

Prions: The Protein of Your Nightmares

Dr. Stanley B. Prusiner was awarded the Nobel Prize in Physiology or Medicine in 1997 for his discovery of prions. Prions, which stand for proteinaceous infectious particles, are defined as infectious agents that cause spongiform encephalopathies or holes in the brain that are reminiscent of a sponge (CDC, 2010). They are transmissible virus-like agents devoid of nuclei acids, composed exclusively of modified proteins (Prusiner, 1998). The genes encoding prion proteins, known as PRNPs, are found in both vertebrates and invertebrates and are active in the brain and other tissues (McGraw-Hill, 2010). The precise function of the prion protein is not yet known, however the National Institutes of Health (NIH) believes they are most likely associated with transport of copper ions into the cell, cell signaling, the formation of cell synapses, and cell protection (US National Library of Medicine, 2008). The PRNP gene belongs to the family of cluster of differentiation genes, or CD genes. CD genes are most well known for providing the instructions to make proteins on the surface of leukocytes in all developmental stages (US National Library of Medicine, 2011). Each prion species is encoded by the sequence on the PRNP gene of the mammal in which it was last replicated (Prusiner, 1998). It is important to note that mice that lack the PRNP gene are unable to be infected with the misfolded prion protein. Prions include strain-specific properties (unique to each prion disease) in the tertiary structure of their abnormal folding configuration, unlike mammals, which contain nucleic acid genomes.

The normal prion protein (PrP^{C}) is bound to the surfaces of neurons. The infectious prion arises when the normal protein becomes altered and misfolds, taking on a different conformation known as PrP^{SC} (prion protein scrapie associated). PrP^{SC} then enters the normal brain and binds to normal cellular PrP^{C} where it induces PrP^{C} to fold to the abnormal confirmation. It is unclear how it causes the conformational change. Newly formed PrP^{SC} molecules then convert more normal PrP^{C} molecules into the abnormal PrP^{SC} form. An alternative hypothesis for PrP^{C} 's change into the abnormal conformation is that PrP^{SC} activates certain enzymes, which modify PrP^{C} structure. Interaction of PrP^{SC} with PrP^{C} cross-links normal PrP^{C} molecules. Cross-linking of these molecules triggers apoptosis or programmed cell death. These normal but cross-linked proteins cause neuron loss while the abnormal (misfolded) proteins act as infectious agents (McGraw-Hill, 2010).

Prions have been dubbed “the perfect pathogen” and are notable for causing transmissible spongiform encephalopathies or TSE’s (prion diseases) in around one in a million people worldwide, or about 300 cases per year in the United States (US National Library of Medicine, 2007). These diseases are a family of rare progressive neurodegenerative disorders that affect both animals and

humans (CDC, 2010). They are notable for causing bubbles to form in brain cells. When these brain cells die the brain becomes filled with gaps or holes and takes on the appearance of a sponge (Gambetti, 2007). Prion diseases occur either through a mutation in the PRNP gene, or in most cases (roughly 85-90 percent) sporadically (Gambetti, 2007). Currently no effective treatment exists. They are distinguished by long incubation periods, failure to induce inflammatory response, resistance to heat, and characteristic spongiform changes that are associated with neuronal loss (CDC, 2010). They can be characterized in humans or animals with loss of motor control, dementia, wasting, paralysis, muscle twitching, permanent insomnia, pneumonia and eventual death. However, humans with such diseases are able to live symptom free for years, at times even decades. They have “no fever, no coughing, no elevated white-blood cell counts-no sign whatsoever of an infection” (Yam xiv). Once prognosis is set, though, the individual has a matter of months to a year before death.

The scientific community was not very interested in prions or the diseases they caused until around 22 years ago when bovine spongiform encephalopathy (BSE) or Mad Cow disease was found in Great Britain. The epidemic in the 90s initially spread after cattle were fed meal that contained parts of ground up cattle including brain tissue. Evidence of the infection in cattle includes a host of symptoms including changes in temperament such as nervousness or aggression, incoordination, difficulty in rising, abnormal posture, decreased milk production, and loss of weight despite continued increases in appetite. Since its inception it has caused around ninety human deaths. Eating cows with this disease was found to cause variant Creutzfeldt-Jakob disease (VCJD) in humans. However it is important to note that the risk of obtaining VCJD even after consuming contaminated meat products is extremely low (CDC, 2010).

Variant Creutzfeldt-Jakob disease was initially discovered in 1996 in Great Britain. Its clinical and pathological characteristics are quite different than those seen within classic Creutzfeldt-Jakob disease. One main difference is the median age of death for each disease with 68 years being the median for classic CJD and 28 years the median for VCJD. Another main difference is that the median duration of illness for classic CJD is five months compared to 14 months in VCJD. A few more characteristic differences are the presence of the prion agent in the lymphoid tissue of VCJD patients with none detected in classic CJD, and marked accumulation of protease-resistance prion protein seen in VCJD compared to variable accumulation noted within classic CJD (CDC, 2010).

In addition to the aforementioned prion diseases noted in humans Gerstmann-Straussler-Scheinker Syndrome (GSS) is another human prion disease worthy of mention. This disease characterized by ataxia and dementia is found to occur between the ages of 35 to 55. It is a slow

progressive illness with a prognosis of around two to ten years. The disease ends with severe disablement and eventually death, as all prion diseases do with the patient often lapsing into a coma or obtaining a secondary infection such as aspiration pneumonia. Currently there is no cure for GSS, however there are treatments to ease the patient's pain. The NIH is actively researching human prion diseases such as GSS, Fatal Familial Insomnia, and Kuru (NINDS-NIH, 2007).

Animal prion diseases include the previously discussed BSE, chronic wasting disease in N. American cervids, Scrapie in sheep, transmissible mink encephalopathy, feline spongiform encephalopathy, and ungulate spongiform. Scrapie, one such disease discovered in sheep around 250 years ago in Western Europe and Great Britain, has been reported all over the world in all but Australia and New Zealand. The first case of Scrapie was found within the United States around 1947 in Michigan. The disease affects the central nervous system of goats and sheep. Flocks that have become infected often lead to significant losses in production if the amount of sheep susceptible is high. Scrapie is spread most often from ewes to their offspring and through placental fluids. Animals begin showing the noticeable signs of infection including but not limited to tremors of the neck and head, scratching and rubbing against fixed objects to relieve itching, weight loss, loss of coordination, hopping like a rabbit, and lip smacking around two to five years after being infected. Veterinarians diagnose animals on farms through biopsy of the lymphoid tissues and appearance of the supposed infected animal's aforementioned symptoms. The eradication program includes steps such as live scrapie testing, genetic testing, and tracing animals back to their flock/herd of origin. The animals are then destroyed (USDA, 2010).

Although discussion of the aforementioned diseases is relevant, development of treatment strategies is most important. Phillip Yam, of *Scientific American*, who wrote the book, "The Pathological Protein" summarizes a few potential treatment strategies he has encountered from various scientists along the road to writing his book. These include stopping or slowing expression of the PrP^C, which would thus give less raw material to make the misfolded prion protein, prevent PrP^{SC} floating around outside of the cell from latching onto the normal PrP^C protein that is setting on the surface of the cell, block the interaction between the chaperone molecules that convert PrP^C to PrP^{SC}, refold misfolded PrP^{SC}, block the accumulated PrP^{SC} toxic effects, and keep the misfolded protein from assaulting the central nervous system (Yam, 215). Although potential therapies may appear promising the reality is that these diseases affect only a handful of individuals. Due to the small number of individuals affected the government is currently only funding a small handful of clinical trials.

Additionally, like all diseases, “the cure could be deadlier than the disease itself” (Yam, 215).

Therefore, diagnoses of such diseases, as in all infections, are the first step in fighting back.

In terms of diagnosis the bioassay is the best diagnostic test. However it is time consuming and can take between a few months to a year to complete. An alternative to the bioassay is the immunological assay such as the Western Blot or ELISA (Enzyme-Linked Immunosorbent Assay), which only takes four to six hours to complete. This assay relies on protease-resistant PrP^{SC}. It involves taking a piece of brain tissue and adding a protease to destroy the normal PrP^C. Scientists then add antibody to the prion protein that remains. However, making antibodies against the prion protein requires a lot of time and money. Man-Sun Sy, an immunologist from Case Western Reserve University, under contract with Prion Developmental Laboratories in Buffalo Grove, Illinois, has developed a wide array of antibodies that each bind to various epitopes along the prion protein. Sy’s idea is that these epitopes residing in the coils of the protein may become exposed when PrP^C conformationally shifts to PrP^{SC} and that with the correct antibody(ies) PrP^{SC} could be tagged.

In addition to the type of assay used, a live test also remains to be found in order to test those individuals who are suspected or may eventually develop one of the numerous prion diseases, to ensure a safe blood, organ, and tissue supply, prevent reuse of surgical tools that are contaminated, and allow physicians to monitor patients’ recovering from therapy. A blood test would help immensely, however prions exist at very small levels in the bloodstream, which no assay can detect. Additionally, a urine test, especially for sheep, would also be helpful, as obtaining sheep’s blood is difficult, even when performed by professionals.

Gene therapy may contain the cure to these diseases as well. By using a technique called dominant-negative inhibition transgenic mice that contained a slightly mutated prion protein (one amino acid on the normal PrP^C is replaced) stayed healthy after being inoculated with Scrapie. These mice, which contained both mutant and normal allelic copies of the prion protein, lived longer than normal mice. Additional genetic techniques that could be used include creation of normal prion dimers that cannot withstand folding of their Beta sheets when they come in contact with PrP^{SC}, or shortening the amino acid sequence that codes for the prion protein. However genetic modification may be better suited to breed prion-disease resistant livestock than for use within human therapies. Regardless of the therapy invented, one must be created in the near future in order to slow the spread and halt this disease in its tracks. However, it is very hard to isolate that which is so readily different than the infectious agents scientists have seen in the past. Regardless, scientists such as those working within

the NIH and the Center for Disease Control and Prevention are working hard to discover a cure (Yam, 2003).

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