Commentary

Building a More Perfect Beast

APP Transgenic Mice with Neuronal Loss

Dennis W. Dickson

From the Department of Pathology (Neuropathology), Mayo Clinic, Jacksonville, Florida

While there have been a number of successful attempts to develop transgenic mice that express mutant amyloid precursor protein (APP) and develop amyloid deposits that have features similar to those in aging and Alzheimer's disease (AD),¹ until now there has been either no neuronal loss or less than convincing evidence in favor of neuronal loss. In this issue of *The American Journal of Pathology*, Schmitz and colleagues² provide compelling evidence for age-dependent neuronal loss in the hippocampus of bigenic transgenic mice expressing a double mutation in APP (Swedish APP mutation APP_{K670N, M671L} (APP_{sw}) and APP_{V717I}, under the mouse Thy1 promoter) and mutant presenilin-1 (PS-1 M146L under the pHMG promoter). The neuronal loss was ascertained by unbiased stereologic methods on Nissl-stained sections. The animals showed significant (30 to 35%) neuronal loss in the hippocampal pyramidal layer compared to controls, which was disproportionate to the degree of amyloid deposition. No neuronal loss was detected in the dentate fascia of the hippocampus, the only other region assessed for neuronal loss. While the regions analyzed are of interest, if the model is to have relevance to AD, it will be important in future studies to extend neuronal counts to limbic and association cortical regions, which are especially vulnerable to amyloid deposition in humans. The behavioral consequences, if any, of neuronal loss in this model are not described in this report or other descriptions of this model³ and are left to future work.

The mechanism of neuronal loss remains to be determined, but previous studies of this model demonstrated intra-neuronal A β as well as neuronal expression of oxidative stress and pro-apoptotic markers.³ This would suggest that mechanisms other than those associated with extracellular amyloid deposits are involved. The poor correlation of neuronal loss with extracellular amyloid deposits fits with previous studies of humans and other animal models of AD. Amyloid burden, as measured by image analysis or point counting methods as in this study, has not proven to be the most robust correlate with cognitive impairment and neuronal loss in humans⁴ or

with behavioral abnormalities in transgenic models of AD.⁵ In contrast, cognitive dysfunction correlates better with markers of neuronal degeneration, such as synaptic loss⁶ or neurofibrillary pathology.⁷ Given the popular belief that amyloid is the cause of neuronal degeneration in AD, these findings have prompted modification of the original "amyloid cascade" hypothesis for AD.⁸ Recent research has changed its focus to possible toxic effects of protofibrillar forms of A β or intra-neuronal A β . The latter is a feature described in this model as well as other AD models that have shown cognitive and synaptic dysfunction.⁹ Unfortunately, the anatomical relationship between neurons with cytoplasmic A β and neuronal loss is not described, albeit the neurons with cytoplasmic A β are described as being restricted in number and distribution.³ One would hope that if intra-neuronal A β is detrimental to long-term neuronal survival, it should antedate and parallel the distribution of neuron loss.

In addition to those mentioned, a number of other areas of investigation remain to establish this bigenic mouse as a valuable model for AD. Given the evidence that synaptic loss is an important structural correlate of cognitive impairment in AD, it is important that synaptic integrity be assessed in future studies. The time course of neuronal and potential synaptic loss may shed light on the mechanism of synaptic degeneration in AD. The role of neuro-inflammation in neuronal loss also is an active area of investigation, and the anatomical and temporal relationships of inflammatory reaction associated with amyloid deposition and neuronal loss will be important to determine. Finally, a detailed time course of changes in the levels of various A β species may also shed light on the mechanism of neuronal loss, as previous studies have shown a predictable sequence of changes in A β solubility that antedate visible extracellular deposits.¹⁰

The question that arises from this report is why this model is associated with neuronal loss, while previous APP models are not. There are a number of factors to

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Address reprint requests to Dennis W. Dickson, M.D., Neuropathology Laboratory, Mayo Clinic, 4500 San Pablo Road, Jacksonville, FL 32224. E-mail: dickson.dennis@mayo.edu.

Table 1. APP + PS-1 Bigenic Transgenic Mice

APP mutation (promoter)	APP genetic background	PS-1 mutation/promoter	PS-1 genetic background	Reference
APP751sw+APP _{V717I} (mo Thy-1)	CBA × C57B6	M146L (pHMG)	C57B6	2
APP695sw (hPrP)	C57B6/SJL × C57B6	M146L (hPrP)	SW/B6D2 × B6D2	5, 20
APP695sw (hPrP)	C57B6/SJL	A246E (mo Thy-1)	C57B6/SJL	21
APP695sw (hPrP)	C3H/HeJ × C57B6	A246E (hPrP)	C3H/HeJ × C57B6	19, 27

mo, mouse; h, hamster.

consider. The genetic constructs used to produce the mice are the most obvious factor. The precise combination of transgenes and promoters in this animal has not been used in any of the other reported APP models of AD. The APP mutation is a double mutation (APPsw and APP_{V717I}) on APP751. APP751 is a form of APP that is found in neurons, but also in glial cells.¹¹ Most APP models have used APP695.¹ The first APP model with convincing amyloid pathology was PDAPP mice,¹² which has a different APP mutation, APP_{V717F}; a different promoter, PDGF; and a construct that leads to expression of APP751, APP770, and APP695 rather than just APP751. The PDAPP mice develop amyloid deposits as early as 6 months of age, and the plaques have many features in common with those seen in aging and Alzheimer's disease, including dystrophic neurites and reactive glia. Initial studies failed to find significant neuronal loss.¹³ In contrast, the model reported by Schmitz and co-workers^{2,3} has amyloid deposits as early as 2.5 months of age. The PDAPP mice have evidence of perturbation of neuronal populations in the vicinity of amyloid deposits,¹⁴ which has also been noted in the APP23 mice, a model based on APP751 with APPsw mutation driven by the Thy-1 promoter.¹⁵

A problem that has plagued assessments of neuronal loss in brain regions that are also susceptible to amyloid deposition is the fact that amyloid deposits, especially in transgenic mice, tend to be dense structures that exclude other tissue elements from the central region of the deposit. They are, in essence, space-occupying lesions that, at the very least, displace neurons and other parenchymal elements that would normally occupy that volume. This certainly complicates the interpretation of neuronal counts in the APP23 model, even with unbiased stereologic methods.¹⁵ To address this issue, Schmitz and co-workers² performed an analysis in which they assumed similar density of neurons within the amyloid deposits as in the immediate surrounding tissue and mathematically added back the missing neurons in this area. With this "reconstructed" measurement, neuronal loss was not as marked, but still significantly different from controls. Thus, neuronal loss was not merely a counting artifact due to local perturbation of cell populations by dense amyloid deposits, which is a methodologic innovation that future quantitative studies of neuronal counts in mice with amyloid deposits should also consider implementing.

In a recent report neuronal loss has been reported in the CA3 region of the hippocampus in older PDAPP mice,¹⁶ which, as mentioned above, was not detected in

previous studies of neuronal integrity in PDAPP mice.¹³ The authors attributed the inconsistency to genetic background differences. The line with no neuronal loss was on an outbred background (C57BL/6 × DBA/2 × Swiss-Webster) whereas the line with hippocampal neuronal loss was maintained on a hybrid background (C57BL/6 × DBA/2). Another difference was the methodology used to count neurons. The negative study was based on unbiased stereology with Nissl-stained sections, while the study that showed neuronal loss used "stereologic counting principles" to count MAP-2 immunostained neurons with confocal microscopy. The possibility of a phenotype change (ie, loss of MAP-2 immunoreactivity), rather than actual neuronal loss, cannot be completely excluded with the latter method.

An APP construct more similar to that used by Schmitz and co-workers² was used to generate the TgCRND8 mice as reported by Chishti and co-workers.¹⁷ The transgene in TgCRND8 is APPsw and APP_{V717I} on APP695, but under a different promoter, the hamster prion promoter (hPrP). This promoter has been successfully exploited in making a number of transgenic animal models;¹⁸ it is widely expressed in the CNS and in peripheral tissues, but is not specific to neurons. Expression is high in the spinal cord, and spinal cord pathology complicates a number of animal models that use the PrP promoter. Nevertheless, the TgCRND8 mouse also has very early amyloid deposition (as early as 3 months) with neuritic dystrophy, reactive glial changes, and behavioral deficits. Evidence for neuronal loss in this model is lacking.

At least three other bigenic PS1/APP transgenic models have been produced^{19–21} (Table 1). As in the model reported by Schmitz and co-workers,² all have had early amyloid deposition, with neuritic dystrophy and reactive gliosis, as well as behavioral deficits. The latter have been the focus of a number of recent studies addressing the role of amyloid deposits on behavior.⁵ All three bigenic mice were generated by crossing APP transgenic mice with PS-1 transgenic mice, but no two are identical in terms of mutations, promoter, or background strain. In the other models APPsw, rather than a double APP mutation, is used and most use the PrP promoter rather than the Thy-1 promoter for both the APP and PS-1 parental transgenic mice. The choice of PS1 mutation also varies in the reported bigenic mice; however, the presence of the additional mutated PS-1 transgene in every case leads to accelerated amyloid deposition compared to the transgenic mice with only APP. Given that many of the transgenic mice reported to date have had robust amyloid pathology at relatively early ages, it would seem

unlikely that the severity of amyloid deposition or time course of amyloid deposits is a major explanation for differences between the models.

At present, neuronal loss has been assessed in only one of three bigenic animals, and this study failed to show neuronal loss, either globally in the cerebrum or in the hippocampal pyramidal cell layer.²² Variance in the data precluded detection of neuronal loss less than 20%, but neuronal loss comparable to that reported by Schmitz and co-workers² (30 to 35%) should have been detectable. In considering the role of experimental variance in the differences between the two reports, it is worth noting that both studies were based on a relatively small number of bigenic animals ($N = 4^{22}$ and $N = 6^2$). A control used by Takeuchi and co-workers²² was APPsw littermates, while this control was not included in the Schmitz study² due to the breeding strategy. The Schmitz study² did use PS-1 littermate controls, however.

A complicated issue to consider in assessing differences between transgenic models is the role genetic background might have had on the observed outcomes. This is an area of increasing research,²³ but often relatively neglected. In some reports of transgenic animals, the genetic background information is not even included in the description; however, it is the consensus that this information should be mandatory in all reports of genetically modified mice.²⁴ The role of genetic background certainly makes use of wild-type mice bred separately or purchased from the supplier suspect. It appears that this may have been the type of control chosen in the study by Schmitz and co-workers.² A better control would have been non-transgenic littermates; however, given that the mating was between homozygous PS-1 mice and hemizygous APPsw/APP_{V717I} mice, a non-transgenic control was not possible. Littermate controls are preferable since they control for genetic background. The controls used by Takeuchi and co-workers²² included non-transgenic littermates. Despite this concern, it is unlikely that use of sub-optimal controls is the explanation for differences between the two studies; however, it is worth noting that the most robust statistical differences were between the wild-type controls and the bigenic mice rather than between the PS-1 littermates and the bigenic mice.²

The effect of genetic background is a recognized factor in determining complex behavioral and neuronal phenotypes in genetically modified mice.²³ Since there is no "normal" mouse strain, it is essential to control for genetic background using strategies that have been recommended by a recent consensus committee, including back-crossing onto standard laboratory strains, such as C57B6.²⁴ An example of the importance of genetic background in transgenic models of AD is the recent report that behavioral deficits observed in outbred APPsw transgenic animals were not detected when the animals were back-crossed to produce congenic mice.²⁵

Another caveat of the study by Schmitz and co-workers² was that the analysis was restricted to female animals. The explanation for limiting the analysis to females is not given, but sex has been shown to influence the phenotype of several transgenic models of AD, most notably the descriptions of more severe amyloid deposi-

tion in female APP mice^{26,27} and more marked neurofibrillary pathology in female tau mice.²⁸ For some transgenic mice, most notably Tg2576,²⁷ male mice are unusually aggressive and need to be housed singly. This creates yet another difference when considering behavioral and pathological phenotypic differences between males and females, since the nature of housing has been shown to influence the phenotype. For example, Jankowsky and co-workers recently showed that environmental enrichment is associated with a greater amyloid burden in APP transgenic mice.²⁹

In summary, the model described by Schmitz and co-workers² has significant value as a model for neuronal loss associated with amyloid deposition if the results can be confirmed in a larger series of animals with appropriate non-transgenic littermate controls and if the neuronal loss can be shown to be anatomically relevant to that found in AD. The temporal sequence of biochemical and cellular pathology, as well as the behavioral correlates of amyloid-associated neuronal loss are important outcome variables that remain to be determined.

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