell body of neurons containing either Aβ (52) or containing dramatic increases in intracellular βPP (51, 52) supports the hypothesis that the amyloid produced by the cell body leaves the cell in soluble, nonfibrillar form. It may act as a toxin in the neuropil, but one must also consider the possibility that deranged intracellular metabolism has already set in motion events that can produce degeneration of nerve endings and dendrites, resulting in tau positive neuritic plaques which also can bind Aβ. In either event, our attention should be drawn back to the pathologic processes within the cell body, and hence back to production and accumulation of Aβ within it.

If the events within the cell body are critical for the cognitive decline in AD, it is not surprising that some workers find an excellent correlation between loss of synaptic components and cognitive decline (53, 54). Whatever the role of extracellular factors may or may not be in attacking synapses, the synapses clearly depend upon the normal functioning of the cell body for their integrity since the metabolism of the cell body is responsible for the protein and transmitter synthesis that maintains both structural and functional characteristics of the synapse. In AD the harmful effect on synapses of pathologic changes in the cell body is illustrated by the finding that neurons with NFT have decreased levels of the RNA message for synaptic proteins (55). This conclusion was based upon comparison with non-tangle bearing neurons in the same cases. It may be that more subtle defects in message levels will be found in neurons without NFT but staining for either βPP or for Aβ.

In AD the immunopathologic evidence and the chemical studies show an intimate relationship between Aβ and neurofibrillary tangles as described above. Further evidence for a causative role of the Aβ is the fact that all of the genetic variations associated with an increased incidence of AD have thus far been associated with an increased production of Aβ (7–10). This includes both the presence of the extra chromosome 21 in Down syndrome and the alterations in the genes controlling expression of so called presenilins. One should not allow debates about whether, and at what loci, the increased Aβ is in the form of an amyloid to obscure the important fact that its production is increased. If we accept the neuron as the source of this amyloid, then we should also consider the potential significance of this aspect of deranged intracellular metabolism for producing a decline of function and additional morphological alterations, like NFT, in the cell body.

With this suggestion in mind, it is important to point out that AD is not the only condition in which a relationship between Aβ “burden” and NFT has been found. NFT are produced by head injury and it is now known that after head injury there is significant extracellular deposition of Aβ especially near neurons containing large amounts of βPP as a result of the head trauma (51). The NFT in the brains of prizefighters contains Aβ itself (34). In the people of Guam, where the Parkinsonian-dementia complex is rife, NFT can also be labeled with anti-Aβ (32, 33). Moreover, only cases that could be so labeled also demonstrated SP (32), detected with the antibody against Aβ. Neurons that were not Aβ labeled were not associated with SP. This strongly implies that the source of Aβ in the plaques was the neuron, and that intraneuronal Aβ was not, at least in Guamanian Parkinson-dementia patients, an intrinsic part of the NFT (which contain tau), but was merely bound to them. At the same time, a second population of neurons was found in Guamanians. These neurons contained NFT without Aβ. Such brains were free of plaques. The presence of NFT without amyloid indicates that epitopes for Aβ are not an intrinsic part of tau and also show that Aβ is not necessary for tangle formation. However, the existence of such tangle bearing neurons does not disprove the hypothesis that Aβ, when present in the cell, is the initiator of NFT formation or a marker for processes that initiate NFT formation.
Aβ PEPTIDE IN THE NEURON

In this regard one should note that in another organ, skeletal muscle, the pathogenesis of inclusion body myositis has now been linked to the intracellular production and deposition of Aβ and these cells, like neurons containing Aβ peptide, contain tau in the form of PHF (56–58).

CONCLUSIONS

Abundant evidence shows that Aβ exists in neurons that also contain tau positive NFT. Neurons that contain NFT have been associated with tau positive neuritic senile plaques. Both NFT and neuritic tau positive plaques have been more readily correlated with cognitive decline than total plaque counts or plaque counts that fail to segregate tau positive neuritic plaques. Aβ, and particularly the 1–42 amino acid variant, can form fibrillar Congo red birefringent material in which the Aβ has a beta pleated sheet tertiary structure. These features define an amyloid. However, attention directed toward the possible toxicity of this amyloid in the extracellular space, and particularly in the vicinity of SP, may have deflected attention from the potential importance of the aberrant processing of the Aβ precursor protein (BPP) in the neuronal cell body. It is this aberrant processing that makes the neuron a source of Aβ within both the neuron and the extracellular space. During this processing the Aβ exists first in a nonfibrillar form. Congo red birefringence of the NFT have been ascribed to a binding of the dyes directly to the PHF, which were thought to possess not only the paired helical structure but also a beta pleated sheet configuration. Later work shows that purified PHF do not, in fact, have a beta pleated sheet configuration or any other characteristic of an amyloid, suggesting that the earlier studies making this assertion used material contaminated with Aβ.

Tangles can be stained with antibodies directed against Aβ. When the PHF of tangles were thought to be an amyloid, the staining by anti-Aβ was thought to reflect a coincidental sharing of epitopes also present in the extracellular amyloid derived from beta amyloid precursor protein and found in extracellular SP. In view of later work showing that PHF are not an amyloid, it seems more likely that the staining of tangles with anti-Aβ is due to the proximity of Aβ molecules to the PHF in the tangle. The sole evidence against this explanation for the amyloid staining properties of tangles is the failure to find, within the neuron, 5–10-nm filaments that characterize typical amyloid on electron microscopy. However, contrary to widespread belief, it has not been definitively established that binding of Congo red and birefringence require the assembly of beta pleated sheets into filaments recognized by the electron microscope. It is theoretically possible that smaller multimers of beta pleated Aβ might bind Congo red and demonstrate birefringence, either alone, or if first bound to the paired helical filaments of the NFT. This provides an alternate explanation for both the Congo red staining of the tangles and the staining of tangles with anti-Aβ.

In any case, it is now well established that the neuron makes Aβ and that this aberrant metabolism of the precursor protein takes place within the cell body where the Aβ will then initially reside. Thus, there is an intraneuronal source for both the Aβ that may decorate the tangles and for the Aβ in senile plaques. Most attention has been given to the possibility that the relatively insoluble 42 amino acid form of Aβ is neurototoxic in the extracellular space. This emphasis has perhaps been responsible for the relative lack of attention to the existence of Aβ within the cell body and the now well-documented demonstration of its production within the cell body. This abnormal processing of the amyloid precursor protein occurs not only in AD, but apparently after head trauma which also leads to tangle formation and in the people of Guam who are prone to develop a distinctive form of dementia accompanied by tangle formation. These associations of intraneuronal beta formation with abnormalities of the cytoskeleton, synaptic loss (in the case of AD), and cognitive decline suggest that the aberrant processing of BPP and the formation of Aβ within the neuronal cell body may be key events in a cascade of pathologic changes leading to dementia. Attention to this possibility should not be deflected by debates as to whether the Aβ initially meets all the structural requirements of an amyloid, or by the failure to find amyloid-like filaments in neurons shown to be labeled by antibodies to Aβ. Nor should attention to the possible toxic effects of supposedly insoluble filaments of Aβ outside the neuron deflect from the recent demonstration that soluble Aβ—which must exist in the neurons producing it—contains highly neurotoxic epitopes.

Finally, attention directed toward the formation of intraneuronal Aβ need not imply belief that the Aβ is toxic. The initial abnormal processing of the precursor protein may have other consequences not yet delineated. Prevention of the abnormal processing might thus be beneficial to potential or present victims of AD for diverse reasons not restricted to the removal of a toxin.

REFERENCES


41. Haass C, Hung AY, Schlossmacher MG, Teplow DB, Selkoe DJ. β-Amyloid peptide and a 3-kDa fragment are derived by distinct cellular mechanisms. Biochem Chem 1993;268:3021-24

42. Xu H, Sweeney D, Greengard P, Gandy S. Metabolism of Alzheimer β-amyloid precursor protein: Regulation by protein kinase A in intact cells and in a cell-free system. Proc Natl Acad Sci USA 1996;93:4081-84


44. Kislinsky R, Fraser, P. Proteoglycans and amyloid fibrillogenesis, In: Bock GR, Goode J, eds. The nature and origin of amyloid fibrils.


56. Askanas V, Engel WK. Does overexpression of bAPP in aging muscle have a pathogenic role and a relevance to Alzheimer’s diseases? Am J Pathol 1998;153:1673–77
