Prions and Transmissible Spongiform Encephalopathies

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Prions

- Prions are normal proteins of animal tissues that can misfold and become infectious: they are not cellular organisms or viruses.
- In their normal noninfectious state, these proteins may be involved in cell-to-cell communication.
- When these proteins become abnormally shaped i.e., infectious prions, they are thought to come into contact with a normally shaped protein and transform that protein into the abnormally shaped prion.
- This process causes a geometric increase of abnormally shaped prion proteins until the number of abnormally shaped protein causes overt illness.
- When consumed by animals, prions are thought to be absorbed into the body during digestion where they begin the process of changing their normal protein counterparts into abnormal proteins; however infectious prions from one species of animal have less of a potential of causing the abnormal shape in the normally shaped prion proteins of another species (the "species barrier").

Human Prion Diseases

- Kuru
- Creutzfeldt-Jacob Disease
- Gerstmann-Straussler-Scheinker Disease

Animal Prion Diseases

- Scrapie (sheep)
- Bovine spongiform encephalopathy (cattle)
- Transmissible mink encephalopathy (mink)
- Chronic wasting disease (mule deer, elk)
- Feline spongiform encephalopathy (cats)
- Exotic ungulate encephalopathy (greater kudu, nyala, oryx)

Nature of Disease (1)

- Prions are associated with a group of diseases called Transmissible Spongiform Encephalopathies (TSEs).
- In humans, the illness suspected of being food borne is variant Creutzfeldt-Jakob disease (vCJD).
- The human disease vCJD and the cattle disease, bovine spongiform encephalopathy (BSE), also known as "mad cow" disease, appear to be caused by the same agent.
- Other similar but not identical TSE diseases exist in animals, but there is no known transmission of these TSEs to humans. Included among these is chronic wasting disease (CWD), and the oldest known of these diseases - scrapie - which occurs in sheep and goats.
- No early acute clinical indications for TSEs have been described.
- After an extended incubation period of years, these diseases result in irreversible neurodegeneration.

Nature of Disease (2)

- **The neurodegenerative phase of vCJD in humans typically involves the formation of "daisy-shaped" areas of damage in the central nervous system.**
- **There is also, in common with other TSEs, vacuolization (formation of holes) that gives brain tissue a spongy appearance when examined under a microscope.**
- **It is thought that the build-up of the abnormally shaped prion proteins causes the observed neurodegeneration.**

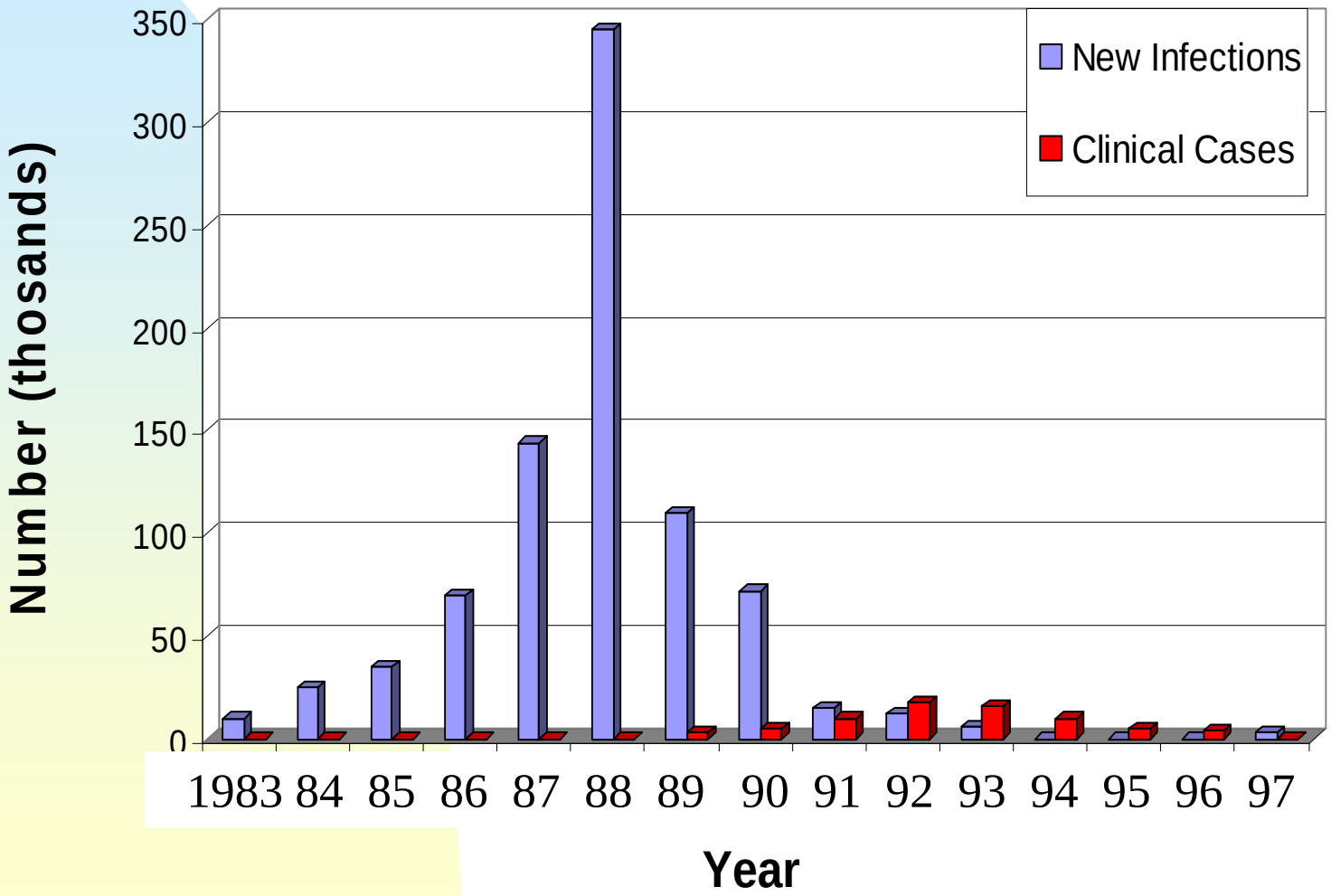
Associated Foods

- **The major concern for consumers is the potential contamination of meat products by BSE contaminated tissues or the inclusion of BSE contaminated tissues in foods, including dietary supplements.**
- **High risk tissues for BSE contamination include the cattle's skull, brain, trigeminal ganglia (nerves attached to the brain, eyes, tonsils, spinal cord, dorsal root ganglia (nerves attached to the spinal cord), and the distal ileum (part of the small intestine).**
- **The direct or indirect intake of high-risk tissues may have been the source of human illnesses in the United Kingdom and elsewhere.**
- **Bovine meat (if free of central nervous system tissue) and milk have, to date, shown no infectivity in test animals.**
- **Gelatin, derived from the bones of cattle, appears to be very low risk , especially with adequate attention to the quality of source material and effectiveness of gelatin-making process.**
- **Based upon many studies, scientists have concluded that forms of CJD other than vCJD do not appear to be associated with the consumption of specific foods.**

Reported Cases

- There is one reported human cases of vCJD in the United States in a woman that appears to have aquired the illness from consumption of contaminated food when growing up in the United Kingdom.
- In the U. K., there have been around 143 human cases of suspected or confirmed vCJD from 1993, when the illness was first recognized, through December 2003.
- There have been six reported cases of vCJD in France and one in Italy.
- Since 1986, more than 180,000 cases of BSE have occurred in the U.K.in cattle, particularly dairy cattle.
- BSE cases have also been identified in 20 European countries, Japan, Israel, and Canada.
- The feeding of rendered TSE-infected animal by-products to cattle is believed to have caused the epidemic of BSE.
- Practices such as this have now been prohibited, resulting in a dramatic decline in the number of cases.
- There is one reported case of BSE in the U.S. which appears to be the result of importing cattle from Canada that may have been exposed to feed which contained meat and bone meal from rendered cattle.

Epidemic curve of BSE in British Cattle



* Adopted from Commun Dis Public Health 1999; 2: 5-13

Course of Disease

- **Cases of vCJD usually present with psychiatric problems, such as depression.**
- **As the disease progresses, neurologic signs appear -- unpleasant sensations in the limbs and/or face.**
- **There are problems with walking and muscle coordination.**
- **Sometimes, late in the course of the disease, experience severe problems with processing information and speaking.**
- **Patients are hospitalized and are increasingly unable to care for themselves until death occurs.**

Food Analysis

- No practical detection methods exist, at present.
- The abnormally shaped prions are resistant to most heat and chemical treatments, however certain food manufacturing processes (e.g. gelatin production) do result in significant decrease in prion infectivity through exclusion.
- There are no known means of reconditioning contaminated foods.
- The key to food protection is obtaining bovine meat and meat byproducts from animals not infected with BSE and protecting against contamination of food with high risk tissues, especially brain and spinal cord tissue.

Prion

(Proteinaceous Infectious Particle)

- 1982, coined by Prusiner
- Unique nature of prions (PrP^{Sc})

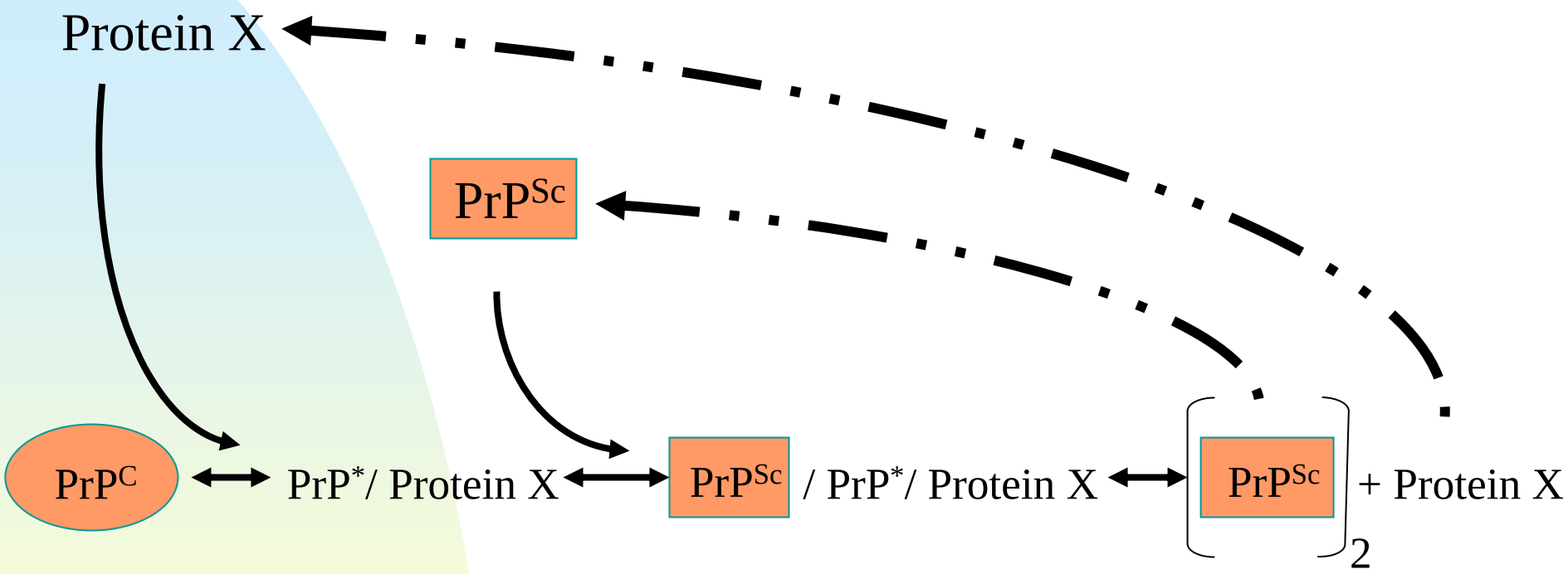
  -- hereditary & transmissible

- Lack of nucleic acid
- Resistant to standard sterilisation methods
- Capable of replication
- The species barrier
- B-cells may be crucial in neuro invasion
- Dissemination within CNS? slow axonal transport

Properties of Cellular and Scrapie Prion Proteins

	PrP^{C}	PrP^{Sc}
Protease K digestion	Sens	Res
Detergent extraction	Sol	Rods
Secondary structure	α-helix	β-sheet
Cellular localisation	Cell surf	Vesicles
Presence in normal brain	Yes	No
Presence in Scrapie-brain	+	+++
Synthesis	Rapid	Slow
Degradation	Rapid	Slow

PrP^{Sc} Formation



*Adapted from Prusiner, SB. et *al*,(1998) *Cell* 93: 337-348

Transmission

- Not contagious
- Intracerebral inoculation, most efficient
- Oral route inefficient (kuru, CJD, BSE, vCJD)
- Knock-out mice are immune to Prion challenge
- Genetically engineered mice that express mo/hu transgenes suggest that Prion formation may depend on a second species specific molecule, designated ‘protein X’--efficiency of binding:

  -protein X & PrP of the same species

  -molecular compositition of the central domain of the PrPgene
- Vertical transmission

Unquantified Risks

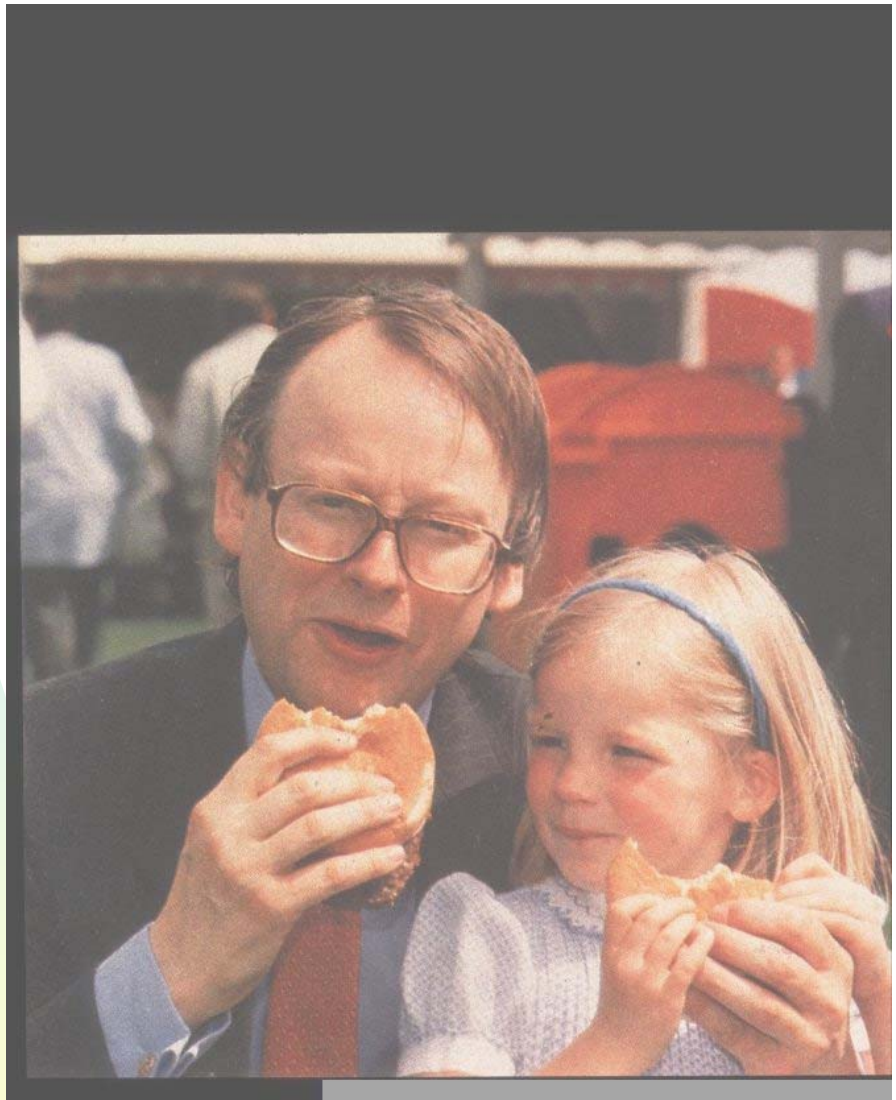
- Blood transfusion
- Tissue donation (risk of CJD in all US corneal donors is 0,005%)
- Contamination of surgical instruments

Inactivation methods

- Resistant to standard methods of disinfection and sterilisation, such as alcohol, phenol, bleach or formalin solutions or UV light or 121ºC for 15´
- Steam autoclaving 1 hour at 132ºC or immersion in 1 N NaOH (1hr at RT) -- Committee on Health care Issues of the American Neurological Association
- Steam autoclaving 4.5 hrs at 121ºC and 15 pounds per square inch or 1 N NaOH immersion 30´ x3 (Prusiner et al)
- Concentrated > 3M guanidine thiocyanate solutions
- Immersion of tissues in 96% formic acid for 1hr after fixation is advised.

Main BSE Control Measures

- 1988, notification of BSE
- 1988, ruminant feed ban
- 1988, slaughter and compensation (50%)
- 1988, milk ban suspect animals
- 1989, specified bovine offal ban in human food
- 1990, compensation 100%
- 1990-1996 EU ban on export of British beef
- 1996, prohibition of cattle>30 months from entering human food chain & heads of goats and sheep
- 1997, beef on the bone ban (lifted now)



Diagnosis

- Clinical presentation
- Routine laboratory tests (e.g. CBC, ESR, etc)
- S100 protein (sensitivity 78%, specificity 81%)
- CSF a cellular, 14-3-3 protein, S100, neuron-specific enolase
- EEG (periodic sharp waves in sCJD)
- CT
- MRI
- BRAIN BIOPSY (histological immune staining of brain for PrP^{Sc})
- **Absence of a pre-clinical test**

Diagnosis of Human Illness

- The most reliable means for diagnosing any TSE is the microscopic examination of brain tissue - a post-mortem procedure.
- Preliminary diagnoses of vCJD are based on patient history, clinical symptoms, electroencephalograms, and magnetic resonance imaging of the brain.

vCJD Control Measures

- Banning of specified ‘risk materials’ in human alimentation and cattle>30 months
- Surveillance of all forms of prion diseases
- Selection of blood donors / withdrawal if from vCJD
- Leucodepletion
- Exclusion of potential corneal donors by using tightened medical record and historical screening criteria by investigating:
  - -cognitive changes
  - -speech abnormalities
  - -cerebellar findings
  - -signs of myoclonus

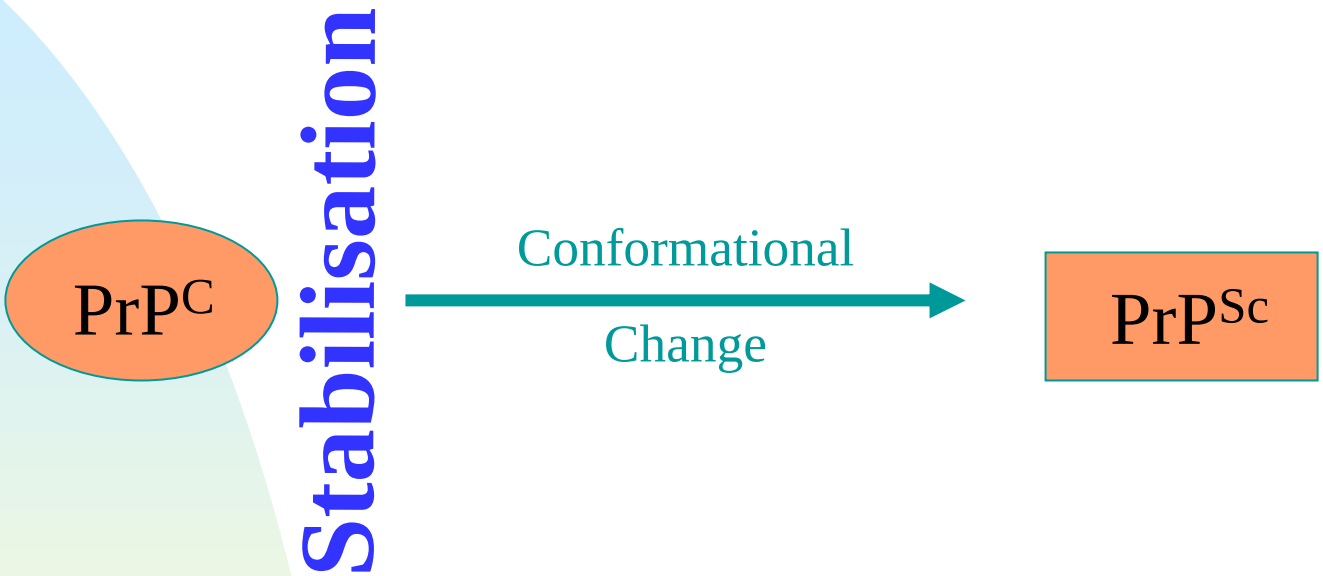
Is vCJD caused by BSE?

Evidence supported by compelling experimental data

- Cases of vCJD followed a massive epidemic of cattle BSE in the UK (peak 1992-93)
- Molecular strain-typing studies

  Same glycosylation pattern of PrP^{Sc} protein from the brains of vCJD patients as that of BSE PrP^{Sc}
- Experimental transmission studies into transgenic and conventional mice

Treatment



- Invariably fatal
- No known treatment
- New anti-Prion drugs (e.g. anion Congo red) on animal models particularly transgenic & on cell culture systems