Research Focus

Alzheimer's amyloid story finds its star

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β-Amyloid peptides (Aβ) have been genetically implicated as the cause of Alzheimer's disease, but the causality of amyloid deposited as plaques has been challenged. The controversial role of amyloid peptides in Alzheimer's disease has been highlighted in a recent paper from Lesne and colleagues, who applied Koch's postulates to cast a specific memory-deficit-inducing oligomer species as a central player causing memory loss. These authors used a transgenic mouse model to identify a specific type of aggregate that emerges with cognitive deficits and is capable of transmitting a spatial memory defect to unimpaired animals.

Amyloid peptides in the Alzheimer story: a confused role

The identified genetic causes of Alzheimer's disease (AD) are all known to increase the production or aggregation of amyloid-β protein 42 (Aβ42), a 42 amino acid peptide with a high propensity to aggregate. Although genetic data argue that Aβ42 has a causal role, they offer no detailed insight into its mechanism. The name amyloid-β protein was chosen because the most-noticeable aggregates are long protein filaments that have the β-pleated-sheet secondary structure that is characteristic of amyloid fibrils. In AD, these filaments, which are deposited as the core component of extracellular patches, are visible to pathologists as amyloid plaques and vascular amyloid using light microscopy and as filaments using electron microscopy. Because high levels of amyloid fibril preparations are directly toxic to cultured neurons, it was initially suggested that amyloid fibrils might cause AD [1]. However, many cognitively normal individuals have died with extensive amyloid-plaque accumulation that is typical of AD patients, raising questions about its causal role. In contrast to the other major AD lesion, known as the neurofibrillary tangle (NFT), which is made up of tau protein filaments, β-amyloid plaques do not correlate well with cognitive decline. If amyloid plaques and vascular amyloid correlate poorly with cognitive decline and are not sufficient to cause AD, how do we interpret the genetic data implicating increased Aβ42?

Casting the amyloid role using memory loss as a bioassay

In a recent paper published in Nature, Lesne *et al.* [2] offered a solution to this apparent conundrum. Their approach was to postulate that some non-plaque species of an Aβ aggregate caused cognitive deficits and to use a bioassay to identify it. For this purpose, the authors used the line of transgenic mice (*Tg2576*) produced in their laboratory that expressed high levels of the human β-amyloid precursor protein (β-APP or simply APP), which carries a familial AD mutation originating in a Swedish lineage (APPsw). This mutation markedly increases cleavage of APP to generate high levels of Aβ. Although the APPsw mutation is sufficient to cause AD in humans and the APPsw transgenic mice have many amyloid plaques and cognitive deficits, the mice lack some important features of AD including neurofibrillary tangles and significant neuron loss. Their cognitive deficits are typically measured in a Morris water maze. Once mice are trained to swim through cloudy water to find a visible platform island and escape the water tank, they are then evaluated on their skill to remember the platform location when it has been hidden by slightly submerging it in subsequent trials. Although APPsw mice could learn to find the submerged platform, they did not remember the platform location the day after a training trial. Thus, the mice showed selective long-term spatial memory deficits analogous to humans in an early stage of cognitive decline who cannot find their car and 'escape' via the parking ramp.

Auditions for an Aβ star produce a specific oligomer finalist

Lesne *et al.* [2] reasoned that some form of Aβ must initiate these cognitive deficits because, in these and other APP transgenic mice, administration of specific anti-Aβ antibodies rapidly ameliorates these memory problems. In the mouse models, memory deficits emerge before plaques, so the authors concluded that the increased production of a particular Aβ species before plaques was the cause of cognitive decline. They designated this hypothetical species Aβ star (Aβ*). Candidates for Aβ* were expected to emerge coincident with cognitive deficits and to correlate with further cognitive change. Mouse forebrains were extracted into fractions enriched in extracellular, cytoplasmic, membrane-associated and insoluble pelleted plaques. Aβ species in the cytoplasmic and membrane-enriched fractions did not correlate with the onset of cognitive deficits in the *Tg2576* model, whereas Aβ in the insoluble pellet accumulated over the next seven months without further spatial reference memory decline. Sodium dodecyl sulphate (SDS)-stable soluble extracellular Aβ species were analyzed on Westerns and included monomer, trimer and higher-order assemblies that might correspond to multiples of trimers, namely hexamer, nonamer and dodecamers. Of these, dodecamers had the strongest correlations with memory performance and emerged as the leading candidate for the hypothetical Aβ*, termed Aβ*56 based on the apparent molecular weight of the Aβ structure.

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Available online 1 August 2006.

Aβ*56 oligomer is characterized by transferring memory deficits

To prove that the soluble Aβ*56 was sufficient to cause memory deficits, it was purified from *Tg2576* brains using anti-Aβ antibodies and confirmed as an Aβ species using mass spectrometry. The candidate Aβ* was then injected via an indwelling cannula into the lateral cerebroventricles of the test subjects, which were healthy young rats pre-trained in the Morris water maze. Compared with vehicle injections, Aβ*56-injected rats showed defective long-term spatial memory but, otherwise, normal acquisition of spatial information. This confirmed that Aβ*56 met the criteria for a cognitive-decline-inducing Aβ assembly, although whether or not Aβ*56 can also impair other cognitive functions remains unknown. Notably, the dose of Aβ* injected (8.5 picomoles) to give low nanomolar levels was in a realistic pathophysiological range. By contrast, because micromolar levels of Aβ fibrils and other Aβ preparations have typically been used to produce toxicity *in vitro* or *in vivo*, the real relevance of acute *in vitro* amyloid fibril toxicity is more questionable. Thus, Lesne *et al.* [2] have produced evidence along the lines of Koch's criteria for a disease-causing agent. Namely, they have shown that Aβ*56 appears only with the emergence of Morris-water-maze deficits, and that it can be purified and injected at relevant doses to transfer these deficits to previously healthy inoculated test animals.

The Aβ star role

These data clearly support the evidence that soluble oligomer preparations of Aβ derived from APP transfected cultured cells [3] or Aβ aggregates made from synthetic Aβ42 [4] can cause deficits in hippocampal long-term potentiation (LTP), a candidate electrophysiological substratum of memory. Because oligomers that are similar in size to Aβ*56 bind directly to synaptic sites [5], it might be suggested that they are a primary cause of AD. Oligomer-induced memory deficits are rapid and reversible and provide a compelling and encouraging rationale for efforts to target the Aβ* oligomer species. Because it is possible to generate oligomer-specific antibodies, one strong candidate is the vaccine approach. Cognitive improvement by passive immunization with an Aβ-aggregate-specific antibody that failed to reduce amyloid plaques supports this view [6]. Novel small molecule drugs and natural compounds can also limit oligomer formation and/or action. An example is the curry spice extract curcumin, which both blocks Aβ aggregation and limits Aβ oligomer toxicity *in vitro* [7]. However, it might be necessary to target other oligomers in addition to dodecamers. Oddo *et al.* [8] have not yet ruled out the possibility that other soluble Aβ species, including trimers [9] or an intra-neuronal or SDS–urea soluble Aβ oligomer species might also contribute to cognitive deficits.

A major caveat in translating these animal model results to humans is that the mouse model lacks the neurofibrillary tangles and significant neuron loss that is associated with progressive dementia in AD. Once developed, these apparently difficult to reverse lesions might produce additional deficits that might not be suppressed by anti-oligomer therapy. Nevertheless, there is accumulating evidence that tangles and neuron loss ultimately lie downstream from some form of Aβ as they can be accelerated by mutant APP transgenes [10], induced by injections of Aβ fibril preparations [11] and reduced by anti-oligomer antibody treatment in models with both tangles and plaques [8]. Furthermore, oligomers can act via a major tau kinase glycogen synthase kinase 3β (GSK-3β) [12], fyn kinase [13] and p21-activated kinase (PAK) [14], and seem to be in equilibrium with intra-neuronal Aβ [15], another candidate cause of neurodegeneration in AD.

Concluding remarks

Although there is rapidly accumulating evidence that Aβ oligomers can induce multiple downstream neurodegenerative pathways and cognitive deficits relevant to AD, as shown by Lesne *et al.* [2], their proximal receptors or other direct actions remain poorly characterized. A better understanding of crucial oligomer species and their receptors, or other activities such as pore formation and the relationship to intra-neuronal Aβ should lead to better drug targets and new therapeutic opportunities.

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1471-4914/$ – see front matter. Published by Elsevier Ltd.
doi:10.1016/j.molmed.2006.07.002