Commentary

Cathepsin Deficiency as a Model for Neuronal Ceroid Lipofuscinoses

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Lysosomal storage disorders arise from genetic alterations in lysosomal function and result in the aberrant accumulation of undigested lipoproteins.¹ Neuronal ceroid lipofuscinoses (NCL), collectively known as Batten disease, are autosomal recessive and classical lysosomal storage disorders and are the most common human pediatric neurodegenerative diseases, occurring in 4 to 8 of every 100,000 children.² NCL is a progressive and fatal neurodegenerative disease currently lacking any effective treatment or cure, thus underscoring the need for a greater understanding of its pathophysiology. In this issue of *The American Journal of Pathology*, Koike and colleagues³ elegantly describe how the cathepsin D (CD)-deficient mouse and the combined cathepsins B and L (CB/CL)-deficient mouse share neuropathological features common to NCL. Although mutations and/or deficiencies in cathepsins have not been described clinically in NCL, striking similarities exist in the neuropathology of these mice compared to established mouse models of NCL and to human NCL, suggesting involvement of similar neurodegenerative pathways.

Previous reports of cell death in NCL have focused on apoptotic cell death as its principal death pathway. However, other death pathways also appear to be involved. Cell death has been defined previously by morphological criteria as either type I apoptotic or type II autophagic.⁴ Apoptotic death is regulated by pro- and anti-apoptotic members of the Bcl-2 family⁵ and culminates in the activation of caspases, which are responsible for causing the nuclear condensation, DNA fragmentation, and cell shrinkage that characterize apoptotic morphology.⁴ Autophagy is a normal cellular process whereby cytoplasmic material and organelles are shuttled to the lysosomes by a complex, regulated series of vesicle fusion events for degradation and ultimate recycling of lysosomal contents.⁶ Autophagic death is defined morphologically by the aberrant accumulation of autophagic vacuoles in a degenerating cell that in addition often displays morphological features of apoptosis.⁴ In agreement with such

mechanisms, reports from Koike and colleagues,^{3,7} and Nakanishi and colleagues⁸ indicate the contribution of both apoptotic and autophagic neuron death, suggesting an interrelationship between these two pathways in neuron death and neurodegeneration in NCL.

Clinical Features, Neuropathology, and Genetics of NCL

Clinical features of NCL include seizures, progressive visual impairment, loss of motor function, and ultimately a persistent vegetative state and death.⁹ NCL results in brain atrophy, neuron loss, and reactive gliosis, and NCL brains exhibit an accumulation of autofluorescent storage bodies, defined ultrastructurally as mutation-specific patterns of granular osmiophilic deposits, fingerprint and curvilinear profiles, and/or rectilinear complexes.² NCLs were classified initially by age of clinical onset as infantile (INCL, within 1 year), late infantile (LINCL, 2 to 4 years), juvenile (JNCL, 4 to 7 years), or the very rare adult (ANCL) form. To date, mutations in six human genes (*CLN1*, -2, -3, -5, -6, and -8) have been identified, each yielding distinct biochemical and neuropathological forms of NCL.¹⁰

Mutations in *CLN1* can generate all four forms of NCL, with INCL representing the predominant form.² *CLN1* encodes palmitoyl protein thioesterase-1 (PPT-1), a soluble lysosomal hydrolase that, on its deficiency, leads to the accumulation of small, fatty-acylated peptides.⁹ The majority of diagnosed LINCL cases are attributed to mutations in *CLN2*, which encodes tripeptidyl peptidase-1 (TPP-1), a soluble lysosomal serine carboxyl peptidase.^{2,9} Mutations in *CLN3* result in JNCL, the most commonly diagnosed form of NCL worldwide, whereas mutations in *CLN5*, -6, and -8 result in variations of LINCL.^{2,9} Little is known about the function of mutated *CLN3*, -5, -6, and -8 proteins, but all are predicted to be transmem-

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brane proteins localized to the plasma membrane, endoplasmic reticulum, Golgi, or lysosomes, suggesting mutation-specific alterations in their intracellular trafficking patterns.^{11,12} Brains of CLN1 patients have increased sphingolipid activator proteins (saposins) A and D within storage bodies, whereas brains of CLN2 through CLN8 patients are characterized by increased storage of subunit c of mitochondrial ATP synthase (SCMAS).⁹ A decrease in the activity of TPP-1 is associated with decreased cleavage and hence accumulation of SCMAS in LINCL.⁹ However, it is unclear how SCMAS accumulates in JNCL because mutations in *CLN3* are associated with increased activity of TPP-1.¹³

NCL Gene-Disrupted Mice

Mouse models of CLN1, -2, -3, -6, and -8 have been generated by targeted gene disruptions or identified as spontaneous mutations. Mice deficient in *CLN1*/PPT-1 recapitulate the aggressive INCL phenotype, displaying seizures, progressive motor dysfunction, and death by 7 to 10 months.¹⁴ PPT-1-deficient mouse brains show severe neuron loss, apoptosis, and increased autofluorescent storage bodies but lack a reported increase in saposins, the primary storage protein in human CLN1. *CLN2*-deficient mice show features of LINCL, including loss in TPP-1 activity, accumulation of autofluorescent storage material concomitant with widespread neuron loss and neurodegeneration, tremors, altered motor function, and a 3- to 6-month life-span.¹⁵ However, they do not show increased SCMAS as reported in human CLN2. Both knockout^{16,17} and knockin¹⁸ mouse models have been generated for the *CLN3* gene, yielding slower progression and longer life-span in comparison to *CLN1*- and *CLN2*-deficient mice and closely emulating the delayed onset and longer life-span of human JNCL. *CLN3*-mutant mice display neuron loss and autofluorescent storage bodies that include SCMAS, but behavioral changes are limited to alterations in gait and limb clasping with a lack of seizure-like activity.

Spontaneous mutations for *CLN6* and *CLN8* have been reported in mice, corresponding to genetically variant LINCL that are homozygous for neuronal ceroid lipofuscinosis (*ncfl*) and motor neuron degeneration (*mnd*) genes, respectively.^{19,20} Both *CLN6*- and *CLN8*-mutant mice display altered motor function, brain and retinal atrophy, and accumulated lipoprotein storage bodies, with mice dying by 12 months of age. *CLN8*-mutant mice display an accumulation of SCMAS similar to that seen for human CLN8, but the predominant storage protein(s) of *CLN6*-mutant mice has not yet been identified.

What Cathepsin Deficiency Has Taught Us about NCL

To date, alterations in levels or function of cathepsins have not been reported in mouse models of NCL or in human NCL. However, analysis of cathepsin-deficient mice clearly indicates NCL-like neuropathology, suggest-

ing either that alterations in cathepsins exist in human NCL but are not yet appreciated or that cathepsin deficiency exacerbates the alteration of downstream signaling molecules known to occur in NCL. Cathepsins are lysosomal proteases responsible for the degradation and turnover of proteins at acidic pH, as in autophagy, and are expressed ubiquitously in mammalian neurons.²¹ Within lysosomes, CD is the major aspartate protease, while CB and CL are ubiquitous cysteine proteases.²²

CD deficiency has been found to induce a lysosomal storage disorder with accelerated neurodegeneration, features very similar to human and other mouse models of NCL.⁷ CD-deficient mice develop seizures and blindness and die at approximately postnatal day (P) 26 from massive intestinal necrosis, thromboembolism, and lymphopenia.²³ A spontaneous mutation in ovine CD also induces a phenotype very similar to human NCL,^{24,25} and experimentally generated CD-deficient *Drosophila* recapitulate NCL morphology.²⁶ The combined CB/CL-deficient mouse, which was reported recently,²⁷ succumbs to a more rapid death at ~2 weeks of age and displays massive brain atrophy, neuron cell death, and reactive gliosis.³ Mice singly deficient for CB or CL appear normal (besides hair loss in the CL-deficient mouse), have a normal life-span and are without neurological dysfunction,^{28–30} suggesting functional redundancy between these two cysteine proteases.

With few exceptions, the neuropathology of CD- and CB/CL-deficient brains is strikingly similar, with neurons from each accumulating autofluorescent storage bodies with granular osmiophilic deposits, SCMAS, and autophagic vacuoles^{3,7} as early as P13. Older CD-deficient brains indicate a clear progression of neuropathology that is not evident in CB/CL-deficient brains due to their shorter life expectancy. Levels and activity of TPP-1 also increase in CD-deficient brain, possibly precluding its role in the accumulation of SCMAS (as predicted in CLN2) and perhaps defining a novel subtype of NCL. Similarly, only brains of CB/CL-deficient mice surviving to P17 or later exhibit increased activity and levels of TPP-1 and PPT-1.²⁷ (The effect of CD deficiency on PPT-1 activity has not yet been reported.) The onset of tremors has been documented as early as P10 in CB/CL-deficient mice and at approximately P20 for CD-deficient mice. CB and CD are increased in CD- and CB/CL-deficient brains, respectively,^{7,27} indicating compensatory regulation of cathepsins in response to the other's absence.

Autophagy and Apoptosis in Cathepsin Deficiency

In this issue of *The American Journal of Pathology*, Koike and colleagues³ illustrate the role of cathepsins in the accumulation of lysosomal structures in NCL and lysosomal storage disorders. The lack of lysosomal cathepsins induces extensive neuron death and neurodegeneration that is preceded by an accumulation of autophagosomes. The presence of autophagosomes can be confirmed either morphologically by electron microscopy or biochemically by an increase in MAP-light chain 3-II

(LC3-II), the membranous form of LC3 specific for autophagic vacuoles.³¹ Cathepsin deficiency induces dramatic increases in the 16-kd LC3-II with little change in the cytosolic 18-kd LC3-I,³ suggesting that the increased immunohistochemical detection of LC3 in neurons is specific for LC3-II.

Autophagosomes appear as early as P8 in cathepsin-deficient brain and continue to increase with age.³ Apoptotic, terminal dUTP nick-end labeling-positive neurons appear as early as P16 and increase dramatically by P23.⁸ *In vitro*, the pharmacological inhibition of autophagy by agents that disrupt vesicular acidification induces an accumulation of autophagosomes that in turn induces Bax-dependent apoptosis.³² Perhaps in NCL the disruption of lysosomal function inhibits the recycling of autophagosomes that leads ultimately to apoptotic death. Together, these findings suggest that the accumulation of autophagic vacuoles precedes and possibly stimulates the induction of neuron apoptosis, a sequence of events that most likely occurs in mouse models of NCL and in human NCL.

Analysis of cathepsin-deficient brain also indicates widespread microglial activation, which has been proposed to induce secondary neuron apoptosis. Treatment of CD-deficient mice with inhibitors of nitric oxide synthase decreases the number of apoptotic neurons but does not reduce the number of autophagic neurons, microglial activation, or the close association of microglia with autophagic neurons, suggesting that an accumulation of autophagic neurons induces an inflammatory state that stimulates nitric oxide-dependent apoptotic death of neighboring neurons.⁸ Preliminary evidence in our laboratory suggests that removal of the intrinsic mitochondrial apoptotic pathway via the targeted deletion of Bax prevents apoptotic neuron death but not autophagosome accumulation or widespread neurodegeneration induced by CD deficiency (Shacka and Roth, unpublished observations). These data suggest again that apoptosis in NCL may be secondary to the accumulation of autophagosomes in neurons.

Summary and Future Challenges

Regardless of the direct or indirect relationship to NCL, the study of cathepsin deficiency has generated a wealth of information regarding the neuropathology and neuronal cell death pathways in NCL and lysosomal storage disorders. An important future challenge will be the further temporal and spatial characterization of apoptosis and autophagy in NCL in the hopes of determining the relative contributions of these two death pathways to NCL-induced neuropathology. Ultimately, effective treatment strategies will need to prevent the accumulation of autophagosomes and the subsequent induction of autophagic and apoptotic neuron death. In addition to NCL and lysosomal storage disorders, other neurodegenerative diseases, such as Alzheimer's, Parkinson's, and Huntington's, have been characterized by an accumulation of storage proteins and elements of autophagy and/or apoptosis.^{1,33–35} Therefore, regardless of the initiating

biochemical event(s), neurodegenerative disease in its broadest scope may involve activation of both autophagic and apoptotic cell death pathways.

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