Prion Infections in Creutzfeldt-Jakob Disease and Its Variants*

JOSEPH C. PARKER, Jr., M.D.,
and JAMES W. SNYDER, Ph.D.

Department of Pathology and Laboratory Medicine,
University of Louisville School of Medicine,
Louisville, KY 40292

ABSTRACT

Prions (PrPSc) are proteinaceous infectious particles that occur as sporadic (85 percent), infectious (iatrogenic) (5 percent) or hereditary (10 percent) diseases in humans and animals. These unique infectious agents produce a spongiform change in the central nervous system without any inflammation, inclusion bodies or apparent antibody response. A helper (X) protein and genetic predisposition appear to be required to establish the infection, which seems associated with a post-translational change of a normal protein (PrPC) encoded by a gene on human chromosome 20. Sporadic human prion disease (Creutzfeldt-Jakob disease) is the most common form of human transmissible spongiform encephalopathy. Nevertheless, it is undoubtedly under-recognized as a result of both low autopsy rates and confusion with other dementing diseases like Alzheimer's disease. Although no therapy is currently available for this infectious dementia, which has a prolonged incubation period, these unfortunate victims should be offered supportive care and postmortem examinations. Universal precautions will protect laboratorians from this infectious, but not contagious, disease.

Overview

Prions are proteinaceous infectious particles, designated PrP, and are derived from a normal cellular isoform PrPC on human chromosome 20 after post-translation modification to PrPSc or PrPCJD. Neurodegenerative prion diseases produce dementia, are transmissible and are associated with prion proteins (PrPSc, PrPCJD or PrPres). The human prion disorders include Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker disease (GSS), fatal familial insomnia (FFI) and kuru. The disease can occur in many animals, including sheep (scrapie), goats, mink, deer, elk, felines and cattle (Mad Cow Disease). These transmissible spongiform encephalopathies with vacuolated neurons and neuropil require several years of incubation and lack inflammation, inclusions, viral particles and antibodies. They may be dominantly inherited, but typically develop sporadically. Rarely, they may be acquired iatrogenically.

Disease Mechanisms

The abnormal prion in human disease, designated PrPCJD, and in animal disease, desig-
uated \(PrP^{Sc}\), is derived from a normal cellular isoform of prion protein \(PrP^c\) following posttranslational modification of its structure. This is an extremely rare event. These infectious prions are resistant to most routine disinfectants and have been called \(PrP^{Res}\).

The human \(PrP\) gene, designated \(PRNP\), is located on the short arm of chromosome 20. Multiple mutations in the open reading frame of the \(PRNP\) gene have been linked to familial (hereditary) human prion disorders. These mutated prion proteins from prion particles are designated \(\Delta PrP\). Prion diseases are characterized by a vacuolar (spongiform) degeneration of the neuropil, cortical neurons and subcortical grey matter with neuronal loss, reactive astroglisis and variable \(PrP\) amyloid plaques. The agent cannot be inactivated by ultraviolet radiation or routine chemical disinfectants.

A necessary component for the infectious prion is a 27-30 kDa protein (table I). No nucleic acid is present. This \(PrP\) 27-30 is derived from a larger 33-35 kDa protein (\(PrP^{Res}\)) found in infected individuals. Naturally occurring prion protein (\(PrP^c\)) is a glycolipid anchored cell membrane glycoprotein with four alpha helical regions and two asparagine-linked carbohydrate side chains at codons 181 and 197. The infectious prion \(PrP^{CJD}\) and the normal prion \(PrP^c\) have the same molecular weight, the same amino acid sequence and similar carbohydrate and glycolipid anchor side chains. The infectious prions, unlike \(PrP^c\), are only partially digested by proteinase to \(PrP\) 27-30 by removing the N-terminal 67 amino acid residues. The \(PrP\) 27-30 aggregates into rod-like amyloid structures when exposed to detergents. The only difference between infectious and non-infectious prion proteins is the acquisition of \(\beta\)-sheet formation during the post-translational conversion of \(PrP^c\) to \(PrP^{Sc}\) (table I). \(PrP^c\) has 3 percent \(\beta\)-sheets and 42 percent \(\alpha\)-helics, whereas \(PrP^{Res}\) has 43 percent \(\beta\)-sheets and 30 percent \(\alpha\)-helics. The \(PrP\) 27-30 has even higher \(\beta\)-sheet content (54 percent) and lower \(\alpha\)-helical content (21 percent) than the \(PrP^{Res}\).

Accumulation of abnormal prions has a profound effect on cell membrane receptors and ion channel formation, producing selective vulnerability in the nervous system. Infectious prions (\(PrP^{Res}\)) derived from the normal cellular prion protein (\(PrP^c\)) can be induced by exposing cells to \(PrP^{Res}\). This can be prevented by releasing \(PrP^c\) from the cell surface with a phospholipase or by blocking the transport of \(PrP^c\) from the Golgi apparatus to the plasma membrane.

The \(\beta\)-sheet acquisition by \(PrP\) can be achieved by mutations of the \(PrP\) gene in

---

### TABLE I

<table>
<thead>
<tr>
<th>Features</th>
<th>(PrP^c)</th>
<th>(PrP^{Sc} (PrP^{Res}))</th>
<th>(PrP^{CJD} (PrP^{Res}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight</td>
<td>33–35 kD</td>
<td>33–35 kD</td>
<td>33–35 kD</td>
</tr>
<tr>
<td>Amino acid sequence</td>
<td>254 amino acids</td>
<td>same</td>
<td>same</td>
</tr>
<tr>
<td>Infectivity</td>
<td>none</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Proteinase digestion</td>
<td>complete</td>
<td>partial</td>
<td>partial</td>
</tr>
<tr>
<td>(PrP) 27–30</td>
<td>none</td>
<td>yes ((\Delta PrP))</td>
<td>yes ((\Delta PrP))</td>
</tr>
<tr>
<td>(\alpha)-sheets</td>
<td>42 %</td>
<td>30%</td>
<td>30%</td>
</tr>
<tr>
<td>(\beta)-sheets</td>
<td>3%</td>
<td>43%</td>
<td>43%</td>
</tr>
</tbody>
</table>

\(PrP^c\) = Native (normal) prion.
\(PrP^{Sc}\) = Scrapie agent.
\(PrP^{Res}\) = Resistant prion (\(\Delta PrP\)).
\(PrP^{CJD}\) = Creutzfeldt–Jakob disease agent.
familial prion diseases, which are much rarer than sporadic CJD. Inherited disorders account for 15 percent of human prion diseases, with an incidence of 1 per 10 million. Exogenous PrPRes, pathogenic ΔPrP, or both, can initiate a self-perpetuating conversion of PrPc to infectious PrPRes in vulnerable cells. This conversion appears to occur in endosomes and may proceed in an exponential fashion. A heterodimer composed of at least one molecule of PrPRes and one molecule of PrPc is formed. A sufficient degree of amino acid homology is required between these two prions.

The continuous formation and accumulation of PrPRes in lysosomes can cause disruption of their membranes, with release of hydrolytic enzymes into the cytoplasm, causing cell death. The process hypothesis, however, suggests that conversion of PrPc to PrPRes and subsequent shunting of PrPRes to secondary lysosomes, with altered protein and lipid trafficking, change the composition and properties of the plasma membrane. Both processes probably play a role in human prion diseases.

The etiology of CJD is variable. Sporadic CJD may be an age-related, acquired mutation with spontaneous conversion of PrPc into pathogenic PrPcJD in one or more subpopulations of cells. Patients with sporadic CJD show 95 percent or more homozygosity for methionine or valine at codon 129 on chromosome 20. In iatrogenic CJD, the infectious prions have been transmitted through corneal transplantations, contaminated EEG electrodes and contaminated operative instruments. The disease has occurred after implantation of dural grafts and after the use of human growth hormones and pituitary gonadotropin. The hereditary prion diseases also include fatal familial insomnia and variants of Gerstmann-Sträussler-Scheinker disease.

**The Diseases**

Infectious prions (PrPRes) are found in prion amyloid plaques in scrapie, CJD and GSS with protease resistant prion protein; in local accumulation of PrPRes in brain sections with spongiform degeneration and reactive astrogliosis of gray matter; in inherited human prion diseases linked to mutations of the PRNP gene; and in PrP knockout mice, designated Prnp<sup>-/-</sup>, that do not synthesize PrPc, do not develop neuropathologic changes, nor do they propagate scrapie activity when inoculated with these prions.

Uptake of PrPRes by neurons requires their synthesis of PrPc with subsequent conversion and/or intrinsic accumulation of PrPRes with neuronal dysfunction and death. The vacuolation of spongiform encephalopathy occurs primarily in synapses and is characterized by neuritic swelling, loss of internal organelles and accumulation of abnormal membranes. Abnormal receptor mediated ion channel function may account for these functions. Three forms of prion diseases in humans include infectious or iatrogenic CJD, inherited forms (GSS, FFI) and the more common sporadic CJD.

**Infectious (Iatrogenic/CJD)**

A peculiar bovine spongiform encephalopathy (Mad Cow Disease) was recognized in the United Kingdom in 1986 in dairy and beef cattle. This disease became an epidemic by July, 1996, when over 160,000 cattle were involved. The spongiform change was particularly severe in the brain stem but was also recognized in the cerebrum. The disorder was similar to scrapie in sheep and goats, which has been transferred to other animals through contaminated feed. Bovine spongiform encephalopathy peaked in 1992 and is decreasing currently. Based upon other iatrogenic or infectious prion diseases, including kuru, the incubation period varies from 5 to 20 years.

Over ten cases of a new variant of Creutzfeldt-Jakob disease (CJD) in the United Kingdom were related to bovine spongiform encephalopathy, which had a greater incidence in dairy cattle than beef cattle, consistent with ingestion of scrapie contaminated meat and bone meal. Dairy cattle commonly were fed commercial concentrate feeds, whereas beef cattle usually grazed in fields. The source of the contamination was traced to a food supple-
ment from sheep and cattle offal in the late 1970s. Prior to the 1970s, techniques used to prepare animal renderings inactivated the scrapie agent; new procedures did not. In 1988, the British government banned animal derived feed supplements, and the epidemic has declined.

This variant CJD in the United Kingdom was observed in individuals under 40 years, including teenagers with dysesthesia or behavioral changes lasting 7 to 30 months. The neuropathological alterations in these patients were similar, and many kuru-type amyloid plaques were seen in the cerebrum and cerebellum surrounded by spongiform degeneration. No patient had a pathogenic mutation of the PRNP gene, and all were homozygous for methionine at residue 129, which seemed to predispose individuals to this disease. Some local environmental factor was postulated in view of the absence of pathogenic mutations in the prion gene as well as the absence of any relationship between victims who lived in the United Kingdom. Heterozygosity at codon 129 seemed to provide some partial protection against prion infection.

**Sporadic and Familial CJD**

CJD is the most common form of transmissible spongiform encephalopathy which affects one person per one million population. This sporadic infection accounts for 85 percent of all CJD cases compared to hereditary cases, which account for 10 percent (1 person per 10 million population), and infectious or iatrogenic cases in only 5 percent. Familial CJD is associated with at least 11 mutations of the PRNP gene but are much rarer than the sporadic variety. Sporadic CJD affects males and females equally. Peak age of onset is about 60 years, with a range of 40 to 90 years. Rarely, a sporadic CJD victim may be 20 years of age. Typically, the disease runs a rapid course, leading to death within 4 to 12 months; occasionally, victims survive 2 to 5 years. Familial CJD cases tend to present at an earlier age than sporadic CJD, with onset averaging 55 years, and has been linked to octapeptide inserts in the PRNP gene, with onset at 23 to 35 years of age, followed by a long progression of over 4 to 13 years to death.

All forms of CJD [infectious (iatrogenic), familial and sporadic] manifest as fatigue, sleep disturbances, memory disturbances, behavioral changes, vertigo and ataxia. Characteristically, the clinical course is rapid with mental deterioration, dementia, myoclonus and widespread motor disturbances, including extrapyramidal, cerebellar, pyramidal and/or anterior horn cell lesions. The EEG reveals periodic short wave activity. The diagnosis of CJD is relatively certain when dementia, myoclonus, periodic EEG activity and rapid progression are present. Variable degrees of atrophy may involve the cerebral cortex, striatum and/or cerebellum. Vacuolization, astroglisis and neuronal loss may involve the cortex, corticostriatum with or without visual loss, corticostriatocerebellum, corticospinal tracts and corticonigral area. Additional manifestations include visual alterations, sensory deficits and nystagmus.

**Pathology**

The spongiform changes manifested by vacuoles tend to be round or oval and vary from 5 to 25 micra (figure 1). They may be associated with reactive astrocytosis (status spongiosis). This degeneration is found predominantly in the gray matter throughout the cerebrum and basal nuclei. Minimal changes are seen in the brain stem and spinal cord. The spongiform degeneration consists of focal swelling of neuritic processes and synapses, with loss of internal organelles and accumulation of lacy abnormal membranes. Vacuolization of nerve cell bodies has been recognized. Lesions in white matter in CJD and other prion diseases are secondary to neuronal loss. Nevertheless, a vacuolar myelopathy is found in some CJD victims. Amyloid plaques with prion particles are seen in only 5 to 10 percent of CJD cases. They are immuno-positive with the PrP antibodies but negative with antibodies to β amyloid. The definitive diagnosis of CJD requires autopsy verification and features of one of the following four criteria: (1) pres-
ence of PrP amyloid plaques; (2) transmission of spongiform encephalopathy to animals; (3) presence of PrP<sup>CJD</sup>; or (4) presence of a pathogenic PRNP gene mutation.

**Laboratory Approach**<sup>1,5,6,19,21,29,31–33</sup>

Although CJD is a transmissible infection, it is not contagious. Victims should not be denied basic services, autopsy examination or even burial. A 25 percent error rate is seen in clinical diagnosis. Infectious prions create a disease primarily found in the central nervous system and eye. Highest levels of infectivity are observed in the brain, spinal cord and eyes, based on rates of transmission to non-human primates. Nevertheless, CJD has been transmitted less consistently from cerebro-spinal fluid, lung, liver, kidney, spleen and lymph nodes. The infectious prions can accumulate in connective tissues associated with the nervous system, including the dura and cornea. There is no evidence that a human prion disease can be transmitted from exposure to breath, saliva, nasopharyngeal secretions, urine, feces or blood. CJD is not a significant health risk to medical personnel or to family and friends of victims. The incidence of CJD among health care workers is the same as the general population. Twenty-four health care workers with CJD included non-surgical physicians, neurosurgeons, a pathologist, dentists, a dental surgeon, nurses, nursing assistants and histopathology technicians. Recurrence of CJD among spouses is exceedingly rare, with only one reported case, and the risk of acquiring CJD by friends or family is no greater than the population at large.

Prion infectivity is influenced by the route of infection. Transferring prions to animals using scrapie infected brain tissue is highest for intracerebral inoculations, whereas it is reduced significantly for intraperitoneal inoculations and oral ingestion. In handling surgical and autopsy materials from CJD cases, caution should be taken to avoid percutaneous exposure, particularly during dissection of brain, spinal cord and reticuloendothelial organs such as the spleen and lymph nodes. Universal precaution should be used routinely for handling any human fluids or tissues. Contamina-
tion of working surfaces and instruments with prions can be effectively disinfected. Solid waste and instruments can be autoclaved for 4.5 hours at 132°C. Liquid waste can be mixed 1 to 1 with 2N sodium hydroxide and then autoclaved for 4.5 hours at 132°C. Spills can be soaked in sodium hydroxide three times for 30 minutes each. Formalin fixation does not destroy prion infectivity; however, all residual infectivity of CJD and scrapie prions in formalin fixed tissue is eliminated if the tissue is immersed for one hour in formic acid followed by further fixation in formalin. There is no evidence for an increased incidence of CJD among families, friends, health care providers, or laboratorians when dealing with patients with sporadic or familial forms of CJD.

References