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Cat and Mouse

The role of cathepsin B, a lysosomal protease implicated in amyloid-β (Aβ1-42) metabolism, in Alzheimer's disease remains controversial. In this issue of *Neuron*, Mueller-Steiner et al. manipulate the expression of cathepsin B in aged APP transgenic mice, observing that increased expression degrades preformed oligomeric and fibrillar amyloid, while inactivation accelerates β-amyloidosis.

Amyloid-β protein (Aβ) is a 40–42 amino acid peptide, accumulating in plaques and blood vessels of Alzheimer's disease (AD) patients. Although Aβ peptides are normally cleaved out of a larger amyloid precursor protein (APP) by endopeptidase "secretase" enzymes and rapidly released and cleared in soluble form, in AD, Aβ peptides accumulate extracellularly as protease-resistant fibrillar aggregates with β pleated sheet conformation. The longer 42 amino acid form (Aβ1-42) has two additional hydrophobic amino acids making it "stickier," causing it to aggregate much more rapidly than the 40 amino acid form and even to accumulate within neurons, an event that may contribute to neurotoxicity. Aβ1-42 is implicated in AD pathogenesis because early-onset familial AD can be caused by many different mutations with the common property of increasing the production of Aβ1-42. However, the majority of AD cases are late onset and of as yet unknown origin.

One contributing and possibly causal factor in Aβ accumulation in late-onset AD is an aging- or disease-related decline in the proteases that normally clear soluble Aβ species. Several proteases have already been identified that contribute to the normal rapid clearance of soluble Aβ. These include insulin-degrading enzyme (IDE), neprilysin (Nep), endothelin-converting enzyme (ECE), and plasmin (Eckman and Eckman, 2005). However, once the β sheet Aβ fibrils have formed, they become resistant to these important normal clearance mechanisms, causing amyloid to accumulate exponentially.

In the current issue of *Neuron*, Mueller-Steiner and colleagues (Mueller-Steiner et al., 2006) at the Gladstone Institute, UCSF, unveil compelling and novel evidence that the endosomal/lysosomal protease cathepsin B (CatB) normally degrades Aβ1-42, including fibrillar Aβ aggregates. Previous research had suggested that CatB might function as a "secretase" and help cleave APP to Aβ. However, when the Gladstone researchers tested the hypothesis by expressing APP in CatB null mice, there was no impact on any APP secretase products. Rather than altering APP processing, loss of CatB resulted in increased amyloid plaque load and increased Aβ1-42 to total Aβ ratios determined by ELISA. The increases in amyloid were accompanied by reduced hippocampal dentate gyrus calbindin, a marker which the authors have previously found to reflect neurodegeneration in their APP transgenic mice. CatB was localized extracellularly to plaques and internally to plaque-associated dystrophic neurites, reactive astrocytes, and microglia (Figure 1). These results are consistent with reports of increased neuronal CatB and extracellular CatB in plaques in AD brain (Nixon and Cataldo, 2006). Within the Tg+ mouse neurons, this Cat was found in lysosomes (as expected). Further, Aβ1-42 levels were decreased in primary neurons overexpressing virally directed CatB, suggesting that intraneuronal Aβ1-42 accumulating in the endosomal/lysosomal system might be a CatB target. Although the mouse model used in the present study has not been reported to accumulate high levels of intraneuronal Aβ, intraneuronal Aβ accumulation has been linked to neurodegeneration (Takahashi et al., 2004) and cognitive deficits (Billings et al., 2005).

CatB Nips at Aβ Better Than Nep

Analysis by SELDI-TOF mass spectrometry demonstrated that the in vitro carboxypeptidase activity of CatB nipped at the Aβ1-42 C terminus, generating the less amyloidogenic C-terminally truncated Aβ1-40 and Aβ1-38. This result explains some selectivity of CatB for long Aβ. Aβ1-33 was also generated, presumably by endopeptidase activity. Most significantly, potentially neurotoxic oligomeric, protofibrillar, and even fibrillar aggregates were equally readily cleaved by CatB, producing the same products. While the activity was higher at pH 6.0, where it might be in intracellular compartments, CatB also effectively degraded all of the Aβ assemblies at neutral pH, suggesting that it can work on extracellular deposits. Consistent with this expectation, expression of stereotaxically injected lentiviral CatB for 28 days in aged plaque-bearing APP transgenic mice cleared preformed Aβ deposits, including thioflavin S-positive plaques, which are known to contain masses of protease-resistant fibrillar Aβ. In contrast, thioflavin S plaques remained unchanged by lentiviral expression of neprilysin (Nep), which only cleared diffuse Aβ deposits compared to viral vector alone. Thus, CatB in mouse was a better Aβ degrader than Nep, although Nep can effectively degrade small Aβ oligomers like dimers that can compromise synaptic function (Huang et al., 2006). In view of the widely held belief that the neurotoxic properties of aggregated Aβ assemblies actually cause AD, the identification of a major protease responsible for their intracellular and extracellular clearance is an exciting result and full of promise. One of the few other options might be to increase matrix metalloproteinase-9, also very recently identified as a fibril protease (Yan et al., 2006). While it is likely not possible to virally express CatB in humans, there may be other ways to increase CatB expression. For example, the authors observed that microglia secrete abundant CatB. This may be regulated by their activation state, which, for example, can be upregulated by the amyloid vaccine or anti-amyloid antibodies, currently one of the most effective methods of reducing preformed thioflavin S-labeled amyloid plaques (Bard et al., 2003). One might consider expressing exogenous CatB in grafted bone marrow cells, which have been shown to migrate

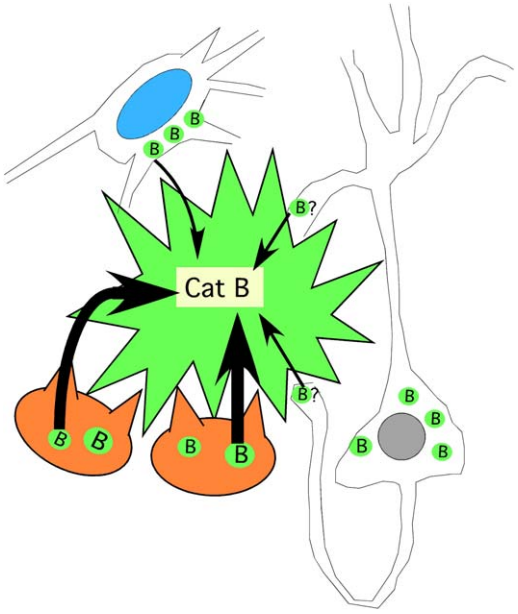

Figure 1. Schematic Diagram Showing the Role of Cathepsin B in Amyloid Clearance

Mueller-Steiner et al. demonstrate that cathepsin B (CatB) can clear fibrillar amyloid-β. CatB can be secreted (arrows) into plaques (green star) and promote the degradation of extracellular amyloid-β. A major potential source of CatB in plaques may be microglia (orange), which secrete avidly in vitro, but other cell types (astrocytes, blue top left) and neurons (gray, right) may also contribute CatB to plaques. The green circles with "B" represent endosomes or lysosomes containing CatB, where it may degrade intracellular Aβ. It is unclear whether CatB secreted from neurons is primarily dendritic or nerve terminal in origin.

to plaques (Simard et al., 2006). However, there is reason to be cautious about overexpression of CatB, which has been reported to contribute to neurotoxic effects of microglia (Gan et al., 2004; Kingham and Pocock, 2001). Of course, there may be other ways to increase intracellular CatB expression with small molecules. Whether or not the new findings lead to CatB-based therapeutics, the evidence for this normal clearance tale for Aβ aggregates is clearly an important new chapter for the AD research field. And with any luck, this will not end as just a Cat in mouse story.

Greg M. Cole[1,2] and Sally A. Frautschy[1,2]
[1]Greater Los Angeles Healthcare System
Veterans Administration Medical Center
Geriatric Research Education Clinical Center
North Hills, California 91343
[2]Departments of Medicine and Neurology
University of California, Los Angeles
Los Angeles, California 90095

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Inherited Neuropathies: New Genes Don't Fit Old Models

Mutations in *GARS* cause dominantly inherited neuropathies in humans. *GARS* encodes glycyl-tRNA synthetase, the enzyme that couples glycine to its tRNA. In this issue of *Neuron*, Seburn et al. have identified and characterized a mutant mouse with a dominantly inherited axonal neuropathy caused by a *Gars* mutation that is inferred to have a gain of function.

In 1886, Charcot, Marie, and Tooth described patients who are now understood as having a dominantly inherited neuropathy that affects myelinated motor and sensory axons in a length-dependent manner. This disorder is usually called Charcot-Marie-Tooth disease, or simply CMT, and is one of the most common inherited neurological diseases (Lupski and Garcia, 2001; Shy et al., 2005; Wrabetz et al., 2004). Like most kinds of neuropathy, CMT is characterized by progressive dysfunction that is related to the length of the affected axons. The longest sensory and motor axons are affected first and are more affected over time. This progressive, length-dependent dying back of motor and sensory axons produces the classic clinical picture of distally accentuated weakness, atrophy, and sensory loss.

By 1980, a few different kinds of CMT were clinically recognized. The demyelinating form was termed CMT1 and is characterized by slowed nerve conduction velocities and evidence of demyelination and remyelination in nerve biopsies. The neuronal/axonal form was termed CMT2, characterized by relatively normal conduction velocities and axonal loss but not demyelination/remyelination in nerve biopsies. The more severe kinds of demyelinating neuropathy that start in infancy or childhood retained different names (congenital hypomyelinating neuropathy or Dejerine-Sottas neuropathy, respectively), and their relationship to CMT was not understood. The terms hereditary motor neuropathies and