Madrid: Beyond Aβ—Learning, Age, DNA Damage, and Pai-1

15 September 2006. This story closes our coverage of the 10th International Conference on Alzheimer's Disease and Related Disorders, held last July in Madrid. Bid adios to this news-rich conference by reading about attempts to branch out beyond the Aβ peptide itself. Two studies summarized here work toward understanding how Aβ functions in a broader context of aging and synaptic plasticity. The final part highlights a novel drug development approach to promote its clearance rather than slow its production.

In a presentation on the continuing analysis of the triple transgenic mouse model of AD, **Frank LaFerla** of University of California, Irvine, focused on the effects of age and learning. Mental activity is thought to protect against AD. In a separate talk, **Serge Gauthier** of McGill University in Montreal, Canada, discussed the promise of cognitive training as a non-pharmaceutical measure that could be used to treat amnestic MCI even while practitioners have to make do without good mechanism-based drugs. But much confusion remains about exactly how this protection works at the cellular level. Synaptic activity appears to drive Aβ production (Cirrito et al., 2005), and too much synaptic activity, that is, excitotoxicity, certainly damages neurons. In his talk, LaFerla addressed the questions of how much good cognitive training can do and what pathology has to do with it. He used his lab's triple transgenic mice to model these questions.

This work is a current focus in the ongoing behavioral characterization of the triple transgenic mouse model beyond the published data (e.g., Oddo et al., 2003; Billings et al, 2005). In brief, starting at 6 months of age, the triple transgenic mice diverge from controls in that they learn but do not remember well, the homozygotes more so than the heterozygotes. Both show a training effect in that repeated learners tend to perform better than transgenics of the same age who must plunge into the Morris water maze the first time. With increasing age, the mice's cognitive performance goes down as intraneuronal Aβ levels go up, a finding made by Claudio Cuello's lab, as well.

In Madrid, LaFerla described a longitudinal study from 2 to 18 months of age, in which his coworkers evaluated triple transgenic mice at multiple time points, at every step comparing them with naïve transgenics. As expected, the trained transgenics performed better at every step. Unexpectedly, however, the training had an effect on the pathology. The repeated learners had less amyloid deposition and soluble Aβ, similar to what Carl Cotman's (Adlard et al., 2005) and Sam Sisodia's groups have found (Lazarov et al., 2005). Unlike the latter study, however, LaFerla's group found no evidence for an enrichment-induced upregulation in enzymes that degrade Aβ monomer. Instead, preliminary data suggest that the repeated learners might have less of certain kinds of Aβ oligomer, particularly the Aβ56* species (Lesne et al., 2006). The mice also had reduced levels of tau phosphorylation and of active GSK3β tau kinase.

Continued training carried the transgenics only so far. By 15 months of age, learners lost their advantage over naïve transgenics, presumably because the induced pathology increases with time and overwhelms whatever activity-induced protective mechanisms are at play. It could also be that aging itself induces gene expression changes that affect learning and pathology, or loss of neurogenesis, LaFerla noted.

The Cdk5 system of tau phosphorylation was unaffected in LaFerla's learning series, but it clearly plays a role in learning- and memory-related synaptic activity. **Li-Huei Tsai**, of the Picower Institute for Learning and Memory at MIT, presented new data for the mechanisms by which this kinase participates in neurodegeneration in a mouse model. Cdk5 has been implicated in AD, Parkinson disease, stroke, and even neurodevelopmental conditions (e.g., Jacobs et al., 2006; Smith et al., 2006; Liu et al., 2005; Venturin et al., 2005). Prior work by Tsai and others has established Cdk5 as a kinase that is normally tightly controlled by its cofactor p35 but becomes hyperactive when the protease calpain cleaves p35 to release p25 (Cruz and Tsai, 2004). An inducible mouse model of p25 shows that a transient p25 sharpens synaptic plasticity and learning, but prolonged p25 exposure leads to a crash and massive neurodegeneration, suggesting that temporary p25 production helps accommodate increased demand for plasticity and learning but that neurons cannot sustain this state (Fischer et al., 2005).

In Madrid, Tsai laid out a temporal sequence of events inside neurons from p25 induction to neuronal death. Like AD patients, the p25 inducible mice suffer severe neuronal loss, astrogliosis, and brain shrinkage. Like the triple transgenics, the p25 inducible mice show increases first and predominantly in intraneuronal Aβ. Data for this include intraneuronal staining with oligomer-specific antibodies and immunogold electron microscopy labeling of filamentous Aβ near the nuclei of forebrain neurons and in distended axons. This led Tsai to suggest that p25 can somehow upregulate Aβ production. The mechanism is unclear, but options include effects on BACE1, on APP C-terminal phosphorylation, and on axonal transport. The mice also show tau hyperphosphorylation (Cruz et al., J. Neuroscience, in press).

Further analysis of the phenotype pointed to DNA damage and cell cycle induction as underlying mechanisms of the neurodegeneration. Tsai's group performed a microarray analysis of brain mRNA preparations to compare gene expression 2 weeks after induction (prior to neuronal loss and gliosis) and 8 weeks after induction (well into degeneration). Of 236 genes, the expression of which was altered at the 2-week point, 74 were related to the cell cycle and DNA damage response. This pattern reflects the induction of neurodegeneration more than its progression, because these genes were much more highly upregulated at the 2-week than the 8-week point, Tsai noted.

Subsequent analyses showed evidence of DNA double-strand breaks in p25 transgenic mice. It also indicated that this damage is still reversible 2 weeks after p25 induction, suggesting that ongoing DNA repair mechanisms can handle damage for a while but that continuous stress will exhaust them. In cultured cortical neurons, p25 induced DNA double strand breaks. It did so 4 hours after p25 expression, but the neurons did not die until many hours later. This echoes the time course in the mice, where DNA damage precedes cell death. (It also fits with longitudinal multiphoton imaging on another severe neurodegeneration mouse model, performed by Tara Spires in Brad Hyman's lab, where doomed neurons that express mutant human tau, bear tangles, and even have activated caspases still manage to survive for weeks.) In Tsai's work, double-staining experiments indicate that those same neurons that accrue DNA damage at the same time also attempt to re-enter the cell cycle. In this sense, the p25 mice confirm earlier data by Karl Herrup, Inez Vincent, and Peter Davies, who suggest that cell cycle re-entry is an early sign of AD. Currently, the Tsai lab is testing human brain samples for evidence of DNA damage and double-strand breaks in AD.

Taken together, Tsai's data would suggest, then, that chronic p25 generation leads to DNA damage, cell cycle reactivation, Aβ induction, and tau phosphorylation, and that these processes feed into each other before the neuron eventually dies. How p25 fits into synaptic activity in aging humans, however, remains a question.

Many scientists across the field share the goal of broadening their vista, and die-hard believers of the amyloid hypothesis practice their own version of this virtue. An example on display in Madrid was **Steve Jacobsen**'s presentation on his company's attempt to reduce Aβ levels by a new mechanism. Wyeth Research, in Princeton, New Jersey, has started a drug development program around the notion that finding a small-molecule inhibitor of a target upstream of an Aβ-degrading enzyme might reduce rising Aβ levels in a way that is different from current attempts, such as secretase inhibition or immunotherapy. Researchers frequently have called for efforts to understand the broader regulation of Aβ metabolism so that targets several steps removed can be tackled for drug development. This strategy has been successful in heart disease, where statin drugs don't attack cholesterol itself but inhibit an enzyme that acts upstream in its synthesis cascade.

In this case, the Wyeth researchers picked up earlier academic work by Steve Estus, Sidney Strickland, and others who had put the plasmin system on the map as one of a handful of Aβ-degrading enzyme systems now known (Tucker et al., 2000; Melchor et al., 2003; Melchor and Strickland, 2005). Plasmin looked attractive because, unlike IDE, ECE, and neprilysin, it cleaves oligomeric Aβ as well as the monomeric form. A growing number of scientists suspect Aβ oligomers of causing acute synaptic dysfunction, and plaques of rousing glial cells and inflammation and impeding impulse transmission in other ways. In AD, Jacobsen said, the plasmin system itself is probably intact but an upstream protein that downregulates it is abnormally increased. That protein is called plasminogen activator inhibitor-1 (Pai-1). Aging and inflammation both somehow elevate Pai-1 in the brain, Jacobsen said. Pai-1 feeds into the biochemical plasmin cascade by inhibiting the tissue plasminogen activator (TPA) complex, which turns into plasminogen and then into active plasmin. Consequently, small-molecule inhibitors of Pai-1 should spur Aβ degradation by plasmin and tip the balance between Aβ production and clearance toward the latter. Such a compound would be the first in a new class, Jacobsen said.

The company scientists have found Pai-1 inhibitors that enter the brain. Some lower plasma and brain Aβ levels in Tg2576 mice, and improve their performance in contextual fear conditioning. A safety concern with this approach is that TPA serves as a treatment for ischemic stroke, so fueling this cascade could cause bleeding. Jacobsen said that TPA is an endogenous molecule that would be induced at much lower concentrations than are given in acute ischemia. Pai-1 knockout mice are normal. Pai-1 loss-of-function mutations known to occur among the Old Order Amish cause no bleeding, though whether these people enjoy protection from AD is unknown, Jacobsen added. A separate genetic tie to AD exists in the form of association studies implicating a tissue plasminogen activator gene in AD; see Alzgene overview. Hasta luego, Madrid.—Gabrielle Strobel.