

Priola

Open Session

May 16, 2001

**Transmissible spongiform encephalopathies (TSE)
or prion diseases**

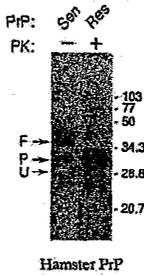
- Slow, fatal, transmissible brain diseases
- Affect humans, sheep, **cattle, deer, other** mammals
- Species barriers to **infection**
- Experimentally transmitted to mice, hamsters
- Different **strains** of TSE agent
- **Infections occur from** ingestion or inoculation
- Disease **can** take **from** months to decades to appear
- Always **fatal**
 - No **pre-clinical** diagnostic test available
 - No effective **pre- or post-clinical** treatments available
- **Infectious agent unclear**
 - **unusually hard to kill**

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PrP and TSE Disease



- **Normal PrP**
 - Proteinase K sensitive (PrP-sen)
 - Detergent soluble
 - Expressed in many different tissues
 - Primarily alpha helix/loop structure
- **Abnormal PrP**
 - Proteinase K resistant (PrP-res)
 - Detergent insoluble, aggregated
 - TSE specific
 - Found in CNS, LRS
 - Mostly beta sheet structure

Role of PrP in TSE diseases

- **Normal PrP (PrP-sen)**
 - Required for infection and disease
 - Mutations in **PrP-sen** can strongly **influence** susceptibility to TSE disease
- **Abnormal PrP (PrP-res):**
 - Associated with toxic events **in the brain**
 - Always associated **with infectivity**

Human TSE Diseases

- Sporadic Creutzfeldt-Jakob Disease (CJD)
 - No known exposure to TSE infectivity
 - Not associated with mutations in PrP
 - Accounts for 95% of all TSE cases (annually 1 case/10⁶ people)
- Familial
 - Familial CJD, Gerstmann-Strausler-Scheinker Syndrome (GSS), Fatal Familial Insomnia (FFI)
 - Associated with mutations in PrP, heritable
 - Extremely rare (~1-10 cases/10⁶ people)
- Infectious
 - Kuru
 - Iatrogenic CJD from contaminated medical procedures
 - Variant CJD (vCJD)

TSE Infectivity as an Issue in Cell Culture

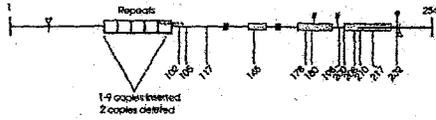
- Development of new cell lines
 - From CNS tissue which is potentially TSE-infected
 - From individual carrying a familial PrP mutation
- Infection of tissue culture cells
 - Exposure to bovine derived products
 - Susceptibility factors (e.g. cell type, PrP sequence)
 - Multiplicity of infection
 - Acute versus persistent infection
- Detection of TSE infectivity
 - Bioassay
 - PrP-res or other markers
 - Sensitivity

TSE Infection can be Maintained in Cells Derived from Brain or Spinal cord of TSE Infected Individuals

TSE	source	Persistence of Infectivity (bioassay)		
		≤300days	>2 passes	>10 Passes
Kuru ^a	Brain	+	-	-
CJD ^a	Brain	-I-	-	-
CJD ^a	S. cord	+	-	-
Scrapie (mouse) ^{a,b,c}	Brain	+	+	+
Scrapie (sheep) ^a	Brain	+	+	+

^a=Asher et al., 1979
^b=Clarke and Haig, 1970
^c=Gustafson and Kenitz, 1965

PrP Mutations Associated with Familial TSE Diseases

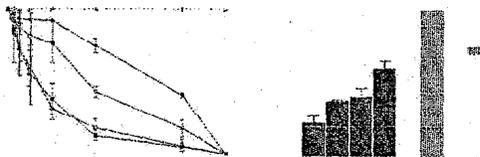


*Not known how specific PrP mutations lead to familial TSE disease

*Hypothesis based on the protein **only** theory of TSE disease:

PrP mutants have altered biochemical properties and spontaneously convert to PrP-res, thus causing disease

Altered Properties of PrP-sen Molecules with Familial TSE Mutations



Mutant PrP-sen in vitro:

- Less PK resistant than PrP-res (by ~500x)
- Does not aggregate to the same extent as PrP-res
- Does not spontaneously form PrP-res

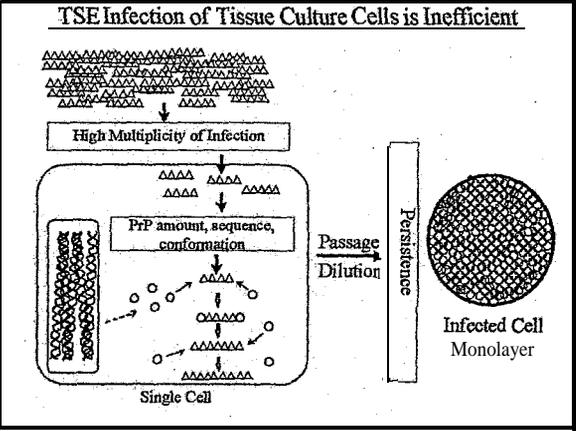
↳ ?TSE disease in vivo?

Analysis of Transgenic Mice Expressing Familial TSE PrP Mutants

PrP Mutation	Overexpression	Disease		
		Neurological	TSE	Infectious
+9 (CJD)	Yes	+	-	-
A117V (GSS)	Yes	+	-	-
P102L (GSS)	Yes	+	+/-	+/-
P102L (GSS)	No	-	-	-
None	Yes	+	-	-

TSE Infectivity as an Issue in Cell Culture

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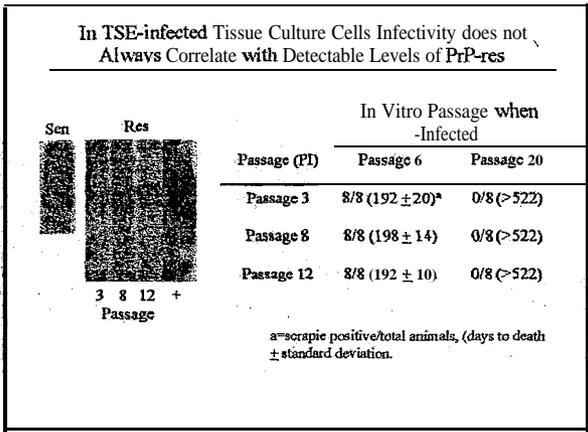


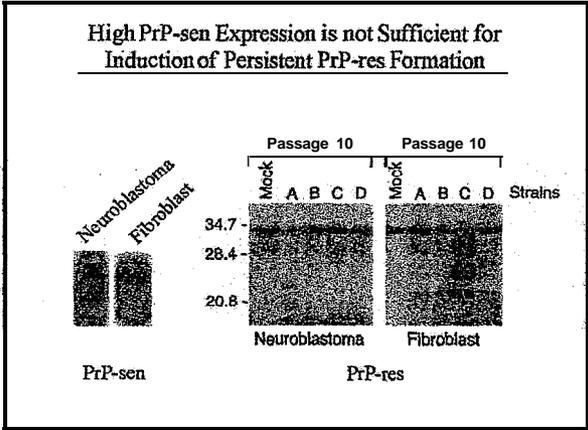
In TSE-infected Tissue Culture Cells Infectivity does not Always Correlate with Detectable Levels of PrP-res

Sen	Res	In Vitro Passage when Infected		
		Passage (PI)	Passage 6	Passage 20
		Passage 3	8/8 (192 ± 20)*	0/8 (>522)
		Passage 8	8/8 (198 ± 14)	0/8 (>522)
		Passage 12	8/8 (192 ± 10)	0/8 (>522)

3 8 12 +
Passage

*=scrapie positive/total animals, (days to death ± standard deviation).





Susceptibility of Different Cell Types to TSE Infection

Susceptible Cell Lines	Non-Susceptible Cell Lines
<ul style="list-style-type: none"> •Neuronal Cells -Mouse neuroblastoma (N2A) -Mouse hypothalamic (GT-1) -Rat pheochromocytoma (PC-12) -Hamster brain (HaB) 	<ul style="list-style-type: none"> •Neuronal Cells •Human neuroblastoma (p12-3) •Human glioma (HIC-15)
<ul style="list-style-type: none"> •Non-Neuronal Cells -Mouse L-fibroblasts -Rabbit kidney epithelial 	<ul style="list-style-type: none"> •Non-Neuronal Cells •Human embryo lung fibroblasts (WI-38, MDC-5) •Human embryo brain fibroblasts •Human embryo kidney •Chinese Hamster Ovary •AGMK (BSC-1)

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Detection of TSE Infectivity

Approved Tests				
Test	Type	Tissue	Sensitivity	
Prionics (Roche Diag.)	Check	Western blot	Brain	Shortly before or at clinical signs
Enfer Scientific (Protherics)		ELISA	Brain, S. cord	Shortly before or at clinical signs
Biorad (CBA)		ELISA	Brain	Same as oral bioassay

Tests in the Approval Process

DELFA test (Fluoroimmunoassay using brain material)

Possible Future Tests

Fluorescence correlation spectroscopy
Capillary immunoelectrophoresis (blood-based)

TSE Diagnostic Tests

Difficulties with **current** tests

- *Post-mortem
- Based only on PrP-res detection
- Maximized to high titer tissue (i.e. brain)
- Sensitivity
 - Shortly before. or at clinical signs
 - *Not as good as bioassay in same species, transgenic mice

Bioassay is the ¹most sensitive test for TSE infection. However, the time required for the assay often makes it impractical.

Summary

• Unlikely that:

- Newly derived human neoplastic cell substrates will be TSE-infected.
- Exposure to potentially BSE contaminated, bovine-derived tissue culture products could lead to persistent TSE infection.

↳ Cannot guarantee zero risk

Analysis of Cell Lines for TSE Infection

Rapid but Less Sensitive Assays

- Commercially available tests (western blot, ELISA), FACS
- Assay for PrP^{scn} and determine its expression level
- Determine sequence of PrP^{scn}
- Assay for PrP^{res}:
 - In multiple cell subclones
 - Periodically at different culture passages

Bioassay for TSE Infectivity

- Most sensitive test but can take a very long time
- For human TSE infectivity:
 - Primate bioassay: up to 5 years or more
 - Human PrP-expressing transgenic mice: up to 2 years
