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Uptake and Degradation of Protease-Sensitive and -Resistant Forms of Abnormal Human Prion Protein Aggregates by Human Astrocytes

Young Pyo Choi,* Mark W. Head,† James W. Ironside,† and Suzette A. Priola*

From the Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, Montana; and the National Creutzfeldt Jakob Disease Research & Surveillance Unit,† School of Clinical Sciences, University of Edinburgh, Edinburgh, United Kingdom*

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Address correspondence to Suzette A. Priola, Ph.D., Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories, NIAID, NIH, 903 S. 4th St., Hamilton, MT 59840. E-mail: spriola@niaid.nih.gov.

Sporadic Creutzfeldt-Jakob disease is the most common of the human prion diseases, a group of rare, transmissible, and fatal neurologic diseases associated with the accumulation of an abnormal form (PrP^{Sc}) of the host prion protein. In sporadic Creutzfeldt-Jakob disease, disease-associated PrP^{Sc} is present not only as an aggregated, protease-resistant form but also as an aggregated protease-sensitive form ($sPrP^{Sc}$). Although evidence suggests that $sPrP^{Sc}$ may play a role in prion pathogenesis, little is known about how it interacts with cells during prion infection. Here, we show that protease-sensitive abnormal PrP aggregates derived from patients with sporadic Creutzfeldt-Jakob disease are taken up and degraded by immortalized human astrocytes similarly to abnormal PrP aggregates that are resistant to proteases. Our data suggest that relative proteinase K resistance does not significantly influence the astrocyte's ability to degrade PrP^{Sc}. Furthermore, the cell does not appear to distinguish between $sPrP^{Sc}$ and protease-resistant PrP^{Sc}, suggesting that $sPrP^{Sc}$ could contribute to prion infection. *(Am J Pathol 2014, 184: 3299–3307; http://dx.doi.org/10.1016/j.ajpath.2014.08.005)*

Prion diseases, or transmissible spongiform encephalopathies, are rare fatal neurologic disorders of mammals that include Creutzfeldt-Jakob disease (CJD) in humans, bovine spongiform encephalopathy in cattle, and scrapie in sheep. Prion diseases are characterized by the conversion of normal prion protein (PrP^{C}) into a disease-associated and aggregated isoform (PrP^{Sc}), which is thought to be the main component of the infectious agent or prion (reviewed in Priola and Vorberg[1]). PrP^{C} is a glycoprotein that contains two N-linked glycosylation sites[2,3] and is bound to the plasma membrane via a glycosyl-phosphatidyl-inositol anchor.[4] Although PrP^{C} is detergent soluble and fully susceptible to proteolytic degradation, PrP^{Sc} has an increased detergent insolubility and partial resistance to proteinase K (PK).[5] The presence of amino-terminally truncated, PK-resistant core fragments of PrP^{Sc} ($rPrP^{Sc}$) after limited proteolysis is considered the most reliable diagnostic marker for prion infection,[6,7] and biochemical profiles of $rPrP^{Sc}$ based on molecular mass and/or the degree of glycosylation are used to help differentiate distinct prion disease phenotypes in humans.[8–11]

In recent years, alternative approaches for analyzing PrP^{Sc} that do not rely on the enzymatic removal of PrP^{C} have indicated that not all forms of PrP^{Sc} are necessarily resistant to proteolytic treatment. The conformation-dependent immunoassay, which uses conformational differences between the N termini of PrP^{C} and PrP^{Sc}, has found that often a majority of PrP^{Sc} present in prion-affected brains is susceptible to proteolytic degradation.[12–15] In sporadic CJD (sCJD), this PK-sensitive species of aggregated PrP^{Sc}, termed $sPrP^{Sc}$, was found in some cases to account for up to 90% of the total PrP^{Sc}.[13,16] Careful analysis of the size distribution of PrP^{Sc} has also found that $sPrP^{Sc}$ forms much smaller aggregates than $rPrP^{Sc}$.[17,18] Thus, $sPrP^{Sc}$ appears to represent a population of PrP^{Sc} aggregates which tends to be both smaller and more protease sensitive than $rPrP^{Sc}$.

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Current address of Y.P.C., Korea Brain Research Institute, Daegu, Republic of Korea.

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Evidence suggests that, like $rPrP^{Sc}$, $sPrP^{Sc}$ has seeding activity and can convert PrP^{C} to protease resistance.[18,19] It has also been associated with prion infectivity[20] and may influence the incubation time of prion disease.[21] Although these data suggest that PK-sensitive disease-associated PrP aggregates may be actively involved in prion pathogenesis, no studies have been performed to determine how this population of PrP aggregates might interact with cells and influence prion infection. In this study, we have looked at the uptake and degradation of PK-sensitive and PK-resistant disease-associated PrP aggregates in an established human astrocyte cell line. Our results indicate that, despite their biochemical differences, PK-sensitive PrP aggregates are taken up and degraded similarly to PK-resistant PrP aggregates, suggesting that relative PK resistance does not significantly influence the cell's ability to degrade PrP^{Sc}. Thus, the astrocyte does not appear to distinguish between $sPrP^{Sc}$ and $rPrP^{Sc}$ aggregates, suggesting that $sPrP^{Sc}$ could be involved in prion pathogenesis.

Materials and Methods

Human Brain Material

Ethical approval for the acquisition and use of human brain material was obtained from the NIH Office of Human Subject Research (Exempt 5480). Human brain tissue derived from four patients with sCJD and two control patients with other non-CJD neurologic disorders were obtained from the National CJD Surveillance Unit Brain and Tissue Bank in Edinburgh, Scotland. All cases had consent for research, and their supply and use in this study was covered by LREC 2000/4/157 (National Creutzfeldt-Jakob disease tissue bank: acquisition and use of autopsy material for research on human transmissible spongiform encephalopathies, Professor James Ironside, amended October 9, 2007). The four sCJD cases used had been fully characterized and comprised two cases of the MM1 subtype and one each of the MV1 and the VV2 subtypes ($CJD1^{MM1}$, $CJD2^{MM1}$, $CJD3^{MV1}$, and $CJD4^{VV2}$, respectively) according to the nomenclature used by Parchi et al.[10] In each case, approximately 2 g of brain tissue taken from the frontal cortex was homogenized in phosphate-buffered saline (PBS; pH 7.4) to a final 10% weight/volume (w/v) homogenate by using a MiniBeadbeater-8 (BioSpec, Bartlesville, OK). The homogenate was divided into aliquots and stored at −80°C until use.

Cells

The established human astrocyte cell line SVG p12 (CRL-8621) was obtained from ATCC (Manassas, VA). SVG p12 cells (hereafter referred to as SVG cells) were maintained in Eagle's Minimal Essential Medium (EMEM; ATCC), supplemented with 10% fetal bovine serum (FBS; Gibco-Life Technologies, Grand Island, NY), 100 U of penicillin, and 100 μg of streptomycin (Gibco-Life Technologies) in a humidified chamber at 37°C with 5% CO_2. On receipt, the SVG p12 cells were passaged four times in medium in a humidified chamber at 37°C with 5% CO_2. At passage 16, a large batch of cells was frozen in 90% FBS/10% dimethyl sulfoxide and stored in liquid nitrogen until use. For each experiment, a vial of cells at passage 16 was thawed and passaged once, and the cells were plated at a density of 2×10^6 cells per well of a six-well plate and allowed to attach overnight. After removal of medium, cells were overlaid with 800 μL of 1:10 dilution (in EMEM) of 10% brain homogenate. After 4 hours, 2 mL of EMEM plus 10% FBS was added. At 2, 8, 24, or 48 hours after exposure, cells were washed thoroughly with fresh medium and directly lyzed with 500 to 550 μL of 2% sarkosyl/PBS. In experiments in which the degradation of PrP^{Sc} was assayed, the initial inoculum was removed at 2 hours after infection, and the cells were washed extensively. After the addition of 3 mL of EMEM plus 10% FBS, cells were incubated for up to 24 hours and then lyzed.

For immunofluorescence studies, 3×10^4 SVG cells were plated into each well of a Lab-Tek Permanox 8-well chambered slide (Thermo Scientific, Rockford, IL) and allowed to attach overnight. After removal of the medium, the cells were overlaid with 125 μL of a 10% brain homogenate from the $CJD1^{MM1}$, $CJD2^{MM1}$, or non-CJD sample diluted 1:10 in Optimem (Gibco-Life Technologies). As an untreated control, cells were incubated in 125 μL of Optimem alone. After 4 hours, 400 μL of EMEM plus 10% FBS was added, and the cells were incubated an additional 20 hours.

NaPTA Precipitation

Sodium phosphotungstic acid (NaPTA; Sigma-Aldrich, St. Louis, MO) precipitation was performed as described previously[22] with minor modifications. Briefly, for SVG cell samples 500 μL of cell lysate was mixed with an equal volume of 2% sarkosyl/PBS. For brain samples, 50 μL of 10% homogenate was mixed with an equal volume of 4% sarkosyl/PBS, and the volume was brought to 1 mL by adding 900 μL of 2% sarkosyl/PBS. In some experiments, the relative amount of cell-associated PrP aggregates was estimated by comparison with an SVG cell lysate spiked with brain homogenate equivalent to 10% of the input inoculum. All samples were treated with 50 U/mL benzonase (Sigma-Aldrich), and NaPTA was added to a final concentration of 0.3% (w/v). The samples were then incubated at 37°C for 1 to 2 hours, followed by 30 minutes of centrifugation at 16,100 × *g*. Pellets were resuspended in 0.1% sarkosyl/PBS and, when appropriate, digested with 50 μg/mL PK for 30 minutes at 37°C. In some experiments, PK digestion was performed before the NaPTA precipitation.

Electrophoresis and Western Blot Analysis

Samples were boiled in urea sample buffer (62.5 mmol/L Tris, pH 6.8, 3 mmol/L EDTA, 5% glycerol, 5% SDS, 0.02% bromophenol blue, and 4 mol/L urea) for 10 minutes.