Specificity of lymphoreticular accumulation of prion protein for variant Creutzfeldt–Jakob disease


Background: Immunocytochemical accumulation of prion protein (PrP) in lymphoid tissues is a feature of variant Creutzfeldt–Jakob disease (vCJD) that has been used both to aid in the diagnosis of patients and as a basis of large scale screening studies to assess the prevalence of preclinical disease in the UK. However, the specificity of this approach is unknown.

Aim: To assess the specificity of lymphoreticular accumulation of PrP for vCJD by examining a range of human diseases.

Methods: Paraffin wax embedded lymphoreticular tissues from patients with several reactive conditions (58 cases), tumours (27 cases), vCJD (54 cases), and other human prion diseases (56 cases) were assessed. PrP accumulation was assessed by immunocytochemistry using two different monoclonal anti-PrP antibodies and a sensitive detection system.

Results: All cases of vCJD showed widespread lymphoreticular accumulation of PrP; however, this was not seen in the other conditions examined.

Conclusion: Lymphoreticular accumulation of PrP, as assessed by immunocytochemistry, appears to be a highly specific feature of vCJD.

Prion diseases, also known as transmissible spongiform encephalopathies, are fatal neurological disorders characterised by the accumulation of a modified host protein, prion protein (PrP), in the central nervous system. These diseases are transmissible both under natural conditions and experimentally, although the precise nature of the transmissible agent remains unclear. Infectivity is closely associated with an abnormal disease associated isoform of PrP (PrPSc), which is postulated to be the sole component of the infectious agent in the prion hypothesis. PrPSc differs from its normal isoform, PrPC, in its increased β sheet content, which renders it relatively resistant to proteolytic degradation, and allows it to accumulate as amyloid plaques in some forms of prion disease, including variant Creutzfeldt–Jakob disease (vCJD). Human prion diseases occur in three main groups: (1) sporadic CJD; (2) familial diseases including familial CJD disease; fatal familial insomnia, and the Gerstmann–Straussler Scheinker syndrome; and (3) acquired disorders including kuru, iatrogenic CJD, and vCJD. vCJD is the only example of a prion disease to humans from another species: there is a large body of evidence to indicate that the transmissible agent in vCJD is identical to its properties to the agent responsible for bovine spongiform encephalopathy (BSE). This transmission is likely to have occurred by the oral route (consumption of BSE contamined meat products).

"PrPSc differs from its normal isoform, PrPC, in its increased β sheet content, which renders it relatively resistant to proteolytic degradation"
human prion disorders, using identical immunocytochemical techniques to those used in the screening studies. 15 16

MATERIALS AND METHODS

Tissue samples

Archival paraffin wax embedded tissue samples from a range of reactive and neoplastic conditions involving lymphoreticular tissue were retrieved from the archives of the pathology departments in Plymouth, UK and Pittsburgh, USA. All such cases from the UK were pre-1986, to ensure that the individuals had not been exposed to BSE contaminated food products. In total, 58 reactive lymph nodes were assessed, which included cases of probable tuberculosis, sarcoid, toxoplasmosis, and cat scratch disease. Thirteen cases of human immunodeficiency virus infection were examined; these were all serologically confirmed and consisted of seven surgically removed tonsils and lymph nodes and six postmortem lymph nodes and spleens, including one case with additional cytomegalovirus infection. A range of 21 lymphomas and six carcinomas, metastatic to lymph nodes, was also examined. All cases were reviewed, and lymphomas were classified using current World Health Organisation guidelines. 17 In addition, paraffin wax embedded samples of spleen and other lymphoid tissues obtained after necropsy were studied in 54 cases of vCJD, 53 cases of sporadic CJD, and three cases of familial prion disease from the archives of the National CJD Surveillance Unit in Edinburgh, UK, some of which had also been assessed by western blot examination for PrP Sc. 5

Immunocytochemistry

Sections (4 μm thick) were cut from paraffin wax tissue blocks. Sections were pretreated by autoclaving at 121 °C for 10 minutes, followed by immersion in 96% formic acid for five minutes and digestion with proteinase K (10 μg/ml) for five minutes at room temperature, to enhance PrP Sc detection and reduce PrP C detection. PrP was detected with the monoclonal antibodies 3F4 (Dako, Ely, Cambridgeshire, UK) and KG9 (IAH, TSE Resource Centre, Newbury, Berkshire, UK), and visualised using the CSA amplification system (Dako), which gives superior results in terms of sensitivity to most other immunohistochemical detection systems. 18 Postmortem tonsil tissues from confirmed cases of vCJD were used as a positive control for each group of slides stained by immunocytochemistry for PrP. Western blot analysis for protease resistant PrP was performed in cases of vCJD and sporadic CJD where frozen samples of lymphoid tissues were available, according to a previously published protocol. 19 Tissues from known patients with CJD were handled in accordance with Department of Health guidance, which allow for derogation from category 3 containment (www.doh.gov.uk/cjd/tseguidance).

RESULTS

All cases of vCJD showed lymphoreticular PrP accumulation by immunocytochemistry with both anti-PrP antibodies in follicular dendritic cells and macrophages (fig 1A). In the nine cases assessed by western blot analysis, all showed detectable protease resistant PrP Sc in one or more of the lymphoreticular tissues that were available for assay.

Of the remaining non-vCJD cases, all were negative with both anti-PrP antibodies by immunocytochemistry (fig 1B).

Table 1 provides a summary of non-vCJD case details. Samples were anonymised before analysis. Local ethical committee approval was obtained for this work.

Table 1 Conditions other than variant Creutzfeldt-Jakob disease (vCJD) assessed

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactive conditions</td>
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<td>Non-specific lymphoid hyperplasia</td>
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</tr>
<tr>
<td>Non-necroasing granulomatous inflammation</td>
<td>6</td>
</tr>
<tr>
<td>Necroaising granulomatous inflammation</td>
<td>9</td>
</tr>
<tr>
<td>Toxoplasmosis pattern</td>
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</tr>
<tr>
<td>Cat-scratch pattern</td>
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</tr>
<tr>
<td>Dermatopathic lymphadenopathy</td>
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<tr>
<td>Human immunodeficiency virus infection</td>
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<tr>
<td>Tumours</td>
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<tr>
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<tr>
<td>Diffuse large B cell lymphoma</td>
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<tr>
<td>Angioimmunoblastic T cell lymphoma</td>
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<tr>
<td>Adenocarcinoma</td>
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</tr>
<tr>
<td>Carcinoma not otherwise specified</td>
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<tr>
<td>Prion disease</td>
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<td>Familial CJD</td>
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<tr>
<td>Total</td>
<td>141</td>
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</table>

Figure 1 (A) Postmortem tonsil from a case of variant Creutzfeldt-Jakob disease showing prominent granular immunoreactivity in follicular dendritic cells and macrophages, following immunocytochemistry with the monoclonal anti-prion protein (PrP) antibody KG9. (B) Reactive lymphoid follicle from a case of cat scratch disease, showing an absence of PrP immunoreactivity.
Take home messages

- Lymphoreticular accumulation of prion protein (PrP) as assessed by immunocytochemistry, appears to be a highly specific feature of variant Creutzfeldt–Jakob disease (vCJD).
- PrP was not found in the other tissues tested: a variety of reactive conditions and tumours involving lymphoreticular tissue, and other human prion diseases.

DISCUSSION

We have confirmed previous observations that lymphoreticular PrPSc accumulation is a consistent and widespread finding in vCJD, occurring in tonsils, spleen, lymph nodes, appendix, and ileum. None of the 56 cases of familial and sporadic CJD showed lymphoreticular PrPSc accumulation, in keeping with two previous studies that investigated a total of 35 prion disorders, including sporadic CJD, iatrogenic CJD, and familial prion disease, all of which were negative. Therefore, these findings suggest that lymphoreticular PrPSc accumulation is a specific feature of vCJD in human prion diseases; the reasons for this are unclear. It is possible that a combination of factors such as the strain type, oral exposure, and crossing the species barrier predispose to lymphoreticular PrPSc accumulation in humans (although it does not appear to be a consistent feature of BSE related diseases in other species).

"The absence of positive cases indicates that PrPC does not accumulate in lymphoid tissues as a "non-specific" reaction to immunological stimulation (as it may do in the brain after oxidative cell stress)."

Screening studies for preclinical scrapie and vCJD have used immunocytochemistry to demonstrate lymphoreticular PrPSc accumulation. Although published data indicate that the PrP immunoreactivity in lymphoreticular tissues in vCJD represents protease resistant PrPSc, and is transmissible, the specificity of immunocytochemistry in the context of large scale screening studies of human tissue samples is unclear. None of the currently commercially available antibodies can reliably differentiate PrP from PrPSc, so it is possible that they could also detect PrPSc accumulation in non-prion disorders, resulting in a "false positive". In addition, immunocytochemical accumulation of PrP in tonsil, and other lymphoid tissues, has been used as an aid to diagnose vCJD during life and at necropsy. Therefore, we have systematically examined a range of reactive and neoplastic non-prion disorders for evidence of PrPSc accumulation in lymphoid tissues. These samples were chosen because the positive sample in our screening study showed PrP accumulation within follicular dendritic cells in secondary follicles, and we wanted to examine the possibility that a reactive condition could cause upregulation of PrP in these cells by examining a wide range of conditions that are known to be associated with prominent follicular dendritic cell networks. The absence of positive cases indicates that PrPSc does not accumulate in lymphoid tissues as a "non-specific" reaction to immunological stimulation (as it may do in the brain after oxidative cell stress). It is clearly impossible to look for PrPSc accumulation in all diseases, and although our study has only investigated a relatively small number of conditions, the findings suggest that lymphoreticular accumulation of PrP is a specific feature of vCJD.

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Refereces