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**Amyloid-β in Alzheimer Disease: The Null versus the Alternate Hypotheses**

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**ABSTRACT**

For nearly 20 years, the primary focus for researchers studying Alzheimer disease has been centered on amyloid-β, such that the amyloid cascade hypothesis has become the “null hypothesis.” Indeed, amyloid-β is, by the current definition of the disease, an obligate player in pathophysiology, is toxic to neurons in vitro, and, perhaps most compelling, is increased by all of the human genetic influences on the disease. Therefore, targeting amyloid-β is the focus of considerable basic and therapeutic interest. However, an increasingly vocal group of investigators are arriving at an “alternate hypothesis” stating that amyloid-β, while certainly involved in the disease, is not an initiating event but rather is secondary to other pathogenic events. Furthermore and perhaps most contrary to current thinking, the alternate hypothesis proposes that the role of amyloid-β is not as a harbinger of death but rather a protective response to neuronal insult. To determine which hypothesis relates best to Alzheimer disease requires a broader view of disease pathogenesis and is discussed herein.

**Amyloid-β and Alzheimer Disease: The Null Hypothesis**

A wealth of evidence implicates amyloid-β (Aβ) in the pathogenesis of Alzheimer disease (AD), leading to the formulation of the amyloid cascade hypothesis (Fig. 1), i.e., the null hypothesis (Hardy and Higgins, 1992; Hardy, 2006). Casual observation of post mortem brain tissue of affected individuals makes it quite obvious why Aβ is the primary suspect in disease pathogenesis, because Aβ is the major constituent in two of the most distinctive histopathologies, namely senile plaques and cerebral amyloid angiopathy (Glenner and Wong, 1984a,b; Masters et al., 1985). The destruction surrounding Aβ is evident from a close examination of histological preparations in the immediate vicinity of senile plaques and amyloid angiopathy, respectively, where degenerative neuronal processes and smooth muscle cells surrounding and infiltrating the Aβ deposits are found, respectively (Geddes et al., 1986; Kawai et al., 1992). Furthermore, areas that are severely affected by disease, namely the hippocampal and frontotemporal cortices, show colocalization between Aβ plaques and neuronal cell death (Rogers and Morrison, 1985). Investigators have explored whether Aβ is toxic to neurons in both in vitro culture assays and in the intact brain of animals. Initially, results of these experiments were extremely inconsistent, stemming from variability among commercial preparation of the peptide and the lack of proper control over whether Aβ was aggregated into fibrils of β-sheet conformation (Cotman et al., 1992). However, it is now firmly established that fibrillation of Aβ is needed to obtain neurotoxic effects (Pike et al., 1993; Lorenzo and Yankner, 1996; Yankner, 1996) and that, under many circumstances, such fibrils are inherently toxic to both neurons and clonal cell lines in culture (Yankner et al., 1990; Pike et al., 1991). The neurotoxicity of Aβ peptide in vivo was likewise measured by infusion of the peptide to various animal
Null Hypothesis

Fig. 1. The null hypothesis. Increased Aβ, often through genetic influences, leads to oxidative stress that then leads to neuronal death.

The strongest evidence for the crucial role played by Aβ in AD pathogenesis has been the characterization of the mutations that underlie familial early onset cases of the disease. All of these inherited mutations directly or indirectly affect both the processing and accumulation of Aβ. Familial Alzheimer disease (FAD) is associated with point mutations in amyloid-β protein precursor (AβPP) in regions that are involved in the proteolytic processing of the peptide (Goate et al., 1991; Lendon et al., 1997). It is thought that these mutations accelerate the onset of AD into the fourth decade by increasing the ratio of Aβ1-42/Aβ1-40, thereby increasing the relative amount of the more fibrillogenic form (Suzuki et al., 1994). A double mutation at positions 670/671 (Swedish mutation) increases the production of total Aβ and thereby increases the load of Aβ1-42 without changing the relative ratio (Citron et al., 1992; Cai et al., 1993). The fact that an increase in total Aβ load accelerates the deposition of Aβ is supported by the neuropathology seen in patients with Down syndrome, a disorder caused by trisomy of chromosome 21, where the AβPP gene is localized. It is thought that the overexpression of AβPP in these individuals (Greenberg et al., 1996) causes the formation of Aβ plaques very similar to those seen in AD. The most common form of FAD is caused by mutations in one of the two presenilin genes (PS1 on chromosome 14 or PS2 on chromosome 1) (reviewed by Gooch and Stennert, 1996). Recently, PS has been identified as one of the component of γ-secretase, and FAD mutations increase the activity of γ-secretase, although the alternate roles of PS other than γ-secretase (Haass and Steiner, 2002) in AD pathogenesis have been suggested as well (Herms et al., 2003; Tu et al., 2006). Most importantly however, missense mutations in the presenilin genes increase the ratio of Aβ1-42/Aβ1-40 (Citron et al., 1997; Tomita et al., 1997). Finally, one allele of the apolipoprotein E gene, namely apolipoprotein e4, predisposes individuals to the development of late-onset AD (Corder et al., 1993). Of the three alleles (also including apolipoproteins e2 and e3), apolipoprotein e4 has the greatest affinity for Aβ, is found associated with senile plaques, and is thought to accelerate fibrillogenesis (Wisniewski et al., 1994). Interestingly, apolipoprotein e2 inhibits fibril formation and is protective against the development of AD (Corder et al., 1994).

The above evidence implicating Aβ in AD pathogenesis led to the supposition that generation of transgenic animals that either overexpress AβPP or a mutation in AβPP that affects processing of the full length protein, thereby leading to an increase in the Aβ1-42/Aβ1-40 ratio, may mimic the pathophysiology that is seen in AD. Taken as a group, the various transgenic mouse strains that have been produced thus far have demonstrated that overexpression of mutated AβPP, but not wild-type AβPP or overproduction of the Aβ1-42 peptide fragment, is alone sufficient to cause deposition of the peptide into senile plaque-like structures (reviewed by Holsher, 1998). Indeed, despite the fact that each of the different constructs yielded somewhat different phenotypes, some aspect of AD pathophysiology is apparent in each of them. For example, Games and colleagues created a transgenic mouse expressing human AβPP with the V717F mutation at 10 times the endogenous level; these animals developed Aβ plaques in the hippocampus, cerebral cortex, and corpus callosum by 6 to 9 months of age and also showed synaptic loss and astrocytosis (Rogers and Morrison, 1985). Another popular model is Tg2576 mouse, which overexpresses AβPP con-
Amyloid-β and Alzheimer Disease: The Alternate Hypothesis

The first and foremost argument that Aβ is not the initiator of AD is that deposition of Aβ into senile plaques is by no means specific to AD patients and is instead a marker of normal aging (Davies et al., 1988). Therefore, the strong association of Aβ in AD may simply mark an acceleration of age-related deterioration. Support for this view can be found with the number of plaques in cognitively normal individuals rivaling those seen in advanced disease (Mann et al., 1992; Schmitt et al., 2000). Furthermore, there exists only a weak correlation between the burden of Aβ and neuronal loss or cognitive impairment (Giannakopoulos et al., 2003; Guillozet et al., 2003). Furthermore, increased Aβ production and deposition are also seen as a response to injury in the central nervous system, especially following ischemia and head trauma (Gentleman et al., 1993; Roberts et al., 1994; Geddes et al., 1997). Aβ deposition in aging and following injury might be a compensation for the primary insult (Lee et al., 2004b). This is not to imply that, in attempting to respond to cellular stresses, Aβ cannot lead to cellular destruction. However, it does require an underlying pre-existing stress, i.e., the presumptive etiology of AD.

A review of the literature would indicate that the underlying stress is of energetic origin, because a shortage of energy supply [and Ca(II) overload] induces an up-regulation of AβPP expression. Ischemia, hypoglycemia, and traumatic brain injury, a condition that has been shown to put neurons under metabolic stress (Xiong et al., 1997), all up-regulate AβPP and its mRNA in animal models and culture systems (Hall et al., 1995; Jendroska et al., 1995; Yokota et al., 1996; Shi et al., 1997; Murakami et al., 1998). Not only does energy deficiency and Ca(II) dysregulation promote AβPP expression, they also route the metabolism of AβPP from the non-amyloidogenic to the amyloidogenic pathway. For example, loss of mitochondrial energy metabolism alters the processing of AβPP to generate amyloidogenic derivatives (Gabuzda et al., 1994; Mattson and Pedersen, 1998), and likewise, oxidative stress increases the generation of Aβ (Frederikse et al., 1996; Neve and Robakis, 1998; Paola et al., 2000). This finding can be applied to the role of AβPP as an acute-phase reactant that is up-regulated in neurons, astrocytes, and microglial cells in response to inflammation and numerous associated cellular stresses, including axonal injury (Gentleman et al., 1993; Blumbergs et al., 1995), loss of innervation (Wallace et al., 1993), excitotoxic stress (Topper et al., 1995; Panegyres, 1998), heat shock (Ciallella et al., 1994), oxidative stress (Yan et al., 1994; Frederikse et al., 1996), aging (Higgins et al., 1990; Nordstedt et al., 1991; van Gool et al., 1994), and inflammatory processes (Brugg et al., 1995). Other proinflammatory stimuli that mediate the synthesis and release of AβPP include IL-1β (Goldgaber et al., 1989; Buxbaum et al., 1992) and tumor necrosis factor-α-converting enzyme (Buxbaum et al., 1998). The increased expression of AβPP by these stresses is probably a result of diminished energy resources. It is interesting that Aβ can regulate glucose metabolism (Ling et al., 2002) and that β islet cells of the pancreas express AβPP and several related enzymes at high levels (Figuerola et al., 2001).

The increased generation of Aβ under conditions of energetic stress may therefore be both a response to oxidative challenge observed in AD and following injury as well as a re-routing of metabolic priorities. In this scenario, Aβ in fact plays a protective role. The Aβ burden of the brain negatively correlates with oxidative stress markers (Nunomura et al., 1999, 2000; Cuajungco et al., 2000). This argues against the neurotoxic role of Aβ in vivo, as does the observation that cultured neurons can be cultured directly on top of isolated Aβ plaques or immobilizing Aβ without any notable toxicity (Canning et al., 1993; Carpenter et al., 1993; DeWitt et al., 1998). Perhaps the in vitro toxicity that is sporadically shown in culture and very unreliable reproduced in animal models may not be an intrinsic property of the peptide itself (Rottkamp et al., 2001). Notably, Aβ appears to attenuate oxidative stress in vivo (Nunomura et al., 2000, 2001) by probably acting as an antioxidant (Cuajungco et al., 2000). Furthermore, at nanomolar concentrations, Aβ can serve as a trophic factor (Yankner et al., 1990) and attenuates metal-induced oxidative damage (Zou et al., 2002). These findings are consistent with the trophic and neuroprotective action of Aβ at physiological concentrations (Whitson et al., 1989, 1990; Yankner et al., 1990; Koo et al., 1993; Singh et al., 1994; Luo et al., 1996). Aβ also protects neurons from death following injection with saline or iron (Bishop and Robinson, 2003) and protects lipoproteins from oxidation in cerebrospinal fluid and plasma (may involve metal ion sequestration) (Atwood et al., 1998; Kontush et al., 2001). Moreover, low concentrations of Aβ possess significant antioxidant activity in an ascorbate-stimulated-lipid-peroxidation assay (Andorn and Kalaria, 2000). In summary, the physiological explanation for increased generation of Aβ in AD and following head trauma is the need to reduce ROS to prevent neuronal apoptosis (Raina et al., 2001) and to promote neuritic repair.

Neurons are especially prone to oxidative stress due to high oxygen consumption, low levels of classic antioxidants, high unsaturated lipid content of neuronal membranes, and lack of mitotic renewal. As a result, the ratio between ROS and antioxidant defenses is essential for proper neuronal function. Normally antioxidant defense systems are sufficient to block ROS-mediated damage. However, in cases of age-related neurodegeneration, where there is consider-
able redox imbalance, oxidative stress overwhelms the system (Zhu et al., 2005). Given that Aβ is associated with the production of free radicals in vitro, it is essential to consider the in vivo temporal relationship between oxidative stress phenomena and Aβ deposition. Notably, oxidative stress, in vivo, is found in morphologically normal neurons in AD and seems to be inversely correlated with Aβ deposition (Nunomura et al., 1999, 2000). Therefore, it therefore seems unlikely that Aβ accumulation, in vivo, by itself is sufficient to explain altered oxidative balance.

The strongest evidence for the role of Aβ in AD comes from the familial forms of this disease and involves mutations of genes that are directly involved in AβPP processing. Although a tremendous amount of effort has been dedicated to determining the mechanism of disease related to these mutations, this has proven only marginally useful to our understanding of sporadic AD, which represents the majority of cases. For example, AβPP mutations have been identified in only 20 to 30 families worldwide and represent far less than 0.1% of the 15 million known cases of AD (Josefsen, 2002; Lleo et al., 2004; Hardy and Crook, 2005). Mutations in both presenilin 1 and 2, which are the most common genetic determinants of AD, only contribute an additional 120 to 130 families. Although it is clear that mutations in these proteins involved in AβPP processing are capable of inducing amyloid deposition and dementia, no aberrant change is observed usually for many decades, and even then, this is likely a result of exacerbation of age-related deposition of Aβ in these individuals (the joint result of increased Aβ concentration and microenvironmental conditions), leading to the chronic neuroinflammation associated with the disease.

The positive correlation between apolipoprotein E ε4 genotype and incidence of AD in supporting a causative role for Aβ is flawed. Although it is true that apolipoprotein E ε4 has the greatest affinity for Aβ, is found associated with senile plaques, and is thought to accelerate fibrillogenesis (Wisniewski et al., 1994), this is not the sole or even the major physiological role of apolipoprotein E proteins. Apolipoprotein E helps to regulate the transport and metabolism of lipids. The level of apolipoprotein E is elevated in response to injury in the peripheral and central nervous system (Horsburgh et al., 2000) and, just like Aβ, apolipoprotein E may thus serve a protective role after ischemia or traumatic brain injury by distributing phospholipids and cholesterol to injured neurons (Poirier et al., 1993). Apolipoprotein E may further protect against oxidative injury and prevent the accumulation of lipid peroxidation end products, such as hydroxynonenal, which are prominent features in AD and acute brain injury (Sayre et al., 1997). In support of this view, various studies show that patients that are homozygous for apolipoprotein ε4 genotype have longer periods of unconsciousness and higher incidence of post-traumatic coma following severe traumatic brain injury (Sorbi et al., 1995; Friedman et al., 1999). Thus, apolipoprotein ε4 predisposes patients for any number of neurodegenerative processes, not specific for AD. Therefore, as in acute injury, apolipoprotein ε4 may be associated with a higher incidence of AD because it is less efficient in protecting neurons from the causative insult and therefore may have very little to do with its affinity for Aβ.

Recently, the original amyloid cascade hypothesis was subsequently forced to be changed to the oligomeric amyloid cascade hypothesis, because a number of discordant findings disprove the amyloid cascade hypothesis as we discussed above. For example, there is a very weak correlation between Aβ and disease state (Giannakopoulos et al., 2003) with very high Aβ loads often seen in cognitively intact old people (Crystall et al., 1988). Moreover, transgenic animal models, including Tg2576, with supraphysiological Aβ levels, show little/no neuronal loss (Irizarry et al., 1997a,b). Despite a relative paucity of solid experimental evidence, oligomeric Aβ has quickly risen to be the new star in the field, seemingly through the momentum of the original hypothesis. Nonetheless, until recently, the pathophysiological identity of oligomeric Aβ was unclear. In this regard, recently, Lesne et al. (2006) showed that Aβ*56 (which may be a dodecameric oligomer of Aβ) is found in cognitively impaired Tg2576 animals without Aβ plaques, but not in unimpaired animals. Aβ*56 correlates with early declines in memory but not later ones (Lesne et al., 2006), and when isolated and injected into rats, Aβ*56 leads to reversible cognitive deficits. Although this is an interesting study that will definitely add fuel to the oligomeric amyloid hypothesis, before we get ahead of ourselves, a few salient aspects bear remembrance. First, different groups have reported that knockout of PS1 (i.e., no Aβ and therefore no Aβ*56, either), while attenuating Aβ pathology in AβPP mutant transgenic mice, does not cure cognitive deficits (Dewachter et al., 2002; Saura et al., 2005). In fact, that PS1 knockout-AβPP double transgenic animals lacking Aβ perform worse than single AβPP animals with Aβ might even indicate that Aβ is beneficial in certain circumstances as we previously indicated (Nunomura et al., 2001; Rottkamp et al., 2001; Lee et al., 2004a,b). In any event, what is clear from this is that cognitive deficits do not relate to Aβ (in any guise, even Aβ*56). Furthermore, the study by Galvan et al. (2006) provides another clear example that Aβ is not responsible for the cognitive and pathological changes that stereotypify AD (Lee et al., 2004a,b, 2006). Specifically, introducing an additional mutation to AβPP mice, that prevents the cleavage of AβPP by caspase, rescues cognitive and pathological deficits but does not affect Aβ plaques. Although these authors did not specifically address whether Aβ*56 was affected, the similarities in other Aβ species would tend to indicate not. Therefore, transgenic manipulations have now clearly demonstrated that cognitive deficits and pathological abnormalities in AβPP transgenic mice bear no relationship to Aβ—both the positive and negative control experiments show this result (i.e., cognitive deficits with no Aβ and rescue of cognitive deficits without change of Aβ).

The alternate hypothesis (Fig. 2) is that Aβ simply represents a bystander or a protector rather than the causative.

![Fig. 2. Alternate hypothesis. Risk factors for AD lead directly to oxidative stress that not only causes neuronal death but also an adaptive response by neurons (amyloid-β) as a protective measure.](image-url)
factor of disease (Smith et al., 2002; Lee et al., 2003, 2004b). Notably, all therapeutic studies that have an effect on Aβ levels in cells or animals have shown extremely poor or no efficacy in subsequent clinical trials. This includes indomethacin (Weggen et al., 2001), ibuprofen (Lim et al., 2000), sulfadiazone (Weggen et al., 2001), a nitric oxide-releasing acin (Weggen et al., 2001), ibuprofen (Lim et al., 2000), suflaxine to evaluate efficacy in subsequent clinical trials. This includes indomethacin (Weggen et al., 2001), ibuprofen (Lim et al., 2000), sulfadiazone (Weggen et al., 2001), a nitric oxide-releasing acin (Weggen et al., 2001), and estrogen (Zheng et al., 2002). Clearly, the alternate hypothesis points to greater therapeutic efficacy by directing efforts to the upstream metabolic and oxidative abnormalities that are what led to Aβ.

References


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