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.

Prior to the identification of the infectious agent in the prion hypothesis,12 the accumulation of PrPSc in lymphoid tissues has been used to diagnose preclinical vCJD, together with the availability of large tissue archives of surgically removed lymphoid tissue, has allowed national screening of appendix and tonsil samples to look for PrPSc accumulation as an estimate of the number of individuals who may be incubating vCJD in the UK.12 In the absence of a blood based test for vCJD, such estimates are the only means of determining the potential risk of iatrogenic spread of vCJD via blood products, organ transplants, or surgical instruments.12 Anti-PrP antibodies that can distinguish PrPSc from PrPC would be extremely useful in this regard. Two groups have reported the development of PrPSc specific antibodies, but these are not yet commercially available, and their selective reactivity appears to be restricted to interactions with native PrP, thus limiting their application to techniques such as immunoprecipitation followed by western blotting.14 Ethical constraints require screening studies to be carried out in an anonymised manner,12 so that positive cases cannot be identified to determine whether they develop vCJD.

Although the absence of lymphoreticular accumulation of PrP has been previously demonstrated in postmortem tissues from a range of neurological disorders,2 a systematic study of its specificity has not been published. To assess further the specificity of lymphoreticular PrP accumulation for vCJD we have investigated a range of reactive and neoplastic conditions, most of which are known to be associated with prominent follicular dendritic cell networks, in addition to vCJD.3–7 Animal models suggest that PrPSc accumulates in lymphoreticular tissues early on in the disease process,4 reliably predicting future neurological disease,5–7 and the accumulation of PrPSc has been used to diagnose prion scrapie, with an estimated sensitivity and specificity of greater than 90%.10 The lymphoreticular accumulation of PrPSc in the preclinical phase of vCJD,11 together with the specificity of lymphoreticular accumulation of PrPSc in the preclinical phase of vCJD, together with the availability of large tissue archives of surgically removed human tissue, has allowed national screening of appendix and tonsil samples to look for PrPSc accumulation as an estimate of the number of individuals who may be incubating vCJD in the UK.12 In the absence of a blood based test for vCJD, such estimates are the only means of determining the potential risk of iatrogenic spread of vCJD via blood products, organ transplants, or surgical instruments.12 Anti-PrP antibodies that can distinguish PrPSc from PrPC would be extremely useful in this regard. Two groups have reported the development of PrPSc specific antibodies, but these are not yet commercially available, and their selective reactivity appears to be restricted to interactions with native PrP, thus limiting their application to techniques such as immunoprecipitation followed by western blotting.14 Ethical constraints require screening studies to be carried out in an anonymised manner,12 so that positive cases cannot be identified to determine whether they develop vCJD.

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Abbreviations: BSE, bovine spongiform encephalopathy; CJD, Creutzfeldt–Jakob disease; PrP, prion protein; vCJD, variant Creutzfeldt–Jakob disease

“PrPSc differs from its normal isofrom, 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.\textsuperscript{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.\textsuperscript{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\textsubscript{Sc}.\textsuperscript{5} Table 1 provides a summary of non-vCJD case details. Samples were anonymised before analysis. Local ethical committee approval was obtained for this work.

**Immunocytochemistry**

Sections (4 \mu m 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 \mu g/ml) for five minutes at room temperature, to enhance PrP\textsubscript{Sc} detection and reduce PrP\textsuperscript{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.\textsuperscript{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.\textsuperscript{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\textsubscript{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). Seven of the sporadic CJD cases were also examined by western blotting and these too proved uniformly negative for the available lymphoreticular tissues. The presence of follicular dendritic cells was confirmed by the identification

<table>
<thead>
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<tr>
<td>Reactive conditions</td>
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<td>Non-specific lymphoid hyperplasia</td>
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<tr>
<td>Human immunodeficiency virus infection</td>
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</tr>
<tr>
<td>Tumours</td>
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</tr>
<tr>
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<tr>
<td>Diffuse large B cell lymphoma</td>
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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.
clearly impossible to look for PrP\textsuperscript{c} 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.

**ACKNOWLEDGEMENTS**

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**REFERENCES**


**Take home messages**

- Lymphoreticular accumulation of prion protein (PrP\textsuperscript{c}), as assessed by immunocytochemistry, appears to be a highly specific feature of variant Creutzfeldt–Jakob disease (vCJD).
- PrP\textsuperscript{c} was not found in the other tissues tested: a variety of reactive conditions and tumours involving lymphoreticular tissue, and other human prion diseases of secondary lymphoid follicles or CD21 immunoreactive cells in all except seven cases (one case of granulomatous inflammation, four cases of diffuse large B cell lymphoma, and two cases of metastatic carcinoma).

**DISCUSSION**

We have confirmed previous observations that lymphoreticular PrP\textsuperscript{Sc} accumulation is a consistent and widespread finding in vCJD,\textsuperscript{1,6} occurring in tonsils, spleen, lymph nodes, appendix, and ileum. None of the 56 cases of familial and sporadic CJD showed lymphoreticular PrP\textsuperscript{Sc} 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.\textsuperscript{2,7} Therefore, these findings suggest that lymphoreticular PrP\textsuperscript{Sc} 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 PrP\textsuperscript{Sc} accumulation in humans (although it does not appear to be a consistent feature of BSE related diseases in other species).\textsuperscript{21}

"The absence of positive cases indicates that PrP\textsuperscript{c} 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\textsuperscript{16} and vCJD\textsuperscript{15} have used immunocytochemistry to demonstrate lymphoreticular PrP\textsuperscript{Sc} accumulation. Although published data indicate that the PrP immunoreactivity in lymphoreticular tissues in vCJD represents protease resistant PrP\textsuperscript{Sc},\textsuperscript{2,7} and is transmissible,\textsuperscript{12} 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\textsuperscript{c} from samples is unclear. None of the currently commercially available antibodies can reliably differentiate PrP\textsuperscript{c} from...