The Socrates Award Lecture 2005
Prions and Public Health

Professor Jill Johnson
Microbiology, Molecular Biology and Biochemistry
University of Idaho
Moscow, Idaho USA

Summary

• Background
• What causes the disease?
• How does the prion protein cause disease?
• Prions and Public Health
• Models of Prion diseases

Background: What are Prion Diseases?

• Prions are infectious pathogens that cause a group of fatal neurodegenerative diseases
  – In humans, main form is Creutzfeld-Jakob disease
     – Rare, 1 per 1,000,000 in U.S.
• Occurrence of Prion diseases may be Genetic
  – Familial Creutzfeld-Jakob disease
• Sporadic- No other family members affected
• Infectious- Transmitted by eating contaminated material or through tissue transplants
  – New Guinea tribe Fore, 10% killed by Kuru, ritualistic cannibalism

Prions Disease Also Present in Other Animals
in all cases due to mutant form of the cellular prion protein

Prusiner PNAS 95:13363-83, 1998

Prion Disease Also Present in Other Animals
in all cases due to mutant form of the cellular prion protein

Prusiner PNAS 95:13363-83, 1998

Cause of Prion Disease is Unique

• Most human diseases caused by:
  – Inheritance of mutant gene
  – Novel DNA mutation that causes disease
  – Viral or bacterial infection

• Prion diseases are rare because they may be genetic or infectious

• What is the nature of the infectious particle?

What Causes Prion Diseases?

• Early studies focused on transmission of scrapie agent
  – Scrapie agent could not be inactivated by UV, ionizing radiation, heat, formalin
    – would kill bacteria and virus
  – Treatment that destroys DNA or RNA

• Scrapie agent could be destroyed by treatments that destroy proteins
  – Scrapie agent lacks any nucleic acid
Principles of Environmental Toxicology

The Prion Hypothesis

- Stanley Prusiner termed the phrase “Prion”
  - Proteinaceous and infectious
  - Nobel Prize in Physiology or Medicine, 1997
- Completely novel mechanism of disease transmission
  - Not mediated by DNA or RNA
- The transmissible agent is a mutant form of a protein.

What Do We know About the Prion Protein?

- The gene encoding the Prion protein was identified in 1982
- Protein is constitutively expressed in adult uninfected brain
  - Also expressed in other tissues, highest in brain
- More than 20 mutations in the PrP gene are known to cause inherited prion disease
- Libyan Jews in Israel develop CJD 30 times more often than other Israelis
  - Libyan patients have specific mutation in their prion protein gene that makes them more susceptible to disease

The Prion Protein from Affected Individuals is Altered

- A fragment of the prion protein was isolated by enriching fractions of brains for scrapie infectivity
- PrP 27-30 is a protease resistant core of PrP(sc)
- PrP 27-30 derived from larger PrP(sc) found in brains of animals with Prion disease not other neurodegenerative diseases

Prion Formation

- Most proteins have one biologically active form, or structure
  - Common structural motifs in proteins are alpha helices and beta sheets
- Cellular form of prion protein (not associated with disease) is comprised of mainly alpha helices, disease form has beta sheets

Formation of Disease Requires Conformational Switch

- Some mutations in prion protein make it more likely to undergo conformational change
Prion Form

Development of prion form due to:
Inherited mutation in prion protein
Sporadic mutation in prion protein

Prion Disease and Public Health

Infection with prion protein (seed) from affected individual/animal is rare due to species-specific differences in protein

Bovine Spongiform Encephalopathy
-Mad Cow Disease

Transmission of Prion Disease Across Species is Very Rare

- Humans have been exposed to scrapie, the probable cause of BSE for at least two centuries with no detection of human risk
  - It is not clear whether natural human/sheep differences prevented a direct species switch.
- Likely that transmission mediated by rare occurrence of prion form in sheep that could infect other species, spread through food.
  - Large number of cattle ‘inoculated’ with prion proteins
- Until this outbreak, there had been no evidence of preexisting prion disease of cattle in Great Britain or anywhere.

Prions from BSE Cattle may be Transmitted

- Brain extracts from BSE cattle cause disease in cattle, sheep, mice, pigs and mink
- 1988 - practice of feeding meat and bone meal to sheep and cattle was banned
- 1994 - First case of vCJD in teenagers and young adults (25 in 1998)
  - Difference between CJD and vCJD is age of patient and pattern of neurodegeneration

Bovine Spongiform Encephalopathy
-Mad Cow Disease

- Estimated that one million cattle were infected with prions
  - Mean incubation time for BSE is 5 years
- Most cattle are normally slaughtered between 2-3 years of age
- However, 160,000 dairy cows died of BSE during epidemic
- Common-source epidemic caused by meat and bone meal fed to dairy cows

Total confirmed cases of bovine spongiform encephalopathy (BSE) in cattle and of human variant Creutzfeldt-Jakob disease (vCJD), United Kingdom, 1988-2003.
Genetic Predisposition to vCJD

- As of October 2005, 180 people in the United Kingdom have died from vCJD
- Most (if not all) of the people that have contracted vCJD have two copies of one particular form of the prion protein (Met 129)
  - These people also have higher incidence of sporadic CJD
- Not yet known if this is the only combination that will lead to disease or if this form just causes disease to occur earlier

17% of the UK is homozygous for M129

Sources of Infectious CJD in Humans

- Improperly sterilized depth electrodes
- Transplanted corneas
- Human growth hormone (90 cases in 1998)
- Dura mater grafts (60 cases in 1998)
- Gondotropin derived from cadaver pituitaries
- Surgical instruments
- Incubation period varies 1-20 years, suggests there are different 'strains' that vary in infectivity
- Unknown if can be transmitted through blood, but American Red Cross bans donations from those who have spent a certain amount of time in the UK

What Can be Done about Prion Diseases?

- BSE numbers are falling dramatically, now only rare cases
- Plan to selectively breed scrapie resistant sheep
  - One particular genotype appears resistant to scrapie and to BSE
- Plan is to breed those sheep selectively, eliminate/reduce future risk

New Cases of CJD in Idaho, 2005

- Nine people in Idaho have died from suspected CJD in 2005
  - 1 per 1,000,000 versus 9 per 1,300,000
- This is not vCJD and not linked to BSE epidemic
- Only 2 of the cases have been confirmed, 2 pending (1 was not CJD)
- Confirmation requires special autopsy, most cases not done
- Special autopsy is expensive
  - Many morticians reluctant to do autopsy on suspected CJD cases
  - Standard sterilization procedures don’t eliminate risk of transmission
  - Equipment must be soaked in chemical solution for more than an hour and then heated

Is There a Common Link Between Affected Idahoans?

- Most lived in one of two counties in Southern Idaho
  - There is one new case in Northern Idaho
- Epidemiology studies of four cases
  - All white females over age of 55
  - No common occupation or place of employment
  - No common travel habits
  - No common unusual eating habits or animal exposures
  - No common surgical procedures or medications
- Still under investigation:
  For current info on this and other scary diseases etc
  http://www.cidrap.umn.edu/index.html

Other Animals Have Prion Diseases

- Deer and elk may have chronic wasting disease (CWD)
  - Affects less than 5% of wild deer and elk
- CWD identified in Wisconsin in February 2002
  - 86-90% of deer in Wisconsin have allele combinations that make them susceptible to infection
- No conclusive evidence that it may be transmitted to humans from deer or elk
  - Some circumstantial evidence
More on Prion Biology

- All prion diseases caused by alteration of Prion protein
- There are other proteins that share biochemical characteristics of prion proteins
  - Protease resistant core
  - Protein rich in alpha helices becomes rich in beta sheets
  - Prion-like protein can form in vitro aggregates that look like Prion rods
  - 'Prion' form of protein can be transmitted from one cell to another without DNA or RNA

Primary Model of Understanding How Prion-like Proteins Form Aggregates is the Budding Yeast Saccharomyces cerevisiae

- Prions are not just single case of aberrations of misfolded Prion protein
  - Similar rod-like structures are observed in other neurodegenerative diseases
  - Alzheimer's disease, Parkinson's disease, Huntington's disease
- Many proteins form prion-like aggregates
  - Budding yeast has at least four proteins that fall into this category makes it possible to use yeast as a model organism to study prion diseases and other protein misfolding diseases
  - Cheaper and faster than alternative methods

Understanding Prion Biology in Yeast

- Under some conditions, presence of prion-like aggregates may be beneficial to yeast (reviewed in Devel. Cell. 2:143-151, 2002)
  - Still unknown whether the formation of the aggregate itself is a protective measure within the cell or whether formation of the aggregate is what leads to disease
- Main use of yeast is to determine whether anything reduces the formation of the aggregates
  - Answer is yes!

Analysis of Yeast Prion-like Proteins

Express prion-like protein fused to Green fluorescent protein in yeast

Can Yeast be Altered to Prevent Aggregates?

- Molecular chaperones are a group of proteins that help other protein attain the correct conformation
- Overexpression of one molecular chaperone, Hsp104, cures the yeast prion
- Overexpression of other molecular chaperones may help stimulate formation
- Chaperones may work against each other in assembling and disassembling the aggregates
- Not-so futuristic use of information about yeast molecular chaperones...