Seeding Neuritic Plaques from the Distance: A Possible Role for Brainstem Neurons in the Development of Alzheimer's Disease Pathology

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Key Words

Alzheimer's disease · Neurodegeneration · β -Amyloid precursor protein · β -Amyloid peptide · Neuritic plaques · Autophagy · Mitochondria · Brainstem neurons · CAD cells

Abstract

Background and Objective: Our goal is to obtain insight into the causes of the pathological lesions in Alzheimer's disease (AD). It is thought that the β -amyloid (A β) deposits within the cerebral cortex and hippocampus of AD brains are initiated by a 'bad seed' of oligomeric A β . The origin of this seed is unknown. Here, we focused on the events that might trigger the formation of neuritic plaques, aiming to explain how these plaques form in cortical and hippocampal regions. **Methods and Results:** Using immunocytochemical and biochemical methods, we showed that brainstem-derived, neuronal cells (CAD) – but not cortical or hippocampal neurons – show large amounts of A β accumulated at the terminals of their processes. This is similar to what is believed to occur in brain neurons, in the early phases of AD. CAD cells that contain A β accumulations also concentrate β -secretase at process terminals. We show that, while the anterograde transport of small vesicles is not significantly affected, the mitochondrial transport is perturbed in CAD cells that contain A β accumulations. We further show that intracellular, neuritic A β accumulations may become extracellular upon neurite degeneration, thus providing the initial 'bad seed' of

A β oligomers that triggers further aggregation of extracellular proteins. **Conclusion:** We propose that brainstem neurons, known to send projections throughout the brain, could provide the 'bad seed' of A β that nucleates plaques in the cerebral cortex and hippocampus of AD brains.

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Background and Objective

Alzheimer's disease (AD), a complex neurodegenerative disorder, is characterized by two major lesions: the neuritic plaques and the neurofibrillary tangles. Neuritic plaques contain extracellular deposits of β -amyloid (A β) peptide, a metabolite of the transmembrane protein, A β precursor protein [1].

A characteristic feature of AD neuropathology is the preferential formation of plaques in cortical and hippocampal brain regions. Yet, the initial events that trigger plaque formation in certain brain regions and not in others are not known. According to the seeded polymerization theory [2], the aggregation of soluble A β , which leads to plaque formation, is nucleated by 'bad seeds' of oligomeric A β . The origin and nature of these hypothetical seeds are not known. As described below, our work with the neuronal cell line CAD suggests that A β oligomers form at the terminals of projections of brainstem neurons and could act as such seeds.

Fig. 1. CAD cells immunolabeled with antibody 6E10 (Signet, Dedham, Mass., USA), showing A β accumulations at neurite endings (A). B An enlargement of a process, showing localization of A β to large particles resembling late endosomes and autophagosomes.

Fig. 2. Diagram showing plaques (*) at the terminals of projections of brainstem (BS) neurons. The drawing was modified from Aston-Jones and Cohen [12], and used with permission from the *Annual Review of Neuroscience*, vol. 28, 2005, by Annual Reviews (www.annualreviews.org).

Methods

CAD cells [a locus coeruleus (LC)-derived cell line] [3] have emerged as an important in vitro experimental system for studying the molecular pathobiology of AD [4–8], and – as highlighted here – may be particularly relevant to the initiation of neuritic plaque formation. Using immunocytochemical and biochemical approaches, we have characterized the CAD cell line with respect to the metabolism of A β precursor protein and generation of A β .

Results

We discovered that CAD cells are prone to accumulation of large amounts of intracellular A β at the terminals of their processes (fig. 1), similar to what may occur in brain neurons, during the initial phases of AD [7]. Using carboxy-terminal-end antibodies to A β species, we showed that these A β accumulations contain both the A β ₄₀ and A β ₄₂ peptides [7]. Cross-reactivity of the accumulations with an antioligomer antibody that preferentially detects species larger than the octamer (A11) [9] indicated that the accumulations include large-molecular-size A β oligomers [7].

The neuritic accumulation of A β in CAD cells is restricted to a small population of cells that show redistribution of β -secretase (BACE1) to the processes, where it colocalizes with A β and markers of late endosomes and autophagic vacuoles [7]. These findings suggest that the A β accumulations could be generated through endocytosis or macroautophagy, two processes previously implicated in the formation of the neuritically localized A β [10, 11]. Importantly, unlike the LC-derived CAD cells, cultured cortical and hippocampal neurons do not show

detectable A β accumulations at their neuritic terminals (data not shown).

Here, we hypothesize that in AD brains, accumulations of A β similar to those observed in CAD cells (fig. 1) could form at the projection terminals of brainstem neurons (which send projections throughout the central nervous system, including the cerebral cortex and hippocampus), and serve as seeds for further A β aggregation. This process leads to plaque formation at locations remote from the brainstem, such as the cerebral cortex and hippocampus (fig. 2). The LC is largely affected by cell death early in AD. However, since this brain region mostly lacks neuritic plaques, the prevailing view was that neuronal loss in the LC is caused by the cortical A β , which ‘poisons’ the projections of brainstem cells [13]. By contrast, our results suggest that the neuropathology of AD may actually begin in the subcortical regions, and then spread to the cortex and hippocampus. This is in line with earlier reports suggesting that, in AD, pathologic alterations in the LC may spread to cortical and hippocampal brain regions [14–16].

How could the intracellular A β accumulations (fig. 1) become extracellular and serve as seeds for A β deposition? The mechanism could be the fusion of the A β -containing autophagosomes and late endosomes with the plasma membrane, or alternatively, neurite degeneration. Indeed, we occasionally found neuritic debris containing A β , in the areas enriched in CAD cells having A β accumulations (fig. 3). A further question raised by this result is what could trigger neurite degeneration in these cells? We found that, while the anterograde transport of small vesicles is not significantly affected [7], the transport and the neuritic localization of mitochondria is evidently perturbed in CAD cells that contain A β accumulations

Fig. 3. A β accumulations in the debris of CAD cell processes (arrows). **A** CAD cells immunolabeled with antibody 6E10. **B** Corresponding phase contrast micrograph.

Fig. 4. Mitochondrial transport into neurites that contain A β deposits is disrupted. Fewer mitochondria are present throughout the neurite of a CAD cell that contains A β deposits (short arrows). By contrast, neurites that lack A β deposits have numerous mitochondria that accumulate at the terminal (long arrows). Mitochondria were detected with an antibody to lipoic acid (**A**) and A β was detected with antibody 6E10 (**B**).

(fig. 4). This could result in diminished supply of ATP in the neurites, which could cause their observed degeneration.

Conclusions

Taken together, our results point to the brainstem neurons as possible initiators of plaque formation in AD. We propose that the initial seeds of aggregated A β are produced at the neurite terminals of the brainstem neurons that project into the brain regions prone to AD pathology (e.g. cerebral cortex, hippocampus). These seeds then trigger further aggregation of soluble, extracellular A β

into plaques. Thus, our work offers a novel perspective on AD pathogenesis. In addition, the CAD cell system that we established is ideally suited for screening of compounds that could prevent accumulation of A β , and thus could be used to treat brainstem neurons at the early stages of AD.

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