ISSN 0976 – 3333

Available Online at www.ijpba.info

International Journal of Pharmaceutical & Biological Archives 2011; 2(5):1380-1390

REVIEW ARTICLE

Prion - A Review

Minakshi Nehete*, Rohini Chandratre, Tanmayee Joshi

C. U. Shah College of Pharmacy, S. N. D. T. Women's University, Juhu, Santacruz (West), Mumbai 400049

Received 19 Jul 2011; Revised 13 Oct 2011; Accepted 23 Oct 2011

ABSTRACT

Prion diseases are incurable neurodegenerative diseases caused by proteinaceous infectious particles affecting both animals and humans. Human prion diseases include Creutzfeldt-Jakob disease (CJD), Kuru**,** Gerstmann-Sträussler-Scheinker Disease (GSS)**,** Fatal Familial Insomnia (FFI), Variant Creutzfeldt-Jakob Disease (vCJD)**.** The spread of human prion diseases is through ingestion of contaminated meat. Animal prion diseases like scrapie of sheep, transmissible mink encephalopathy, chronic wasting disease of cervids, and bovine spongiform encephalopathy all seem to be laterally transmitted by contact with infected animals or by consumption of infected feed. There is no current treatment for prion diseases. The different modes of transmission of different prion diseases, the unpredictable species barriers, the variable distribution of infectivity in tissues, and strain variations found in some diseases all make risk assessment and predictions of future events difficult.

Key Words: Prion, Kuru, Mad cow diseases.

INTRODUCTION

Structure:

Prions are proteins that can adopt two different forms, a normal form and a misfolded form. This may not seem unusual, since many proteins are flexible and adopt different shapes. However, prions have another unusual characteristic that the misfolded form of the prion can force normal prions to change into the misfolded shape. In this way, a few misfolded prions can corrupt a whole population of normal prions, converting them one-by one into the misfolded shape. These misfolded prions are proteinaceous infectious particles, so a small dose of misfolded prions can infect and corrupt an entire organism[1].

Prions are composed exclusively of a single sialoglycoprotein called PrP 27-30. They contain no nucleic acid. PrP 27-30 has a mass of 27,000 - 30,000 daltons and is composed of 145 amino acids with glycosylation at or near amino acids 181 and 197[2]. This protein polymerizes into rods possessing the ultrastructural and histochemical characteristics of amyloid.

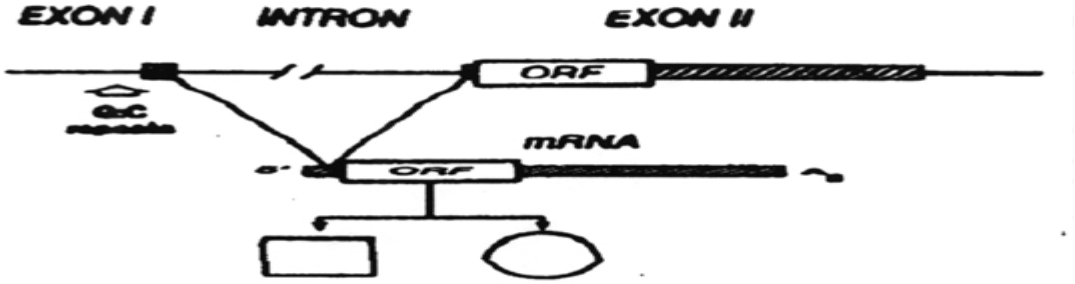
Amyloid is deposited intercellularly and/or intracellularly in many human diseases such as Alzheimer's disease, Creutzfeldt-Jakob disease, Down's syndrome, fatal familial insomnia, Gerstmann-Straussler syndrome and Kuru Leprosy.

Evidence suggests that a prion is a modified form of a normal cellular protein known as PrPc (for cellular), a single copy gene. This protein is found predominantly on the surface of neurons attached by a glycoinositol phospholipid anchor, and is protease sensitive. The modified form of PrPc which may cause disease known as PrPsc (for scrapie) 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[3]. This process is unknown but it could involve a chemical or conformational modification.

Replication:

The prion is a product of a human gene, termed the PrP gene, found on chromosome 20. This gene contains two exons separated by a single intron. Exon I and Exon II are transcribed and the two RNAs ligated into a single mRNA. This mRNA contains an open reading frame (ORF) or protein coding region which is translated into the PrP protein. The PrP protein is a precursor of the prion protein. It is termed PrP 33-35.

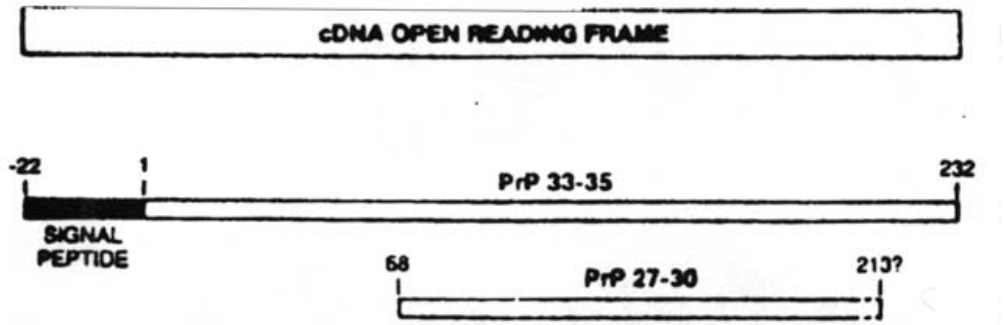
***Corresponding Author:** Minakshi Nehete, **Email:** minakshi1277@yahoo.co.in



Organization and Expression of the PrP Gene. The features presented were deduced from the nucleotide sequences of PrP genomic and cDNA clones. Untranslated regions of the mRNA are indicated by hatched boxes. An open reading frame or protein coding region is indicated by the open box. The diagonal lines show a splicing event that joins the 5' leader sequences to the remainder of the coding sequences.

The PrP 33-35 undergoes several post-translational events to become the prion protein (PrP 27-30):

1. Glycosylation - at two sites.
2. Formation of a disulfide bond between two cysteine residues.
3. Removal of the N-terminal signal peptide.
4. Removal of the C-terminal hydrophobic segment.
5. Addition of a phosphatidylinositol glycolipid at the C-terminal.
6. Removal of the N-terminal first 57 amino acids.



Structure of the prion protein (PrP). The open reading frame encodes a protein of 254 amino acids. The first 22 amino acids comprise a signal peptide that is cleaved during synthesis of PrP 33-35. Digestion of the scrapie isoform of PrP 33-35^{Sc} with proteinase K generates a smaller protease-resistant polypeptide designated PrP 27-30.

In normal cells only the PrP 33-35 protein is synthesized. It is found in the neural cell membrane where it's function is to sequester Cu++ ions. In abnormal ("infected") cells, the PrP 27-30 is produced from the PrP 33-35 protein. The PrP 27-30 triggers a series of reactions that produce more PrP 27-30 proteins, i.e., PrP 27-30 induces its own synthesis. In addition to the post translational modifications, the PrP 27-30 protein differs from the PrP 33-35 protein in a single amino acid residue. Residue 178 in the PrP 27-30 contains an asparagine residue whereas the PrP 33-35 protein has an aspartate residue at this position[4]. This causes a conformational change in the PrP 27-30 protein from an a-helix to a b-sheet. This conformational change in the PrP 27-30 protein has three effects:

1. It imparts to the PrP 27-30 protein the ability to induce the same α-helix to β-sheet conformation in the PrP 33-35 protein. This is a permanent conformational change. It thus induces its own "replication."
2. The β-sheet-forming peptides aggregate to form amyloid fibrils.
3. The amyloid fibrils kill thalamus neurons through apoptosis, a programmed series of events that leads to cell death.

Pathologies induced by prions:

All diseases caused by prions in animals and humans are neurodegenerative diseases.

The pathological and clinical signs of these diseases suggest that they are closely related. In fact they may be variants of the same disorder. All pathological features are confined to the central nervous system. The prion protein accumulates selectively and abnormally in CNS nerve cells during the course of the disease. PrP 27-30 accumulates within the neuropil where it causes:

1. Astrocyte gliosis (an increase in the number of astrocytes).
2. Depletion of dendritic spines in neurons.
3. Formation of numerous vacuoles in the cerebellar cortex because the accumulation of aberrant prions causes the neurons (nerve

cells) to die. It is these characteristic vacuoles that give the diseases their names as spongiform (sponge-like) encephalopathies (spongiform encephalopathy)[5].

4. Amyloidosis - deposition of amyloid in the cerebellar cortex, thalamus, brain stem and in the lumen of blood vessels within the brain. These amyloid plaques consist of discrete eosinophilic glassy-appearing masses, often having radiating amyloid fibrils at their periphery. The plaques are primarily subependymal, subpial and perivascular. The pathology does not include any signs of inflammation or fever. This is evidence that the immune system does not respond to the prion protein.

These pathologies give rise to the clinical symptomology seen in these patients. These are:

1. A long incubation period (several years) which has given rise to the term "slow infection."
2. Loss of muscle coordination which leads to a difficulty in walking, indicating a functional disorder of the cerebellum.
3. Dementia characterized initially by loss of memory, diminished intellect and poor judgement.
4. Progressive insomnia characterized by a marked reduction or loss of the slow-wave and rapid-eye-movement phases.

Prion Diseases:

Animal:

1. Scrapie
2. Chronic Wasting Disease
3. Bovine Spongiform Encephalitis
4. Feline spongiform encephalitis
5. Transmissible mink encephalitis

Human:

1. Kuru
2. Gerstmann-Sträussler-Scheinker Disease (GSS)
3. Fatal Familial Insomnia (FFI)
4. Alpers' Disease
5. Creutzfeldt-Jakob Disease (CJD)
6. Variant Creutzfeldt-Jakob Disease (vCJD)

ANIMAL DISEASES

Scrapie:

Scrapie was the first prion disease to be identified and has been recognised by shepherds for over 200 years [6]. Scrapie is a fatal, degenerative disease affecting the central nervous system. The disease is characterised by scraping of fleece, stumbling, and behavioural changes and has a progressive course, leading to death in 3–6 months. Other symptoms include loss of coordination, weight loss, hopping like a rabbit and swaying of the rear end. Post-mortem examination shows changes only in the CNS with neuronal loss, gliosis, and vacuolisation of neural cells. The precise route of transmission is unclear, but lambs exposed in the pasture show infectivity first in tonsils, retropharyngeal lymph nodes, and intestine, which suggest infection via the alimentary tract[7]. Vertical transmission may occur, but exposure of young lambs to infected flocks seems to be the major risk factor[8]. The infection crosses species barriers to goats that share pastures with affected sheep, but no evidence implicates natural spread to other livestock or to people. The disease has been experimentally transmitted to primates, rodents, and other species. Diagnosis is made based on the animal's physical symptoms, the animal's history and finally by exam of brain tissue. A diagnostic test is undergoing evaluation by USDA's Animal and Plant Health Inspection Service for the detection of Scrapie in live animals. Increased concern over this disease has caused packers and producers to have difficulty in disposing of sheep offal and dead sheep, causing increases in disposal costs. In addition, other countries are hesitant to purchase sheep products from the U.S. Control programs are focusing on developing a diagnostic test, investigating transmissibility, and providing effective cleanup strategies that are economic for packers and producers.

Chronic Wasting Disease (CWD):

It was first discovered in Colorado in the mule deer population and manifested itself as a "wasting" syndrome, resulting in severe weight loss, unsteadiness, and excessive salivation after entering the facility as fawns or young adults. Animals became emaciated, and developed behavioural changes, and consequent death. Death occurred within weeks to months, and pathological examination of brains showed widespread spongiform changes in grey matter[9]. Elk in contact with mule deer developed the disease, and the disease was experimentally transmitted to various species, including limited evidence of transmission to squirrel monkeys and cows [10,11]. The disease has now been found in deer in New York State to the east, in Utah to the west, in New Mexico to the south, and in Canada to the north. The origin of this prion disease is unknown[12] and the mode of transmission among cervids is mysterious, because deer have been infected after entering paddocks in which sick deer had been removed over 1 year before[13]. The potential risk to cattle sharing ranges with wildlife and to people hunting and eating venison are

evident, but there is no evidence of natural transmission to non-cervids[14]. Transmission of the disease is thought to be from animal to animal but may occur through birth. There is also evidence that it can be spread through exposure to prions in the environment. Currently researchers are in the process of developing a live-animal diagnostic test. As in other spongiform diseases, brain lesions occur and current diagnosis is made after the animal has died. Prevention of CWD is by elimination of infected animals and limiting the distribution of the disease to the endemic area for free-range animals and surveillance of farmraised animals. Hunters should contact state wildlife officials to avoid endemic areas. Precautions to be taken when field-dressing these animals include using gloves, boning-out the meat from the animal, and minimizing handling of the brain and spinal cord.

Bovine Spongiform Encephalopathy (BSE) or Mad Cow Disease:

This disease is named as "Mad Cow Disease" due to the behavior exhibited by the cattle when they are infected. The origin of the disease appears to be cattle feed that contained Scrapie infected sheep brain tissue that had been treated in a new way that did not destroy the infectiousness of the Scrapie prions.

In 1985, the first cases of BSE were observed in the UK; in the next decade a massive epidemic throughout the country led to infection of about 1 million cows. Export of cattle and feed spread the disease to Europe and to scattered countries around the world. This was apparently an extended common source outbreak, and the source was evidently the contamination of meat-and bone meal fed to young calves. The initial hypothesis assumed that scrapie in infected sheep carcasses rendered into bone meal crossed a species barrier causing infection in calves and disease in cows age 4–5 years. Subsequent rendering of cattle carcasses fuelled the epidemic. An alternative hypothesis is that a sporadic case of BSE in a cow could have initiated the epidemic. In 1988, ruminant carcasses were banned in cattle feed in the UK. A 4–6 year incubation period was estimated by age of affected cows, and 4 years after the ban, cases peaked at over 36 000 and began decreasing about 40% per year thereafter[15]. Several cases appeared in exotic ruminants in zoos presumably from feeding of bone meal; and cases developed in house cats and zoo felines fed pet foods and meat. Interestingly, dogs fed similar pet food (bone meal) do not seem to be susceptible. The long experience that scrapie infected lamb or mutton had not caused CJD was reassuring that BSE would not be transmitted to people; but in 1990, transmissions to cats were an ominous forewarning of the human cases.

Transmissible mink encephalopathy:

Transmissible mink encephalopathy has occurred as confined outbreaks in mink ranches. First reported in Wisconsin, USA, most outbreaks have been traced to mink feed suppliers with the assumption that scrapie infected sheep were included in the feed[16]. The epidemics in some cases may have been exacerbated by the feeding of adults that had had their pelts removed to the young mink. Doubt has been cast on traditional views by the report of one rancher who had an outbreak despite his claimed exclusion of sheep from his feed; he prepared his own feed using only sick or dead horses and cows[17]. This single observation is widely cited to support the thesis that spontaneous BSE may occur.

HUMAN DISEASES

Kuru:

Kuru is a prion disease that was discovered in the early 1900s in the people of New Guinea[18]. Kuru was a progressive cerebellar ataxia leaving victims helpless within a few months; cognitive changes developed only in advanced stages of disease[19]. The disease manifests itself as a neurodegenerative disorder starting with unsteadiness, deterioration ten to one; children over 5 years of age were affected at an intermediate rate with of speech, and tremor. It then moves on to cause more severe tremors, shock-like muscle jerks, and uncontrolled bursts of laughter. In the final stage, all the symptoms become severe, and difficulty in swallowing and inability to feed oneself lead to starvation. No remission or survival was ever recorded. The adult ratio of women to men with the disease was about boys and girls being infected equally. The incubation period for Kuru was determined to be from 2 years to 23 years from exposure.

In 1959, Hadlow[20] pointed out the similarities in epidemiology, clinical signs, and pathological findings between kuru and scrapie. Because scrapie had been transmitted to dairy goats after an incubation period of many months, he suggested that brain tissue from patients with kuru be inoculated into non-human primates that were held for long-term observation. Acting on this suggestion, Gajdusek and colleagues [21] successfully transmitted a "kuru-like" syndrome to chimpanzees after incubation of 18–21 months. Disease was subsequently transmitted in limiting dilutions to replication, transmission was possible

by multiple routes of inoculation, and transmission to some other species was successful [22]. During this time the epidemic of kuru subsided; disease disappeared first in young children. The human disease had been transmitted by ritual endocannibalism, a bereavement ceremony in which dead relatives are eaten primarily by women and shared with the children. In sorrow they also rubbed tissue over their bodies allowing subcutaneous inoculation via ubiquitous tropical sores, or into conjunctiva or olfactory pathways.

Gerstmann-Sträussler-Scheinker Disease (GSS):

GSS Disease is an inherited neurodegenerative disorder caused by an accumulation of a mutated prion protein amyloid. It is inherited as an autosomal dominant disease, which means that both sexes are affected and there are no carriers of the mutant gene. GSS slowly progresses with symptoms beginning between the ages of 30 and 70. Patients experience lack of muscle coordination and have difficulty walking. As the disease progresses, symptoms include slurring of speech, involuntary movements of the eyes, rigid muscle tone and eventually dementia, which is less common than in Creutzfeldt-Jakob Disease (CJD). In some cases the disease progresses rapidly and consequently cannot be distinguished from CJD. In GSS, spongiform changes in the brain tissue may or may not occur. Patients with GSS can live from 2 to 10 years with treatment aimed at alleviating symptoms[23]. Currently there is no cure for this rare inherited disease. Current research is focused on the prion that causes the disease, attempting to characterize it, clarify the disease mechanism, and then developing ways to prevent, treat, and cure GSS disease.

Fatal Familial Insomnia (FFI):

FFI is a rare autosomal dominant hereditary disease caused by a prion that results in amyloid plaques that affect the thalamus, causing severe selective atrophy. The thalamus is a center in the brain that is responsible for regulation of sleep. As a result of the degradation of the thalamus, there is an interruption of the body's circadian rhythms. Consequently, patients with FFI lose sleep, can have hallucinations, and eventually go into coma, with death in about 18 months. The age of onset ranges from 30 to 60. The four stages of FFI are: Progressive insomnia, panic attacks, and bizarre phobias developing over a four month period characterize the first stage. The second stage lasts about five months with symptoms including hallucinations, panic, agitation, and sweating. The stage three, total insomnia is paired with weight loss and lasts about three months. The final stage, which lasts six months, includes dementia, total insomnia, loss of hearing and sudden death. New techniques such as DNA sequencing or molecular hybridization should be developed to make an early diagnosis, as the disease does not begin progression until after childbearing years[24]. Currently there is no cure for this disease, but gene therapy could be promising to prevent FFI. In this case, the correct gene could be inserted to cause the correct protein to be developed, consequently allowing for the thalamus to function normally, thus preventing insomnia and subsequent deterioration.

Alpers' Disease:

Unfortunately, Alpers' Disease affects infants and children. It is an autosomal recessive disorder that can be seen in siblings and is known also as Christensen's disease or Christensen-Krabbe disease. Alfons Jakob first recognized it in the early 1900s and his students, Souza, Freedom, and Alpers further described cases. It is manifested by convulsions, developmental delay, mental retardation, and dementia. Only thirteen cases have been identified since 1931, but others may have been missed due to chronic liver dysfunction being present, which may mask diagnosis of Alpers' Disease. Liver failure is usually the ultimate cause of death within the first two years of life. Final diagnosis is at autopsy when spongiform plaques are identified in the gray matter of the brain[25]. There is no current treatment for the disease, only for the symptoms, such as anti-convulsants for the seizures.

Creutzfeldt-Jakob disease (CJD):

CJD is also referred to as subacute spongiform encephalopathy due to the formation of microscopic vacuoles or holes in the neurons that appear "sponge-like." The disease is named for Drs. Hans Creutzfeldt and Alfons Jakob who documented the first cases in the 1920s. This disease affects both men and women in the 50 to 75 year age range, with one case per million per year. Cases in persons under 30 years of age are extremely rare, with fewer than 5 cases per billion. A person can acquire CJD in one of three ways. Firstly, the disease can appear sporadically, without any apparent cause. Secondly, it can be inherited as an autosomal dominant pattern. This type of transmission occurs in about 10-15 % of the cases. Thirdly, an infectious agent can transmit the disease. Iatrogenic transmission is an unintended consequence of a medical procedure using instruments tainted by contaminated human

growth hormone (about 100 cases), by corneal grafts from asymptomatic infected individuals, or by infected neural material. In 1976, more stringent sterilization procedures were put into place. Additionally, recombinant DNA technology is now used for producing human growth hormone. Because of these advances, no further documented cases of CJD have occurred from iatrogenic transmission. There are no known instances of transfusion-related CJD.

Symptoms begin with insomnia, depression, confusion and problems with memory, coordination, and sight. As the disease progresses, patients experience progressive dementia and involuntary jerking movements. In the final stages of the disease, patients lose all mental and physical functions, lapse into coma and die, usually from pneumonia due to the unconscious. CJD patients will succumb within one year of diagnosis. There are no known effective treatments for CJD, so treatment focuses on easing symptoms.

CJD is difficult to diagnose, so the first step is to rule out other diseases that might have similar symptoms. It may be mistaken for Alzheimer's disease, Pick's disease, Huntington's disease, cerebral hematomas and vascular irregularities. An EEG can detect a characteristic abnormal brain pattern associated with the later stages of the disease, but cannot confirm a CJD diagnosis. A new test to detect a specific protein (14-3-3) in cerebrospinal fluid (CSF) has been developed, but again this does not give a definitive diagnosis. CJD can definitively be diagnosed by performing a brain biopsy or autopsy. However, a brain biopsy can be a dangerous procedure, can result in a false-negative result if the wrong area of the brain is chosen, and is quite costly. In addition, there is a risk to healthcare workers if strict sterilization and infection control precautions are not taken. When available, disposable equipment should be used in suspected cases of CJD and then incinerated. If equipment is to be reused, steam sterilization or cleaning with 1 N Sodium hydroxide (followed by steam sterilization) can be utilized. If this cannot be accomplished, the equipment must be disposed of by incineration. Contaminated skin surfaces are to be washed with 1 N sodium hydroxide or 10% bleach followed by rinsing with copious amounts of water. Splashes to the eyes may be treated using copious amounts of water or saline. Contaminated dry waste or sharps waste should be autoclaved for 4.5 hours prior to incineration.

Sporadic CJD:

Sporadic CJD occurs throughout the world without overall geographic or seasonal clustering[26,27]. The disease affects men and women equally, average age at onset is 60 years, and it is rare in people under age 40 years or over age 80 years.[28,29].The initial symptoms are systemic complaints of fatigue, disordered sleep, and decreased appetite; about a third of patients present with behavioural or cognitive changes; and the final third have focal signs such as visual loss, cerebellar ataxia, aphasia, or motor deficits[30].15 The disease progresses rapidly with prominent cognitive decline and the development of myoclonus, particularly startlesensitive myoclonus. The median time to death from onset is only 5 months, and 90% of patients with sporadic CJD are dead within 1year[28]. Characteristic patterns on MRI, the synchronised biphasic or triphasic sharp-wave complexes on the electroencephalogram, and the finding of 14-3-3 protein in CSF all support the diagnosis of CJD[31,32] but none are 100% sensitive or specific. MRI and electroencephalogram changes are commonly found only with repeated examination, and 14-3-3, a normal neural protein released with rapid neuronal loss, is present in CSF after strokes or during encephalitis. The pathological findings in CJD are limited to the brain and spinal cord. There is neuronal loss, and vacuolization within cell bodies and dendrites that gives a spongiform appearance to the cortex and deep nuclei. The pathogenic isoform of prion protein can be demonstrated in brain by immunocytochemical staining and by western-blot analysis.

The mode of infection is unknown. Exposure to people with the illness, even the intimate exposure of years of marriage, does not seem to increase the risk, and there has been only one documented conjugal case pair[33]. Occupational exposures have not been incriminated: surgeons, pathologists, abattoir workers, butchers, ranchers, and cooks are not overrepresented[34]. Diet, including the eating of brains, has been suggested in some case-control studies[35,36], but these are subject to recall bias. Evidence of consumption of sheep with scrapie was sought for many years, but sporadic CJD is as common in countries such as Australia and New Zealand that are scrapie free as in the UK, France, and the USA where scrapie persists.

Lifelong vegetarians have developed sporadic CJD[37]. However, sporadic CJD can be transmitted among humans by medical procedures. If sporadic CJD follows exogenous infection, few clues to route of entry have been

found. Tonsilar and gastrointestinal tissues contain the abnormal prion protein, as they do in the variant form, for which ingestion is the proposed route of infection[38]. In one of sporadic CJD, staining for abnormal protein was found in neuroepithelium of the olfactory mucosa suggesting an olfactory pathway[39], but this was an autopsy study, and the presence of the protein could have represented centrifugal spread late in disease rather than an initial route of nervous-system invasion. Many people think that sporadic CJD results from endogenous generation of prions. Random misfolding of the prion protein might lead to a cascade of misfolding of normal prion protein into the pathogenic isoform. Others blame sporadic CJD on somatic mutation of the gene that encodes the prion protein, *PRNP*.

Familial diseases:

Familial CJD cases show autosomal dominant inheritance of mutations in *PRNP*. Over 50 different mutations in *PRNP* have been found in kindreds with familial CJD; but four point mutations—at codons 102, 178, 200, and 210—and insertions of five or six octapeptide repeats account for 95% of the familial cases[40]. In addition, a polymorphism at codon 129 leads to the protein containing either methionine or valine. This polymorphism influences susceptibility to or phenotype of CJD[41].26 For example, over 80% of patients with sporadic CJD are homozygous at this site compared with 49% of healthy controls[41,42].

In general, familial CJD has earlier age of onset and longer clinical course than sporadic CJD. The commonest familial form of CJD results from mutation at codon 200,and the phenotype in patients with this mutation resembles that of sporadic CJD[42].Several other mutations result in a phenotype sufficiently different from sporadic

CJD that distinct names have been used. Gerstmann- Sträussler-Scheinker disease is characterised by onset at age 20–40 years with progressive cerebellar ataxia and, in many patients, spastic paraparesis. In some families myoclonus is not prominent, and dementia develops late. In contrast to sporadic CJD the course may last 5–11 years. The pathological changes are also unique with amyloid plaques throughout the brain[43]. Most kindreds have a point mutation at codon 102, but the phenotype has been described with several other mutations. Fatal familial insomnia is the strangest phenotype of familial prion diseases. The clinical course is dominated by progressive insomnia, autonomic dysfunction and dementia. Polysomnography shows little sleep, loss of sleep spindles, and near absence of rapid-eye-movement sleep. The neuropathological changes are localized largely to neuronal loss in the thalamus—particularly the anterior ventral and mediodorsal nuclei, and the olivary nuclei of the brainstem—and there is little vacuolisation. In fatal familial insomnia, there is a mutation in *PRNP* at codon 178[44,45]. However, this mutation had been found in typical familial CJD[42,46]. The phenotype is determined by the polymorphism at codon 129; those homozygous for methionine had fatal familial insomnia and those homozygous for valine or heterozygous had typical CJD[46]. Presumably the polymorphism can change the tertiary structure of the pathogenic isoform of the prion protein.

Latrogenic CJD:

Transmission of CJD among people has occurred with corneal transplants, dural grafts, injections of hormones extracted from human pituitary glands, and contaminated neurosurgical instruments. The concern about surgical tools arose when CJD developed in two young patients 16 months and 20 months after they underwent surgery to excise epileptic foci. Silver electrodes, used for stereotactic electroencephalography during surgery, had previously been implanted to record abnormal movements in a patient with CJD. After each use, the electrodes had been "sterilised" in alcohol and formaldehyde vapour[47]. 2 years after the apparent transmission the electrodes were retrieved and implanted into a chimpanzee that subsequently developed spongiform encephalopathy. Sizeable outbreaks of iatrogenic CJD have occurred after distribution of contaminated dural graft material and human growth hormone. Since 1985, over 100 cases of CJD have occurred 16 months to 18 years after surgical use of human cadaveric dura mater[48]. In 1985, four patients under 40 years of age developed CJD[49], all of whom had previously received human growth hormone made from pooled human cadaveric pituitary glands. Over 8000 children and adolescents in the USA had received this preparation. The product was withdrawn in most countries, and a recombinant human growth hormone was quickly licensed. Since then, however, over 130 young adults have developed CJD 5–30 years after discontinuing injections[48]. The long incubation period after growth hormone injections presumably reflects the peripheral route of inoculation in contrast to intracerebral placement of contaminated dura mater. In both situations, homozygosity at codon 129 seems to increase susceptibility to iatrogenic disease[48].

Variant CJD:

In 1996, a disturbing fact emerged that showed a causal relationship between BSE and a new disease called variant Creutzfeldt - Jakob disease (now referred to simply as vCJD). Young adults were dying after exhibiting clinical symptoms of CJD, including dementia and muscle jerks. Cases were predominately coming from Britain, but several cases were documented from patients outside of Britain. These patients were found to have lived in the British Isles for at least five years during the epidemic (1980-1995). As of June, 2007 there have been 161 deaths in Britain from definite or probable vCJD with four probable cases still alive. Thirty-nine other cases have occurred outside Britain, primarily in France and other European countries that imported meat from Britain. Of the three documented cases in the U.S, two had lived in Britain and one had lived in Saudi Arabia. Patients have a course and pathology distinctive from sporadic CJD young age at onset, prominence of psychiatric and sensory symptoms, and long disease course. Neuropathological examination shows widespread vacuolisation with many plaques of abnormal prion protein. All cases tested have been homozygous at codon 129 for methionine[49,50]. The incubation period for vCJD is still unknown. Documented patient incubation times have been six or more years.

The prions from the variant disease seem to be of common origin: they have similar localisation and incubation periods in strains of inbred mice and similar western blot patterns. Prions from patients with variant CJD share these signatures with those from cattle with BSE, indicating that they have a common source[51,52].Cattle were likely infected by feed and people were probably infected by the consumption of beef. Although this route is probably true, human beings, like cattle, are exposed directly to products of the rendering industry[53]. The derived tallow is used in cosmetics that could lead to conjunctival or mucous membrane exposure, in soap that can come into contact with skin abrasions, and in gelatin that can lead to oral exposure. Furthermore, bone meal produced by rendering is a component of gardening products, such as dusting powder for roses, which could lead to olfactory exposure.

The current risk of acquiring vCJD from eating beef cannot be determined for travelers to Britain. However, the risk decreases by avoiding beef or beef products or selecting beef or beef products that are solid muscle pieces (versus calf brains or burgers or sausages). Public health preventive measures have been put into place including enhancement of BSE surveillance, culling of sick animals, and using the "over thirty months scheme." This excludes animals over 30 months of age from both the human and animal food chain. In 2002, there was a case of person-to-person, blood-borne transmission of vCJD. This occurred in a 69 year-old man who had received, six years previously, several units of blood. One of those units came from a 24 year-old donor who developed vCJD three years after donation. Taking into consideration all other factors, the conclusion was made that the recipient indeed did contract vCJD from this donor. Due to the fact that vCJD can be easily detected in lymphoid tissues and the existence of a possible blood phase had led researchers to believe that -borne transmission of vCJD was possible.

TRANSMISSION

Spread of the disease is via horizontal transmission, i.e., transmission from one person to another, either directly or by fomites or by ingestion of contaminated meat. The disease may also be spread by surgical instruments: infectious prions are resistant to high temperatures, irradiation and common chemical treatments that destroy other known pathogens. Furthermore, prions have also been found in the epididymis and seminal plasma tissues of rams[54] . Such findings led to concern about prion infection via sperm donations and even resulted in a US ban on sperm donations by Europeans and men who have lived in Europe. Nonetheless, a survey of experts worldwide estimated that the chance of prion transmission via sperm was less than 1:10 000 000. But tissue contact – whether via sperm or blood or on surgical instruments – is not the only potential source of transmission: most of us consume milk and dairy products. In 2006, a team of scientists from Switzerland detected low levels of the normal form of prions in milk bought in European shops . Another study found that aberrant prions were able to replicate within mammary glands of scrapieinfected sheep[55]. Taken together, these results suggest that aberrant prions might be present in the milk of animals suffering from prion diseases. As prion disease symptoms take several years to develop, aberrant prions may be present in milk that is sold before the animals are diagnosed with the illness. Nonetheless, although it is not yet possible to give a clear estimate of the risk, it is generally thought that milk is safe until proven otherwise.

DIAGNOSIS

In the past, diagnosis of prion disease was made through examination of brain biopsies taken from patients in advanced stages of the disease or, more commonly, after they had died. In January of 1999 it was found that the prion protein accumulated in the tonsils and could be detected by an immunofluorescence test on tonsilar biopsies. A second test was simultaneously developed which was based on a Western blot. Later that year a third test was developed that had the high sensitivity necessary to detect the prion protein in blood. This test is based on capillary electrophoresis with laser-induced fluorescence. It detects as little as 10^{-18} mole.

TREATMENT

PrPc overexpression facilitates the development of prion diseases. It may therefore follow that agents which reduce PrPc expression will delay the onset of prion diseases. One can speculate that chemicals which bind to and stabilize the PrPc conformation may be beneficial. Similarly agents destabilizing the PrPsc conformation may be effective. In this regard several vaccines to Alzheimers amyloid plaques are in clinical trials. 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 PrPc increases, the chances of rationally deducing effective therapeutics based on these ideas increases.
- Finally we have seen that PrPc expression is required for pathology. Chemicals affecting the endocytosis, exocytosis, intracellular trafficking and degradation of proteins and in particular PrPc may also be effective. Amphotericin for instance is reported to delay prion disease in hamsters (although it apparently has little effect in humans).

CONCLUSION

Surveillance, along with advances in detection, and prevention are needed to eliminate these prion caused diseases. Research into how prions are formed and transmitted may be the key to unlocking the mystery. There is no current treatment for prion diseases. Once there is more information about how prions work, treatment modalities may be discovered.

REFERENCE

1. Zahn R, Liu A, Luhrs T, Riek R, Von Schroetter C, Lopez Garcia F et al. NMR solution structure of the human prion protein. Proc.Natl.Acad.Sci.USA 2000; 97: 145-150.
2. McKintosh E , Tabrizi SJ, Collinge J, Prion diseases, Journal of NeuroVirology 2003; 9:183-193.
3. Priola SA, Chesebro B, Caughey B. Biomedicine. A view from the top--prion diseases from 10,000 feet. Science 2003; 300 (5621): 917–9.
4. Cohen FE, Pan KM, Huang Z, Baldwin M, Fletterick RJ, Prusiner SB. Structural clues to prion replication. Science 1994; 265 (5178): 530–531.
5. Ligios C. PrPSc in mammary glands of sheep affected by scrapie and mastitis. Nature Medicine 2005; 11: 1137–1138.
6. Prusiner SB. Novel proteinaceous infectious particles cause scrapie. Science 1982; 216: 136–44.
7. Hadlow WJ, Kennedy RC, Race RE. Natural infection of Suffolk sheep with scrapie virus. J Infect Dis 1982; 146: 657–64.
8. Hourrigan JL, Klingsporn AL. Scrapie: studies on vertical and horizontal transmission. In: Gibbs CJ Jr, ed. Bovine spongiform encephalopathy: the BSE dilemma. New York: Springer; 1996. p. 59–83.
9. Williams ES, Young S. Chronic wasting disease of captive mule deer: a spongiform encephalopathy. J Wildlife Dis 1980; 16: 89–98.
10. Bartz JC, Marsh RF, McKenzie DI, Aiken JM. The host range of chronic wasting disease is altered on passage in ferrets. Virology 1998; 251: 297–301.
11. Hamir AN, Kunkle RA, Culip RC, et al. Experimental transmission of chronic wasting disease agent from mule deer to cattle by the intracerebral route. J Vet Diagn Invest 2005; 17**:** 276–81.
12. Miller MW, Williams ES. Chronic wasting disease of servide. Curr Top Microbiol Immunol 2004; 284: 193–214.
13. Miller MW, Williams ES, Hobbs NT, Wolfe LL. Environmental sources of prion transmission in mule deer. Emerg Infect Dis 2004; 10: 1003–06.
14. Belay ED, Maddox RA, Williams ES, et al. Chronic wasting disease and potential transmission to humans. Emerg Infect Dis 2004; 10: 977–84.

15. Collee JG, Bradley R. BSE: a decade on—part I. Lancet 1997; 349: 636–41.
16. Hartsough GR, Burger D. Encephalopathy of mink: epizootiologic and clinical observations. J Infect Dis 1965; 115: 387–92.
17. Marsh RF, Bessen RA, Lehmann S, Hartsough GR. Epidemiological and experimental studies on a new incident of transmissible mink encephalopathy. J Gen Virol 1991; 72: 589–94.
18. Gajdusek DC, Zigas V. Degenerative disease of the nervous system in New Guinea: the endemic occurrence of "kuru" in the native population. N Engl J Med 1957; 257: 974–78.
19. Hornabrook RW. Kuru—a subacute cerebellar degeneration: the natural history and clinical features. Brain 1968; 91: 53–74.
20. Hadlow WJ. Scrapie and kuru. Lancet 1959; 2: 289–90.
21. Gajdusek DC, Gibbs CJ Jr, Alpers M. Experimental transmission of a kuru-like syndrome to chimpanzees. Nature 1966; 209: 794–96.
22. Gajdusek DC. Infectious amyloids: subacute spongiform encephalopathies as transmissible cerebral amyloidoses. In: Field BN, Knipe PM, Howley PM, et al, eds. Field's Virology, 3rd ed. Philadelphia: Lippincott-Raven; 1996. P. 851–900.
23. National Institute of Neurological Disorders and Stroke, Gerstmann-Starussler-Scheinker Disease Information Page, February 14, 2007. Available at: http://www.ninds.nih.gov/disorders/gss/gss.htm. Accessed January 7, 2008
24. Akroush, Ann M., Fatal Familial Insomnia. 1996-1997. Available at: http://wwwpersonal.umd.umich.edu/~jcthomas/JCTHOMAS/1997%20Case%20Studies/AAkroush.html. Accessed January 7, 2008.
25. National Institute of Neurological Disorders and Stroke, Alpers' Disease Information Page, February 14, 2007. Available at: http://www.ninds.nih.gov/disorders/alpersdisease/alpersdisease.htm. Accessed January 7, 2008.
26. Ladogana A, Puopolo M, Croes EA, et al. Mortality from Creutzfeldt-Jakob disease and related disorders in Europe, Australia, and Canada. Neurology 2005; 64: 1586–91.
27. Linsell L, Cousens SN, Smith PG, et al. A case-control study of sporadic Creutzfeldt-Jakob disease in the United Kingdom: analysis of clustering. Neurology 2004; 63: 2077–83.
28. Brown P, Gibbs CJ, Rodgers-Johnson P, et al. Human spongiform encephalopathy: the National Institutes of Health Series of 300 cases of experimentally transmitted disease. Ann Neurol 1994; 35: 513–29.
29. Will RG, Mathews WB. A retrospective study of Creutzfeldt-Jakob disease in England and Wales 1970–79, I: clinical features. J Neurol Neurosurg Psychiatry 1984; 47: 134–40.
30. Bernoulli CC, Masters CL, Gajdusek DC, Gibbs CJ Jr, Harris JO. Early clinical features of Creutzfeldt-Jakob disease (subacute spongiform encephalopathy) In: Prusiner SB, Hadlow WJ, eds. Slow transmissible diseases of the nervous system, vol 1: clinical, epidemiological, genetic and pathological aspects of the spongiform encephalopathies. New York: Academic Press; 1979. p. 229–41.
31. Tschampa HJ, Kallenberg K, Urbach H, et al. MRI in the diagnosis of sporadic Creutzfeldt-Jakob disease: a study on inter-observer agreement. Brain 2005; 4: 641-45.
32. Steinhoff BJ, Zerr I, Glatting M, Schulz-Schaeffer W, Poser S, Kretzschmar HA. Diagnostic value of periodic complexes in Creutzfeldt-Jakob disease. Ann Neurol 2004; 56: 702–08
33. Brown P, Cervenakova L, McShane L, et al. Creutzfeldt-Jakob disease in a husband and wife. Neurology 1998; 50: 684–88.
34. Harries-Jones R, Knight R, Will RG, Cousens S, Smith PG, Matthews WB. Creutzfeldt-Jakob disease in England and Wales, 1980–1984: a case-control study of potential risk factors. J Neurol Neurosurg Psychiatry 1988; 51: 1113–19.
35. Bobowick AR, Brody JA, Mathews MR, Roos R, Gajdusek DC. Creutzfeldt-Jakob disease: a case control study. Am J Epidemiol 1973; 98: 381–94.
36. Davanipour Z, Alter M, Sobel E, Asher DM, Gajdusek DC. A case-control study of Creutzfeldt-Jakob disease: dietary risk factors. Am J Epidemiol 1985; 122: 443–51

37. Matthews WB, Will RG. Creutzfeldt-Jakob disease in a lifelong vegetarian. Lancet 1981; 2: 8252–937.
38. Hill AF, Butterworth RJ, Joiner S, et al. Investigation of variant Creutzfeldt-Jakob disease and other human prion diseases with tonsil biopsy samples. Lancet 1999; 353: 183–89.
39. Zanusso G, Ferrari S, Cardone F, et al. Detection of pathologic prion protein in the olfactory epithelium in sporadic Creutzfeldt-Jakob disease. N Engl J Med 2003; 348: 711–19.
40. Capellari S, Cardone F, Notari S, et al. Creutzfeldt-Jakob disease associated with the R208H mutation in the prion protein gene. Neurology 2005; 64: 905–07
41. Palmer MS, Dryden AJ, Hughes JT, Collinge J. Homozygous prion protein genotype predisposes to sporadic Creutzfeldt-Jakob disease. Nature 1991; 352: 340–42.
42. Mastrianni JA. Prion diseases and dementia. In: Power C, Johnson RT, eds. Emerging neurological infections. Boca Raton: Taylor and Francis; 2005. p. 77–113.
43. Masters CL, Gajdusek DC, Gibbs CJ. Creutzfeldt-Jakob disease virus isolations from the Gerstmann-Sträussler syndrome with an analysis of the various forms of amyloid plaque deposition in the virus-induced spongiform encephalopathies. Brain 1981; 104: 559–88.
44. Medori R, Tritschler HJ, LeBlanc A, et al. Fatal familial insomnia: a prion disease with a mutation at codon 178 of the prion protein gene. N Engl J Med 1992; 326: 444–49.
45. Montagna P, Gambetti P, Cortelli P, Lugaresi E. Familial and sporadic fatal insomnia. Lancet Neurol 2003; 2: 167–76.
46. Goldfarb LG, Petersen RB, Tabaton M, et al. Fatal familial insomnia and familial Creutzfeldt-Jakob disease: disease phenotype determined by a DNA polymorphism. Science 1992; 258: 806–08.
47. Bernoulli C, Siegfried J, Baumgartner G, et al. Danger of accidental person-to-person transmission of Creutzfeldt-Jakob disease by surgery. Lancet 1977; 1: 478–79.
48. Brown P, Preece M, Brandel JP, et al. Iatrogenic Creutzfeldt-Jakob disease at the millennium. Neurology 2000; 55: 1075–81.
49. Brown P, Gajdusek DC, Gibbs CJ , Asher DM. Potential epidemic of Creutzfeldt-Jakob disease from human growth hormone therapy. N Engl J Med 1985; 313: 728–31.
50. Peden AH, Ironside JW. Review: pathology of variant Creutzfeldt- Jakob disease. Folio Neuropathol 2004; 42 (suppl A): 85–91.
51. Bruce ME, Will RG, Ironside JW, et al. Transmissions to mice indicate that 'new variant' CJD is caused by the BSE agent. Nature 1997; 389: 498–01.
52. Hill AF, Desbruslais M, Joiner S, et al. The same prion strain causes vCJD and BSE. Nature 1997; 389: 448–50.
53. Burnham FA. Rendering: the invisible industry. Fallbrook, CA: Aero, 1978.
54. Gatti JL et al. Prion protein is secreted in soluble forms in the epididymal fluid and proteolytically processed and transported in seminal plasma. Biology of Reproduction 2002; 67: 393-400.
55. Ligios C et al. PrPSc in mammary glands of sheepaffected by scrapie and mastitis. Nature Medicine 2005; 11: 1137–1138.