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Prion Diseases

This document describes infectious agents which (almost certainly) do not have a nucleic acid genome. It seems that a protein alone is the infectious agent. The infectious agent has been called a **prion**. A prion has been defined as **"small proteinaceous infectious particles which resist inactivation by procedures that modify nucleic acids"**. The discovery that proteins alone can transmit an infectious disease has come as a considerable surprise to the scientific community.
Prion diseases are often called **spongiform encephalopathies** because of the post mortem appearance of the brain with large vacuoles in the cortex and cerebellum. Probably most mammalian species develop these diseases. Specific examples include:

- **Scrapie**: sheep
- **TME** (transmissible mink encephalopathy): mink
- **CWD** (chronic wasting disease): muledeer, elk
- **BSE** (bovine spongiform encephalopathy): cows

Humans are also susceptible to several prion diseases:

- **CJD**: Creutzfeld-Jacob Disease
- **GSS**: Gerstmann-Straussler-Scheinker syndrome
- **FFI**: Fatal familial Insomnia
- **Kuru**
- **Alpers Syndrome**

These original classifications were based on a clinical evaluation of a patients family history symptoms and are still widely used, however more recent and accurate molecular diagnosis of the disease is gradually taking the place of this classification.

The **incidence** of sporadic CJD is about 1 per million per year.
GSS occurs at about 2% of the rate of CJD.

It is estimated that 1 in 10,000 people are infected with CJD at the time of death.
These figures are likely to be underestimates since prion diseases may be misdiagnosed as other neurological disorders.

The diseases are characterised by **loss of motor control, dementia, paralysis wasting and eventually death, typically following pneumonia**. Fatal Familial Insomnia presents with an untreatable insomnia and dysautonomia. Details of pathogenesis are largely unknown.

Visible end results at post-mortem are **non-inflammatory lesions, vacuoles, amyloid protein deposits and astrogliosis**.

GSS is distinct from CJD, it occurs typically in the 4th-5th decade, characterised by cerebellar ataxia and concomitant motor problems, dementia less common and disease course lasts several years to death. (Originally thought to be familial, but now known to occur sporadically as well).
CJD typically occurs a decade later has cerebral involvement so dementia is more common and patient seldom survives a year (originally thought to be sporadic, but now known to be familial as well).

FFI pathology is characterised by severe selective atrophy of the thalamus.
Alpers syndrome is the name given to prion diseases in infants.

Scrapie was the first example of this type of disease to be noticed and has been known about for many hundreds of years. There are two possible methods of transmission in sheep:

1. Infection of pasture with placental tissue carrying the agent followed by ingestion,or direct sheep-lamb transmission i.e. an acquired infection.

2. Parry showed considerable foresight by suggesting that it is not normally an infectious disease at all but a genetic disorder. He further suggested that selective breeding would get rid of the disease.

Humans might be infected by prions in 2 ways:

1. Acquired infection (diet and following medical procedures such as surgery, growth hormone injections, corneal transplants) i.e. infectious agent implicated.

2. Apparent hereditary mendelian transmission where it is an autosomal and dominant trait. This is not prima facie consistent with an infectious agent.

This is one of the features that single out prion diseases for particular attention. They are **both** infectious **and** hereditary diseases (?see below). They are also sporadic, in the sense that there are also cases in which there is no known risk factor although it seems likely that infection was acquired in one of the two ways listed above.

Kuru is the condition which first brought prion diseases to prominence in the 1950s. Found in geographically isolated tribes in the Fore highlands of New Guinea. Established that ingesting brain tissue of dead relatives for religious reasons was likely to be the route of transmission. They ground up the brain into a pale grey soup, heated it and ate it. Clinically, the disease resembles CJD. Other tribes in the vicinity with same religious habit did not develop the disease. It is speculated that at some point in the past a tribe member developed CJD, and as brain tissue is highly infectious this allowed the disease to spread. Afflicted tribes were encouraged not to ingest brain tissue and the incidence of disease rapidly declined and is now almost unknown.

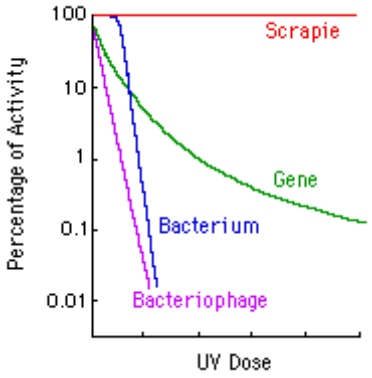
Evidence suggests that a prion is a modified form of a normal cellular protein known as **PrPc** (for cellular), a normal host protein encoded by a single exon of a single copy gene. This protein is found predominantly on the surface of neurones attached by a glycoinositol phospholipid anchor, and is **protease sensitive**. Thought to be involved in synaptic function.
The modified form of PrPc which may cause disease i.e. the prion is known as **PrPsc** (for scrapie) which is **relatively resistant to proteases** and accumulates in cytoplasmic vesicles of diseased individuals.

It has been proposed that PrPsc when introduced into a normal cell causes the conversion of PrPc into PrPsc. Process is unknown but it could involve a chemical or conformational modification.

Several lines of evidence support protein only model of infection:

1. Nucleic acid is not necessary for infectivity:

- unusually small target size for ultraviolet and ionising radiation:



- the low ratio of nucleic acid to infectious material.
- resistance of infectivity to agents which modify or damage nucleic acids but infectivity is susceptible to reagents which destroy proteins:

Stabilities of the scrapie agent and viriods (PSTV):

Chemical Treatment:	Concentration	PSTV	Scrapie
Et2PC	10-20mM	(-)	+
NH2OH	0.1-0.5mM	+	-
Psoralen	10-500µg/ml	+	-
Phenol	Saturated	-	+
SDS	1-10%	-	+
Zn2+	2mM	+	-
Urea	3-8M	-	+
Alkali	pH 10	(-)	+
KSCN	1M	-	+
Enzymatic Treatment:	Concentration	PSTV	Scrapie
RNAse A	0.1-100µg/ml	+	-
DNAse	100µg/ml	-	-
Proteinase K	100µg/ml	-	+
Trypsin	100µg/ml	-	+

+ = inactivated; - = no change in infectivity

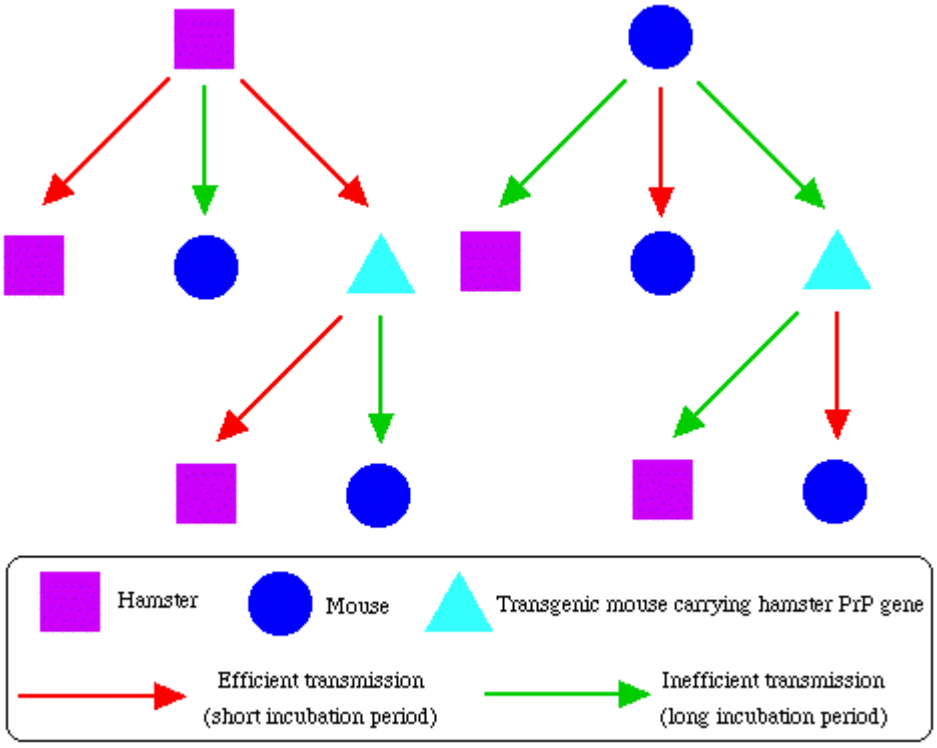
- failure to identify a scrapie specific nucleic acid either in prion preparations or infected brains using a variety of sophisticated techniques.

2. PrPsc is associated with scrapie infectivity:

- purification of scrapie infectivity results in preparations highly enriched for PrPsc
- purification of PrPsc results in enrichment of scrapie activity
- purification of PrPsc by SDS-PAGE also recovers infectivity
- PrPsc can be denatured and renatured without loss of infectivity

3. Susceptibility of a host to prion infection is co-determined by the prion inoculum and the PrP gene:

- disease incubation time for a single prion isolate various between mouse strains, this variation depends on the Sinc gene, this is very closely linked to or coincident with the PrP gene itself, suggesting some forms of PrPc may be more easily converted to PrPsc than others.
- when prions are transmitted from one species to another disease develops only after a very long incubation period, if at all, but on serial passage in the new species the incubation time often decreases dramatically and then stabilises. This species barrier can be overcome by introducing a PrP transgene from the prion donor i.e. hamster PrPc but not murine PrPc is a suitable substrate for conversion to hamster PrPsc by hamster prions and vice versa:



4. Mutated gene can cause susceptibility to disease without apparent infection:

- Homozygosity at the polymorphic amino acid position 129 of PrP protein predisposes an individual to acquired and sporadic CJD
- 2 unrelated GSS families have the same double mutation i.e. 178 D-N; 200 E-K
- disease tightly linked to a P-L mutation at 102 in some familial GSS cases. 100 controls did not have this mutation nor did another 15 sporadic victims of CJD
- other familial cases have been shown to carry this or several other mutations e.g. 117-A-V; 198 F-

S; N178; V129 = CJD; N178 M129 = FFI

One possibility, **the genetic explanation**, was that these mutations enhance the rate of spontaneous conversion of PrPc to PrPsc which permits disease manifestation within the lifetime of an individual. Suggests that some sporadic incidents can be accounted for by somatic mutation of the PrP gene. **An alternative explanation is that these mutations confer susceptibility to infection**

5. Crucial experiment:

Mice carrying a murine transgene with the 102P-L GSS mutation spontaneously develop a lethal scrapie like disease. Brains also contain infectious prions because transmission to recipient animals has been demonstrated.
Mice lacking the PrPc gene develop normally, no evident physiological or behavioural problems. Suggests that loss of PrPc function is unlikely to be the cause of disease rather accumulation of PrPsc is responsible. When inoculated with prions they do not develop disease . There are obvious implications here for the livestock and pharmaceutical industries and treatment of familial cases. Animals expressing reduced levels of PrPc are also resistant to infection - perhaps more immediately relevant to human disease. Animals overexpressing PrPc are more likely to develop prion diseases.

Evidence against the prion model:

The existence of many different strains of scrapie (at least 15: latency, lesion patterns differ) which can be propagated in the same inbred mouse strain. These can transmit serially without changes in properties in the same mouse strain homozygous for a single PrP genotype. Cannot argue that there a distinct PrPc is converted to distinct PrPsc. Must mean that a common PrPc is corrupted in a different way, seems improbable but there is now experimental support for it.

Are prion diseases genetic disorders?

Is the genotype of an animal or human the direct cause of a prion disease or a susceptibility factor? It has long been known that some genotypes of sheep often develop scrapie, e.g. in the UK the genotypes:

- Ala136Ala, Arg154Arg, Gln171Gln
- Val136Val, Arg154Arg, Gln171Gln

are almost always identified in scrapie infected sheep. In contrast only one sheep with Ala136Ala,Arg154Arg,Arg171Arg has been identified with scrapie, these animals are resistant to both a scrapie and BSE challange. Surprisingly (if the genetic explanation is correct), these scrapie-susceptible genotypes are common in Australia and New Zealand which are thought to be free of scrapie. Antipodean sheep have also been bought back to the UK and maintained in quarantine conditions and not developed scrapie. In other words, the genotype does not confer scrapie on the animal but susceptibility to scrapie infection. **Scrapie would appear to be an infectious disease, not a genetic one.** This observation may have implications for families carrying a mutant prion gene.

So how can a protein be infectious?

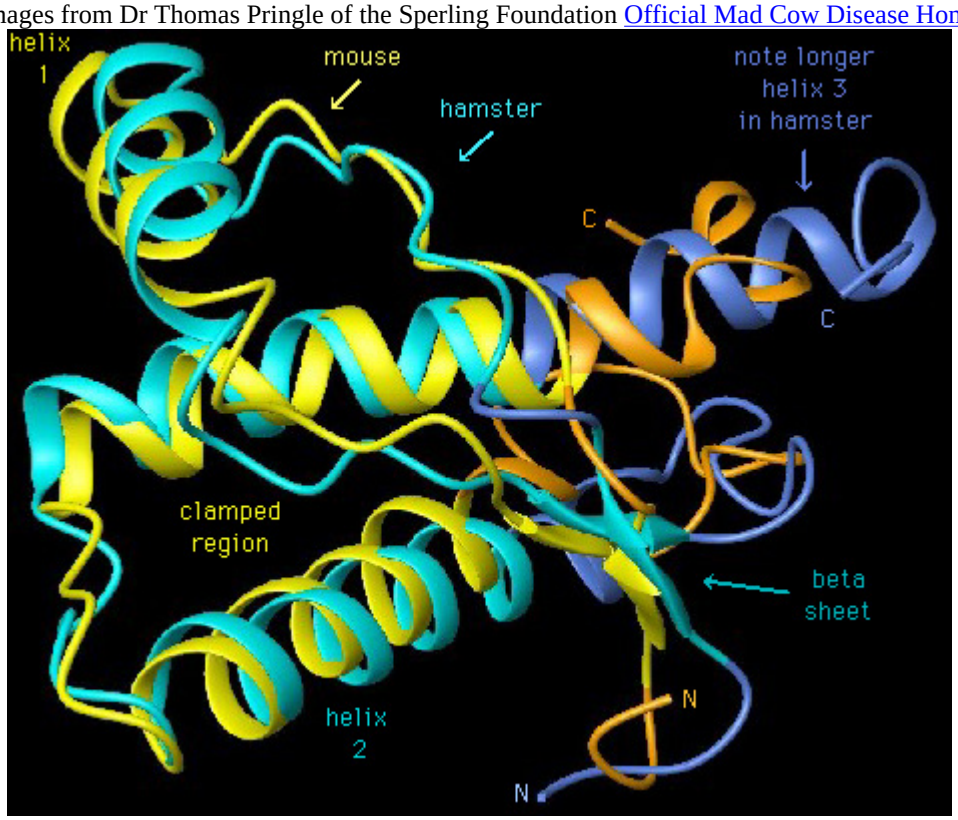
PrPsc is a protease resistant form of PrPc, both are extensively post translationally modified. No chemical differences between the two forms of the protein have been detected. Clearly there must be

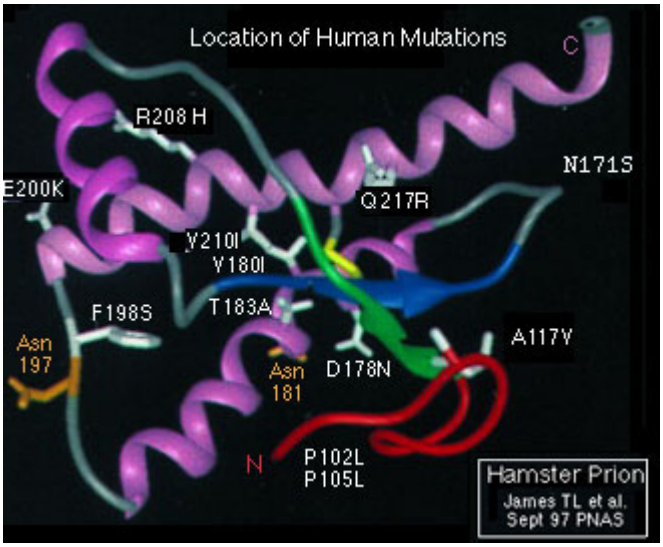
some difference. One great problem is that infectivity ratio is about 100,000:1, so infectious agent even if derived from PrPc may not be PrPsc and it could be chemically modified.

However, a more likely possibility is that the difference between PrPc and PrPsc is conformational. 3D structure of part of the murine PrPc expressed in E. coli has recently been determined. As expected from spectroscopy measurements PrPc is predominantly alpha helical and contains almost no beta sheet. The structure of PrPsc has not yet been determined but is predicted to be predominantly B- sheet. Proposed therefore that this protein can adopt 2 quite different stable conformations. The safe PrPc form is normally adopted but rarely it can switch to the PrPsc form. Mutations favour this switch. Propose that PrPsc is transdominant and converts PrPc to PrPsc in an exponential fashion. Precedents for this model do exist. There is a yeast mutant phenotype which doesn't correlate to any mutational difference in the gene structure but does correlate to a different protein structure. The Ure2p protein converts to an inactive conformation. Mutations in the tumour suppressor protein p53 which are associated with the onset of neoplastic disease have a different conformation to the normal protein. When normal protein is incubated with mutant protein its conformation is altered to the mutant form.

The first image below shows the structure of part of the hamster and mouse PrPc molecules superimposed. The close similarity in structures is obvious, as is the preponderance of alpha helical structure.The second shows the position of various mutations important for prion disease development *in humans* modelled on the hamster structure PrPc.Many of these mutations are positioned such that they could disrupt the secondary structure of the molecule.

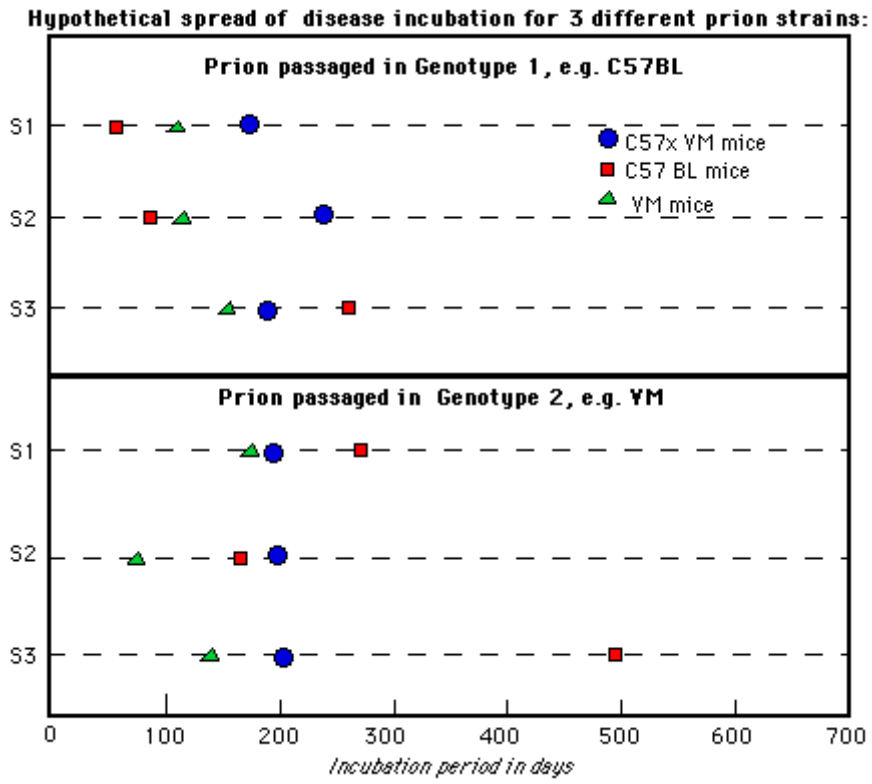
Both images from Dr Thomas Pringle of the Sperling Foundation Official Mad Cow Disease Home Page



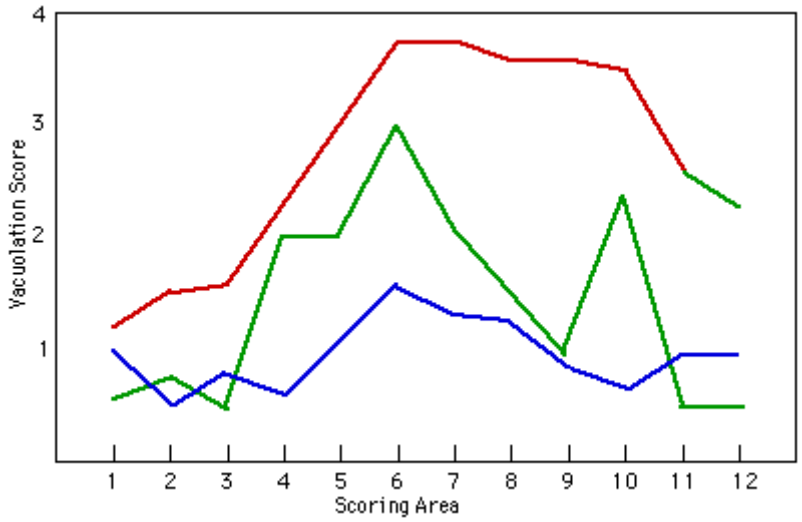


Strain Typing:

The existence of multiple strains of prion agents by definition means that the agents carry strain specific information. The virino hypothesis states that this is a small nucleic acid molecule. The prion hypothesis states that it is due to differences in the chemical or tertiary structure of the prion protein.
Strain discrimination relies on the use of mice of defined genotype and having different PrP gene sequences.
Measurement of parameters such as incubation period in these mice and the pathological changes occurring in the brain are made.
Measurement of Incubation period: Precise quantities of brain homogenates from clinically affected animals are injected intracerebrally into mice. Animals are then assessed regulary for the definite appearance of disease using a defined set of observations. This measurement is very precise with errors of less than 2% of the mean:



Pathological changes: Strains show very different and reproducible differences in the pattern of vacuolar degeneration of different inbred mice. These effects are scored in a variety of different brain sections. Up to 12 topologically specific sites of the brain are scored for vacuolation e.g. cortex, cerebellum, brain stem etc. This semiquantitative assessment or lesion profile is then plotted. Individual strains have a characterisitc and highly reproducible lesion profile in a given mouse genotype e.g. Using this technique it is very clear that the recent outbreaks of new TSE diseases in various animals is caused by a prion with very similar characteristics ie BSE and quite distinct from previously recognized scrapie prion strains. Strain typing has also demonstrated beyond reasonable doubt in the last few months that nv-CJD or v-CJD are caused by a strain very much like the BSE agent. Some people now call this disease Human BSE:



BSE and NV-CJD:

This section will continue to be overtaken by events. (The time of writing is 20th August 1998.)
Many people have been concerned since the recognition of the BSE epidemic in 1987 that it may pose a risk to humans.
Strain typing studies show one major strain. BSE can transmit to many species experimentally e.g. sheep, pigs and macaque monkeys.
Strain typing studies have confirmed that domestic cats, big cat and exotic ungulates have been infected by eating tainted beef.
About 2 years ago a new variant of CJD was recognized in the British population. At the time of writing 26 cases have been confirmed. People have developed this form of CJD at strikingly early ages 17-55. The neuropathology and early clinical symptoms are also very distinct from 'classical' CJD. The CJD Surveillance Unit at Edinburgh and the committee charged with giving the government recommendations to deal with this threat to human health, SEAC (Spongiform Encepahalopathy Advisory Committee) have concluded that the most likely cause is ingestion of infected beef.
Strain typing studies of NV-CJD as described above have confirmed that this is very probably the case..
Earlier experiments involving the production of a "protease fingerprint" of PrPsc showed that 10 or so of the NV-CJD cases so far analysed had a unique form and distribution characteristic of a BSE infection and completely different to 'classical' CJD.
It seems therefore that BSE has infected humans by oral ingestion.
An epidemic? It is currently unclear whether the incidence of NV-CJD will remain very low or become very high.
It is likely that a large proportion of the UK population has been exposed to the infectious agent.
The size of the epidemic will depend on infectious dose exposure via the gastric route which may be cumulative size of the species barrier: for a more detailed discussion of these points, click here.

Infection and Pathogenesis

Ingested prions may be absorbed across the gut wall at Peyers patches. These are a part of the MALT, or mucosal associated lymphoid tissue. It is thought that the MALT presents microorganisms to the immune system in a contained and ideal fashion, facilitating a protective immune response. Prions could be taken up in the same way. Lymphoid cells then phagocytose the particle and travel to other lymphoid sites such as nodes, the spleen and tonsils. The prion can replicate at these sites. Many of these sites are innervated and eventually the prion gains access to a nerve and then propagates back up the axon to the spinal cord and eventually the brain.

- SCID mice are resistant to a prion challenge, confirming the importance of the lymphoid system.
- PrP null mice ie those in which both alleles have been disrupted, $PrP^{o/o}$, cannot be infected.
- $PrP^{o/o}$mice carrying a $PrP^{+/+}$ brain graft can develop pathology following an intracerebral injection but only in the graft.
- $PrP^{o/o}$ mice + graft + a reconstituted $PrP^{+/+}$ lymphoreticular system are resistant to a prion challenge.

This indicates another compartment in addition to the brain and the LRS must express PrP, if a peripheral prion challenge is to be successful. That compartment is probably a nerve. Mature B lymphocytes are also now known to be required for the development of the disease following infection from a peripheral route.

Tg PrP mice and knockout mice have been fantastically informative in prion research. In a further series of recent experiments $PrP^{o/o}$ mice have had a hamster PrP transgene incorporated. This transgene has been put under the transcriptional control of either the glial fibrillary acidic protein promoter, or the neuron specific enolase promoter such that hamster PrP is only expressed in either the glial cells or neuronal cells. Following an intracerebral prion challenge both groups of animals can replicate the prion agent and develop disease pathology. So either glial cells or neural cells can propagate the disease independently. The fact that PrPsc intracerebral injection alone in $PrP^{o/o}$ mice, does not cause pathology means that cells must be making PrP for a pathological result. It is known that cytokine levels are elevated in the later stages of a prion infection. Astrocytes and other glial cells produce these and presumably have a key role in pathogenesis. This conclusion is supported by the fact that murine retroviruses are known which infect glial cells and result in a spongiform degeneration of the brain.

Treatment:

PrP overexpression facilitates the development of prion diseases. It may therefore follow that agents which reduce PrP expression will delay the onset of prion diseases.
One can speculate that chemicals which bind to and stabilise the PrPc conformation may be beneficial. Similarly agents destabilising the PrPsc conformation may be effective.
Agents which interfere with the putative PrPc-PrPsc interaction might similarly be effective.

A number of reagents showing affinity for amyloid proteins are known e.g congo red. As our knowledge of the structure of PrP increases, the chances of rationally deducing effective therapeutics based on these ideas increases.

Finally we have seen that PrP expression is required for pathology. Chemicals affecting the endocytosis, exocytosis, intracellular trafficking and degradation of proteins and in particular PrP may also be effective. Amphotericin for instance is reported to delay prion disease in hamsters (although it apparently has little effect in humans).

Further Information:

Books about:

Prions | Mad Cow Disease | BSE (technical)

- **References**
- **How Now Mad Cow:** Online tutorial.

BSE: Latest News:
Visit The Microbiology Newsroom

Latest publications on BSE/CJD:

Click Here

Other BSE links:

- John Pattison: The Emergence of Bovine Spongiform Encephalopathy & Related Diseases. Emerging Infectious Diseases 4(3):390-394, 1998.
- Ministry of Agriculture, Fisheries & Food (MAFF)
- I.A.O.
- USDA Veterinary Services
- Official Mad Cow Disease Home Page
- Centre for the Study of Health, Brunel University
- C.A.B. INTERNATIONAL
- BSE information, gossip
- BMJ's BSE-CJD Homepage
- BSE and Scrapie in the UK

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