Prions

The mysterious infectious agents

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ABSTRACT

Prions, a novel biological entity are causative agents of fatal neurodegenerative diseases. Such diseases gain importance because of its effect on both humans and animals and because of the unique biological features of the infectious agent. Since its discovery the agent responsible has remained mysterious in its mechanism of action, pathogenesis and the ability to produce disease. In this review, the considerable evidence regarding the molecular biology, pathogenesis, epidemiology, diagnosis and therapeutic approaches are being discussed. The advances in understandings of fundamental biology of prion diseases may open the possibilities for the prevention and treatment of these unusual diseases.

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The term prion was coined by Stanley Prusiner in 1982 to distinguish the mysterious infectious agent responsible for several neurodegenerative diseases in animals and humans from other typical infectious agents.[1] Prion diseases are fatal neurodegenerative diseases that are important because of their impact on public health and because they exemplify a novel mechanism of infectivity and biological information transfer.[2] Such diseases as: Scrapie in sheep; Bovine spongiform encephalopathy (BSE) in cattle; Transmissible mink encephalopathy; Creutzfeldt-Jakob disease (CJD), Kuru, fatal familial insomnia and Gerstmann-Straussler-Scheinker disease in humans are collectively known as transmissible spongiform encephalopathies (TSE). The prion hypothesis initiated by Prusiner faced sharp criticism from the scientific community, but the extensive studies he conducted to support his hypothesis awarded him the Nobel Prize in Medicine in 1997 for the discovery of prions "a new biological principle of infection." Interest in this agent has risen remarkably following his award achievement and scientists all over the world have become more and more engaged in prion research. The number of publications that appeared in the science citation indexes since 1982 have increased greatly, particularly in the last 7 years (**Figure 1**). The word prion itself derives from "proteinaceous infectious particle." The agent consists of only protein, with no nucleic acid genome. All previously known pathogens, such as bacteria and viruses, contain nucleic acids, which enable them to reproduce. Prions shows remarkable resistant to inactivation by heat, nucleases, irradiation and chemical disinfectants.[3] Prions can be transmitted, possibly by eating and certainly by inoculation either directly into the brain or into skin and muscle tissue. Naturally occurring TSE diseases such as BSE in cattle are probably transmitted by oral or other peripheral routes of infection.[4] The prion has been found only in brain tissue, spinal cord and retina but not in meat or milk of cattle naturally affected with BSE. However, a recent study showed that mouse skeletal muscle could propagate prions and accumulate substantial titers of these pathogens. The study demonstrated that factors in addition to the amount of prion protein expressed determine the tropism of prions for certain tissues.[5] Considerable evidence argues that consumption of beef products from cattle infected with BSE prions causes new variant Creutzfeldt-Jakob disease (vCJD). Prion diseases can arise in 3 different ways, through

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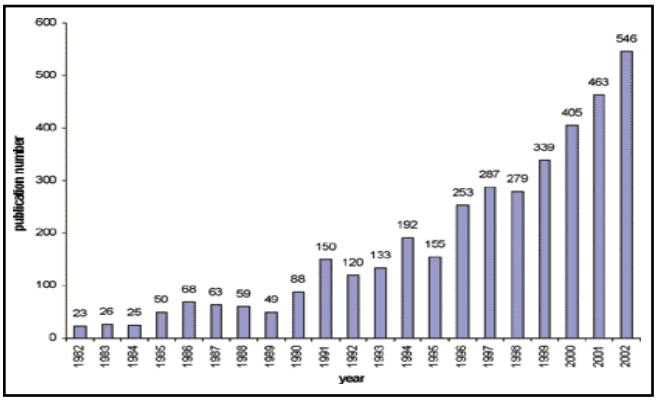

Figure 1 - The number of prion publications appearing in the science citation indexes since 1982.

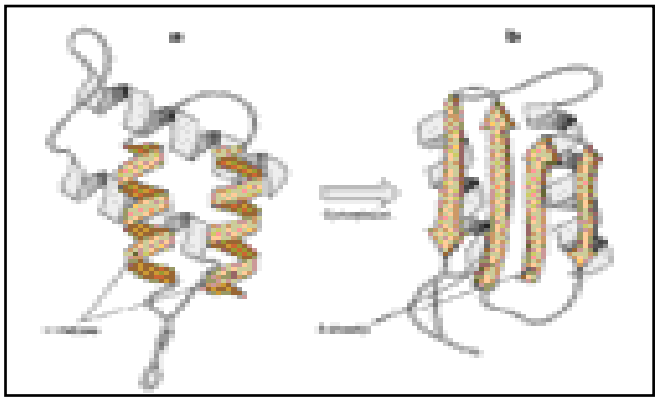

Figure 2 - Proposed three-dimensional structure **a)** PrPc and **b)** PrPSc. (Source: Prior Biology and Diseases, New York (NY): Cold Spring Harbor Laboratory Press; 1999.)

horizontal transmission from for example, a sheep to a cow (BSE), in inherited forms, mutations in the prion gene are transmitted from parent to child and they can arise spontaneously by mutation.[6]

Molecular biology. A major breakthrough occurred when researchers discovered that the infectious agent (prion) consists primarily of a protein found in the membranes of normal cells. The normal cellular protein (PrPc) is a highly conserved cell surface glycoprotein expressed in the neurons and glia of the brain and spinal cord, as well as in several peripheral tissues and in leukocytes.[7] This protein consists of approximately 250 amino acids and is characterized by 4-helices, upon conversion to the disease-associated form of prion protein (PrPSc), results in the loss of 2 of the helical structures, which are converted to linear structures known as beta-sheets that are associated with the acquisition of prion infectivity (**Figure 2**). This conversion provided a mechanism for disease transmission and accounted satisfactorily for the long incubation time and the natural history of the disease.[7] So now we know that the normal cellular protein, PrPc, which is found in all of us, is centrally involved in the spread of prion diseases. It was thought that during prion infection, a highly specific physical interaction that is responsible for generating new molecules of PrPSc occurs between PrPc and PrPSc. This conclusion was based on several types of evidence. First, and perhaps most compelling, mice that do not synthesize PrPc because their endogenous PrP gene has been ablated are completely resistant to prion infection. Second, expression of genetically engineered forms of PrP in transgenic mice and cultured cells profoundly alters their susceptibility to prion infection. For example, mice are not normally susceptible to prions derived from hamsters, but expression of a hamster PrP transgene in mice renders them susceptible to infection.[3] Polymorphisms in the prion protein (PrP) gene are associated with phenotypic expression differences of TSE in animals and humans. In sheep, at least 10 different mutually exclusive polymorphisms are present in PrP. This finding indicates a linkage of these alleles with a reduced susceptibility or resistance for scrapie. In addition, PrPSc with the codon 171 (Gln-to-His) polymorphism, is the first variant reported to induce higher conversion efficiencies with heterologous rather than homologous PrP variants.[8] More than 20 mutations of the PrP gene are now known to cause the inherited human prion diseases, and significant genetic linkage has been established for 5 of these mutations.[9] In TSE, disease is closely associated with the conversion of the normal proteinase K-sensitive host prion protein (PrP-sen) to the abnormal proteinase K-resistant form (PrP-res). Amino acid sequence homology between PrP-res and PrP-sen is important in the formation of new PrP-res and thus in the efficient transmission of infectivity across species barriers.[7] It was previously shown that the generation of mouse PrP-res was strongly influenced by homology between PrP-sen and PrP-res at amino acid residue 138, a residue located in a region of loop structure common to PrP molecules from many different species. However, such homology was not critical for the formation of protease-resistant hamster PrP rather, homology between PrP-sen and hamster PrP-res at amino acid residue 155 determined the efficiency of formation of a protease-resistant product induced by hamster PrP-res. It was suggested that PrP-res molecules isolated from scrapie-infected brains of different animal species have different PrP-sen structural requirements for the efficient formation of protease-resistant PrP.[10] In vivo under pathological conditions, the normal cellular form PrPc (residues 23-231) misfolds to the pathogenic isoform PrPSc, beta-rich aggregated pathogenic multimer. Proteinase K digestion of PrPSc leads to a proteolytically resistant core, PrP 27-30 (residues 90-231) that can form amyloid fibrils. Multiple pathways of misfolding and the formation of distinct beta-sheet-rich abnormal isoforms may explain the difficulties in refolding PrPSc in vitro, the need for a PrPSc template, and the

significant variation in disease presentation and neuropathology.[11]

Conversion to the infectious conformer is particularly associated with major structural rearrangement in the central portion of the protein (residues 90-120), which has an extended flexible structure in the PrPc isoform. Using a panel of recombinant antibodies reactive with different parts of PrPc, showed that equivalent major structural rearrangements occur spontaneously in this region of PrPc immobilized on a surface. In contrast, regions more towards the termini of the protein remain relatively unaltered.[12] The rearrangements occur even under conditions where individual PrPc molecules should not contact one another. The propensity of specific unstructured regions of PrPc to spontaneously undergo large and potentially deleterious conformational changes may have important implications for prion biology.[13] Only one epitope region at the C terminus of PrP was well presented on both PrPc and PrPSc, while epitopes associated with most of the specific antibodies developed in one study were present on PrPc but absent from PrPSc.[11] The identification of proteins in yeast and filamentous fungi that behave like prions makes it likely that inheritance of biological information via protein conformation will prove to be a general phenomenon in nature.[14-16] It is now becoming widely accepted that prions are elements that impart and propagate variability through multiple conformers of a normal cellular protein. Two notable prion-like determinants, [URE3] and [PSI$^+$], have already been described in yeast and one in another fungus denoted [Het-s]. The determinant [URE3] makes cells derepressed for nitrogen catabolism, while [PSI$^+$] elevates the efficiency of weak suppressor transfer RNA.[17] Studies of candidate prion proteins in yeast may prove particularly helpful in the dissection of some of the events that feature in PrPSc formation. Interestingly, different strains of yeast prions have been identified.[9] The yeast [PSI$^+$], [URE3] and [PIN$^+$] genetic elements are prion forms of Sup35p, Ure2p and Rnq1p. Over expression of Sup35p, Ure2p or Rnq1p leads to increased de novo appearance of [PSI$^+$], [URE3] and [PIN$^+$].[18] The Ure2p of *Saccharomyces cerevisiae* normally functions in blocking utilization of a poor nitrogen source when a good nitrogen source is available.[19] Propagation of [PSI], a prion-like form of the release factor Sup35, was regulated by the interplay between chaperone proteins Hsp104 and Hsp70.[9,20,21] These findings showed that various members of the yeast Hsp70 family have diverged from each other in regard to their roles in prion propagation and suggest that Ssb could serve as a proofreading component of the enzymatic system, which prevents formation of prion aggregates.[21]

Transgenetic studies argue that PrPSc acts as a template upon which PrPc is refolded into a nascent PrPSc molecule through a process facilitated by another protein.[9] It has been suggested that additional cellular factors might be involved in the physiological function of PrPc and in the propagation of PrPSc. Screening of a HeLa cDNA library identified heat shock protein 60 (Hsp60), a cellular chaperone as a major inter-actor for PrPc.[22] The highly abundant molecular chaperone Hsp90 functions with assistance from auxiliary factors, collectively referred to as Hsp90 co-chaperones, and the Hsp70 system. The Hsp104, a molecular chaperone required for stress tolerance and for maintenance of [PSI$^+$] prions in the budding yeast *Saccharomyces cerevisiae*, appears to collaborate only with the Hsp70 system. These results showed that several co-chaperones previously thought to be dedicated to Hsp90 are shared with Hsp104 and suggested that co-chaperone sharing may favor adaptation to altered metabolic conditions.[23] The role of chaperones in the formation of PrPSc appears to be quite significant. The idea is appealing from a theoretical point of view, since PrPSc formation involves changes in protein folding and possibly intermolecular aggregation, processes in which chaperones are known to play a role. Chaperones are proteins that normally facilitate the folding of polypeptides during their biosynthesis and transport into organelles and help prevent protein aggregation during cellular stress.[24] They bind to their substrates and prevent the formation of unproductive folding intermediates. Chaperones are found in several cellular compartments, although the endoplasmic reticulum is the only organelle through which PrP passes during its cellular trafficking that contains known chaperone molecules.[25,26] Experimental evidence in favor of chaperone involvement in prion phenomena came from studies of transmission patterns in transgenic mice.[27] Human prions propagate poorly in mice carrying a human PrP transgene, but the effect was alleviated either by eliminating the expression of endogenous mouse PrP or by modifying the human PrP transgene to include N- and C-terminal segments from the mouse PrP gene. These results and others[28] have been interpreted to imply the existence of cellular chaperones, collectively referred to as protein X, that interact in a species-specific way with the C terminus of PrPc during prion propagation. If such chaperones were also cell specific, this might explain the selective propagation of prions in distinct neuronal populations and in particular types of peripheral cells such as those of the lymphoreticular system. Evidence from scrapie-infected neuroblastoma cells also implicates chaperones in prion biogenesis. Several "chemical chaperones" such as glycerol and dimethyl sulfoxide, which stabilize protein conformation, inhibit PrPSc production in infected cells.[29] The most direct evidence that chaperones can affect generation of PrPSc is provided by experiments in which the yeast chaperone Hsp104 and the bacterial chaperone GroEL have been shown to enhance the formation of PrPSc in a cell-free conversion system.[30]

Pathogenesis. The classical hallmarks of prion diseases include vacuolation, gliosis, accumulation of a protease-resistant, abnormally folded isoform of the prion protein (PrPSc) and neuronal cell death. Although the mechanism of the cell death is poorly understood, accessory cells, including astrocytes and microglia, appear to participate in neural loss through the release of soluble mediators and toxic metabolites.[31,32] Certain properties of the prion protein such as the apparent form of its glycosylation and conformational properties reflected by the preferential site of digestion with proteinase K are associated with particular phenotypes of prion disease.[33] Since the immune system does not recognize prions as foreign, no natural protection develops. This hypothesis explains why infection with PrPSc did not stimulate an immune reaction, as the infective agent was not foreign to the host but the product of the host's own prion gene.[7]

The normal function of PrPc remains unknown, although its localization on the cell surface would be consistent with roles in cell adhesion and recognition, ligand uptake, or trans-membrane signaling.[3] Recently PrPc was found to be able to bind copper ions in vitro and could exist in a copper-bound state in vivo. Findings suggest that these copper-binding properties of PrPc may be important in the protection of cells from 'oxidative stress'. It is the destructive effect of highly charged, toxic oxygen free radicals, which are produced in the body in a number of normal biochemical reactions. It appears that PrPc may influence the activity of a copper/zinc superoxide dismutase enzyme, which mops up and deactivates harmful free oxygen radicals. Thus, it is possible that the toxicity of PrPSc may be tightly associated with the loss of activity of PrPc in the cell.[34]

Prion infection relies on a continuous chain of PrPc-expressing tissues to spread from peripheral sites to the central nervous system (CNS). Direct neuroinvasion via peripheral nerves has long been considered likely. However, the speed of axonal flow is incompatible with the lengthy delay prior to the detection of PrPSc in the brain. It was demonstrated that PrPc is mainly localized at the cell membrane of the Schwann cell and that infection and replication of PrPSc were shown for the first time in a peripheral glial cell line.[35] There is also biochemical evidence that PrPc in neurons is axonally transported to nerve terminals that is consistent with the localization of the protein in synaptic profiles as shown by immunoelectron microscopy. Light microscopic immunocytochemistry showed that PrPc is concentrated primarily in synaptic fields of the olfactory bulb, limbic structures, and striato-nigral complex.[3] The PrPSc was detected at 3 weeks post-infection in the lumbar spinal cord and ascended to the brain at a rate of approximately 3.3 mm per day. At 6 weeks post-infection, PrPSc was detected in the lateral vestibular nucleus and the interposed nucleus of the cerebellum ipsilateral to the site of sciatic nerve inoculation and in the red nucleus contralateral to the inoculation site. At 9 weeks post-infection, PrPSc was detected in the contralateral hind limb motor cortex and reticular thalamic nucleus.[36]

Current evidence suggests that infection occurs initially in the lymphoid tissues and subsequently in the CNS. The identity of infected lymphoid cells remains controversial, earlier studies point to the involvement of both follicular dendritic cells (FDC) and $CD11c^+$ lymphoid dendritic cells; however, a recent study suggested that neither FDC nor $CD11c^+$ cells are essential for neuro-invasion.[37]

To investigate the role of the pathogenic prion protein PrPSc in controlling susceptibility to foreign prions, 2 Syrian hamster (SHa) prion strains, Sc237 and DY, were transmitted to transgenic mice expressing chimeric SHa/mouse PrP genes, Tg (MH2M). First, passage of SHa (Sc237) prions exhibited prolonged incubation times, diagnostic of a species barrier. The PrPSc of the new MH2M (Sc237) strain possessed different structural properties from those of SHa (Sc237), as demonstrated by relative conformational stability measurements. The results suggested a causal relationship between species barriers, changes in PrPSc conformation, and the emergence of new prion strains.[38]

Several cell types are thought to produce PrPSc following prion infection in vivo, including neurons, astrocytes, and lymphoreticular cells. There is surprisingly little information available about how extracellular PrPSc is taken up by cells during the initial stage of infection. If this uptake were to occur via an endocytic mechanism, PrPSc may interact with PrPc on the plasma membrane or in endosomes and key events in the conversion process may take place in these locations. Several pieces of evidence suggest that an endocytic pathway is involved in the generation of PrPSc in scrapie-infected N2a and HaB cells. One, that the idea is consistent with the localization of at least some PrPSc molecules in endosomes and lysosomes. Two, surface iodination of infected cells results in incorporation of radiolabel into PrPSc after a chase period, arguing that PrPc molecules transit the plasma membrane prior to conversion into PrPSc. Three, treatment of cells with PI-PLC or proteases inhibits the production of PrPSc, presumably by removal of the PrPc precursor from the cell surface.[3]

Epidemiology. Until the sudden occurrence in the mid-1980s of an epizootic of a formerly unknown disease, bovine spongiform encephalopathy popularly named 'mad cow disease', in cattle in the United Kingdom (UK), very little attention had been paid to these rather obscure diseases.[39] Prion disease in man was first described as CJD in the 1920s.[40] Creutzfeldt-Jakob disease the first TSE occurs in a sporadic, familial, or iatrogenic form.[41,42] Sporadic CJD is the most common form of CJD and occurs with an incidence of around one per million in most parts of the world. Familial CJD accounts for approximately

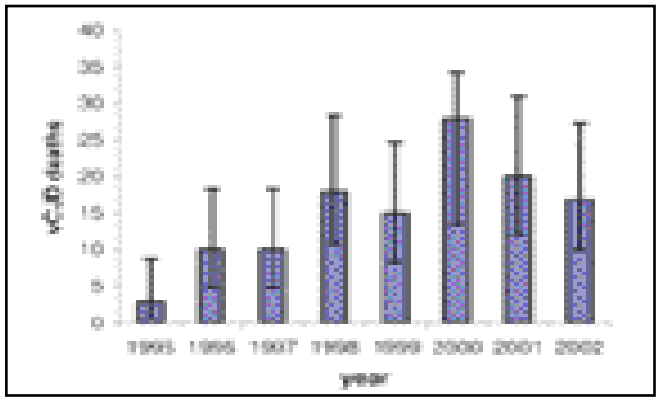

Figure 3 - Time series of 121 deaths from variant Creutzfeldt-Jakob disease (vCJD) to the end of 2002 and 95% Poisson confidence intervals 2001. (Source: Ghani AC, 2003).

Table 1 - Chronology of variant Creutzfeldt-Jakob disease in the United Kingdom and other European countries as of December 2000.

Year of onset	United Kingdom	France	Ireland
1994	8	1	
1995	10		
1996	11		
1997	14		
1998	17		
1999*	20 (+4)	1 (+1)	1
2000*	1 (+2)		

*Parentheses indicate still-living persons with probable variant Creutzfeldt-Jakob disease (vCJD) or deceased persons whose diagnoses have not yet been confirmed by neuropathologic examination. In 2000, additional cases have been identified that do not yet meet the minimum clinical criteria for a premortem diagnosis of "probable" vCJD. Dates are for year of onset of illness, not year of death. (Source: Brown et al, 2001).

10% of all European cases of CJD, and is associated with inherited mutations of the prion protein gene, caused by one of the 24 single amino acid substitutions or insertions of octapeptide repeats.[40] Iatrogenic CJD has occurred due to exposure to infectious brain material as in kuru, where a variant of CJD was transmitted by cannibalism. More than 2500 have died of kuru between 1957 and 1982.[40] Creutzfeldt-Jakob disease is also transmitted by medical interventions such as neurosurgery or injection of human-derived growth hormone.[43] In 1996, a newly recognized variant form of CJD among young patients with unusual clinical features and a unique neuropathologic profile was reported in the UK.[41,43,44] More than 115 people have died from this disease and it has rapidly become a global concern.[43] **Figure 3** shows the time series of vCJD cases diagnosed in the UK by date of death. This new form could be due to transmission to humans of the agent causing bovine spongiform encephalopathy.[44] The exposure of humans to orally ingested BSE agent in contaminated meat products presumably led to the emergence of vCJD.[45] One distinguishing feature of vCJD compared to other classical forms of CJD is that cases have arisen predominantly in the young. The median age at onset of the cases to the end of 2000 was 26 years at onset and 28 years at death[46] and ranged from 12 to 74 years at onset. In contrast, the median age at death for sporadic CJD in the UK between 1995 and 2000 was 65 years, with few cases occurring in those under 40 years.[46] To date, the majority of cases of vCJD have occurred in the UK, although 2 have been diagnosed in France[47,48] and one in Ireland. The chronology of vCJD in the UK and other European countries are shown in **Table 1.**[49]

Precise estimates of the length and variability of the incubation period for CJD are difficult to obtain since they require knowledge of the time of infection, whereas exposure might have occurred over several years. For iatrogenic CJD, case reports indicate a relatively short incubation period (median length of approximately 8 years) when the infectious agent was inoculated through dura mater grafts.[50] Studies are currently under way to obtain more precise estimates of the prevalence of asymptomatic infection through testing tonsil and appendix tissues for the abnormal prion protein.[51] Past projections of the future course of the vCJD epidemic in the UK have shown considerable uncertainty, with wide confidence bounds. However, recent vCJD case data have indicated a decrease in the annual incidence of deaths over the past 2 years.[52]

Diagnosis. Unfortunately, at present the only way to diagnose prion diseases, BSE in cattle or the human form of the disease is after the symptoms have developed and the disease is entering its late stages. The only surefire way now to test for BSE, is to check an animal's brain after it has been killed. The same goes for the human version of the disease, CJD and the new human strain, nvCJD.[6] Samples, collected from the spleen, palatine tonsil, ileum, and 5 different lymph nodes, immunohistochemically stained showed PrPSc deposited in a reticular pattern in the center of both primary and secondary lymphoid follicles. In addition, granules of PrPSc were seen in the cytoplasm in macrophages associated with the lymphoid follicles. It was concluded that tonsils are the best-suited lymphoid tissue to be biopsied for the detection of PrPSc in the diagnosis of clinical scrapie in living sheep.[53] In humans, pre-mortem diagnosis of vCJD is currently based on clinical presentation, characteristic appearances on magnetic resonance imaging of the brain, and the exclusion of other causes.[43] Unlike the other human TSE, where the abnormal protein is largely confined to the CNS, a distinctive feature of vCJD is the widespread distribution of the abnormal protein in peripheral lymphoid tissue. In addition to the CNS, abnormal protein is detectable in the spleen, lymph nodes, tonsils, optic nerve and retina of vCJD cases.[43] Studies of tonsil tissues using a highly sensitive immunoblotting assay have accurately been able to detect vCJD in patients with clinical symptoms.[54-56] Immunocytochemistry has also been

used to detect the presence of abnormal PrPSc deposition in the appendix of a vCJD patient removed 3 years prior to the onset of clinical symptoms.[56] Definitive diagnosis of vCJD requires histopathological demonstration of the abnormal protein in the CNS.[43] Several companies were recently engaged to develop a blood test to identify the "novel agents" that recognize the prion protein. Recently, one company has announced the development of a live test for prion diseases that is based on the recent discovery of disease associated prion protein in the urine of both animals infected with BSE and humans infected with CJD.

Therapeutic approaches. Efforts have been greatly intensified in recent years to fight the prion agent. Several compounds are being tried and have shown an effect on these agents. These include: 1. Branched polyamines were the first class of compounds shown to cure prion infection in living cells and may prove useful as therapeutic, disinfecting, and strain-typing reagents for prion diseases. Such polyamines, including polyamidoamine and polypropyleneimine (PPI) dendrimers, can purge PrPSc, from scrapie-infected neuroblastoma (ScN2a) cells in culture. The susceptibility of PrPSc to proteolytic digestion was strain dependent.[57] 2. Polyene antibiotics such as amphotericin B have been shown to delay the accumulation of PrPSc and to increase the incubation time of the disease after experimental transmission in laboratory animals. It was shown for the first time that amphotericin B can inhibit PrPSc generation in scrapie-infected GT1-7 and N2a cells.[58] 3. Tumor necrosis factor alpha (TNF-α) secretion by lymphocytes is important for maintaining follicular dendritic cell (FDCs) networks, and signaling is mediated through TNF receptor 1 (TNFR-1) expressed on FDCs, their precursors, or both. Studies of a mouse scrapie model have shown that treatments that specifically inhibit the TNFR signaling pathway might present an opportunity for early intervention in peripherally transmitted TSEs.[59] 4. Tricyclic compounds with an aliphatic side chain at the middle ring moiety were reported to constitute a new class of antiprion reagents. Because quinacrine and chlorpromazine have been used in humans for many years as antimalarial and antipsychotic drugs, and are known to pass the blood-brain barrier, it was suggested that they are immediate candidates for the treatment of CJD and other prion diseases.[60] 5. The ability of several recombinant antibody antigen-binding fragments (Fabs) to inhibit prion propagation in cultured mouse neuroblastoma cells (ScN2a) infected with PrPSc was examined. Antibodies binding cell-surface PrPc inhibit PrPSc formation in a dose-dependent manner. In cells treated with the most potent antibody, Fab D18, prion replication is abolished and preexisting PrPSc is rapidly cleared, suggesting that this antibody may cure established infection. The potent activity of Fab D18 is associated with its ability to better recognize the total population of PrPc molecules on the cell surface, and with the location of its epitope on PrPc. These observations support the use of antibodies in the prevention and treatment of prion diseases and identify a region of PrPc for drug targeting.[61] 6. Gene therapy with mutant PrPc studied in transgenic mice showed that peripheral expression of heterologous PrPc completely protected most mice from clinical disease after oral or intraperitoneal scrapie infection. The role of tissue-specific PrPc expression of foreign PrPSc in interference with scrapie infection may be effective in preventing TSE diseases.[4] 7. Tetracycline hydrochloride or doxycycline hyclate was shown to reduce prion infectivity through a direct interaction with PrPSc and were potentially useful for inactivation of BSE- or vCJD-contaminated products and prevention strategies.[62]

The heterogeneity and complexity of the etiopathogenesis of prion diseases suggest that various strategies and a combination of several compounds with different modes of actions are necessary for prevention and treatment. Major efforts should be focused on the development of preclinical diagnostic tests in conjunction with immunization strategies for diseases acquired by peripheral route and identification of more effective compounds for the other etiological forms.[63] Recently, researchers have shown that it is possible to delay the onset of a prion-based disease in mice, by immunizing with a recombinant protein. Since these aberrant proteins do not elicit a classical immune response, presumably because they are not seen as foreign, Sigurdsson and colleagues[64] have overcome this obstacle by vaccinating mice with recombinant PrP (recPrP) mixed with a powerful adjuvant (heat-killed mycobacteria). Following inoculation with PrPSc, mice immunized with the mixture develop prion disease later than unvaccinated controls. Whether this approach will have any relevance to humans still needs to be investigated.

In conclusion, the extensive studies that have been conducted so far on the prion agent have shed some light on its mode of action, pathogenesis and the ability to develop disease, which might open the possibilities for the prevention and treatment of these unusual diseases.

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