Misfolded, protease-resistant proteins in animal models and human neurodegenerative disease

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Neurodegeneration in the substantia nigra has been known to be a constant feature of Parkinson disease (PD) since the early part of the twentieth century, when Trétiakoff (1) correlated the clinical findings of patients with PD with postmortem pathology. A few years earlier, Lewy had described concentric hyaline inclusions within neurons, which are now referred to as Lewy bodies (LBs) (2). LBs are a sine qua non for the neurodegeneration that characterizes PD, but they are also found in nonparkinsonian disorders, most notably dementia with Lewy bodies (3). LBs are composed of filamentous aggregates of α-synuclein (4), and immunohistochemistry for synuclein is currently the most sensitive method for detecting LBs in brain tissue. Ubiquitin is another key component of LBs (5), and widespread use of immunohistochemical methods for detecting LBs, first with ubiquitin and later with synuclein antibodies, demonstrated that pathology in these disorders extended beyond LBs within neuronal perikarya to fibrillar lesions within neuritic processes, so-called Lewy neurites. Neuritic pathology is more widespread than LBs and affects regions of the brain, such as the hippocampus (6), that were not previously considered affected in PD. More recently, using antibodies to modified forms of α-synuclein (7) or sensitive antigen retrieval methods, it has been discovered that LBs are more prevalent and Lewy neurites more widespread than previously recognized. For example, LBs are present in a high proportion of Alzheimer brains (8), and the basal ganglia contain many Lewy neurites (7). The report by Neumann et al. in this issue of the JCI moves the field forward another step (9). The authors have employed a method for detecting abnormal forms of α-synuclein that may not necessarily be composed of fibrillar structures, which are the feature that permits ready detection of α-synuclein in LBs and Lewy neurites in tissue sections.

**Histoblots detect protease-resistant protein**

In this report (9) Neumann and coworkers applied a modification of a method, the histoblot (10), originally developed for detection of the pathologic form of prion protein (PrP\(^{res}\)), which evidence suggests is the causative agent in transmissible spongiform encephalopathies such as Creutzfeldt-Jakob disease (11). The histoblot method takes advantage of the unusual solubility properties of PrP\(^{res}\) (derived from the nonpathogenic cellular form of prion protein, PrP\(^{c}\)) that make it resistant to proteases. Sections of brain tissue are cut and applied to a nitrocellulose membrane support and subsequently treated with protease, which digests PrP\(^{c}\) and leaves an imprint of the distribution of PrP\(^{res}\) in the remnants of tissue adherent to the nitrocellulose. The nitrocellulose is then immunostained much like a Western blot, but the result is an immunohistochemical imprint of the pathologic form of the protein freed from the staining of normal cellular protein, which is abundant for PrP\(^{c}\). Instead of prion protein antibodies, Neumann and coworkers have used antibodies to α-synuclein, and instead of transmissible spongiform encephalopathy tissue, they have used Lewy body disease and other disorders, such as multiple system atrophy, with abnormal α-synuclein aggregates (Figure 1). As might be expected given the harsh treatment of the tissue, the method lacks fine resolution, but it does illustrate the general distribution of pathologic forms of α-synuclein without the confound of normal cellular α-synuclein, which is very abundant in synaptic termini in the neuropil of gray matter. The approach is not novel and has also been used to show abnormal forms of β-amyloid in human brains after ischemic stress (12). This is, however, the first use of a modification of histoblots to detect abnormal forms of α-synuclein.

In addition to studies of human tissue, Neumann and coworkers also mapped α-synuclein pathology in transgenic mice expressing human α-synuclein (9). The method proved to be very sensitive and specific for illustrating the distribution of the abnormal form of α-synuclein in the mouse brain, but the distribution does not map with the distribution of pathologic in humans. A notable difference is that the midbrain tectum contained abundant protease-resistant α-synuclein but the substantia nigra contained hardly any. In humans, the reverse would be expected. The problems with this transgenic model are not unique. Initial attempts to generate α-synuclein transgenic mice that modeled PD met with limited success (13), but more recent models, including the one reported by Neumann and coworkers (9), have developed Lewy neurites and, occasionally, perikaryal inclusions that share some features with LBs from human brains (14, 15). Thus, α-synuclein transgenic mice are models for α-synuclein cytopathology,
but not faithful models for the clinical disorder. Regions of the brain that are vulnerable to α-synuclein–related pathology in transgenic mice overlap only loosely with human Lewy body disease, and behavioral abnormalities more often resemble motor neuron disease than they do an extrapyramidal disorder. Motor neurons are not vulnerable in human Lewy body disease.

**Antigen retrieval and abnormal forms of α-synuclein**

Beyond demonstration of the distribution of pathologic forms of α-synuclein, the study (9) confirms the results of previous studies that found altered forms of α-synuclein in both animal models and humans. Previous studies have shown that α-synuclein had abnormal solubility properties in Lewy body disease (16) and multiple system atrophy (17), but protease resistance had not been widely appreciated. Nevertheless, among the antigen retrieval methods that have been championed for demonstrating α-synuclein pathology in brain tissue, protease treatment has been claimed to be among the most sensitive (8, 18). While it is possible that protease treatment reveals hidden epitopes in α-synuclein, it is easier, especially in light of the observations of Neumann et al. (9), to interpret the effects of protease treatment as enhanced detection of pathologic forms of α-synuclein, because the normal cellular form is digested (Figure 2). The distinction between more widely used antigen retrieval methods and the histoblot method is that the histoblot may also detect a soluble, protease-resistant form of α-synuclein that would be washed away in the traditional immunostaining approach. This issue might be addressed, but not completely resolved, if histobLOTS could be studied with electron microscopy to determine whether the protease-resistant α-synuclein is nonfilamentous.

**Lewy body disease is a β-fibrillosis**

The biochemical basis for the protease resistance of α-synuclein and PrP<sup>res</sup> is not entirely known, but most current evidence suggests that protein conformation may play a significant role. Creutzfeldt-Jakob disease is the archetype of a neurologic disorder caused by an abnormal conformation of a normal cellular protein. Comparative investigations of PrP<sup>res</sup> and PrP<sup>res</sup> have shown that conformation rather than posttranslational modification is the best explanation for the differences (19). The abnormal conformer that is protease-resistant has a high content of β-sheet secondary structure, and these properties fit well with the predicted properties of a “self-replicating” molecule (20). Fibrillar forms of α-synuclein have also been shown to have high β-sheet content, while the soluble, nonfilamentous form of α-synuclein is a natively unfolded protein with very little secondary structure (21). It is of more than passing interest that tau protein, the microtubule-associated protein that is the major structural component of neurofibrillary tangles in Alzheimer disease, shares a number of properties with α-synuclein (22). Tau is also a natively unfolded protein with little secondary structure that forms pathologic filaments with unusual solubility properties and protease resistance. Unlike the fibrillar forms of synuclein, however, tau appears to contain little β-sheet secondary structure (23).

The characteristics of PrP<sup>res</sup> and protease-resistant α-synuclein are also the characteristics of amyloid. Despite the
wide clinical diversity and pathogene-
sis of amyloidoses, they share common
properties, including increased fre-
cquency with age, defective proteolytic
processing, and association with acidic
macromolecules (24). The various amy-
loid molecules are structural variants
of a normal precursor protein, and the
major structural feature common to
amyloid is β-sheet conformation, which
led Glenner to refer to these dis-
orders as the β-fibrilloses (25). There
are many paths to amyloid, but in most
cases the conversion of the normal pre-
cursor protein to the amyloid form of
the protein involves overcoming a ther-
modynamic energy barrier. The size of
the energy barrier and thus the likeli-
hood of conversion of the normal to
the pathologic form may be modified by
a number of factors, such as post-
translational modification (e.g., prote-
ylosis and phosphorylation) and asso-
ciation with chaperone proteins, lipids,
divalent cations, or acidic macromole-
cules. A fundamental difference
between prion diseases and the other
disorders has been shown to
be transmissible.

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