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REVIEW

Amyloid-β in Alzheimer's disease: the horse or the cart? Pathogenic or protective?

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Summary

While the pathogenesis of Alzheimer's disease (AD) is unclear, amyloid-β plaques remain major lesions in the brain of individuals with AD. Likewise, amyloid-β is one of the best-studied proteins relating to the pathogenesis of AD. Indeed, the pathological diagnosis of AD tends to be congruous with the quantity of amyloid-β. However, it is important to recognize that pathological diagnosis merely represents the association of a pattern of pathological changes with a clinical phenotype. Therefore, it should be acknowledged that, although amyloid-β detection and semiquantification have some diagnostic utility, the simple presence of amyloid plaques, as with proteinaceous accumulations in essentially all neurodegenerative diseases, does not presume aetiology. Thus, in this review, we discuss the role of amyloid-β in the pathogenesis of AD and provide an alternative view to the widely accepted dogma.

Keywords
Alzheimer's disease, amyloid-β, antioxidant, oxidative stress, senile plaque

Amyloid-β pathology in Alzheimer's disease (AD)

Senile plaques, and their major protein component amyloid-β (Glenner and Wong 1984a, b; Masters *et al.* 1985), comprise one of two principal microscopic lesions in AD (Mirra *et al.* 1991; Hyman and Trojanowski 1997). Senile plaques contain a central core made of 6–10 nm amyloid-β protein filaments arranged as bundles radiating from the centre (Kidd 1964). The core is surrounded by an argyrophilic rim of dystrophic synapses and neurites (mainly axons) often containing paired helical filaments and altered membranes. Such alterations in close proximity to the plaque core are often viewed as evidence of the destructive nature of amyloid-β (Geddes *et al.* 1986). Indeed, regions severely affected by disease, including the hippocampus and frontotemporal cortices, show a spatial correlation, albeit not perfect, between amyloid-β plaques and neuronal cell death (Rogers and Morrison 1985). Three stages in the gradual evolution of the disease can be distinguished (Braak and Braak 1991) with amyloid-β plaques, being first seen in the basal temporal neocortex (stage A). From there,

the alterations spread to adjoining neocortical areas, initially sparing the 'belt' regions and primary motor and sensory cortices. The perforant pathway then becomes studded with amyloid-β deposits as it extends through the hippocampal formation (stage B). The end stage of AD exhibits amyloid-β plaques in virtually all neocortical areas (stage C).

Amyloid-β: facts or artifacts?

The level of amyloid-β is increased in AD

The widely accepted Amyloid Cascade Hypothesis suggests that amyloid-β is the aetiological or rate-limiting factor for development of AD (Selkoe 2001). The increased number of amyloid-β plaques in AD, exceeding those found in 'normal ageing', and their localization in brain regions related to cognitive deficits that occur in AD tend to support this hypothesis. The relevant fact that serves as the foundation for this argument is 'the increase of amyloid-β plaques in AD'. This fact (*sic*) nevertheless needs to be interpreted in the light of an expanding knowledge of AD pathogenesis. With this in mind, the point of view that amyloid-β is 'consequence' rather than 'cause' is gaining more and more support. This alternative to the Amyloid Hypothesis predicts that factors that cause AD, via oxidative stress, would also lead to increased amounts of amyloid-β.

Oxidative stress has been shown to specifically increase the generation of amyloid-β (Frederikse *et al*. 1996; Misonou *et al*. 2000; Paola *et al*. 2000) and experimental conditions that induce oxidative stress (Xiong *et al*. 1997) such as ischaemia, hypoglycaemia and traumatic brain injury, all up-regulate amyloid-β protein precursor (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; Shi *et al*. 1998). Further, the role of AβPP and amyloid-β as acute phase reactants to cellular stress is supported by increases following 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), ageing (Higgins *et al*. 1990; Nordstedt *et al*. 1991; van Gool *et al*. 1994) and inflammatory processes (Goldgaber *et al*. 1989; Buxbaum *et al*. 1992; Brugg *et al*. 1995; Buxbaum *et al*. 1998). Therefore, in AD, where there is cellular stress early in disease [e.g. mild cognitive impairment (Pratico *et al*. 2002)], one would predict consequent increases in amyloid-β that colocalize with 'affected' areas. Such a notion is consistent with AβPP/superoxide dismutase (SOD) knockout mice where absence of SOD leads to increases in amyloid-β (Li *et al*. 2004).

Unbiased stereological counting indicates that during normal ageing there is little or no cell loss despite, as pointed out above, the presence of an increasing number of senile plaques (Long *et al*. 1999). Even the hyper-physiologic levels of amyloid-β in AD transgenic mice (Hsiao *et al*. 1996) only lead to senile plaque formation in middle-aged mice and do not lead to inevitable neuronal death (Irizarry *et al*. 1997; Takeuchi *et al*. 2000). Moreover, like their human counterparts, senile plaques in these experimental models are preceded by oxidative stress (Pappolla *et al*. 1998; Smith *et al*. 1998; Pratico *et al*. 2001). This again suggests that amyloid-β is not driving the pathogenic process but is rather a consequence of the disease state.

All familial AD mutations increase amyloid-β

The existence of germline mutations in AβPP that lead to familial, autosomal dominant AD is central to the Amyloid Cascade Hypothesis. All mutations in AβPP (as well as presenilin 1/2) increase the production of amyloid-β 1–42. On the other hand, it should be noted that the double mutant AβPP, not only with the FAD-linked V642I mutation but with the deletion of the 41st and 42nd residues in the amyloid-β region, is able to effectively induce neuronal cell death (Yamatsuji *et al*. 1996). Also, in response to anti-AβPP antibody binding, wild type AβPP without the 41st and 42nd residues in the amyloid-β region causes neuronal cell death as strongly as intact wild type AβPP. The Swedish mutation in AβPP (NL-AβPP) also can cause neuronal cell death, even when NL-AβPP dose not have the 41st and 42nd residues in the amyloid-β region (Hashimoto *et al*. 2001). Therefore, increased amyloid-β via AβPP and presenilin 1/2 germline mutation would not be a 'toxic' process but instead suggests another mechanism of cytotoxicity in neuronal cell culture. Among the support for this concept are double transgenic, mice studies that show that neuronal deficiency of presenilin 1 inhibits amyloid-β formation but has no effect on cognition (Dewachter *et al*. 2002). Therefore, it would appear that, at least in transgenic animals and cell cultures, amyloid-β is not the underlying problem; rather, it is the presence of mutated AβPP that results in cognitive deficits and neurodegeneration. In this regard, it is interesting to note that antioxidants are able to prevent behavioural deficits in the AβPP transgenic animals often independently of alterations in amyloid-β pathology (Joseph *et al*. 2003). These data are consistent with the idea that oxidative stress temporally precedes amyloid-β (Pratico *et al*. 2001) at the time point where behavioural alterations first manifest.

The notion of amyloid-β deposits *per se* as neurotoxic lesions may be called into question (Smith *et al*. 2000) in the light of the early appearance of sequelae of oxidative stress relative to amyloid-β deposits (Nunomura *et al*. 2000; Nunomura *et al*. 2001), while the concept of amyloid-β deposits as protective in nature makes mechanistic sense in both familial autosomal dominant and sporadic AD. Neurones respond to oxidative stress, both *in vitro* and *in vivo*, by increasing amyloid-β production (Yan *et al*. 1995), and this increase in amyloid-β is associated with a consequent reduction in oxidative stress (Nunomura *et al*. 2000; Nunomura *et al*. 2001). Proteins, such as amyloid-β, that are induced under oxidative conditions and act to lessen oxidative damage are typically thought of as antioxidants, and we have likewise demonstrated that amyloid-β is a *bona fide* antioxidant that can act as a potent SOD (Cuajungco *et al*. 2000). By this logic, AD kindreds with AβPP mutations lose, by virtue of mutation, effective antioxidant capacity, while the prodigious amyloid-β deposits themselves are signatures not of neurotoxicity *per se* but of oxidative imbalance and an oxidative stress response. This is consistent with the data that virtually everyone over the age of 40 years contain detectable amyloid-β deposits, an age, not coincidentally, where redox alterations first manifest (Nunomura *et al*. 2001). The alternate view that everyone at mid-life is on the verge of developing AD is manifestly extreme and not supported by the fact that a large percentage of cognitively intact, aged individuals contain amyloid-β loads equivalent to patients with AD (Davis *et al*. 1999). Thus, the current interpretation of the relationship between AβPP mutations and disease, which is explained as a gain of function process, may require some revision. We suggest that the relationship between amyloid-β and disease is explained as a loss of function process, resulting in increased susceptibility to oxidative stress or loss of antioxidant protection. The prodigious amyloid-β deposits in brain and blood vessels are thus the pathological signatures of the loss of function and reflect an altered steady state as a result of the mutation. With this paradigm in mind, it is not surprising that free radicals are among the best inducers of AβPP protein expression and consequent amyloid-β production (Yan *et al*. 1995).

Amyloid-β is toxic

Fibrillar or aggregated forms of amyloid-β, like those present in the senile plaques, are toxic to cultured neurones *in vitro* (Pike *et al*. 1991). However, *in vivo*, the presence and density of amyloid-β correlates weakly with the onset and severity of AD (Davies *et al*. 1988), and therefore there was a shift towards determining whether the presence of the soluble form of amyloid-β in the brain may be a better predictor of the disease (McLean *et al*. 1999). Specifically, the oligomers, not monomers, of this form of amyloid-β seem to play an important role, as shown by augmented presence of these oligomers during the expression of mutations in AβPP or presenilin (Xia *et al*. 1997), as well as by their capacity to interfere cognitive function *in vivo* when microinjected into the brains of rodents (Walsh *et al*. 2002; Cleary *et al*. 2005). However, detracting from the importance of these species, the temporal relationship between oligomeric amyloid-β and oxidative stress is unclear. Even in the transgenic Tg2576 mouse model, which produces large amounts of amyloid-β, oligomeric amyloid and oxidative stress appear at approximately the same age (Kawarabayashi *et al*. 2001; Pratico *et al*. 2001). In fact, it has recently been shown that amyloid growth occurs by the addition of monomers in a reaction distinct from, and competitive with, formation of potentially toxic oligomeric intermediates (Collins *et al*. 2004). If this finding can also be proved for amyloid-β, the formation of amyloid-β plaques could represent the protective process against toxic oligomeric amyloid-β; the level of oligomeric amyloid-β in transgenic mice and AD brain would thus be of limited significance because there are large amounts of amyloid-β plaques in both brains. Therefore, while oligomers have certainly rejuvenated the amyloid-β hypothesis, their role in disease is uncertain. Further study is required to adequately assess the relationship between oxidative stress and oligomer formation as well as between fibril and oligomer formation.

Neurotoxicity in cultured cells may also be an artifact of *in vitro* conditions (Rottkamp *et al*. 2001), an idea further supported by the findings that neither isolated senile plaques nor immobilized amyloid-β elicit neurotoxicity *in vivo* or *in vitro* (Frautschy *et al*. 1992; Canning *et al*. 1993; DeWitt *et al*. 1998). Thus, the capacity of amyloid-β to induce oxidative stress remains controversial (Walter *et al*. 1997). Recent data suggest that the oxidant properties of amyloid-β may stem from its capacity to interact with transition metals and mediate toxicity via redox-active ions that precipitate lipid peroxidation and cellular oxidative stress (Rottkamp *et al*. 2001).

Conclusion

While amyloid-β is associated essentially with aetiology by various laboratories, the observed decrease in oxidative damage with amyloid-β accumulation suggests, rather, a mechanism of survival (Perry *et al*. 2000; Smith *et al*. 2000; Joseph *et al*. 2001; Rottkamp *et al*. 2002; Smith *et al*. 2002a; Smith *et al*. 2002b; Arrasate *et al*. 2004; Lee *et al*. 2004). Moreover, as a consequence of age-related oxidative stress, there is an up-regulation of amyloid-β resulting in senile plaques. Amyloid-β lesions serve antioxidant functions and limit

age-related neuronal dysfunction. In AD, this age-related oxidative stress is compounded by macromolecules (Sayre *et al.* 1997) and heavy metals (Calingasan *et al.* 1999; Takeda *et al.* 2000) as additional sources of oxidant stress that overcome antioxidant effects of enhanced amyloid-β production, leading to neurodegeneration and consequent dementia. In the light of these observations, efforts aimed solely at eliminating amyloid-β appear short-sighted.

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