Lecture 11
Prion Diseases
(Mad Cow disease, Creutzfeldt-Jakob disease and others)

• Required readings:
  – Article: Prion disease Jucker and Walker Nature
Summary

• Overview and Classification
• Clinical Features
• Relationship Between variant Creutzfeld Jakob Disease (vCJD) and Bovine Spongiform Encephalopathy (BSE; ‘mad cow’)
• Histopathological Features
• Mechanism of Infection/Pathogenesis
  – Characteristics of the PrP$^{Sc}$
  – PrP$^{Sc}$ Formation
  – Requirements for infection and Variables
  – Route of Invasion for PrP$^{Sc}$
Introduction: the clinical problem

• Identification of a disease that appeared to be a transmissible dementia in the inhabitants of New Guinea (cannibals); the ‘Fore people’; clearly associated with cannibalism; Dr. Carlton Gajdusek investigated this outbreak; Epidemiology interesting; also could be transferred to primates (inter- species transfer).

• Very different from Alzheimer’s disease (AD); death within a year; rapidly progressive; pathology different; confusion, memory impairment, myoclonus (large-amplitude jerking of body, or limbs). Similar to a disease sometimes seen in the general population of Western countries called Creutzfeldt-Jakob disease (CJD). Incidence roughly 1/1,000,000.

• If transmissible, what was the infectious agent? ‘Slow’ virus?

• Resistant to UV and ionizing radiation, ? No evidence of DNA

• ‘Organism’ now believed to be a protein (well accepted; Dr. S. Prusiner – associated with this hypothesis) ‘Prion hypothesis’

• There is an endogenous protein PrP (prion protein), called PrPc
Prion Diseases – Overview

• Prion = proteinaceous infectious particle only
  – Prion diseases are characterized by infectious agents which very likely do not have a nucleic acid genome; rather, it is the protein alone that seems to be the infectious agent (highly unusual).
  – Prion diseases are also known as transmissible spongiform encephalopathies (TSEs) because of the post-mortem appearance of the brain with large vacuoles.

• It is likely that most mammals are capable of developing TSEs
  – all TSEs include neuronal degeneration, gliosis and the accumulation of prion protein (Pr\text{sc})
Prion Disease – Classification

- Prion diseases have many variants and exist in a wide variety of species
  - In various mammals:
    - Bovine Spongiform Encephalopathy (BSE) (aka Mad Cow Disease): Cattle
    - Scrapie: Sheep
    - Chronic Wasting Disease: Elk
    - Transmissible Mink Encephalopathy: Mink
  - Prion diseases also occur in human which are classified and sub-classified into various forms:
    - Kuru (form seen in New Guinea)
    - Fatal Familial Insomnia
    - Creutzfeldt Jacob Disease (CJD)
      - further sub-classified into 4 families of TSE
Classification

– **Kuru**
  - first brought prion diseases to prominence in the 1950s;
  - found in tribes of New Guinea due to cannibalism

– **Fatal Familial Insomnia**
  Largely familial (autosomal dominant) 18-61 years; lasts 7-36 months; severe insomnia, impaired autonomic function, (sweating, hyperthermia), reflects thalamic damage; Prp gene mutation, codon 178 with M at codon 129. Not much spongiform change.

– **Creutzfeldt Jacob Disease (CJD)**
  - Most common variant of TSE in humans; can be sub-classified into 4 families:
    – **Sporadic CJD**: 85-90% of all CJD cases; occur at the rate of about one per million per year; average age of affected individual ~65 yrs
    – **Familial CJD** (aka Gerstmann-Straussler-Scheinker Disease - GSS): 5–10% of all CJD cases; autosomal dominant.
    – **Iatrogenic CJD**: <5% or all CVD cases; result from the accidental transmission of the causative agent (e.g., via contaminated surgical equipment; corneal or dura mater transplants etc.)
    – **Variant Creutzfeldt-Jakob disease (VCJD)**: affects younger patients (average age 29 yrs), has a relatively longer duration of illness and is strongly linked to exposure, probably through food, to (BSE)
Variant CJD (vCJD) and BSE

- vCJD is strongly linked to exposure to BSE, which itself first came to prominence in the UK in the 80s.
- Timeline in Britain:
  - 1970s: Hydrocarbon-solvent extraction of meat and bone meal (MBM) for cattle feed.
  - 1987: First cases of BSE confirmed.
  - 1988: Epidemiology suggested a prion disease, and MBM use was abandoned; estimated that over 1,000,000 cattle infected.
  - 1989: Human consumption of bovine CNS tissue (thought to have the highest prion concentration) banned based on fears of transmission to humans.
  - 1996: a new type of CJD appeared in Britain and France; young patients (<40 years old) and different neuropathology.
vCJD and BSE

• BSE spread to other areas, but it did not become epidemic as it was in Great Britain
  – Major concern because discovery of BSE cattle in a given country carries with it a severe economic toll (i.e., may result in quarantines against beef from the country)

In 2012; 27 deaths from CJD in Canada; 1 vCJD in 2011
Clinical Findings

- Different brain regions are selectively vulnerable to different forms of TSEs; damage to a given brain region likely accounts for differential signs and symptoms:
  - (CJD) – Cerebral cortex: loss of memory and mental acuity; visual impairment is also possible
  - (FFI) – Thalamus: insomnia (fatal familial insomnia)
  - (Kuru, GSS) – Cerebellum: difficulty coordinating body movements and difficulty walking
  - (BSE) – Brain stem is affected
Clinical Findings

• In addition to the specific clinical effects associated with select forms of TSE, CJD in general has several well characterized effects:
  – Dementia
    • progresses rapidly, often worsening day-by-day
    • can take many forms: memory loss, hallucinations, delusions, disturbances to both mood and emotions
  – Neurological symptoms
    • dysarthria, gait abnormalities, pain and paresthesias, progressive weakness and immobility, tremor, myoclonus (jerking movements of limb, or trunk)
    Almost always fatal within 1 year of onset
Histopathological Features

• Prion diseases are classified as spongiform encephalopathies
  – Lots of vacuoles both in neurons and astrocytes leads to “swiss cheese” appearance
  – In addition, there is considerable neuronal loss and proliferation of astrocytes but without much inflammation
Pathogenesis

• The prion protein exists in two forms:
  – Normal, innocuous protein (PrP\textsuperscript{c}) can change its shape to a harmful, disease-causing form (PrP\textsuperscript{Sc}).
    • PrP\textsuperscript{Sc} may then be endocytosed from outside the cell
    • Misfolded PrP\textsuperscript{c} accumulating in the cytosol may also trigger PrP\textsuperscript{Sc} formation
    • The conversion from PrP\textsuperscript{c} to PrP\textsuperscript{Sc} then proceeds via a chain-reaction.
  – When enough PrP\textsuperscript{Sc} proteins have been made they form long filamentous aggregates that gradually damage neuronal tissue.
Pathogenesis – PrP^{Sc}

Characteristics

- “Infectious agent” is composed of a post-translational modified form of the PrPc protein (PrP^{Sc}) derived from the host’s normal prion protein (PrP^{c})
  - The harmful PrP^{Sc} form is very resistant to high temperatures, UV-irradiation and strong degradative enzymes (e.g. bleach)
  - Normal prion protein is sensitive to proteinase K digestion whereas PrP^{Sc} shows partial resistance to proteinase K digestion.

Note: The function of normal endogenous PrP^{c} remains unknown, though mice in which PrP^{c} is knocked out have altered sleep/wake cycles and circadian rhythm.
The dogma is that in Prion disease there is the conversion of endogenous Prion protein (PrPc) (c for cytosolic) to PrPsc (sc for scrapie).
FIGURE 4-2  Western blot comparing the major isoforms observed in the four principal subtypes of prion disease. To the left of the blot displays the prion protein (PrP) segment that is represented in the adjacent blot. The highest molecular weight of PrP is the diglycosylated fraction of PrP, whereas the monoglycosylated and unglycosylated fractions run faster in the gel because of their lower molecular weight. In Jakob-Creutzfeldt disease, fatal familial insomnia, and variant Jakob-Creutzfeldt disease, proteinase-K cleaves approximately the first 67 amino acids of protease-resistant scrapie-related isofrom (PrP$^{Sc}$), leaving the PK-resistant core, PrP90-231. In the Jakob-Creutzfeldt disease case shown, the unglycosylated (lowest) band migrates at 21 KDa, indicating Type 1 prions, whereas the fatal familial insomnia case migrates lower, at 19 KDa, indicating Type 2 prions. In most cases of Gerstmann-Sträussler-Scheinker syndrome, a second C-terminal cleavage that removes the glycosylated segment occurs endogenously, leaving a unglycosylated central segment, which usually runs at 7 to 8 KDa.

FFI = fatal familial insomnia; GSS = Gerstmann-Sträussler-Scheinker syndrome; JCD = Jakob-Creutzfeldt disease; vJCD = variant Jakob-Creutzfeldt disease.

Pathogenesis – PrP<sub>Sc</sub> Formation

• According to the **protein-only hypothesis**, the conversion from PrP<sub>c</sub> to PrP<sub>Sc</sub> proceeds via a chain-reaction:
  - PrP<sub>c</sub> → PrP<sub>Sc</sub> → PrP<sub>Sc</sub> fibrils → PrP<sub>Sc</sub> plaques

The change in PrPc to PrPsc; PrP sc has Beta-pleated organization and protein accumulates with a labelling with stains for amyloid (but not beta-amyloid)
Pathogenesis – Requirements and Variables

• For a prion (PrP$^{Sc}$) to infect a host, the host must have a recognizable cellular form (PrP$^{c}$) of that prion
  
  – Generally, the closer the phylogenetic relationship between the donor host and the recipient, the greater the chance for infection, and the more rapidly symptoms occur
    
    • However, level of accumulation of PrP$^{Sc}$ does not necessarily correspond to level of disease.

  – Several additional variables influence the course and vulnerability to the disease:
    
    • PRNP gene isoform
    • In the case of vCJD:
      
      – amount of infected tissue consumed
      
      – origin of infected tissue (e.g., brain, muscle etc.)
Pathogenesis – Route of Invasion for PrP<sub>Sc</sub>

- It remains uncertain how PrP<sub>Sc</sub> migrates to the brain
- Current Dogma:
  - 1) Ingest food or receive tissue (e.g., blood transfusion) containing PrP<sub>Sc</sub>
  - 2) Protease resistant PrP<sub>Sc</sub> fragments absorbed into blood/lymph
  - 3) PrP<sub>Sc</sub> begins recruiting PrP<sub>c</sub> in lymph
  - 4. PrP<sub>Sc</sub> migrates to CNS and brain via peripheral nerves (mainly sympathetic NS)
  - 5. Brain neurons produce plaque & die
Pathogenesis – Route of Invasion for PrP\textsuperscript{Sc}

- The blood-brain-barrier (BBB) may serve as a possible route for neuro-invasion
- Three recipients of donated blood developed variant CJD
  - Also, in sheep experiments, BSE was transmitted by blood transfusions and prion has been detected in the circulation of hamsters with scrapie
    - PrP\textsuperscript{Sc} has been detected on intra-cranial vessel walls in the terminal stages of human prion disease
How do prions move from cell to cell?

Figure 1. Steps in Trans-cellular Propagation

Trans-cellular propagation of prions is likely to involve escape from a first-order cell, binding to a second-order cell, uptake into a second-order cell, seeding of native monomer, and fragmentation and amplification of the seeded aggregates. (A) Escape of prions from a first-order cell could occur by direct release into the extracellular space. This may be driven by unconventional secretion, cell death, or membrane penetration. (B and C) Alternatively, prions could escape in exosomes (B) or directly move to neighboring cells via tunneling nanotubes (C). (D) In the exosomal pathway, cell entry would presumably occur via vesicle fusion. (E) More likely, prions bind to heparan sulfate proteoglycans (HSPGs) to trigger macropinocytosis. (F) It is theoretically possible that prions gain entry by another form of receptor-mediated endocytosis, although there is not clear evidence for this. (G) Prions escape the lumen of vesicles to encounter cognate monomer. (H) The seed acts as a template for recruitment of monomer to amplify the prion structure. This likely involves a replication machinery that may also be involved in fibril fragmentation to amplify the number of seeds, which then repeat the cycle of propagation to other cells.
Susceptibility: the PRNP gene

- Prp gene called PRNP gene
- Lots of polymorphisms of the PRNP gene
- E.g. codon 129 of PRNP gene site (methionine/valine polymorphism in Caucasians) 37% M/M; 51% M/V, 12% V/V; 129 V/V long duration disease; M/M and M/V-typical ‘short duration’ disease
- Familial: Some people have mutations in PRNP gene (chromosome 20), at least 23 known mutations and 3 polymorphisms; virtually all mutations pathogenic for some form of disease (point mutations, insertional mutations, stop codons and truncated protein)
- What does the PRPc do. We do not know. Synaptic protein?
How might PrPsc kill cells?
Evidence that prion proteins cause disease

Box 1. Prions: the protein-only infectious agents that transmit disease

The prion or protein-only hypothesis proposes that the sole component of the infectious agent in TSEs is a misfolded version of the prion protein (PrP\text{Sc}), which replicates by converting the normally folded host-associated cellular prion protein (PrP\text{C}) into the misfolded form [2]. Compelling data have established that PrP\text{Sc} is indeed the basic element of the infectious agent [2,108].

- The infectious agent is too small to be a bacteria or virus.
- Infectivity is not removed by treatments that destroy nucleic acids.
- Injection of highly pure PrP\text{Sc} induces prion disease in animals.
- Infectivity is proportional to the concentration of PrP\text{Sc}.
- In general, PrP\text{Sc} is detectable only in individuals affected by the disease.
- All inherited cases of prion disease are associated with mutations in the PrP gene.
- Transgenic animals overexpressing the mutant PrP gene develop neurologic dysfunction, spongiform brain degeneration and symptoms that mimic those observed in scrapie-infected animals.
- PrP knockout mice are resistant to prion infection.
- PrP\text{Sc} self-propagates \textit{in vitro} by inducing the misfolding of PrP\text{C}.
- \textit{In vitro} replication of PrP\text{Sc} generates infectious material using PrP\text{C} from entire brain homogenates, purified mammalian PrP\text{C} or recombinant PrP\text{C}.
- \textit{De novo} production of PrP\text{Sc} either from mammalian or bacterial origin produces infectious material with novel characteristics.
- The phenomena of prion strains, species barrier, strain adaptation and prion memory are encribed in the folding of PrP\text{Sc} and can be reproduced during \textit{in vitro} prion replication.
Prion biology

- For a prion ($\text{PrP}^{\text{Sc}}$) to infect a host, the host must have a recognizable cellular form ($\text{PrP}^c$) of that prion.
- Generally, the closer the phylogenetic relationship between the donor host and the recipient, the greater the chance for infection, and the more rapidly symptoms occur.
- Level of accumulation of prion does not necessarily correspond to level of disease.
- Mice in which $\text{PrP}^c$ copy is knocked out have altered sleep/wake cycles and circadian rhythm.
IMPLICATIONS:
- In this course we have talked about mutant proteins leading to CNS disease
- Prion diseases have served as a paradigm where a mutant protein appears to aggregate (as detected by immunocytochemistry, or other methods), and the aggregated protein seems to lead to CNS disease.
- The prion agent is remarkable as it appears to become amplified using a ‘template-directed assembly’, where the mutant protein will alter the configuration of native proteins and ‘spread’ the disease.
- This ‘prion-like’ spread of disease may occur for other disorders even though the proteins are not ‘prion’ proteins.
- The proteins that may ultimately become damaging may be “intrinsically disordered proteins”, which inherently lack a tertiary protein structure.
- The protein misfolding may occur because of a variety of triggers including amino acid substitutions, cleavage, phosphorylation, or partial unfolding.
- The protein “chaperones” (e.g heat shock proteins, etc.) may be needed to assist in protein refolding.
- It is likely that the protein misfolding renders the protein more likely to self-assemble (aggregate) and also make it more resistant to cellular clearance mechanisms like proteosomal destruction.