

Mouse Models of Human Neurodegenerative Disorders

Requirements for Medication Development

Floyd E. Bloom, MD; John F. Reilly, PhD; Jeff M. Redwine, PhD; Chi-Cheng Wu, PhD; Warren G. Young, PhD; John H. Morrison, PhD

Central nervous system diseases constitute a major target for drug development. Transgenic mouse models, in which genes identified in familial forms of human brain diseases are expressed in mouse neurons and glia, offer opportunities to detect and follow pathologic progression and provide potential biomarkers by which to assess therapeutic interventions. Evidence for Alzheimer disease suggests some starting requirements for the experimental data that could enhance the likelihood of developing medications in these mouse models that would also be effective in humans.

Arch Neurol. 2005;62:185-187

Diseases of the central nervous system remain a major burden of illness to society and a major opportunity for drug development. Despite the completion of the human and mouse genome inventories, the molecular bases for most major brain diseases are unknown, aside from the relatively rare single-gene diseases. Furthermore, given the very large numbers of genes used exclusively or predominately by the nervous system, the ability to detect genes whose expression is altered either by the disease's process or by the adaptations cells of the brain undertake to resist the disease process becomes a major technological challenge. Nevertheless, the detailed genomic information available provides the preparatory tools for establishing molecular mechanisms of neuropathology.

Familial forms of human neurodegenerative diseases offer a starting point to recapitulate the pathological processes in transgenic (Tg) mouse models. Because it is possible to define pathologic processes early in the course of a Tg mouse's life, before signs of functional deficit emerge, finding the earliest possible biomarkers for pathologic conditions can be useful for testing hypothetical disease mechanisms and refining optimal treatments based on the progression of biomarkers.

The classic method for defining cellular neuropathology has been microscopy. While the tools for neurochemistry, non-invasive neuroimaging, and neuronal gene discovery have advanced rapidly, the same cannot be said for current methods of chemical and structural neurocytology. Furthermore, the absence of a standardized regimen for the collection and organization of neuroanatomic data makes accurate comparisons such as those achieved in the analysis of DNA or protein sequences problematic. Thus, unfortunately, the products of many current comprehensive analyses of gene expression patterns in the human and experimental animal brain are approximate rather than precise and quantitative.

Over the past 3 years, we have developed high-throughput, rigorous, and standardized methods for research on brain structures, from cells to macroregions to whole brains, and devised protocols for analysis of gene expression patterns within the 3-dimensional context of the brain's structure, circuits, and cells.

ALZHEIMER DISEASE: A PROTOTYPE FOR TECHNOLOGICAL INNOVATION

Alzheimer disease (AD) has been found to have highly inheritable familial forms, as do several other neurodegenerative diseases. Transgenic mouse models of the mutated forms of one likely AD candidate protein,

Author Affiliations: Neurome Inc, La Jolla, Calif (Drs Bloom, Reilly, Redwine, Wu, and Young); and Neurobiology of Aging Laboratories, Mount Sinai School of Medicine, New York, NY (Dr Morrison).

Table. Single, Double, and Triple Transgenic Mice in Which Familial Alzheimer Disease Genes Have Been Examined for the Time Course of Amyloid Deposition

Source	APP Mutation (Promoter)	APP Genetic Background	PS-1 Mutation (Promoter)	PS-1 Genetic Background	Tau Mutation	Tau Background	Age at Amyloid Detection
Single Transgenic APP Mutant Mice							
Games et al, 1995*	APP695, 751, and 770 V717F (PDGFβ)	C57B6xDBA xSwiss Webster					6 mo
Hsia et al, 1999*	APP695, 751, and 770 V717F (PDGFβ)	C57B6xDBA					6 mo
Hsiao et al, 1996*	APP695 K670N, M671L (hPrP)	C57B6/SJLxC57B6					9 mo
Strurchler-Pierrat et al, 1997*	APP751 K670N, M671L (mouse Thy-1)	C57B6xDBA					6 mo
Moechars et al, 1999*	APP695 V642I (mouse Thy-1)	FVB/N					12-15 mo
Chisti et al, 2001*	APP695 K670N, K671L, and V717F (hPrP)	C57B6 xC3H					3 mo
Richardson et al, 2003 ⁶	APP695 K670N, M671L (mouse Thy-1)	C57B6 xC3H					12 mo
Buttini et al, 2002 ³	APP695, 751, and 770 V717F (PDGFβ)	C57B6 (>95%)					12 mo
Kulnane and Lamb, 2001 ⁴	APP 695/751/770, K670N/M671L, endogenous promoter	C57BL6x129/Sv					14-16 mo
Double Transgenics: APP and PS-1 or tau							
Schmitz et al, 2004†	APP751sw APPV717I (mouse Thy-1)	CBA C57B6	M146L (pHMG)	C57B6			2.5 mo
Holcomb et al, 1998, 1999†	APP695sw (hPrP)	C57B6/SJL C57B6	M146L (hPrP)	SW/B6D2 B6D2			16 wk
Dinely et al, 2002†	APP695sw (hPrP)	C57B6/SJL	A246E (mo Thy-1)	C57B6/SJL			7 mo
Borchelt et al, 1997†	APP695sw (hPrP)	C3H/HeJxC57B6	A246E (hPrP)	C3H/HeJxC57B6			"Accelerated" to 12 mo from 18 mo
Lewis et al, 2001 ⁵	APP695 K670N, M671L (hPrP)	C57B6/SJLxC57B6			P301L	JNPL3	6 mo (neurofibrillary tangles, 3 mo)
Triple Transgenics: APP, PS-1, and tau							
Oddo et al, 2003†	APP695 K670N, M671L (mouse Thy-1)	C57B6x129	M146V		P301L		Intraneuronal: 3 mo; extracellular: 6 mo

Abbreviations: APP, amyloid precursor protein; hPrP, hamster prion protein; PDGFβ, platelet-derived growth factor β; PS-1, presenilin 1.

*Modified from Dodart et al, 2002.¹

†Modified from Dickson, 2004.²

the amyloid precursor protein (APP), have been achieved, alone or in combination with known mutations in other familial forms, including presenilin and tau (**Table**).¹⁻⁶ Each of the mutated APPs shows amino acid substitutions around the proteolytic cleavage sites of the β or γ secretases. Therefore, the resulting abnormal fragments of APP, Aβ1-40, or Aβ1-42 are thought to be the neurotoxic agents of the disease, and drugs developed to block these proteases or means to blunt their effects (eg, absorption by antibodies) have been pursued as treatments.

Comparison of the different mouse models for the earliest time at which diffuse or compact amyloid deposits are detected reveals that the mutation, the transcriptional promoter used to drive its expression, the background strains in which the mutation is fostered, and several other incompletely known factors combine to produce highly variable courses across Tg mouse models (Table). Rothstein⁷ has drawn similar conclusions for mouse models of amyotrophic lateral sclerosis. However, aside from the intracerebral accumulations of the diffuse or compact aggregates of Aβ, little other mouse neuropathology aside from loss of synaptic proteins has yet been described.

In addition, it is unclear when Tg mice begin to show the pathological effects of the transgene and when the

optimal times of intervention might be, or whether the structural alterations precede or follow the behavioral dysfunctions. Therefore, we sought to define the earliest point at which alterations could be detected and whether such changes were progressive in 1 model. We used quantitative magnetic resonance microscopy and stereology to compare the volumes of the hippocampus in Tg mice at different ages compared with wild-type (WT) mice of the same strain.⁸ Although Tg and WT mice were statistically identical at 40 days, hippocampal volume was statistically significantly smaller by 12.3% in Tg vs WT mice at 100 days and did not progress. Furthermore, the volume loss was restricted to the dentate gyrus (DG), where the difference between the Tg and WT mouse brains was nearly 30% at 100 days.⁸ The human DG is also a site of major neuropathology in the postmortem AD brain.

MAPPING THE PATHWAYS FROM GENES TO DISEASE

All neurodegenerative disorders display selective cellular vulnerability, leaving some neuron classes and circuits devastated and many others unaffected. We next applied our quantitative technologies to define quantitatively the spa-

tial and temporal progression of the age-dependent accumulation of A β in the most vulnerable regions.⁹ Minimal deposition of A β was detected at 6 months of age in PDAPP mice, primarily as thioflavin-positive, compact amyloid, widely dispersed in hippocampus and cortex, that increased only moderately with age. Diffuse amyloid immunostaining increased dramatically between 12 and 15 months in all subfields, particularly in the DG. Amyloid load in the DG was significantly higher than all other hippocampal subregions at 15, 18, and 22 months. The A β appeared to be distributed in a lamina-specific pattern within the DG, highest in the inner and outer molecular layers. Inputs to the outer and inner molecular layers derive from the lateral and medial entorhinal cortices, respectively, and input to the inner molecular layer derives from the dentate hilus. Although total A β loads in the entorhinal cortex prior to 15 months were negligible, between 15 and 22 months there was a progressive increase in diffuse A β deposition selectively in the lateral entorhinal cortex (reaching 16.4% of the volume), while loads in the medial entorhinal cortex remained below 2.3%, demonstrating a circuit-specific accumulation of A β .

More recently, we have pursued the cellular explanation for the volume reduction in the 90-day-old Tg mouse DG by reconstructing dentate granule cell (GC) dendritic complexity¹⁰ with high-throughput diolistic cell loading and 3D neuronal reconstruction. In 90-day-old PDAPP mice, analysis of all sampled GC types revealed a 12% reduction of total dendritic length in PDAPP mice compared with WT littermate controls. Further analysis, performed with refined subgroups, found that superficially located GCs in the dorsal blade were most profoundly altered, exhibiting a 23% loss in total dendritic length, whereas neurons in the ventral blade were unaffected. Superficial GCs of the dorsal blade were particularly vulnerable (a 32% reduction) in the posterior region of the DG. Thus, substantial dendritic pathology is evident in 90-day-old PDAPP mice for a spatially defined subset of GCs well before amyloid accumulation occurs.

CONCLUSIONS FROM PDAPP NEUROPATHOLOGY

These data establish several important links between the temporal progression of amyloid pathology and neural circuitry in this Tg mouse model. First, compact amyloid is deposited early and begins to be detectable between 6 and 12 months of age; however, the proportion of the amyloid load represented by compact (or fibrillar) amyloid remains low and does not increase as do the diffuse amyloid deposits between 12 and 15 months of age. Second, amyloid deposits are selectively present in the inner and outer molecular layers of the DG as defined by the laminar distribution of the zinc transporter. Third, the locations where amyloid is deposited in earnest between 9 and 15 months of age match the sites of volumetric and dendritic complexity differences observed at 90 days, suggesting that certain circuits, such as the lateral (but not medial) entorhinal cortical connections to the DG, are the predetermined targets of the amyloid deposition. Clearly, it is now possible to greatly accelerate the acquisition, analysis, and comparison of morphological data in a detailed, accurate 3-di-

mensional graphical manner to facilitate comparisons of data across experimental groups and to exploit morphological data to assess therapeutic intervention.

REQUIREMENTS FOR MEDICATIONS DEVELOPMENT IN TG MOUSE MODELS

Knowing the earliest form of pathology in one Tg model could be bolstered by taking advantage of the high degree of temporal and behavioral variation in the other Tg models. Transgenic mouse models with more than 1 genetic factor known to affect the human disease should be evaluated (ie, APOE ϵ 4, presenilin 1, and tau) for detection of common earliest pathologic process and its temporal and spatial progression. Defining a common pathologic sequence with different timelines could provide more compelling evidence on which to seek trials in humans based on mouse brain effects after determining dose-response efficacies, time-response efficacies, and pharmacokinetic differences between mice and humans.

Accepted for Publication: July 27, 2004.

Correspondence: Floyd E. Bloom, MD, Neurome Inc, 11149 N Torrey Pines Rd, La Jolla, CA 92037 (fbloom@neurome.com).

Author Contributions: *Study concept and design:* Bloom, Reilly, Wu, Young, and Morrison. *Acquisition of data:* Bloom, Reilly, Redwine, Wu, Young, and Morrison. *Analysis and interpretation of data:* Bloom, Reilly, Wu, Young, and Morrison. *Drafting of the manuscript:* Bloom, Reilly, Wu, Young, and Morrison. *Critical revision of the manuscript for important intellectual content:* Bloom, Reilly, Redwine, Wu, Young, and Morrison. *Statistical analysis:* Redwine. *Obtained funding:* Bloom. *Administrative, technical, and material support:* Bloom, Reilly, Wu, Young, and Morrison. *Study supervision:* Bloom.

REFERENCES

1. Dodart JC, Mathis C, Bales KR, Paul SM. Does my mouse have Alzheimer's disease? *Genes Brain Behav.* 2002;1:142-155.
2. Dickson DW. Building a more perfect beast: APP transgenic mice with neuronal loss. *Am J Pathol.* 2004;164:1143-1146.
3. Buttini M, Yu GO, Shockley K, et al. Modulation of Alzheimer-like synaptic and cholinergic deficits in transgenic mice by human apolipoprotein E depends on isoform, aging, and overexpression of amyloid beta peptides but not on plaque formation. *J Neurosci.* 2002;22:10539-10548.
4. Kulnane LS, Lamb BT. Neuropathological characterization of mutant amyloid precursor protein yeast artificial chromosome transgenic mice. *Neurobiol Dis.* 2001;8:982-992.
5. Lewis J, Dickson DW, Lin WL, et al. Enhanced neurofibrillary degeneration in transgenic mice expressing mutant tau and APP. *Science.* 2001;293:1487-1491.
6. Richardson JC, Kendal CE, Anderson R, et al. Ultrastructural and behavioural changes precede amyloid deposition in a transgenic model of Alzheimer's disease. *Neuroscience.* 2003;122:213-228.
7. Rothstein JD. Of mice and men: reconciling preclinical ALS mouse studies and human clinical trials. *Ann Neurol.* 2003;53:423-426.
8. Redwine JM, Kosofsky B, Jacobs RE, et al. Dentate gyrus volume is reduced before onset of plaque formation in PDAPP mice: a magnetic resonance microscopy and stereologic analysis. *Proc Natl Acad Sci U S A.* 2003;100:1381-1386.
9. Reilly JF, Games D, Rydel RE, et al. Amyloid deposition in the hippocampus and entorhinal cortex: quantitative analysis of a transgenic mouse model. *Proc Natl Acad Sci U S A.* 2003;100:4837-4842.
10. Wu CC, Chawla F, Games D, et al. Selective vulnerability of dentate granule cells prior to amyloid deposition in PDAPP mice: digital morphometric analyses. *Proc Natl Acad Sci U S A.* 2004;101:7141-7146.