Review: pathology of variant Creutzfeldt-Jakob Disease

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Variant Creutzfeldt-Jakob disease (vCJD) is a novel human prion disease that results from exposure to the bovine spongiform encephalopathy (BSE) agent, probably by the oral route. The pathological features of vCJD are unique, with extensive involvement of lymphoid tissues in addition to the central nervous system. This article reviews the histopathology and biochemistry of vCJD, emphasising diagnostic features and indicating several areas of active research. The widespread distribution of infectivity in lymphoid tissues in vCJD has lead to concerns over the possibility of iatrogenic disease transmission by contaminate surgical instruments, or by blood transfusion. VCJD has so far only occurred in individuals within a genetic subset defined by the natural polymorphism at codon 129 in the prion protein gene. It remains uncertain if this disease will occur in other genetic subgroups within the population. Continuing surveillance of vCJD ain the UK and other countries in which BSE has been identified will be necessary for future estimations of disease numbers worldwide.

key words: neuropathology, variant CJD, prion protein, immunocytochemistry, morphometry, florid plaque, biochemistry

INTRODUCTION

Prion diseases are fatal neurodegenerative disorders occurring in mammals, including scrapie in sheep and goats, bovine spongiform encephalopathy (BSE) in cattle, chronic wasting disease in deer and elk and Creutzfeldt-Jakob disease (CJD) in man [19, 27]. These diseases are transmissible and are associated with the conversion of the normal isoform of prion protein (PrP\text{C}) in the brain to an abnormal disease-associated isoform (PrP\text{Sc}). The prion hypothesis states that the transmissible agent or prion in these disorders is composed entirely of PrP\text{Sc} and is devoid of nucleic acid [see 27 for review].

An ever-widening spectrum of human prion diseases has been reported since CJD was initially described in the 1920s (Table 1) [19]. This includes sporadic, familial and acquired diseases, the commonest of which is sporadic CJD. The naturally occurring polymorphism at codon 129 of the prion protein gene (PRNP) influences susceptibility to sporadic CJD (Table 2). In comparison with normal population there is an excess of methionine homozygotes at codon 129 in the PRNP in sporadic CJD, with a reduction in the percentage of heterozygotes [18, 26]. The neuropathological phenotype of sporadic CJD is variable and appears to be influenced by the isotype of PrP\text{Sc} in the brain as determined by Western blotting studies and the PRNP codon 129 genotype [20, 26].

Surveillance of Creutzfeldt-Jakob disease (CJD) was reinstated in the United Kingdom in 1990 following the identification of BSE in cattle. BSE was first reported in 1987 in the UK [33], and was spread by contaminated meat and bonemeal animal feed [34]. A ban on the use of this feed allowed the disease to come under control, but it has still not been eradicated. Around 180,000 clinical cases of BSE have been identified in the UK [10], but the total number of infections (including ani-
mals slaughtered in the preclinical stage of the illness) is likely to have been much higher [1]. Until the identification of BSE, there was no evidence that other prion diseases occurring in animals (particularly scrapie in sheep) were pathogenic to humans. However, the observation that BSE can be transmitted by the oral route [10] has renewed concerns that it might represent a hazard to human health by the consumption of BSE-contaminated meat products.

In the UK, all cases of suspected CJD are reported to the National CJD Surveillance Unit in Edinburgh by clinicians and pathologists. These cases are investigated and subject to detailed clinical and neuropathological assessment whenever possible.

**Neuropathology of human prion diseases**

Human prion diseases are characterised histologically by spongiform change, neuronal loss, glial proliferation, and (in some cases) amyloid plaque formation in the brain [18]. Since the identification of PrPSc in the brain in prion diseases, a histological diagnosis can be confirmed by using techniques to identify this abnormal protein. Western blot analysis on fresh or frozen brain tissues and immunocytochemistry on paraffin sections of the brain are the commonest techniques used to identify PrPSc, and these have been supplemented recently by the PET blot technique [6, 26, 29]. Since most of the available antibodies to PrP recognise both the normal and abnormal isoforms of the protein, a limited protease digestion (usually with Proteinase K) is required to degrade PrPC, leaving the partially digested PrPSc to react with the antibody [20, 26].

Immunocytochemistry has enabled the detection of numerous different patterns of PrP accumulation in the brain [6], which have enabled the identification of different pathological subtypes of sporadic CJD [26]. In most human prion diseases there is little evidence that PrPSc is present in tissues outside the brain, although this has been recently observed in the spleen and skeletal muscle in a subset of patients with sporadic CJD [12]. This is in contrast to other prion diseases, particularly scrapie in sheep, where PrP accumulation and infectivity in lymphoid tissues is readily detectable [17], and is important in relation to the pathology of variant CD (see below).

**Neuropathology of variant CJD**

In 1996, the National CJD Surveillance Unit in the UK described a novel form of human prion disease in series of 10 patients; this disease has subsequently become known as variant CJD [35]. By the end of January 2004, 146 cases of variant CJD had been identified in the UK on the basis of clinical criteria and/or neuropathology. In contrast to sporadic CJD, the neuropathological features of variant CJD are relatively uniform. These features are summarised in Table 3 and discussed in detail below.

**Macroscopic features**

Macroscopic examination of the brain in variant CJD usually shows no specific abnormalities. Cerebral and cerebellar cortical atrophy are common in cases with a prolonged clinical history (2 years or longer), but these

**Table 1. Classification of human prion diseases**

<table>
<thead>
<tr>
<th>Type</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Idiopathic</td>
<td>Sporadic Creutzfeldt-Jakob disease</td>
</tr>
<tr>
<td></td>
<td>Sporadic fatal insomnia</td>
</tr>
<tr>
<td>Inherited</td>
<td>Familial Creutzfeldt-Jakob disease</td>
</tr>
<tr>
<td></td>
<td>Gerstmann-Sträussler-Scheinker syndrome</td>
</tr>
<tr>
<td></td>
<td>Fatal familial insomnia</td>
</tr>
<tr>
<td>Acquired</td>
<td>Human source:iatrogenic Creutzfeldt-Jakob disease</td>
</tr>
<tr>
<td></td>
<td>Bovine source: Variant Creutzfeldt-Jakob disease</td>
</tr>
</tbody>
</table>

**Table 2. Codon 129 PRNP polymorphisms in CJD and normal Caucasian population**

<table>
<thead>
<tr>
<th>Codon 129 polymorphism</th>
<th>Methionine/methionine</th>
<th>Methionine/valine</th>
<th>Valine/valine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>37%</td>
<td>51%</td>
<td>12%</td>
</tr>
<tr>
<td>Sporadic CJD</td>
<td>71%</td>
<td>15%</td>
<td>14%</td>
</tr>
<tr>
<td>Variant CJD</td>
<td>100%</td>
<td>–</td>
<td>–</td>
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</tbody>
</table>

**Table 3. Pathological diagnostic features of variant CJD**

1. Multiple florid plaques in H&E sections; numerous small cluster plaques in PrP stained sections
2. Amorphous pericellular and perivascular PrP accumulation in the cerebral and cerebellar cortex
3. Severe spongiform change; perineuronal and axonal PrP accumulation in the caudate nucleus and putamen
4. Marked astrocytosis and neuronal loss in the posterior thalamic nuclei and midbrain
5. Reticular and perineuronal PrP accumulation in the grey matter of the brainstem and spinal cord
6. Predominance of di-glycosylated PrPRES in central nervous system and lymphoid tissues
7. PrPRES accumulation in germinal centres within lymphoid tissues throughout the body

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features may be absent in cases with a short clinical duration of illness [21]. The subcortical white matter, hippocampus, basal ganglia, thalamus, hypothalamus, brain stem, spinal cord and cranial nerves show no noticeable features. The ventricular system in cases with cerebral cortical atrophy may show compensatory dilatation, but this is usually only a mild to moderate degree.

**Microscopic features**

Spongiform change occurs in a microvacuolar pattern in a widespread distribution within the cerebral cortex, often in relation to amyloid plaques. The occipital cortex is often most severely affected. Although the entorhinal cortex shows patchy microvacuolar spongiform change, this is seldom detected in the hippocampus. In contrast, the caudate nucleus and putamen are severely affected by confluent spongiform change, which is not related to the number of amyloid plaques in these nuclei. Focal spongiform change is present in the globus pallidus, hypothalamus and most of the thalamic nuclei, but the posterior thalamic nuclei (including the pulvinar) are spared. Mild spongiform change is detected in the periaqueductal grey matter in the midbrain and in the pontine nuclei. The cerebellar cortex is variably affected by spongiform change, which is occasionally confluent and often associated with amyloid plaques.

Neuronal loss in the cerebral cortex is most severe in the primary visual cortex within the occipital lobe. In cases with a lengthy clinical history there is a severe loss of neurones and astrocytosis throughout the cerebral cortex, but the hippocampus is relatively well preserved. Neuronal loss in the basal ganglia is most evident in cases with severe and confluent spongiform change. In the thalamus, astrocytosis and neuronal loss are most severe in the posterior nuclei, particularly in the pulvinar [36]. Neuronal loss and astrocytosis are not conspicuous in the brainstem and spinal cord, but are variable in the cerebellum, and usually most severe in the vermis.

Perhaps the most distinctive histological feature of variant CJD is the presence of large fibrillary amyloid plaques in the cerebral and cerebellar cortex. These are known as florid plaques (Fig. 1A), which are defined as a fibrillary amyloid structure with a dense core surrounded by a pale region of radiating fibrils, and surrounded by spongiform change in an otherwise intact neuropil [35]. These plaques can also be identified using periodic acid/Schiff and Alcian blue stains and are particularly well visualised by the Gallyas silver impregnation technique [21]. Florid plaques occur in all layers of the cerebral cortex, but are most conspicuous at the bases of the gyri. They tend to be most numerous in the occipital and cerebellar cortex (in the molecular layer). Similar plaques can usually be identified in the granular layer of the cerebellum as aggregates without surrounding spongiform change in the granular layer.

Ultrastructural studies of the amyloid plaques in variant CJD have shown masses of radiating fibrils at the periphery of the plaques, with abnormal neurites similar to those seen in Alzheimer's disease [22]. However, neurofibrillary tangles and paired helical filaments have not been identified in variant CJD, and immunocytochemistry for tau gives negative results. Immunocytochemistry has shown the PrP accumulation at the ultrastructural level in both in the amyloid fibrils and in some of the abnormal cell membranes surrounding the plaques [11].

**Immunocytochemistry**

Immunocytochemistry for PrP gives an intense positive reaction on the florid plaques in the cerebral (Fig. 1B) and cerebellar cortex [21]. Smaller “cluster plaques” (which cannot be identified in sections stained by haematoxylin and eosin) are revealed by immunocytochemistry for PrP in all cases. PrP immunocytochemistry also shows a widespread amorphous pericellular deposition of PrP around glial cells and small neurones in the cerebral and cerebellar cortex.

In the basal ganglia there is a predominantly perineuronal pattern of PrP accumulation. A synaptic pattern of immunoreactivity with occasional plaques was detected in the thalamus, but the linear pattern of PrP accumulation was absent. In the hippocampus, there is a dense synaptic accumulation in the dentate fascia, subiculum and entorhinal cortex. PrP positivity is present in the brainstem and spinal cord at all levels in the grey matter, particularly in the substantia gelatinosa. The leptomeninges (including the arachnoid granulations) and dura mater show no evidence of PrP accumulation on immunocytochemistry.

Immunocytochemistry for glial fibrillary acidic protein shows the severe astrocytosis in the posterior thalamus (Fig. 1C) [36]. This technique also showed astrocyosis in relation to areas of severe neuronal loss and less frequently around the margins of amyloid plaques in other brain regions.

**Quantitative pathology in variant CJD**

Quantitative studies on the first cases of variant CJD confirmed that the measurable histological accumulation of abnormal PrP deposits in the cerebellum was far greater than in sporadic CJD cases [22]. The severe gliosis in the posterior thalamus was also demonstrated, with measured levels of astrocytosis far in excess of sporadic CJD cases [22]. Subsequent quantitative studies have shown that the relationship be-
between the spongiform change and the presence of PrP amyloid plaques varies in different brain regions in variant CJD [3, 4]. Analysis of the spatial patterns of abnormal PrP deposition in variant CJD has found no significant differences between different regions of the cerebral cortex [5]. There is the prospect of developing textural analysis techniques to investigate the differences in patterns of abnormal PrP deposition in the brain in variant CJD [25].

**Non-CNS tissues**

PrP accumulation is identified in the retina and optic nerve, spinal dorsal root ganglia and in the trigeminal ganglia in variant CJD, but peripheral sensory and motor nerves contain no detectable PrP [13, 14]. The pineal and the posterior pituitary gland show synaptic positivity for PrP and contain a few small plaques, but the anterior pituitary gland shows no PrP accumulation. PrP immunocytochemistry in other organs (adrenal gland, thyroid gland, parathyroid gland, skeletal muscle, bladder, testes, female pelvic organs, heart, lung, liver, kidney, oesophagus, stomach, pancreas, gall bladder, salivary gland and skin) is negative [14, 20, 21].

In contrast, PrP accumulation is identified in follicular dendritic cells and macrophages within many germinal centres in the tonsils (Fig. 1D) and within germinal centres in the appendix, Peyer’s patches in the ileum, spleen and lymph nodes from the cervical, mediastinal, para-aortic and mesenteric regions and the thymus [16, 20, 21]. A recent quantitative study of PrP accumulation in lymphoid tissues in variant CJD indicated that the tonsil and lymph nodes are most likely to contain a high percentage of PrP-positive germinal centres than the spleen or gut-associated lymphoid tissues [14].

**Figure 1A.** A florid plaque in the occipital cortex in a patient with a 20-month clinical history of variant CJD. The plaque has a dense eosinophilic core with a pale fibrillary periphery and is surrounded by a rim of spongiform change; **B.** Immunocytochemistry for PrP in the occipital lobe in variant CJD (same case as Fig. 1A) shows intense labelling of the florid plaques, but also (KG9 anti-PrP antibody); **C.** Widespread astrocytosis in the pulvinar is characteristic of variant CJD, with no amyloid plaque formation and relatively little spongiform change (immunocytochemistry for glial fibrillary acidic protein); **D.** Immunocytochemistry for PrP in the tonsil shows labelling of follicular dendritic cells within a germinal centre (KG9 anti-PrP antibody).
Biochemistry

Biochemical analysis of PrP\textsuperscript{Sc} by Western blot can be used to classify CJD by comparing the relative abundance of the non-glycosylated, mono-glycosylated and di-glycosylated portions of PrP\textsuperscript{Sc} and determining the mobility of the non-glycosylated portion [21, 26]. In sporadic CJD, two major PrP\textsuperscript{Sc} isoforms have been described [26]; the non-glycosylated fragment of type 1 has a molecular weight of 21 kDa (termed type 1), or 19 kDa (termed type 2). The type 2 pattern is further sub-classified as 2A if the middle (monoglycosylated) or bottom (nonglycosylated) bands predominate or type 2B if the top (*, diglycosylated) band predominates.

Discussion

Variant CJD is a unique human prion disease because it results from an acquired infection from a non-human species [8, 9, 19, 30]. It is also distinct from other human prion diseases in terms of the widespread distribution of the infectious agent in the body. PrP\textsuperscript{Sc} was identified by immunocytochemistry and Western blot examination in lymphoid tissues in variant CJD, and experimental transmission studies have recently confirmed that infectivity is present in these tissues, although at levels which are around 2–3 logs lower than in the brain [7]. This has caused concerns that variant CJD may be transmitted accidentally, by surgical instruments used on lymphoid tissues (such as in tonsillectomy procedures), or by blood transfusion or blood products [32]. The concerns over potential infectivity in blood in variant CJD were reinforced by the experimental transmission of BSE by blood transfusion in a sheep model, at a preclinical stage in the infection [17]. More recently, a case of variant CJD has been identified in the UK in an individual who received blood from a donor who had died from variant CJD, representing the first possible case of “iatrogenic” variant CJD [23]. Given the uncertainties over the likely incubation period for variant CJD, it is too early to conclude that further iatrogenic transmissions of this disease are unlikely to occur.

The diagnostic pathological features of variant CJD are summarised in Table 3. It is important to note that florid plaques alone are not diagnostic of variant CJD; florid PrP amyloid plaques have been described in cases of iatrogenic CJD following dura mater graft procedures [31], although the number and distribution of these lesions in the brain is more restricted than in variant CJD. In contrast to sporadic CJD, where multiple PrP\textsuperscript{Sc} isoforms have been identified, and can co-exist even within a single case [28], the biochemical features of PrP\textsuperscript{Sc} in the brain in variant CJD on Western blot examination are relatively uniform [20]. However, a similar PrP\textsuperscript{Sc} isotype has been identified in a recent atypical case of sporadic CJD [15]. This reinforces the need for detailed characterisation of human prion diseases by clinical, pathological and biochemical studies. This approach was undertaken on the first cases of variant CJD, and allowed the early recognition of the link between BSE and variant CJD [8, 9, 30]. As BSE continues to spread across the world, it can be anticipated that future cases of variant CJD will be identified in other countries.

Prediction of the future numbers of variant CJD cases in the UK and elsewhere remains difficult because of the uncertainties concerning the number of individuals incubating the disease and the likely incubation period. Although there was earlier evidence of an increase in the incidence of the disease in the UK, this has not been sustained, and the rate of increase in the number of cases appears to be declining [2]. Continued surveillance for all forms of CJD is required to answer these questions, and neuropathology is a key part in the investigation and characterisation of such cases.
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REFERENCES


