Commentary

Neuropathological Verisimilitude in Animal Models of Alzheimer's Disease

Key to Elucidating Neurodegenerative Pathways and Identifying New Targets for Drug Discovery

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Although fibrillar A β deposits in the extracellular space, known as senile plaques (SPs), and intraneuronal aggregates of τ fibrils, known as neurofibrillary tangles (NFTs), exhibit properties of amyloid and are the defining neuropathological hallmark lesions of the Alzheimer's disease (AD) brain, most patients with familial or sporadic forms of AD as well as elderly Down's syndrome patients with AD also exhibit a third type of amyloid lesion, known as a Lewy body (LB), which is formed by intraneuronal accumulations of α -synuclein fibrils.^{1–3} Thus, AD is a neurodegenerative dementia in which clinical manifestations may arise from a triplet of brain amyloidoses caused by the pathological fibrillization of at least three different building block peptides or proteins (ie, A β , τ , and α -synuclein) that form three distinct types of amyloid deposits (ie, SPs, NFTs, and LBs, respectively) within or outside neurons. However, there are a host of other pathologies that also are consistently associated with AD brain degeneration including neuron and synaptic loss, gliosis, microglial proliferation, as well as other evidence of inflammatory processes, oxidative/nitrative damage, lipid peroxidation, and cholinergic deficits.⁴ Although a direct causal or mechanistic link between these other abnormalities and the diagnostic hallmark SPs and NFTs of AD remain primarily speculative, several of these pathological processes (eg, inflammation and cholinergic deficits) have emerged as potential targets of therapeutic intervention in AD.^{5–8} Indeed, the first Food and Drug Administration-approved AD-specific therapies were directed at correcting the cholinergic neurotransmitter abnormalities in AD, and, although later generation cholinesterase inhibitors have less toxicity than the original prototype compound, the therapeutic efficacy of this class of drugs has been modest at best to date.^{6,7} Thus,

further progress toward optimizing this therapeutic strategy for the treatment of AD patients, as well as further insights into the role of cholinergic neurotransmitter failure in the cognitive and other clinical impairments in AD could benefit from studies of the cholinergic system in animal models of AD-like neuropathology.

To that end, in the current issue of *The American Journal of Pathology*, Gau and colleagues⁹ report studies examining presynaptic cholinergic markers and β -secretase activity during the progressive accumulation of AD-like A β amyloidosis in one of the most well-characterized transgenic mouse models of this neuropathology (Tg2576 mice), which was established by Hsiao and collaborators¹⁰ by engineering these mice to overexpress the human amyloid precursor protein (APP) with the Swedish double familial AD (FAD) mutation (APP^{swe}). Indeed, these mice show many features of AD-like neuropathology including such abnormalities as SPs and other forms of A β deposits, increased levels of soluble and insoluble A β , abnormal synaptic plasticity, microgliosis, inflammation, oxidative stress, lipid peroxidation, and so forth.^{10–18} Further, although they do not develop NFTs or LBs, these transgenic mice do show evidence of τ , ubiquitin, and α -synuclein-positive neurites similar to those seen in AD brains,¹⁹ but, in remarkable contrast to the AD brain, the Tg2576 mice show little or no neuron loss in the central nervous system, even at the end of their life span when SPs, other A β deposits, and brain A β peptides are highly abundant.²⁰ Thus, these transgenic mice recapitulate most of the features of the A β

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amyloidosis typical of classic AD, thereby making them attractive animal models for many types of studies of degenerative processes in AD, as well as for the screening and testing of anti-A β amyloid therapies, but they do not show extensive verisimilitude to the full spectrum of AD neurodegenerative pathology. Moreover, in the studies of these mice at 14, 18, and 23 months of age conducted by Gau and colleagues⁹ reported herein, the authors noted that there were no significant differences between wild-type and transgenic mice with respect to four separate measures of central nervous system cholinergic neurotransmission, ie, choline acetyltransferase and acetylcholinesterase activities, binding to vesicular acetylcholine transporter and Na⁺-dependent high-affinity choline uptake sites. Although an enzyme-linked immunosorbent assay designed to measure the secreted human β -secretase cleavage product (APPs β swe) of APPswe did not demonstrate any abnormalities with aging in the brains of these transgenic mice, Gau and colleagues⁹ did detect an age-dependent increase in soluble A β 40 and A β 42 levels and progressive deposition of A β into SPs and other plaque-like lesions, and these findings are primarily consistent with those described in several earlier reports.^{10,14,15,17} Based on their findings of presynaptic cholinergic integrity in aging Tg2576 mice, Gau and colleagues⁹ suggest that these mice may show more verisimilitude to the early stages of AD with preserved presynaptic cholinergic innervation rather than to fully developed or end stage AD. Nonetheless, the authors point out that some lines of transgenic mice that model AD amyloidosis do show evidence of a certain degree of cholinergic abnormalities.^{21–23}

However, among the large array of pathological abnormalities seen in the AD brain, it seems increasingly likely that brain amyloidosis is the driving force underlying the neurodegeneration and clinical impairments in AD. Accordingly, it would seem highly plausible that the well-documented cholinergic deficits in AD could be because of deposits of amyloid formed from τ and/or α -synuclein fibrils, if they are not caused by deposits of fibrillar A β amyloid. Indeed, recognition of a common mechanistic theme shared by AD and many other seemingly unrelated neurodegenerative disorders (eg, synucleinopathies, tauopathies, prion disorders, trinucleotide repeat diseases) has begun to emerge with the growing realization that a large number of these disorders are characterized neuropathologically by intracellular and/or extracellular aggregates of proteinaceous fibrils many of which show the properties of amyloid including thioflavin staining as well as Congo Red birefringence.¹ Thus, these disorders may share similar physicochemical targets for drug discovery, and despite differences in the molecular composition of the structural elements of these filamentous amyloid lesions, an expanding body of evidence supports the hypothesis that similar pathological mechanisms (ie, aberrant protein folding, fibrillization, and aggregation) may underlie all of these disorders. Specifically, the onset and/or progression of neurodegeneration in AD and other degenerative disorders characterized by prominent brain amyloidosis may be linked mechanistically to abnormal interactions between brain proteins that lead to their assembly into filaments and the

aggregation of these filaments within and/or outside brain cells as fibrous amyloid deposits.¹

These filamentous lesions are exemplified by NFTs as well as SPs in sporadic and familial AD. Moreover, although LBs are regarded as hallmark intracytoplasmic neuronal inclusions of Parkinson's disease, they also occur in the most common subtype of AD known as the LB variant of AD, and it is now known that FAD mutations and trisomy 21 lead to abundant accumulations of LBs composed of α -synuclein filaments in the brains of most FAD and elderly Down's syndrome patients, respectively.^{1–3} Thus, the aggregation of brain proteins into potentially toxic lesions is emerging as a common mechanistic theme in a diverse group of neurodegenerative diseases that share an enigmatic symmetry, ie, missense mutations in the gene encoding the disease protein cause a familial variant of the disorder as well as its hallmark brain lesions, but the same brain lesions also can be formed by the corresponding wild-type brain protein in a sporadic form of the disease. Thus, clarification of this enigmatic symmetry in any one of these disorders is likely to have a profound impact on understanding the mechanisms that underlie all of these disorders as well as on efforts to develop novel therapies to treat them. Nonetheless, because most progress in the last decade of AD research has been made toward identifying therapeutic targets to prevent or eliminate amyloid deposits formed by A β fibrils, many of the most promising emerging therapies for AD have been or are directed at these targets.⁸ For example, as described elsewhere⁸ and in the Alzheimer Research Forum website (<http://www.alzforum.org>), there is a growing number of proposed potential AD therapies that target the disruption of filamentous A β lesions in the AD brain or they are designed to prevent formation of them, and it is possible that similar principles could be exploited to treat other forms of brain amyloidosis. Indeed, it is now feasible to screen large libraries of compounds in high-throughput *in vitro* assays to identify small numbers of drugs that then can be selected for more focused testing in animal models of neurodegenerative diseases.²⁴ Moreover, novel therapeutic approaches that use peptide building blocks of the abnormal fibrils that form brain deposits of A β amyloid in AD as vaccines to prevent or reverse AD amyloidosis⁸ could be extended to treat other neurodegenerative disorders characterized by brain amyloidosis, and, quite remarkably, this seems plausible to accomplish even for a seemingly intractable group of diseases such as prion disorders.²⁵

However, the availability of transgenic mice that model multiple brain amyloidoses (due for example to A β , τ , and α -synuclein fibrils *versus* only one form of amyloidosis resulting from the fibrillization of only a single fibrillizing peptide/protein) should enhance efforts to develop more specific therapies for the different forms of amyloid in AD brains.^{26,27} Indeed, one might envision the generation of transgenic mice that separately model A β amyloidosis, τ amyloidosis, and α -synuclein amyloidosis, as is currently the case, as well as other transgenic mice that model all three of these amyloidoses, similar to the LB variant of AD, or transgenic mice with admixtures of these various amyloids to model AD without LBs or tauopathies with

some A β deposits such as Marianna Island dementia, and so forth. Additionally, these mouse model systems will prove exceptionally valuable in dissecting out the molecular and cellular mechanisms that lead to cholinergic deficits in AD. Finally, whether or not the interpretations and speculations by Gau and colleagues⁹ of their current findings prove to be fully correct, it is increasingly clear that neuropathological verisimilitude of animal models of AD to the entire spectrum of AD brain degeneration (ie, from the prodromal to the end stages of this disorder) will provide the necessary model systems with which investigators can dissect out the entire cascade of cellular and molecular pathways that underlie AD brain degeneration. With these models in hand, it also should be possible to develop an array of therapeutic interventions that might benefit patients regardless of where they lie along the AD neurodegeneration continuum.

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