Journal Club

Amyloid Plaques and Amyloid-β Oligomers: An Ongoing Debate

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Review of Hartman et al. (http://www.jneurosci.org/cgi/content/full/25/26/6213)

Amyloid-β (Aβ) peptides, which accumulate in Alzheimer's disease (AD), are mainly produced by neurons as a soluble protein that aggregates into insoluble amyloid plaques under specific conditions, notably aging. This feature of AD has been reproduced in numerous transgenic models (e.g., PDAPP) that overexpress mutant amyloid precursor protein (APP) (Games et al., 1995). Transgenic mice have been useful in better understanding the pathology of AD and in testing therapeutic strategies. In contrast to strategies targeting β- or γ-secretase activity, immunotherapy could theoretically improve the cognitive status of AD patients without altering physiological APP/Aβ function. In animal models, immunization reportedly lowers cerebral Aβ levels and amyloid burden, in addition to improving memory deficits (Schenk, 2002). In the June 29, 2005 issue of *The Journal of Neuroscience*, Hartman et al. (2005) (http://www.jneurosci.org/cgi/content/full/25/26/6213) sought to determine whether age-dependent cognitive decline in PDAPP mice is attributable to aggregated Aβ accumulation and whether reducing Aβ deposition would improve learning and restore synaptic plasticity.

To assess learning, the authors used the Morris water maze. This water maze is widely used to determine cognitive status in transgenic mice modeling AD and to measure the effects of prospective therapies. This swim test is used to assess hippocampal-dependent memory, but it is also sensitive to other memory systems, including procedural learning. In addition, performance depends highly on genetic background and environmental factors (Wolfer and Lipp, 2000). Thus, although simple in principle, the water maze is complex with regard to experimental design and data interpretation. Appropriately, Hartman et al. (2005) distinguished between an age-independent and age-dependent deficit in PDAPP mice on a pure C57BL/6 background [Hartman et al. (2005), their Figs. 1–3 (http://www.jneurosci.org/cgi/content/full/25/26/6213/FIG1, http://www.jneurosci.org/cgi/content/full/25/26/6213/FIG2, http://www.jneurosci.org/cgi/content/full/25/26/6213/FIG3)]. The observed early learning deficit was attributed to mutant APP overexpression, although it would have been helpful to compare wild-type (WT) APP transgenic mice with equivalent protein expression levels to exclude nonspecific transgene effects. The authors moved the hidden platform location each week and thus incorporated a switching task. Contrary to WT littermates, PDAPP mice failed to display a spatial bias for platform location during probe trials, suggesting that WT and PDAPP mice may use different memory systems or strategies to solve the task (Wolfer and Lipp, 2000). Alternatively, as the authors suggest, performing probe trials at shorter intervals after the final training trial would have improved the performance of PDAPP mice.

The authors hypothesized that Aβ accumulation was responsible for the age-dependent impairment in C57BL/6– PDAPP mice and sought to test this postulate by evaluating total Aβ immunoreactivity, Aβ species levels, and conformations. ELISAs were used to quantify Aβ levels. Reliable and sensitive, Aβ ELISAs are useful in studying Aβ metabolism, although soluble oligomeric Aβ species and fibrillar species are not detected by such assays (Stenh et al., 2005). Plaque load (diffuse and dense core) was estimated by immunohistochemistry and by thioflavine-S staining for β-sheet pleated structures. Because Aβ deposition starts between 6 and 9 months of age in PDAPP mice (Games et al., 1995), no plaques were found in young animals. Amyloid burden and thioflavine S-positive deposits were slightly elevated in middle-aged PDAPP mice and reached ~25% of the hippocampal area in old mice, coinciding with the onset of age-dependent cognitive impairment [Hartman et al. (2005), their Fig. 4 (http://www.jneurosci.org/cgi/content/full/25/26/6213/FIG4)]. These observations were confirmed by Aβ ELISAs for both soluble and insoluble $A\beta_{40}$ and $A\beta_{42}$ [Hartman et al. (2005), their Table 1 (http://www.jneurosci.org/cgi/content/full/25/26/6213/TBL1)].

The authors postulated that immunization with the Aβ antibody 10D5 and a

subsequent modulation of amyloid deposition would restore learning in PDAPP mice [Hartman et al. (2005), their Fig. 5 (http://www.jneurosci.org/cgi/content/full/25/26/6213/FIG5)]. Despite using a different behavioral testing protocol, 10D5 administration partially restored cognitive function. Amyloid burden was also reduced by ~50% and plasma Aβ levels were dramatically increased [Hartman et al. (2005), their Fig. 6 (http://www.jneurosci.org/cgi/content/full/25/26/6213/FIG6)], suggesting that Aβ species were cleared from the brain. Interestingly, Aβ ELISAs confirmed the augmentation of plasma Aβ, but parenchymal levels of all Aβ_{1-x} species were unaltered [Hartman et al. (2005), their Table 2 (http://www.jneurosci.org/cgi/content/full/25/26/6213/TBL2)]. Based on these findings, the authors concluded that amyloid plaques contribute to the learning deficits (Fig. 1). The discrepancy between amyloid burden and Aβ ELISA levels is surprising because parenchymal monomeric Aβ levels might be expected to change after immunotherapy. Moreover, the biochemical methodology used (i.e., non-conformational-specific Aβ antibodies for both ELISAs and vaccination paradigms) cannot discriminate between effects caused by soluble Aβ oligomers and Aβ fibrils because it is likely that 10D5 detects multiple Aβ conformations. Using the Aβ oligomer-specific A11 antiserum (Biosource, Camarillo, CA) would be useful to quantify Aβ oligomers across ages in PDAPP mice, as would a comparison of 10D5- and A11-mediated immunotherapy. In addition, if 10D5 could rescue learning deficits in young mice (when plaques are absent), this would provide additional support for the postulate that Aβ burden, not soluble Aβs, is responsible for the age-dependent behavioral alterations. Biochemical and histological assessments of the effects of 10D5 were performed after 4 – 8 weeks of treatment. Therefore, caution is warranted in relating these changes to the subacute (5 d of 10D5) improvements in behavior.

The authors also determined that immunization restored one measure of neuronal function, long-term potentiation (LTP) [Hartman et al. (2005), their Fig. 7 (http://www.jneurosci.org/cgi/content/full/25/26/6213/FIG7)]. At 1 h after stimulation, hippocampal LTP was sixfold lower in old PDAPP animals compared with WT mice. In contrast, 10D5-treated PDAPP mice showed LTP similar to controls. Although the authors conclude that "Aβ is contributing to functional abnormalities in the PDAPP mice," Aβ oligomers inhibit LTP, a phenomenon that can be rescued by immunotherapy (Klyubin et al., 2005). Thus the possibility that 10D5 administration reduced Aβ dimers and trimers cannot be excluded. In sum, Hartman et al. (2005) demonstrated that PDAPP mice on a pure genetic background display age-independent and age-dependent cognitive deficits, the latter coinciding with amyloid deposition. Immunotherapy partially restored cognitive function and lowered amyloid deposition. The contribution of oligomeric Aβ species to this learning deficit was not specifically addressed, and may be of interest in future studies.

Figure 1. Summary of the behavioral, neuropathological, and functional characterization of C57BL/6 –PDAPP mice. ND, Not determined; ↓, lower than WT; →, equivalent to WT; +, low; +++, high; green areas indicate thioflavine-S (ThioS)-positive Aβ deposit.

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