

Review

Prions and neuro degenerative diseases

Rahul R. Nair* and Jerin K. Johnson

II MTech Biotechnology, School of Biotechnology, Karunya University, Karunya Nagar, Coimbatore, Tamil NADU-641114, India.

Accepted 2 June, 2009

Prion is a disease-causing agent that is neither bacterial nor fungal nor viral and contains no genetic material. A prion is a protein that occurs normally in a harmless form. By folding into an aberrant shape, the normal prion turns into a rogue agent. It then co-opts other normal prions to become rogue prions. Prions have been held responsible for a number of degenerative brain diseases, including scrapie (a fatal disease of sheep and goats), mad cow disease, Creutzfeldt-Jacob disease, fatal familial insomnia, kuru, an unusual form of hereditary dementia known as Gertsman-Straussler-Scheinker disease, chronic wasting disease in deer and elk, bovine spongiform encephalopathy (BSE, commonly known as "mad-cow" disease), exotic ungulate spongiform encephalopathy, Transmissible Mink Encephalopathy (TME), and feline spongiform encephalopathy, albino tigers, pumas, and cheetahs. Currently, definite diagnosis of prion diseases is still considered to be possible only after histopathological examination of biopsied or autopsied brain material. Prion diseases do not stimulate immunity. Therefore, a vaccine that prevents them is unlikely. No treatment is available either, although research in this area is progressing.

Key words: Prion, transmissible spongiform encephalopathies, diagnosis, treatment.

INTRODUCTION

A prion has been defined as "small proteinaceous infectious particles which resist inactivation by procedures that modify nucleic acids" (Anonymous, 1996). Abnormal forms of the prion protein (a ubiquitous protein of unknown function) cause these neurodegenerative diseases. The disease occurs when the normal cellular prion protein undergoes a conformational change to the abnormal form. This may occur spontaneously at an extremely low rate or at a higher rate if there is a defect in the gene. The agent can "replicate" when the abnormal form crosses the path of the normal, cellular prion protein and the abnormal prion induces the normal form to adopt a similar abnormal form. Prions accumulate in the brain as an insoluble complex of proteins called an amyloid (Prusiner, 1995). The interaction of prions with the normal cellular prion proteins damages the cell and leads to its slow degeneration and death. This releases prions, which can then induce more prions on the surface of surrounding cells, causing more degeneration and death of cells. This process of spreading cell death accounts for the

holes in the brain. The current idea is that other tissues are not much affected because prion protein is mainly produced in nerve cells. Lymphoid cells also have a lot of prion protein and they are important in spreading the infection to nerve cells. However, lymphoid cells are readily replaced, whereas a process that destroys neurones, even a slowly progressive one, will lead to disease, since nerve cells cannot normally be replaced (Prusiner, 1996).

Prion diseases, also known as transmissible spongiform encephalopathies (TSEs), are a group of animal and human brain diseases that are uniformly fatal and often characterized by a long incubation period and a multifocal neuropathologic picture of neuronal loss, spongiform changes, and astrogliosis (Prusiner, 1996). TSEs in humans include Creutzfeldt-Jacob disease (CJD), kuru, Gerstmann-Straussler-Scheinker syndrome (GSS), fatal familial insomnia (FFI), and new variant CJD (nvCJD). TSEs described in animals include scrapie in sheep and goats, chronic wasting disease in deer and elk, bovine spongiform encephalopathy (BSE, commonly known as "mad-cow" disease), exotic ungulate spongiform encephalopathy, and feline spongiform encephalopathy, albino tigers, pumas, and cheetahs. The reported ungulate and feline spongiform encephalopathies appear to repre-

*Corresponding author. E-mail: rahulravi777@yahoo.co.in.

sent transmission of the BSE agent to these animals (Prusiner, 1997).

The prion theory

A current definition of a prion is a proteinaceous infectious particle that lacks nucleic acid. Prion protein is capable of having more than one tertiary structure. In one of these structures the protein carries out its normal cellular function. Other tertiary structures can be pathological causing spongiform degeneration. The prion theory states that the normal PrP^c can be converted into PrP^{sc}. Portions of alpha-helical and coil structure in PrP^c is converted in to beta-sheet in PrP^{sc}. The theory argues that PrP^{sc} can act as a template on which PrP^c is folded into a PrP^{sc} molecule (Prusiner et al., 1993). In contrast to pathogens with nucleic acid, prion enciphers strain specific properties in their tertiary structure. The PrP protein is a cell surface glycoprotein expressed at highest levels in neurons (Gajdusek, 1985). The function of this normal protein is not known although knockout mice without the gene show a range of subtle abnormalities that are not consistent between mice. The prion theory explains the apparent paradox of TSE diseases being both genetic and transmissible. More than 20 mutations of the PrP gene have been genetically linked to the development of familial prion disease (Heaphy, 1998). The mutation in the gene causes the resulting protein to be more likely to form the pathogenic prion protein. This protein converts other proteins causing the prion disease. Likewise if pathogenic prion protein is introduced to the brain of a normal person this may cause the same reaction. Therefore prion diseases are also transmissible.

Structure of prion

The prion protein found in infectious material has a different folding pattern and is resistant to proteases. The normal form of the protein is called PrP^c, while the infectious form is called PrP^{sc}-the C refers to 'cellular' or 'common' PrP, while the Sc refers to 'scrapie', a prion disease occurring in sheep. Structural organization of PrP^c is well-defined, but the same for PrP^{sc} defined at a relatively poor level. PrP can be induced to fold into other more-or-less well-defined isoforms *in vitro*. PrP^c is a normal protein (35-36KDa) found on the membranes of cells. It has 209 amino acids (in humans), one disulfide bond, and a mainly alpha-helical structure. Several topological forms exist; one cell surface form anchored via glycolipid and two trans membrane forms. Its function has not been fully resolved. PrP^c binds copper (II) ions with high affinity (Prelli et al., 2000). The significance of this is not clear, but it presumably relates to PrP structure or function. PrP^c is readily digested by proteinase K and can be liberated from the cell surface *in vitro* by the enzyme phos-

phoinositide phospholipase C (PI-PLC), which cleaves the glycoposphatidylinositol (GPI) glycolipid anchor (Prusiner, 1997). The infectious isoform of PrP^c, known as PrP^{sc}, is able to convert normal PrP^c proteins into the infectious isoform by changing their conformation. Although the exact structure of PrP^{sc} is not known, there is increased β -sheet content in the diseased form of the molecule, replacing normal areas of α -helix. Aggregations of these abnormal isoforms may form highly structured amyloid fibers. The end of a fiber acts as a template for the free protein molecules, causing the fiber to grow (Heaphy, 1998).

Small differences in the amino acid sequence of prion-forming regions lead to distinct structural features on the surface of prion fibers. As a result, only free protein molecules that are identical in amino acid sequence to the prion protein can be recruited into the growing fiber.

PRION DISEASES IN ANIMALS

Scrapie in sheep and goats

Scrapie is a neuro degenerative disease that affects the nervous systems of sheep and goats. It is one of several transmissible spongiform encephalopathies (TSEs). Scrapie is caused by a prion. The name scrapie is derived from one of the symptoms of the condition, wherein affected animals will compulsively scrape off their fleece against rocks, trees or fences (Palsson, 1979). Scrapie is infectious and transmissible among similar animals, and so one of the most common ways to contain scrapie (since it is incurable) is to quarantine and destroy those affected. Recent studies suggest that prions may be spread through urine and persist in the environment for decades (Parry, 1983). Scrapie occurs in Europe and North America, but to date Australia and New Zealand (both major sheep-producing countries) are scrapie-free. A test is now available which is performed by sampling a small amount of lymphatic tissue from the third eyelid (Cullie and Chelle, 1939). Out of fear of BSE, many European countries banned some traditional sheep or goat products made without removing the spinal cord such as smalahove and smokie. The agent responsible for scrapie and other TSEs is smaller than the smallest known virus and has not been completely characterized. There are three main theories on the nature of the scrapie agent: (1) the agent is a prion, which is an abnormal form of a normal cellular protein, (2) the agent is a virus with unusual characteristics, and (3) the agent is a virino, a very small piece of DNA that acts like a virus. The scrapie agent is extremely resistant to heat and to normal sterilization processes (Ondera et al., 1993). It does not evoke any detectable immune response or inflammatory reaction in sheep and goats. The scrapie agent is thought to be spread most commonly from the ewe to her offspring and to other lambs through

contact with the placenta and placental fluids. Signs or effects of the disease usually appear 2-5 years after the animal is infected but may not appear until much later. Sheep may live 1 to 6 months or longer after the onset of clinical signs, but death is inevitable. There is no scientific evidence to indicate that scrapie poses a risk to human health. There is no epidemiologic evidence that scrapie of sheep and goats are transmitted to humans, such as through contact on the farm, at slaughter plants, or butcher shops. Due to damage to nerve cells, affected animals usually show behavioral changes, tremor (especially of head and neck), rubbing, and locomotor in coordination that progress to recumbency and death. Early signs include subtle changes in behavior or temperament. These changes may be followed by scratching and rubbing against fixed objects, apparently to relieve itching. Other signs are loss of coordination, weakness, weight loss despite retention of appetite, biting of feet and limbs, lip smacking, and gait abnormalities, including high-stepping of the forelegs, hopping like a rabbit, and swaying of the back end (Parry, 1962). An infected animal may appear normal if left undisturbed at rest. However, when stimulated by a sudden noise, excessive movement, or the stress of handling, the animal may tremble or fall down in a convulsive-like state. Several other problems can cause clinical signs similar to scrapie in sheep, including the diseases ovine progressive pneumonia, listeriosis, and rabies; the presence of external parasites (lice and mites); pregnancy toxemia; and toxins. On the farm, veterinarians diagnose scrapie based on the appearance of its signs combined with knowledge of the animal's history. Scrapie can be diagnosed in the live animal by biopsy of the lymphoid tissues on the inside of the third eyelid. This test is used by the U.S. Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS) to determine whether exposed flocks are infected. Scrapie is most often diagnosed by microscopic examinations of brain tissue at necropsy or by procedures that detect the presence of the abnormal prion protein in brain tissue.

Bovine spongiform encephalopathy (BSE)

BSE is a transmissible, neuro-degenerative fatal brain disease of cattle. BSE is named because of the spongy appearance of the brain tissue of infected cattle examined under a microscope. The disease has a long incubation period of 4 - 5 years and it is fatal for cattle within weeks to months of its onset. Strong evidence currently available supports the theory that the agent is composed largely, if not entirely, of a self-replicating protein, referred to as a prion (Williams et al., 2001). It is transmitted through the consumption of BSE-contaminated meat and bone meal supplements in cattle feed. Current scientific research indicates that cooking will not kill the BSE agent. It is believed by most scientists that the disease

may be transmitted to human beings who eat the brain or spinal cord of infected carcasses (Wijeratne and Curnow, 1990).

In humans, it is known as new variant Creutzfeldt-Jakob disease (vCJD or nvCJD) (Matthew, 1994). Different hypotheses exist for the origin of prion proteins in cattle.

Two leading hypotheses suggest that it may have jumped species from the disease scrapie in sheep, or that it evolved from a spontaneous form of "mad cow disease" that has been seen occasionally in cattle for many centuries (Wells and Scott, 1987). There is no scientific evidence to suggest that milk and dairy products carry the agent that causes BSE (Taylor, 1989). Currently, there is no test to detect the disease in a live animal or in muscle meat. Veterinary pathologists confirm BSE by postmortem microscopic examination of brain tissue using sophisticated laboratory techniques, such as a histopathological examination to detect sponge-like changes in the brain tissue and immunohistochemistry to examine the BSE fibrils (Hussein, 1998). These are "gold-standard" tests, and they take more than a week to run. More rapid tests that provide results within 36 to 48 hours have been developed to detect the abnormal prion in brain or spinal cord tissue of dead animals (Abiola et al., 2002). Rapid tests can be used to determine if BSE exists in a population and to obtain an indication of its prevalence or detect animals with the disease which are not yet showing clinical signs (Hoinville, 1994). Cattle affected by BSE experience progressive degeneration of the nervous system. Affected animals might display changes in temperament, such as nervousness or aggression, abnormal posture, in coordination and difficulty in rising, decreased milk production, or loss of body weight despite continued appetite (Wilesmith et al., 1992).

Chronic wasting disease (CWD) in elk and mule deer

Chronic wasting disease (CWD) is a progressive, fatal disease of the nervous system of cervids such as mule deer, white-tailed deer and elk. Although the exact cause of CWD is unknown, it is associated with the presence of an abnormal protein called a prion. There is no treatment or vaccine currently available for the disease.

The most likely means of transmission is between animals that are in close contact with each other. In addition, the elk and mule deer placed in paddocks that had housed infected cervids for many years became infected, even though there were no other cervids on the premises, leading to the assumption that the environment of a facility could transmit the disease on premises with multiple confirmed cases of CWD (Williams et al., 2001). There is currently no scientific evidence that CWD affects humans, but we must exercise caution since there is evidence to suggest that BSE can affect humans, which is another TSE also known as mad cow disease. In

studies using mice experimentally infected with scrapie, another TSE, muscle and skin tissues were not found to be infectious at any detectable level (Williams and Miller, 2002). However, we have to be very cautious in using these results to predict the safety of products from infected or exposed elk, since test results from one species do not necessarily apply to another (Williams and Young, 1980). Velvet and other products or by-products from elk or deer known to be infected with CWD are not allowed to enter the human or animal food chain. Velvet is used as a medicinal alternative. Most cases of CWD occur in adult animals (Williams and Young, 1982). The disease is progressive and always fatal (Prusiner, 1996). The most obvious and consistent clinical sign of CWD is weight loss over time (Williams and Young, 1992). Behavioral changes also occur in the majority of cases, including decreased interactions with other animals, listlessness, lowering of the head, blank facial expression, and repetitive walking in set patterns. In elk, behavioral changes may also include hyper excitability and nervousness (Williams and Young, 1993). Affected animals continue to eat grain but may show decreased interest in hay. Excessive salivation and grinding of the teeth also are observed. Most deer show increased drinking and urination (Heaphy, 1998).

Feline spongiform encephalopathy

Feline spongiform encephalopathy (FSE) is a transmissible spongiform encephalopathy associated with the consumption of feedstuffs contaminated with tissue from bovine spongiform encephalopathy-affected cattle and characterized by the accumulation in the central nervous system of an abnormal isoform of the prion protein PrP^{Sc} (Hussein, 1998). Clinically, it presents as a progressive fatal neurologic syndrome that is not easily distinguished from other feline neurologic conditions. Most cases of FSE have been reported in England, where it was first detected in 1990, but a few cases have been reported from other European countries (Anonymous, 1990). There is no breed predisposition. The disease appears in adult cats with most appearing at about 5 years of age. Unfortunately a specific diagnosis can only be confirmed at post-mortem. Suspicious cases based upon clinical signs should always be submitted for examination. Typical pathological changes include bilaterally symmetrical vacuolation of the neuropil and vacuolation in neurons (Fraser, 1991). The signs of FSE progress over a long period (months to years), depend upon the site of brain involvement, and include behavioral changes, abnormal gait and death (Prusiner, 1996).

Exotic ungulate encephalopathy (EUE)

Exotic ungulate encephalopathy is a transmissible spon-

giform encephalopathy associated with contaminated feed that caused the disease in cows. The etiological agent is an isoform of prion protein. However, some cases appeared in animals born after total ban on the use of animal offal in animal feeds (Prusiner, 1995). In general, the clinical signs comprised in coordination of movement, tremors, excessive salivation, loss of weight, licking of lips, tilting of head to one side, sleepiness, vague looks and sometimes aggressive behavior (Heaphy, 1998). Typical spongiform changes were found in the brains of the animals. Sometimes the diseases in wild cats and exotic ungulates are grouped under the name "Zoological Spongiform Encephalopathies" (Hussein, 1998).

Spongiform encephalopathy of the ostrich

This disease affects the red-necked ostrich (*Struthio camelus*). The condition was found in an adult female ostrich in a German zoological garden. The affected ostrich showed symptoms of central nervous involvement and locomotion disorders (Manuelidis and Rorke, 1989).

Histological examination of the brain showed spongiform encephalopathy involving the brain stem and medulla oblongata (Hussein, 1998). A male ostrich also died of similar symptoms, but no examination of the brain was made. It is also not known if the condition was caused by a transmissible agent or not. Behavioral changes also occur in the majority of cases, including decreased interactions with other animals, listlessness, lowering of the head, blank facial expression, and repetitive walking in set patterns (Prusiner et al., 1996). In elk, behavioral changes may also include hyper excitability and nervousness. Excessive salivation and grinding of the teeth also are observed. Most deer show increased drinking and urination.

Transmissible mink encephalopathy (TME)

This disease is usually seen in minks. TME is limited to minks, found in commercial ranches and has not been found in the wild. TME is associated with eating contaminated feed, such as meat of scrapie-infected sheep (Marsh and Hadlow, 1992). The disease has been produced experimentally in minks by intracerebral injection of brain material from scrapie-infected sheep and by feeding tissues from infected sheep (Kimberlin et al., 1986). TME affects both male and female minks. The average incubation period is more than 7 months and the most important signs of the disease are behavioral changes (Marsh, 1979). Clinical signs include soiling their cages, step on food and experience difficulty in eating, aggressive and hyper-excitable by noise. They show in coordination of movement, circling, tail arching, tail chewing, stumbling and clenching of the jaw. Finally, the

affected minks isolate themselves and become sleepy, inactive and unresponsive. Usually, they stop eating and may die within just one week after the appearance of symptoms, but sometimes the disease lasts for about a month before the animal dies (Anonymous, 2002).

PRION DISEASES IN MAN

Creutzfeldt-Jakob disease (CJD) and its varieties

CJD is the most common form of TSEs in humans and occurs worldwide, with an estimated incidence of one case/1 million populations per year (Prusner et al., 1996). CJD is reported in almost equal ratios between the sexes, although older males (60 years of age) appear to have a higher incidence of disease. Analysis of the multiple cause-of-death data compiled by the National Center for Health Statistics, Centers for Disease Control and Prevention (CDC), from 1979 through 1994, showed an average annual CJD death rate of 0.95 deaths/1 million populations and a median age at the time of death of 68 years in the United States (Almond et al., 1997). The age-adjusted CJD death rate for whites was significantly higher than that for blacks.

CJD has been recognized to occur sporadically, or through iatrogenic transmission, or as a familial form. Affected patients usually present with a rapidly progressive dementia, visual abnormalities, or cerebellar dysfunction, including muscle incoordination and gait and speech abnormalities. During the course of the disease, most patients develop pyramidal and extra pyramidal dysfunction with abnormal reflexes, spasticity, tremors, and rigidity; some patients may also show behavioral changes with agitation, depression, or confusion. Myoclonus, the most constant physical sign, is present in nearly 90% of CJD patients. CJD is invariably fatal, with a median illness. The sporadic form of CJD accounts for >85% of all CJD cases (Brown, 1995). Although the exact cause is unknown, two hypotheses have been suggested to explain the occurrence of sporadic CJD. The first one is the possibility of an age-related somatic mutation of the prion protein gene that might result in the formation of PrP-res. Such mutations can randomly occur in the population at a rate of nearly one per million, which roughly corresponds to the incidence of sporadic CJD. The second suggested explanation is the spontaneous con-version of PrP-C into PrP-res in a single neuron or a group of neurons, possibly after a chance error during prion protein gene expression (Gore, 1995). The PrP-res is then believed to initiate a chain reaction resulting in the spread of disease to other susceptible.

Although the annual incidence of the sporadic form of CJD is 1 per million or less, there are certain, isolated groups of people in which the incidence is much higher, averaging 30-40 per million. This is due to the fact that about 10-15% of CJD cases have a genetic basis (inhe-

rited form) (Kahana et al., 1974). About 1% of CJD cases are due to iatrogenic infection. Several cases were recorded following treatment with pituitary hormones extracted from human cadavers. The familial form of CJD accounts for 5-15% of CJD patients (Hill et al., 1997). Because of its autosomal dominant inheritance pattern, there is commonly a family history of CJD in these patients. Familial CJD is most frequently associated with mutations at codon 200 and less frequently with mutations at codon 178, 208, or 210 of the prion protein gene (Medori et al., 1992). Compared to the classical CJD, vCJD-variant form of the disease affected a much younger age group (median age 26.5 years), and was characterized by a slower course than CJD (13 months versus 7 month) and atypical clinical manifestations (Almond, 1995). Unlike CJD, in which dementia is the most prominent sign of the disease, the early symptoms of vCJD involved psychological disturbances such as irritability, depression and aberrant behavior, which later gave way to mental disorder and neurologic abnormalities including ataxia, dementia and terminal myoclonus (Brown, 1995).

Gertsman-straussler-scheinker syndrome (GSS)

This disease was originally described in a German family in 1928 (Prusner, 1996). It is a very rare disease, with an incidence of 1-10 per 100 million/year. Its most important clinical manifestations are in coordination of movement and mental disorders. There is involvement of the medulla oblongata and brain stem, with consequent ataxia, stumbling, dysarthria, swallowing and speech difficulties, amnesia and finally dementia (Heaphy, 1998). These symptoms are most frequently observed in patients at the age of 20-30 years, but sometimes at older age. GSS is a slowly progressive disease with duration ranging from one to ten years. In GSS prion protein mutations are seen in codons 102,105,117,198 (Prusiner, 1995).

Fatal familial insomnia (FFI)

This disease was first described in some Italian families in 1986, and is one of the rarest types of human prion diseases, with an incidence rate of 1 per 50 million/year (Medoni et al., 1992). FFI is a primarily hereditary prion disease, characterized by a mutation at codon 178 of the PrP gene, along with methionine polymorphism at codon 129 of the mutated allele. Usually, it occurs between the ages of 40 and 60 years, and the affected person may survive for 7 to 33 months (average one year) after the onset of symptoms, depending on the type of genetic change (Tateishi et al., 1995). The patient loses many of the brain functions relating to sleep, and exhibits hallucinations, illusions, restlessness, poor memory and inability to concentrate. Other symptoms include depression and

a wide range of motor disturbances such as ataxia, dysarthria, muscle tremors, myoclonus and seizures.

Profuse sweating, increased heart rate and hormonal disturbances, especially growth hormone, prolactin, melatonin and some corticosteroid hormones, have also been reported. As in other prion diseases, FFI is characterized by the presence of PrP^{Sc} in the affected neurons (Manetto et al., 1992).

Kuru

This disease appeared in the earlier part of the 20th Century among members of the "Fore" tribe, in the Eastern Highlands of Papua New Guinea, to the east of the Pacific Ocean (Gajdusek et al., 1966). The name "Kuru" is the local name used by fore people to describe the condition. It means "laughing death" in their language because it is accompanied by uncontrollable laughter. Kuru is an invariably fatal disease, and like other TSEs it affects both mental and motor functions. Its incubation period ranges between 2-40 years, but is usually several years long. However, the clinical course of the disease is relatively short - the patient dies within 3 months to one year, at the most, after the appearance of symptoms (Gajdusek and Zigas, 1959). The symptoms include: in coordination of movement, stumbling, muscle tremors, difficulty in articulating words, involuntary oscillation of the eyes (nystagmus), difficulty to swallow, inability to hold things and finally dementia and death. The disease was associated with cannibalism (eating the brain of dead humans) and spread between members of the tribe, affecting more women and children than male adults. It is generally believed that Kuru is now extinct.

METHODOLOGIES FOR DETECTION

Human prion diseases have been traditionally classified into Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker disease (GSS), fatal familial insomnia (FFI) and kuru. About 75% of all human prion diseases are sporadic forms of CJD. Sporadic CJD is a rapidly progressive, multifocal dementia, usually with myoclonus (involuntary muscle twitching without unconsciousness). Dominant clinical features include fatigue, insomnia, depression, weight loss, headaches, general malaise, and ill-defined pain sensations. In addition, neurological features including extra pyramidal signs, cerebellar ataxia, pyramidal signs, cortical blindness and psychiatric features are frequent. Currently, definite diagnosis of prion diseases is still considered to be possible only after histopathological examination of biopsied or autopsied brain material (Gajdusek, 1996). The clinical diagnosis of sporadic CJD is supported by a variety of clinical tests, including detection of 14-3-3 and other proteins in cerebrospinal

fluid, the patterns of electroencephalogram (EEG) and neuro-imaging technologies² such as computer tomography and magnetic resonance imaging (Collinge J, 1996). More recently, the spectrum of human prion diseases has been widened by a new variant form of Creutzfeldt-Jakob disease (vCJD) that has been attributed to the consumption of BSE-contaminated meat products. Historically, TSEs have been diagnosed by their most prominent hallmarks including the histological features of spongiform changes, astrocytic gliosis and, albeit not consistently seen in all TSEs, amyloid plaques. Post-mortem neuropathological examination of brain tissue from an animal or human has remained the 'gold standard' of TSE diagnosis (Lantos et al., 1992). This analysis includes detection of vacuolation within specific brain regions by light microscopy and can be applied to the analysis of different prion strains (Harding et al., 1995). Most remarkably, prion strain typing in mice by analysis of the neuronal lesion profiles in brain tissue has provided strong evidence that vCJD in humans is caused by the same prion strain as BSE in cattle (Deeber et al., 2002). Similar studies to assess the presence of a BSE strain in sheep have not yielded conclusive results, due to the extensive strain variation in natural scrapie.

The extent of astrocytic gliosis in brain tissue can be assessed by immunohistochemical staining using antibodies to the astrocytic marker protein glial fibrillary acidic protein (GFAP) (Kneipp et al., 2002). In a recent study, it was shown that pathological PrP can be detected in lymphoid tissues as early as 42 days following oral exposure of deer to CWD prions. Similarly, vCJD can be diagnosed by detection of characteristic PrP immune staining and PrP^{Sc} on tonsil biopsy. Animal bioassays have been used extensively in TSE research and diagnostic testing. However, bioassays are severely limited in their widespread use by the length of time it takes to obtain results and the species barrier effect (Schemm, 2000). Yet animal bioassays remain the only method to measure directly the infectious agent and are, therefore, the most sensitive assay available for the detection of prions. A breakthrough in the detection of prions by bioassay was achieved by the genetic engineering of PrP-deficient (knockout), mutant and transgenic mice (Bueler et al., 1993). In particular, transgenic mice expressing high levels of heterologous PrP^C (e.g. human or bovine) on an otherwise PrP null background have shown great potential as a diagnostic tool for the detection of human or bovine prions (Cashmann, 2000). Transgenic mice expressing high levels of bovine PrP are about 10 times more sensitive than cattle and more than 1000 times as sensitive as RIII wild-type mice to infection with BSE prions.

Presently, the most widely used diagnostic tests exploit the relative protease resistance of PrP^{Sc} in brain samples to discriminate between PrP^C and PrP^{Sc}, in combination with immunological (anti-PrP-antibody mediated) detection of the proteinase K-resistant part of

PrP^{Sc} (PrP27-30) (Hope et al., 2000). Sample preparation is one crucial part of such diagnostic tests and influences the diagnostic sensitivity and specificity as well as the through-put. Because of the membrane attachment of PrP^C and PrP^{Sc}, tissue solubilisation has to include detergent extraction. In addition, conditions for proteinase K digestion have to be set such that the N-terminus of PrP^{Sc} and the whole PrP^C peptide can efficiently be digested while PrP27-30 re-mains resistant (Saborio et al., 2001).

TREATMENT

Immunity cannot be stimulated by prion diseases. Therefore, vaccines have no role in TSEs. No treatment is available either, although research in this area is progressing (Beck and Daniel, 1987). Removal of the PrP gene renders mice insusceptible to intracerebral inoculation with the scrapie-agent, while PrP over-expression enhances the development of disease (Taylor, 1989). Therefore, it has been suggested that either gene therapy or the use of antisense oligonucleotides that interfere with replication and reduce the amount of mRNA transcription of the PrP gene might be beneficial, although the delivery of these oligonucleotides to the brain might be difficult (Caughy et al., 1993). Current research focusing on drugs can prevent the formation of PrP^{Sc} without affecting PrP^C or bind protein X, thereby preventing PrP^{Sc} replication (Korth et al., 2001). It has been found that the amyloid stain Congo red and certain sulfated glycans strongly and selectively inhibited the production of PrP^{Sc}, without apparently interfering with PrP^C metabolism, in scrapie-infected cells (Peretz, 2001). Amphotericin-B derivative and Anthracyclines were also shown to prolong the incubation period and reduce the infectivity of brain tissue in hamsters infected with the scrapie agent, but does not prevent the disease from developing (Tagliavini et al., 1997). It has also been found that treatment of scrapie infected mice with a synthetic peptide homologous to a small region of the prion protein has reversed the abnormal structure of PrP^{Sc} to its normal (PrP^C) counterpart, delayed the clinical symptoms in the infected mice significantly and reduced brain infectivity by more than 90 - 95% (Priola, 2000). Recent evidences suggest that a number recombinant antibody antigen-binding fragments (Fabs) are useful to inhibit prion propagation in cultured mouse neuroblastoma cells infected with PrP^{Sc} (Weiss, 2001). Reaction mechanism associated with binding of these antibodies to a specific region in normal prion (PrP^C), where the latter interacts with PrP^{Sc}, and in that manner inhibit prion propaga-tion and clear prion clumps out of the cultured cells (Lantos et al., 1992). These studies offer the possibility of using genetically engineered antibodies to prevent and treat prion diseases, and identify sites for drug targeting.

PERSPECTIVES

Prion diseases, also known as the transmissible spongiform encephalopathies (TSEs), are a group of fatal neurodegenerative disorders that affect humans and animals. These diseases are intimately associated with conformational conversion of the cellular prion protein, PrP^C, into an oligomeric β -sheet-rich form, PrP^{Sc}. A growing number of observations support the once heretical hypothesis that transmission of TSE diseases does not require nucleic acids and that PrP^{Sc} alone can act as an infectious agent (Dealler, 1996). The view that misfolded proteins can be infectious is also supported by recent findings regarding prion phenomena in yeast and other fungi. One of the most intriguing facets of prions is their ability to form different strains, leading to distinct phenotypes of TSE diseases. The process of the conformational change remains enigmatic, but a large number of researches on the therapeutic intervention have been done in experimental models over the past 30 years. Against the background of the occurrence of variant CJD in the UK in the 1990s, which is considered to occur by transmission of the BSE agent and to possibly spread through secondary infection via blood transfusion, the research on the development of therapeutic agents has been accelerated using an increasing number of disease models in animals, cells and in vitro system (Deeber et al., 2002).

Within the context of the "protein-only" model, prion strains are believed to be encoded in distinct conformations of misfolded prion protein aggregates. This review described recent advances in biochemical aspects of prion research, with a special focus on the mechanism of conversion of prion protein to the pathogenic form, the emerging structural knowledge of fungal and mammalian prions, and our rapidly growing understanding of the molecular basis of prion strains and their relation to barriers of interspecies transmissibility (Prusiner, 1995). In order to control the spread of prion diseases, effective treatments are crucial. Current research has shown that no psychoactive cannabidiol (CBD) inhibits the accumulation of prion proteins, which gives rise to the possibility of an effective treatment. Hopefully, a better understanding of the function of CBD in hindering the aggregation of infectious prions will allow for the development of more treatments that prevent prion diseases. Overall, despite the advances made in prion research, there are still many questions left unanswered (Cashmann, 2000).

Conclusion

Prions are indeed responsible for transmissible and inherited disorders of protein conformation. They also cause sporadic disease, in which neither transmission between individuals nor inheritance is evident. Moreover, there are hints that the prions causing the diseases ex-

ploded thus far may not be the only ones. Prions made of rather different proteins may contribute to other neurodegenerative diseases that are quite prevalent in humans. Ongoing research may also help determine whether prions consisting of other proteins play a part in more common neurodegenerative conditions, including Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis. There are some marked similarities in all these disorders. As is true of the known prion diseases, the more widespread ills mostly occur sporadically but sometimes "run" in families. All are also usually diseases of middle to later life and are marked by similar pathology: neurons degenerate, protein deposits can accumulate as plaques, and glial cells (which support and nourish nerve cells) grow larger in reaction to damage to neurons. In spite of the amount and quality of the work that has been carried out to prove that an abnormal prion protein is the sole cause of prion diseases and the acceptance of this theory by the majority of the scientific community, the prion hypothesis still faces some crucial problems.

Many scientists argue that a definitive proof that prions could cause disease by themselves is still lacking and that an associated factor such as a virion cannot be ruled out. There are also several other newly emerging diseases that represent a risk to human or animal health that should also be given similar attention. While this is highly desirable in order to protect man and animals from these emerging diseases, older maladies like tuberculosis, malaria, bilharziasis, sleeping sickness and river blindness, which are devastating millions of people and their animals in many parts of the world, should not be overlooked.

REFERENCES

- Abiola OO, Iyegbe C, Lantos P, Plomin R, Anderton BH, Whatley SA (2002). Profound sex-specific effects on incubation times for transmission of bovine spongiform encephalopathy to mice. *Int. Virol.* 45(1): 56-58.
- Almond J, Pattison J (1997). Human BSE. *Nature*, 389: 437-438.
- Almond J (1995). Creutzfeldt-Jakob disease and bovine spongiform encephalopathy; any connection? Will bovine spongiform encephalopathy transmit to humans. *British Med. J.* 311: 1415-1416.
- Anonymous (1990). Prion disease-spongiform encephalopathies unveiled. Editorial. *Lancet*, 336: 21-22.
- Anonymous (1996). Special News Report: Prions. Putting Prions to the test. *Science*, 273: 184-189.
- Anonymous (2002). Transmissible mink encephalopathy. Report by Veterinary Services, United States Department of Agriculture, APHIS, Riverdale, MD 20737, USA.
- Beck E, Daniel PM (1987). Neuropathology of transmissible spongiform encephalopathies. In: *Prions, Novel Infectious Pathogens Causing Scrapie and Cruetzfeldt-Jakob Disease* (editors: Prusiner SB and McKinley MP), San Diego, Academic Press; pp. 331-383.
- Brown P (1995). Creutzfeldt-Jakob disease and bovine spongiform encephalopathy; any connection? The link is unproved, but no better explanation is presently forthcoming. *British Med. J.* 312: 790-791.
- Bueler H, Aguzzi A, Sailer A, Griener RA, Autenried P, Aguet M, Wiessmann C (1993). Mice devoid of PrP are resistant to scrapie. *Cell*, 73: 1339-1347.
- Caughy B, Ernest D, Race RE (1993). Congo red inhibition of scrapie agent replication. *J. Virol.* 67: 6270-6272.
- Cashmann NR (2000). Novel prion diagnostic reagents. In: *Cambridge Healthtech Institute's Second Annual Conference on Biocontaminants and Biological Production Issues* (October 2-3, 2000) Alexandria, Virginia, USA.
- Collinge J (1996). New diagnostic tests for prion diseases. *New England J. Med.* 335(13): 963-965.
- Cullie J, Chelle PL (1939). Experimental transmission of trembling to the goat. *C.R. Seances Acad. Sci.* 208: 1058-1060
- Dealler S (1996). *Lethal Legacy; BSE. The Search For The Truth.* Bloomsbury Publishing PLC, London, UK.
- Deeber SO, Baron TG, Bencsik AA (2002). Transmissible spongiform-encephalopathy diagnosis using PrPsc immunohistochemistry on fixed but previously frozen brain samples. *J. Histochem. Cytochem.* 50(5): 611-616
- Fraser CM editor (1991). In: *The Merck Veterinary Manual*, 7th edition, Merck and Co., Inc., Rahway, N. J., USA. pp. 577-578; 622-623, 1054
- Gajdusek DC, Gibbs CJ, Alpers M (1966). Experimental transmission of a kuru-like syndrome to chimpanzees. *Nature*, 209: 794-796.
- Gajdusek DC (1985). *Subviral Pathogens of Plants and Animals: Viroids and Prions* (editors: Maramorosch K and McKelvey J), Academic Press, New York, p. 483.
- Gajdusek DC, Zigas V (1959). Kuru: clinical, pathological and epidemiological study of an acute progressive degenerative disease of the central nervous system among natives of the Eastern Highlands of New Guinea. *Am. J. Med.* 26: 442-469.
- Gajdusek DC, Zigas V (1957). Degenerative disease of the central nervous system in New Guinea: the endemic occurrence of "kuru" in the native population. *N. Engl. J. Med.* 257: 974-978.
- Gajdusek DC (1996). Infectious Amyloids: Subacute Spongiform Encephalopathies as Transmissible Cerebral Amyloidoses. In: *Fields Virology*, 3rd edition, Lippincott-Raven Publishers, Philadelphia, Pa, pp. 2851-2900.
- Gore S (1995). Creutzfeldt-Jakob disease and bovine spongiform encephalopathy; any connection? More than happenstance: Creutzfeldt-Jakob disease in farmers and young adults. *British Med. J.* 311: 791-793.
- Harding BN, Alsanjari N, Smith SJ, Wiles CM, Thrush D, Miller TD, Scaravilli H (1995). Progressive neuronal degeneration of childhood with liver disease (Alpers' disease) presenting in young adults. *J. Neurol. Neurosurg Psychiatry*, 58(3): 320-325.
- Heaphy S (1998). Prion Diseases. Available on line in: [http://www/micro.msb.le.uk/335/Prion.html](http://www.micro.msb.le.uk/335/Prion.html) (updated 2/9/2000).
- Hill AF, Zeidler M, Ironside J, Collinge J (1997). Diagnosis of new variant Creutzfeldt-Jakob disease by tonsil biopsy. *Lancet*, 349: 99-100.
- Hoinville LJ (1994). Decline in the incidence of BSE in cattle born after the introduction of the feed ban. *Vet. Rec.* 134: 274-2756.
- Hope J, Tahiri-Alaoui A, Rhie A, Bullock M, Wellesley R, Bennett A, Birkett CR, James W (2000). Development of Nucleic Acid Ligands for the Detection of Prions. In: *Cambridge Healthtech Institute's Second Annual Conference on Biocontaminants and Biological Production Issues* October 2-3, 2000, Alexandria, Virginia, USA
- Hussein MF (1998). Bovine spongiform encephalopathy (Mad cow disease) and other prion diseases. *J. Saudi Biol. Soc.* 5(1): 3-24, (in Arabic; An abstract)
- Kahana E, Wilber N, Brahma J, Sofa D (1974). Creutzfeldt-Jakob disease: focus among Libyan Jews in Israel. *Science*, 183: 90-91.
- Kimberlin R, Cole S, Walker C (1986). Transmissible Mink Encephalopathy (TME) in Chinese Hamsters: Identification of Two Strains of TME and Comparisons with Scrapie. *Neuropath. Appl. Neurobiol.* 12: 197-206.
- Korth C, May BCH, Cohen FE, Prusiner SB (2001). Acridine and phenothiazine derivatives as pharmacotherapeutics for prion disease. *Proc. Natl. Acad. Sci. USA*, 98: 9836-9841.
- Kneipp J, Beekes M, Lasch P, Naumann D (2002). Molecular changes of preclinical scrapie can be detected by infrared spectroscopy. *J. Neurosci.* 22(8): 2989-2997.
- Lantos PI, McGill IS, Janota I, Doey LJ, Collinge J, Bruce MT, Whatley SA, Anderton BH, Clinton J, Roberts GW, Rossor MN (1992). Prion disease immunocytochemistry helps to establish the true incidence of

- prion diseases. *Neurosci. Lett.* 147: 67-71.
- Manuelidis EE, Rorke LB (1989). Transmission of Alpers' disease (chronic progressive encephalopathy) produces experimental Creutzfeldt-Jakob disease in hamsters. *Neurology*, 39(5): 615-621.
- Marsh RF (1979). On the Origin of Transmissible Mink Encephalopathy, *Slow Transmissible Diseases of the Nervous System*, vol. 1, edited by Prusiner SB and Hadlow WJ, Academic Press, N.Y., pp. 451-460.
- Marsh RF, Hadlow WJ (1992). Transmissible mink encephalopathy. *Rev. Sci. Tech. Off. Int. Epiz.* 11(2): 539-550.
- Marsh RF (1992). Transmissible mink encephalopathy. In: *Prion Diseases of Humans and Animals* (Editors: Prusiner SB, Collinge J, Powell J and Anderton B) Ellis Horwood, London. pp. 300-307.
- Matthews WB (1994). Bovine spongiform encephalopathy. The safety of beef has not yet been tested and may never be testable. *British Med. J.* 300: 412-413.
- Manetto V, Medori R, Cortelli P, Montagna P, Tinuper P, Baruzzi A, Rancurel G, Hauw JJ, Vanderhaeghen J, Mailloux P, Bugiani O, Tagliavini F, Bouras C, Ruzzuoto N, Lugaresi E, Gambetti P (1992). Fatal familial insomnia: clinical and pathological study of five new cases. *Neurology*, 42: 312-319.
- Medori R, Montagna P, Tritschler HJ, LeBlanc A, Cortelli P, Tinuper P, Lugaresi E, Gambetti P (1992). Fatal familial insomnia: a second kindred with mutation of prion protein gene at codon 178. *Neurology*, 42: 669-670.
- Ondera T, Ikeda T, Muramatsu Y, Shinagawa M (1993). Isolation of scrapie agent from the placenta of sheep with natural scrapie in Japan. *Microbiol. Immunol.* 37: 311-316.
- Parry HB (1962). Scrapie: a transmissible and hereditary disease of sheep. *Heredity*, 17: 75-105.
- Parry HB (1983). In: *Scrapie Disease in Sheep* (Editor Oppenheimer DR), Academic Press, U.K., pp. 31-51
- Palsson PA (1979). Rida (Scrapie) and its epidemiology. In: *Slow Transmissible Diseases of the Nervous System, Volume I*, Academic Press, N.Y., pp. 357-366.
- Prelli RE, Kascak DA, Mendez J, Harris F, Ironside R, Tagliavini I, Carp I (2000). Reversion of prion protein conformational changes by synthetic B-sheet breaker peptides, *Lancet*, 355: 192-97.
- Priola SA, Raines A, Caughey WS (2000). Porphyrin and phthalocyanine anti-scrapie compounds. *Science*, 287: 1503-1506.
- Peretz D, Anthony Williamson R, Kaneko K, Vergara J, Leclerc E, Schmitt-Ulms G, Mehlhorn IR, Legname G, wormalds MR, Rudds PM, Dwek RA, Burton DR, Prusiner SB (2001). Antibodies inhibit prion propagation and clear cell cultures of prion infectivity. *Nature*, 412: 739-743.
- Prusiner SB, Groth S, Serban IA, Stahl W, Gabizon R (1993). Attempts to restore scrapie prion infectivity after exposure to protein denaturants. *Proc. Natl. Acad. Sci. USA*, 90: 2793-2797
- Prusiner SB (1996). Prions. In: *Fields Virology*, 3rd edition, Lippincott-Raven Publishers, Philadelphia, Pa, pp. 2901-2950.
- Prusiner SB, Telling G, Cohen FE, DeArmond SJ (1996). Prion diseases of humans and animals. *Seminars in Virology*, 7: 150-173.
- Prusiner SB (1997). Prion Diseases and the BSE crisis. *Scientific American*, 278: 245-248.
- Prusiner SB (1995). The Prion Diseases. *Scientific American*, (January 1995), 276(1): 48-57.
- Schmerr MJ (2000). Detection of Abnormal Prion Protein in the Blood and the Cerebral Spinal Fluid of Animals Infected with a Transmissible Spongiform Encephalopathy. In: *Cambridge Healthtech Institute's Second Annual Conference on Biocontaminants and Biological Production Issues* (October 2-3, 2000) Alexandria, Virginia, USA (Abstract).
- Saborio GP, Permanne B, Soto C (2001). Sensitive detection of pathological prion protein by cyclic amplification of protein misfolding. *Nature*, 411: 810-813.
- Tagliavini F, McArthur RA, Canciani B, Giaccone G, Porro M, Bugiani M, Lievens PM, Bugiani O, Peri E, Dall'Ara P, Rocchi M, Poli G, Forloni G, Bandiera T, Varasi M, Suarato A, Cassutti P, Cervini MA, Lansen J, Salmons M, Post C (1997). Effectiveness of Anthracycline Against Experimental Prion Disease in Syrian Hamsters, *Science*, 276: 1119-1122.
- Taylor DM (1989). Review Article: Bovine spongiform encephalopathy and human health. *Vet. Rec.* 123: 413-415.
- Tateishi J, Brown P, Kitamoto T, Hoque ZM, Roos R, Wollman R, Cervenakova L, Gajdusek DC (1995). First experimental transmission of fatal familial insomnia. *Nature*, 376: 434-435.
- Weiss S (2001) A selected RNA aptamer that specifically binds PrP^{Sc}. In: *Pitt Symposium*, March, 4-9, 2001, New Orleans, La, USA.
- Wells GAH, Scott AC, Johnson CT, Gunning RF, Hancock RD, Damson M, Bradley R (1987). A novel progressive spongiform encephalopathy in cattle. *Vet. Rec.* 121: 419-420.
- Williams ES, Kirkwood JK, Miller MW (2001). Transmissible spongiform encephalopathies. pp. 292-301. In *Infectious Diseases of Wild Mammals*, 3rd edition, Williams ES and Barker IK (eds.). Iowa State University Press, Ames, Iowa.
- Williams ES, Miller MW (2002). Chronic wasting disease in deer and elk in North America. *Rev. Sci. Tech.* 21(2): 305-316.
- Williams ES, Young S (1980). Chronic Wasting Disease of captive mule deer: A spongiform encephalopathy. *J. Wildlife Dis.* 16: 89-98.
- Williams ES, Young S (1982). Spongiform encephalopathy of Rocky Mountain elk. *J. Wildlife Dis.* 18: 465-471.
- Williams ES, Young S (1992). Spongiform encephalopathies in Cervidae. *Revue Scientifique et Technique Office International des Epizooties*, 11: 551-567.
- Williams ES, Young S (1993). Neuropathology of chronic wasting disease in mule deer (*Odocoileus hemionus*) and elk (*Cervus elaphus nelsoni*). *Vet. Pathol.* 30: 36-45.
- Wilesmith JW, Ryan JBM, Hueston WD, Hoinville LJ (1992). Bovine spongiform encephalopathy: epidemiological features 1985-1990. *Vet. Rec.* 130: 90-94.
- Wijeratne WVS, Curnow RN (1990). A study of the inheritance of susceptibility to bovine spongiform encephalopathy. *Vet. Rec.* 130: 90-94.