Commentary

Linking Selective Vulnerability to Cell Death Mechanisms in Parkinson's Disease

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Two of the major unresolved questions in neurodegenerative diseases are the factors that determine which neurons degenerate and by what mechanism this occurs. There are several leads when it comes to Parkinson's disease, and the studies reported by Zhu et al¹ in this issue of *The American Journal of Pathology* suggest that there may be a link between oxidative stress and non-apoptotic cell death involving autophagic cellular mechanisms.

Neurodegenerative diseases are characterized by selective loss of specific populations of neurons. The distribution of neuronal loss determines the clinical phenotype and is a more powerful determinant than underlying molecular pathology. For example, degenerative diseases that affect the frontal lobe have diverse molecular biology, but they all are capable of producing a similar clinical syndrome.² Likewise, damage to the dopaminergic neurons in the substantia nigra that project to the putamen, known as the nigrostriatal pathway, are affected in a number of disorders that produce parkinsonism, the clinical syndrome associated with slowing of movements, rigidity, tremors, and postural instability. The most common neurodegenerative disorders associated with parkinsonism are listed in Table 1, which demonstrates diversity with respect to both molecular biology and histopathology. It is increasingly clear that neuronal vulnerability in Parkinson's disease extends far beyond the substantia nigra and includes specific neurons in autonomic ganglia, spinal cord, brainstem, basal forebrain, limbic lobe, and even the neocortex,³ but degeneration of the nigrostriatal pathway remains the feature that is most relevant for the parkinsonian clinical syndrome. There are hints as to why the dopaminergic neurons would be selectively vulnerable in Parkinson's disease (see below), but as yet there is no plausible explanation for vulnerability of other neuronal populations.

The basis of selective vulnerability in neurodegenerative disease is largely unknown. Specific features of neuronal phenotype are most often implicated. Several ex-

amples illustrate the state of current knowledge. It has been suggested that distribution of cortical neurodegeneration in Alzheimer's disease correlates with patterns of developmental myelination, with neurons in association areas with minimal myelination being more vulnerable than neurons in primary cortices with more extensive and later myelination.⁴ Stress-associated neuronal loss in the hippocampus has been shown to correlate with the distribution of glucocorticoid receptors in this brain region.⁵ Neurons deficient in calcium buffering proteins, such as parvalbumin and calbindin, may render them vulnerable in motor neuron disease.⁶ Specific glutamate receptor subtypes in hippocampal neuronal subpopulations may account for selective neuronal vulnerability mediated by excitotoxic cell death in epilepsy and hypoxia.⁷ Finally, the susceptibility of dopamine and its metabolites to produce reactive oxygen intermediates may explain vulnerability of substantia nigra neurons.⁸

Support for oxidative stress mechanisms in dopaminergic degeneration in the substantia nigra in Parkinson's disease⁹ comes from a growing body of evidence, indicating that this region has a high propensity for oxidative stress and is also deficient in protective mechanisms. In addition to dopamine and its metabolites, which may have an intrinsic tendency to form reactive oxygen species, the substantia nigra is also rich in iron and copper, essential cofactors in biosynthetic enzymes involved in catecholamine metabolism such as tryptophan hydroxylase and dopamine β -hydroxylase. The oxidation-reduction cycle of iron can generate free radicals and toxic metabolites (eg, hydrogen peroxide). Mitochondrial abnormalities (eg, deficiency in the mitochondrial respiratory chain complex 1), which lead to uncoupling of redox reactions and generation of reactive oxygen species, have also been implicated in Parkinson's disease.¹⁰ There is also

Supported by the Morris K. Udall Parkinson's Disease Research Center of Excellence grant P50-NS40256.

Accepted for publication October 25, 2006.

This commentary relates to Zhu et al, *Am J Pathol* 2007, 170:75–86, published in this issue.

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Table 1. Molecular Pathology of Parkinsonism

Disorder	Molecular pathology	Major morphologic feature
Parkinson's disease	α -Synuclein	Lewy bodies
Dementia with Lewy bodies	α -Synuclein	Lewy bodies and Lewy neurites
Multiple system atrophy	α -Synuclein	Glial cytoplasmic inclusions
Progressive supranuclear palsy	Tau	Neurofibrillary tangles and tufted astrocytes
Corticobasal degeneration	Tau	Neuropil threads and astrocytic plaques
Guam Parkinson dementia complex	Tau	Neurofibrillary tangles
Frontotemporal dementia and parkinsonism	TDP43 ³⁸	Neuronal cytoplasmic inclusions

evidence that the substantia nigra in Parkinson's disease may be deficient in antioxidant molecules such as glutathione.¹¹

Many neurodegenerative disorders, including most of the parkinsonian disorders, are characterized by neuronal or glia inclusions or both (see Table 1). The hallmark histopathological lesion in Parkinson's disease is the Lewy body, a neuronal cytoplasmic filamentous aggregate composed of the presynaptic protein α -synuclein. Although recent evidence from experimental models suggests that inclusion formation may play an adaptive or protective response,¹² in human neurodegenerative diseases, the presence and distribution of inclusions closely parallels neuronal loss. This argues that if inclusions are an adaptive response, the response is ultimately ineffective. There are reasons to suspect that inclusion bodies contribute to disease pathogenesis through one of several means. The inclusions are for the most part composed of abnormal conformers of normal proteins, and pathological protein conformers have toxic properties in cell culture experiments and experimental models. The mechanisms for toxicity include loss of function due to sequestration of proteins essential to normal cellular physiology, disruption of cellular functions due to mass effects of the inclusions (eg, disrupting axoplasmic transport), disruption of proteolytic processing (eg, lysosomal or proteasomal), and induction of cell stress through unfolded protein response or oxidative stress related to posttranslational modifications of the abnormal conformers.¹³

In addition to selective vulnerability, the other major unsolved research question related to neurodegenerative disorders is the mechanism of neuronal death. Although apoptotic cell death is an appealing mechanism for neuronal loss in degenerative disease,¹⁴ and cells with morphological features consistent with apoptosis are sometimes detected in Parkinson's disease,¹⁵ there is increasing experimental evidence that apoptosis probably does not play a major role in neuronal loss in the substantia nigra in Parkinson's disease.^{16,17} Postmortem artifacts, specifically postmortem DNA fragmentation, may account for at least some of the reports of apoptosis in the substantia nigra that relied on terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling techniques.¹⁸

The major nonapoptotic form of cell death is necrosis, which is not likely to play a major role in neurodegenerative diseases because it is associated with energy failure, cell swelling, and rupture with ensuing inflammation, fea-

tures that are largely absent in neurodegenerative disorders. Increasing interest has focused on autophagy and lysosomal pathways in cell death.¹⁹ Autophagy is a fundamental cell process associated with phagocytosis of cellular organelles and other cytoplasmic constituents that can be initiated internally, as in nutrient deprivation, or by external signaling cascades.²⁰ In Parkinson's disease, there is increasing evidence that autophagy plays a role in degeneration and cell death. For example, neurons in the substantia nigra display autophagic features,¹⁵ cellular models with overexpression of mutant α -synuclein show signs of autophagy,²¹ dopamine can induce autophagic cell death as well as α -synuclein expression,²² and axonal dystrophy, which is common in Parkinson's substantia nigra, can be induced by autophagy.²³

A link between oxidative stress and autophagic cell death has been the focus of several recent reviews.^{24–26} Oxidative stress has been implicated in cellular models for Parkinson's disease using the neurotoxin 1-methyl-4-phenylpyridium, the active metabolite of 1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine (MPTP).¹ MPTP was discovered as a toxin that produced parkinsonism in users of illicit drugs, and subsequently, it has been widely used in animal models that faithfully reproduce a number of the clinical and pathological features of nigrostriatal degeneration in Parkinson's disease.^{27–29} The most probable mechanism of MPTP toxicity is oxidative stress because it has been shown to be an inhibitor of the mitochondrial respiratory chain complex 1, leading to uncoupling and generation of reactive oxygen species.²⁹ The study by Zhu and coworkers¹ in this issue of the *AJP* provides compelling evidence, using cell biological as well as ultrastructural analyses, that exposure of both neuroblastoma cell lines and primary dopaminergic neurons to MPTP induces autophagy. Of interest is the fact that the authors were able to determine that the autophagy was distinct from that induced by nutrient withdrawal, a process that has been extensively studied in yeast and has lead to a number of useful biological tools for dissecting this process, because it is dependent on proteins that are shared among a wide range of eukaryotic species.³⁰ The autophagic toxicity induced by MPTP, which presumably is mediated by oxidative stress, is dependent on extracellular signal related kinases such as mitogen-activated protein kinases.

The wider significance of this observation is that the most common genetic basis of Parkinson's disease is mutation in a mitogen-activated protein kinase. Specifi-

cally, mutations in the gene for leucine-rich repeat kinase 2 (*LRRK2*)^{31,32} account for about 3% of seemingly sporadic Parkinson's disease, with as high as 40% of familial Parkinson's disease depending on the cohort.^{33–36} Although much work remains to be done to determine whether the mechanism of *LRRK2* mutations is through kinase-dependent signaling pathways, preliminary evidence suggests that this is the case.³⁷ The intriguing possibility raised by the study of Zhu and coworkers is that this kinase activation lies downstream of autophagic processes that may lead to neurodegeneration. In susceptible neurons in the substantia nigra, this process may be inextricably linked to oxidative stress, although it remains to be determined whether similar mechanisms apply to nondopaminergic neurons widespread throughout the neuraxis that are selectively vulnerable to α -synuclein-associated neurodegeneration and cell death in Parkinson's disease.

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