Cell-associated variants of disease-specific prion protein immunolabelling are found in different sources of sheep transmissible spongiform encephalopathy

Martin Jeffrey, S. Martin and L. González

Scrapie and bovine spongiform encephalopathy (BSE) are transmissible spongiform encephalopathies (TSEs) or prion diseases affecting domestic and exotic ruminants. In previous immunohistochemical studies, we have shown that different sheep TSE sources may be distinguished by both the proportion of disease-specific prion protein (PrP\text{d}) accumulation relative to different cell types in the brain (the ‘PrP\text{d} profile’) and by different labelling patterns for PrP peptide sequences within phagocytic cells. In the present study, we have further characterized the intracellular accumulation patterns of PrP\text{d} in the lymphoreticular system (LRS) and in the brain of sheep clinically affected with scrapie or BSE. BSE-infected PrP\text{ARQ/ARQ} sheep of different breeds were compared with scrapie-infected sheep of different PrP genotypes. Cases of BSE infection could be distinguished from scrapie cases by a marked reduction in labelling of PrP\text{d} containing the 84–105 amino acid residues in phagocytic cells of the LRS and in neurones and glia of the brain. These results therefore indicate that TSE agent-dependent processing of PrP in specific cell types within the brain and LRS can be used to distinguish between BSE in PrP\text{ARQ/ARQ} sheep and scrapie in sheep of several PrP genotypes. Three different N-terminal peptide antibody labelling patterns were recognized for different cell types in different tissues of BSE-infected sheep, suggesting that different truncated forms of PrP\text{d} are formed following infections with this agent strain. These variations in the cleavage sites of BSE PrP\text{d} may be due to cell-specific variation in endosomal–lysosomal digestion or to cell- and tissue-specific differences in BSE PrP\text{d} conformation.

INTRODUCTION

Scrapie is the prototype of the so-called transmissible spongiform encephalopathies (TSEs) or prion diseases, which also include bovine spongiform encephalopathy (BSE), Creutzfeldt–Jakob disease (CJD) and its BSE-linked variant form (vCJD). Ovine TSEs are characterized by the accumulation in both the central nervous system (CNS) and lymphoreticular system (LRS) of an abnormal form of the prion protein (PrP), which is formed by a post-translational modification of the host-encoded normal prion protein (PrP\text{c}). A partially protease-resistant form of PrP (PrP\text{res}) can be detected in detergent-treated extracts of TSE-infected tissues, and it generally correlates with accumulations of disease-specific forms of PrP (PrP\text{d}) demonstrated in the same tissues by immunohistochemical (IHC) methods.

TSEs are widely believed to be caused by a structurally modified isomer of PrP\text{c} designated PrP\text{sc} (Prusiner, 1982, 1999a, b). This infectious prion would acquire pathogenic and infectious properties following its conversion from the normal isof orm, but its precise nature still eludes definition. The structural and biochemical properties of PrP\text{sc} include increased β-pleated sheet content, decreased solubility and increased resistance to degradation by proteolytic enzymes. This latter property (PrP\text{res}) is widely used for diagnostic purposes and PrP\text{res} is often used as the operational definition of PrP\text{sc}. In many instances there is close correlation between PrP\text{sc} and infectivity, but sometimes infectivity and disease are associated with a PrP fraction lacking the biochemical properties of PrP\text{sc} (Lasmezas et al., 1997; Somerville et al., 1991). This has led to a modification of the prion hypothesis according to which only a subfraction of PrP\text{sc} molecules would be infectious. Nevertheless, the term prion continues to be used to describe simultaneously the causal agent and the abnormal PrP protein. Some biochemical variations of abnormal forms of PrP correlate with infections by particular TSE agent strains, but the way in which an abnormally modified host protein may code for the wide diversity of clinico-pathological phenotypes of different TSE strains remains a puzzle (Chesebro, 1998; Farquhar et al., 1998).
Strain diversity has long been recognized in experimental murine scrapie, but the extent to which strains occur in natural sheep scrapie is uncertain. Murine scrapie strains are isolated after cloning by limiting dilution in different strains of inbred mice and are principally characterized by their consistent incubation periods, patterns of vacuolation (Dickinson, 1976; Bruce & Fraser, 1991) and PrP<sub>d</sub> deposition in the brain (Bruce et al., 1976, 1989). More recently, strains or sources of other TSE agents, including those of CJD and vCJD, have been characterized by N-terminal sequencing of the PrP<sup>res</sup> fragment (Bessen & Marsh, 1994; Parchi et al., 2000) or on the biochemical profile of the PrP<sup>res</sup> fraction. These biochemical changes consist of variation in the size, electrophoretic mobility, degree of protease resistance and glycosylation ratio of PrP<sup>res</sup> (Hill et al., 1997; Kuczius & Groschup, 1999; Somerville, 1999; Somerville et al., 1997). Only limited differences in biochemical features of PrP<sup>res</sup> (Hope et al., 1999) and vacuolation profiles (Begara-McGorum et al., 2002; Ligios et al., 2002) between different sheep scrapie sources have so far been described. The biochemical properties of abnormal PrP extracted from infections by different scrapie strains generally overlap, preventing their definitive usage in strain characterization. However, in two separate studies, we have shown that IHC detection of PrP<sup>d</sup> may reveal differences between different scrapie sources. Different cellular patterns of PrP<sup>d</sup> deposition are found in the brain of sheep affected with different TSE sources, including scrapie and BSE (González et al., 2002). Moreover, differences in intracellular PrP<sup>d</sup> labelling of phagocytic cells in the LRS and CNS are found when scrapie and ovine BSE are compared (Jeffrey et al., 2001b).

Electron microscopy studies have shown that the majority of PrP<sup>d</sup> accumulation occurs at the plasmalemma of infected cells and in the adjacent extracellular space. Extracellular PrP<sup>d</sup> accumulations, including amyloid fibrils and plaques, found in rodent and sheep scrapie react with antibodies that recognize the extreme N terminus of the PrP molecule and are therefore assumed to be full-length proteins (Giaccone et al., 1992; Jeffrey et al., 1996, 1998). Some forms of PrP<sup>d</sup> accumulation are, however, intracellular and are located within lysosomes in CNS and LRS phagocytic cells of murine scrapie-infected tissues (Jeffrey et al., 1994, 2000). In sheep, these intracellular PrP<sup>d</sup> accumulations appear to lack the downstream segments closest to the signal peptide of the PrP molecule (Jeffrey et al., 2001a).

In this study, we have further compared the effects of the TSE source or agent, PrP genotype, tissue, cell type and extracellular and intracellular locations of PrP<sup>d</sup> on antibody affinity. In so doing, we have defined more accurately differences in the IHC labelling patterns of the BSE agent and experimental scrapie sources. We conclude that there are consistent differences in the truncation patterns of intracellular PrP<sup>d</sup> between ovine BSE and infections by other UK scrapie sources. These differences are not, however, constant but change with the tissue and cellular site of infection, suggesting that the processing and truncation of PrP<sup>d</sup> depends on both the infecting agent and the tissues and cells where PrP<sup>d</sup> accumulation takes place.

**METHODS**

**Animals.** A total of 60 sheep were subjected to IHC examination as described below. Details of the groups of sheep and tissues available for examination are given in Table 1.

**Table 1.** Details of the 60 sheep examined for detection of PrP<sup>d</sup> in LRS and CNS tissues

<table>
<thead>
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<th>Sheep group</th>
<th>TSE agent</th>
<th>Breed</th>
<th>PrP genotype</th>
<th>No. tested</th>
<th>Clinical disease*</th>
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<th>Brain tissue</th>
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<tr>
<td>1</td>
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<td>Romney</td>
<td>ARQ/ARQ</td>
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*Age (months) at onset of clinical disease.
†Four oral challenge and six intracerebral challenge.
‡Tonsil only examined.
for study are given in Table 1. Groups 1–3 consisted of Romney Marsh, Texel and Suffolk sheep of the PrP<sub>ARQ/ARQ</sub> genotype, orally dosed with 5 g of the same pool of BSE brains as previously reported (Jeffrey et al., 2001c); another six Romney Marsh sheep were infected intracerebrally as described elsewhere (Foster et al., 1996). Sheep of groups 4 and 5 were Cheviot and Poll-Dorset animals, also of the PrP<sub>ARQ/ARQ</sub> genotype, orally infected with a different pool of BSE brains. Groups 6–10 consisted of natural or experimental scrapie cases. Group 6 were Suffolk sheep of the PrP<sub>ARQ/ARQ</sub> genotype from a heavily infected, closed flock in Scotland; Group 7 were Shetland sheep of two PrP genotypes obtained from three farms in a confined geographical area; further details of these animals have been reported previously (Jeffrey et al., 2001a, c, 2002). Group 8 consisted of PrP<sub>VRQ/VRQ</sub> Welsh mountain sheep from a single flock in Wales. Group 9 consisted of a miscellany of natural field cases of scrapie and group 10 was composed of Cheviot sheep experimentally inoculated with SSBP/1 by the subcutaneous route, as previously described (Houston et al., 2002).

**Tissues and IHC procedure.** Brains and a range of LRS tissues, including spleen, tonsil, pre-scapular lymph node, mesenteric lymph node, retropharyngeal lymph node, mediastinal lymph node, spleen and gut-associated lymphoid tissue (mainly from the ileum and colon), were obtained from the animals described above. Tissue samples of the LRS were not available from groups 4 and 9 and CNS tissues were not available from group 2 (Table 1).

Tissues were fixed in 10% neutral phosphate-buffered formalin, trimmed, post-fixed and embedded according to standard procedures. Tissue sections (5 μm) were cut on a microtome, mounted on treated glass slides (Superfrost Plus; Menzel-Glaser) and dried overnight at 60°C. Sections were dewaxed and hydrated by routine methods and then subjected to a retrieval procedure of formic acid and autoclaving in citrate buffer antigen and to an IHC protocol for the detection of PrP<sup>d</sup>, as described previously (González et al., 2002). Briefly, endogenous peroxidase activity was quenched using 1% hydrogen peroxide in methanol. Blocking serum raised against the same species as the secondary antibody was applied for 1 h prior to overnight (16 h) application of optimally diluted primary antibody. Antigen/antibody interaction was visualized using biotinylated secondary antibody (Vector Laboratories) and ABC complex. The chromagen used was DAB. All dilutions were determined using standard checkerboard titration methods against a range of sheep scrapie- and BSE-affected control tissues. Biotinylated secondary antibodies and the ABC complex were used at 1:200.

A range of primary antibodies recognizing specific amino acid sequences of the prion protein were selected according to observations made previously (Jeffrey et al., 2001b). Most antibodies recognized segments of the N-terminal domain of the flexible tail of PrP, with two antibodies recognizing regions of the globular domain and C terminus of the protein (Riek et al., 1997). The antibodies used and the sequences to which they were raised (where known) are shown in Table 2. Primary antibodies were diluted at 1:400 (P4), 1:1000 (FH11, BG4, R145), 1:6000 (505) or 1:8000 (521, R486).

In the present study we have avoided the use of the terms PrP<sup>res</sup> and PrP<sup>d</sup> in the description of our findings, as none of the antibodies used was capable of discriminating between the normal and abnormal isoforms of PrP and also because the sheep examined were infected with either scrapie or BSE agents. We refer to positive labelling in tissue sections as disease-specific PrP accumulation (PrP<sup>d</sup>), because these immunolabelling patterns are not found in tissues of normal animals. Some PrP immunolabelling can be found in uninfected sheep brains (González et al., 2002; Ryder et al., 2001), but these patterns were not considered and are excluded from further comment in this paper. The range of PrP<sup>d</sup> immunolabelling patterns found in sheep brain has been comprehensively described (González et al., 2002; Ryder et al., 2001;

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</tr>
</tbody>
</table>

*Peptide sequence of the PrP molecule used to raise the antibody.
†Single-granule labelling of TBM.
‡Single-granule, multigranular or crescent-shaped immunolabelling of TBM (for detailed description of the morphology of PrP<sup>d</sup> accumulation, refer to the text).
Van Keulen et al., 1995). There is a detailed description of the distribution and relative intensity of neuronal labelling in different neuro-anatomical regions in González et al. (2003).

RESULTS

The detection and characteristics of PrP\textsuperscript{d} accumulation in the LRS and CNS of scrapie- and BSE-affected sheep is shown in Table 2.

LRS

Follicular dendritic cells (FDCs). Within the light zone of secondary follicles, PrP\textsuperscript{d} accumulation associated with FDC processes was observed with all N- and C-terminal antibodies in both scrapie-affected (Fig. 1) and BSE-affected (Fig. 2) sheep. The intensity of FDC labelling was variable across different follicles of the same or different lymph nodes, but was maintained across the different antibodies. Within this series, FDC labelling of lymph nodes of BSE-infected sheep was often less intense than in scrapie-affected animals.

Tingible body macrophages (TBMs). Aggregates of PrP\textsuperscript{d} were observed in TBMs within the light zones of lymphoid follicles in both scrapie and BSE cases. In scrapie-affected sheep, the morphology of those deposits was different when labelled with N-terminal antibodies (i.e. FH11, BG4, P4, 521 and 505) than when C-terminal antibodies (i.e. R145, R486) were employed. Single, intensely labelled and widely separated granules were seen within TBMs with each of the N-terminal antibodies, while multiple small granules were observed with C-terminal antibodies (Fig. 1). The labelled granules could be clearly associated with TBMs because of the co-localization of pyknotic nuclear remnants within the cytoplasm of such cells. In BSE-infected sheep, labelling with each of the same five N-terminal antibodies showed either single granules (Fig. 2) as in scrapie cases or multiple small granules as with C-terminal antibodies. In addition, a curvi-linear pattern of labelling was observed, often arranged in a crescent around the nucleus (Fig. 3), but never extending to involve large parts of the cytoplasm. These three patterns often co-existed within the same lymph node, but individual follicles would usually show only one of them (Figs 2 and 3). For example, a single follicle might show a curvi-linear pattern when labelled with the five antibodies, whereas other follicles within the same node might show only single granule labelling of TBMs.

As within the light zones, intense multigranular labelling of TBMs was observed in the dark zones of secondary follicles in both scrapie-infected (Fig. 1) and BSE-infected (Fig. 2) sheep when antibodies to the C terminus were applied. Granules were numerous and occupied most or all of the cytoplasm and often obscured part of or the entire nucleus. However, in all scrapie and BSE cases, multigranular labelling of TBMs was more intense within the dark zones than in the light zones of the follicles. When antibodies to the N terminus were used, no labelling of TBMs in the dark zones of secondary follicles was observed in either scrapie or BSE cases with FH11 and BG4 antibodies. At this location, multigranular labelling, similar to that seen with the C-terminal antibodies, was observed with P4, 521 and 505 in scrapie-affected sheep (Fig. 1), but no TBM labelling was seen when BSE-infected tissues were examined with any of these antibodies (Fig. 2). Only the P4 antibody gave occasional single granules of immunolabelling in TBMs located in the dark zone/light zone interface.

Other LRS immunolabelling. In addition to the above FDC and TBM patterns of PrP\textsuperscript{d} accumulation in follicles, other immunolabelling patterns were found in the spleens. Several antibodies, particularly P4, gave PrP immunolabelling in the marginal zone of the white pulp and in individual cells within the peri-arteriolar lymphoid sheath. These immuno-labelling deposits took the form of single, intense, intracytoplasmic granules, similar to that seen in some follicular TBMs, and were detected in all scrapie and BSE sheep, irrespective of genotype or source of infection (data not shown).

CNS

Glial cells. Intense granular deposits of immunolabelling were found in close association with microglial or astrocytic nuclei and were interpreted as intracytoplasmic accumulations of PrP\textsuperscript{d} in glial cells. They were apparent at several neuroanatomical sites in all scrapie-affected sheep, irrespective of breed or PrP genotype, though their magnitude varied depending on the infecting source. Intraglial PrP\textsuperscript{d} was not observed in any of these scrapie-affected sheep with FH11 or BG4, but it was detected with all of the other antibodies tested (Fig. 4, Table 2). Irrespective of their breed or route of infection, sheep experimentally infected with BSE showed intraglial PrP\textsuperscript{d} accumulation with C-terminal antibodies and with antibody 505 (Fig. 5), but not with any of the remaining N-terminal antibodies (i.e. FH11, BG4, P4 and 521) (Fig. 5, Table 2).

Neurones. Multigranular intracytoplasmic deposits of PrP\textsuperscript{d} in the neurones were detected in all scrapie- and BSE-affected sheep examined. Intraneuronal PrP\textsuperscript{d} was not detected with FH11 or BG4 in either scrapie or BSE cases, while all other antibodies recognized greater or lesser amounts of intracellular PrP\textsuperscript{d}, with the exception of P4, which produced insignificant labelling in BSE-infected sheep (Fig. 6).

DISCUSSION

In previous electron microscopy studies of murine scrapie we have shown that most PrP\textsuperscript{d} is present as extracellular accumulations around infected cells of the CNS and LRS. A small fraction of PrP\textsuperscript{d} is present as intracellular deposits within the lysosomes of macrophages, glia and Kohler cells.
Fig. 1. Serial sections of a lymph node from a scrapie-affected Suffolk sheep labelled with five antibodies: BG4 (a), P4 (b), 521 (c), 505 (d) and R486 (e). All five antibodies showed FDC labelling patterns to varying degrees of intensity and also labelling of granular structures within the light zone (labelled L). All but the BG4 antibody labelled TBMs within the dark zone (labelled D). There was no immunolabelling in the dark zone of the BG4-labelled secondary follicle. Bar, 26 μm.
Fig. 2. Serial sections of a tonsil from a BSE-infected Texel sheep labelled with five antibodies: BG4 (a), P4 (b), 521 (c), 505 (d) and R486 (e). All five antibodies showed varying levels of FDC labelling as well as granular labelling within the light zone (labelled L). Only antibody R486 showed significant immunolabelling within the dark zone (labelled D) of the secondary follicle. Bar, 26 μm.
Fig. 3. Serial sections of the light zone of the same secondary follicle within a tonsil from a BSE-infected Texel sheep labelled with five antibodies: BG4 (a), P4 (b), 521 (c), 505 (d) and R486 (e). The pattern of labelling with each antibody was consistently different from that shown in Fig. 2, but essentially the same pattern was seen with each antibody. The pattern was of smaller granules located around the nucleus of a macrophage. Apoptotic nuclei (tingible bodies) could be seen within some of these semi-circular bands of immunolabelling confirming the labelled cells as TBM. In addition to the TBM labelling, some labelling between lymphocytes was consistent with extracellular FDC PrP<sub>d</sub> accumulation. This was most conspicuous in (c). Bar, 11 μm.
(macrophages scavenging the surface of the choroid plexus) (Jeffrey et al., 1994, 2000). We have similarly observed that ovine LRS macrophages and CNS neurones also accumulate intralysosomal PrP$^d$ (M. Jeffrey, C. M. Goodsr & G. McGovern, unpublished observations), whereas intraneuronal PrP$^d$ accumulation is not a feature of the murine scrapie strains previously studied. The present study confirms our previous observations (Jeffrey et al., 2001b) that PrP extreme N-terminal peptide antibodies (such as FH11 or BG4) do not immunolabel intracellular PrP$^d$ deposits within neurones, glial cells or most TBMs. This suggests that PrP$^d$ is digested and truncated within the lysosomal compartment of cells following its internalization from the extracellular space or following abnormal intracellular processing of PrP. This is consistent with the intralysosomal PrP$^\text{res}$ truncation observed when infected cells are maintained in vitro (Caughey et al., 1991). In addition, these results also confirm our previous suggestion that the truncation sites of the PrP$^d$ molecules are different in sheep scrapie and ovine BSE. However, as we have not yet been able to perform detailed studies of BSE infections in sheep bearing VRQ alleles and have examined only some UK scrapie sources, the possibility that some naturally occurring sheep scrapie strains may not be differentiated from ovine BSE on this basis cannot be discounted.

The present results differ from our previous observations in one important respect. In our previous studies, no TBM labelling was detected with antibodies recognizing downstream sequences of the flexible tail near the N terminus of PrP$^d$ (BG4, FH11), irrespective of LRS tissue or TSE source. In addition, BSE-infected LRS tissues did not show any TBM labelling with antibodies to upstream sequences (those nearest the globular domain) of the flexible tail of the PrP

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**Fig. 4.** Panel showing serial sections taken from the dorsal (parasympathetic) motor nucleus of the vagal nerve from Welsh mountain scrapie labelled with antibodies P4 (a), 521 (b), 505 (c) and R486 (d). In addition to fine punctate or diffuse labelling there were larger granules of intense labelling. These were closely associated with glial nuclei (arrows). This pattern of labelling was present for all four of the antibodies shown. Bar, 11 μm.
molecule (P4, 521, 505). The present results are in complete agreement with those previous observations as far as the dark zone of the secondary follicles is concerned, so that fundamental differences between BSE and scrapie are maintained on the basis of the different PrP immunoreactivity of dark zone TBM s. However, morphologically distinct forms of TBM labelling were detectable in this paper in the light zone of the follicles with all N-terminal antibodies regardless of the infectious agent or source, albeit with reduced intensity when compared with C-terminal antibody labelling. We attribute this difference in PrP\textsuperscript{d} labelling patterns to subtle changes in pretreatments and equipment performance between our previous and current IHC protocols and therefore suggest that the precise morphological appearance of the TBM labelling may vary according to tissue processing methods and IHC techniques, due to preservation or unmasking of particular epitopes.

The biology of normal secondary follicles suggests that TBM s phagocytose apoptotic B cells and fragments of the FDC processes with associated trapped antigen–antibody complexes. Our current IHC results suggest that in scrapie- and BSE-infected secondary follicles there is a progressive fragmentation and digestion of PrP\textsuperscript{d} within macrophage endosomes and lysosomes. Thus, full-length PrP\textsuperscript{d} (recognized by all antibodies) released by infected FDCs in the light zone of the follicles would be internalized by TBM s. While still in the light zone, TBM s would accumulate PrP\textsuperscript{d} either without any enzymatic cleavage and digestion occurring or with it involving only the most downstream sequences of the molecule, so that the remaining protein would still be

Fig. 5. Panel showing serial sections taken from the dorsal (parasympathetic) motor nucleus of the vagal nerve from BSE-affected Romney sheep labelled with antibodies P4 (a), 521 (b), 505 (c) and R486 (d). The panels labelled with 505 and R486 antibodies showed fine punctate or diffuse labelling as well as larger granules of intense labelling and were therefore similar to those of Fig. 4. The granular labelling is associated with glial nuclei (arrows). The sections labelled with the P4 and 521 antibodies showed only diffuse patterns of immunolabelling. Bar, 11 \mu m.
detected by all antibodies used in our study. We also infer that these initial events are very similar in scrapie and experimental sheep BSE. TBMs within the dark zone show further progressive PrP<sub>d</sub> fragmentation and digestion of internalized PrP<sub>d</sub>. This progressive fragmentation would initially involve the amino acid sequences recognized by FH11 and BG4 antibodies, a common step in both scrapie and sheep BSE infections. While PrP<sub>d</sub> digestion of scrapie-infected macrophages would stop at this point, it would continue in BSE-infected sheep TBMs, so that the sequences recognized by P4, 521 and 505 antibodies would be removed.

Enzymatic digestion of intracellular PrP<sub>d</sub> would also occur in the brain, as revealed by the failure of N-terminal antibodies to label intraneuronal and intraglial PrP<sub>d</sub> accumulations. However, according to our IHC results, differences in intracellular PrP<sub>d</sub> truncation between scrapie and sheep BSE are also present. Thus, BSE-associated intraneuronal PrP<sub>d</sub> would be of shorter molecular length than scrapie-associated PrP<sub>d</sub>, as revealed by the differential reactivity to P4 antibody. Furthermore, intraglial PrP<sub>d</sub> of BSE-infected sheep would also lack the epitopes recognized by the 521 antibody, suggesting it to be not only shorter than its scrapie counterpart, but also than BSE intraneuronal PrP<sub>d</sub>. Although specific results are not shown in this paper, extracellular PrP<sub>d</sub> accumulations in the brain of sheep appear to be full-length, irrespective of the infecting agent, as they were recognized by all antibodies used.

Taking together the LRS and CNS observations derived from this study, we would like to conclude the following (Fig. 7): (i) Extracellular PrP<sub>d</sub> is full-length protein, both in the LRS (FDC-derived) and in the CNS and both in scrapie-and BSE-infected sheep. (ii) Intracellular PrP<sub>d</sub> in light zone
TBMs is either full-length or is truncated at some point in the initial sequence of the flexible tail (downstream of the epitope(s) within the amino acid sequence 54–60). No differences in this respect were found between BSE- and scrapie-affected sheep. (iii) All other intracellular PrP\(\text{d}\) in sheep scrapie examined so far (TBMs in the dark zone, neurones and glial cells) is truncated at some point approximately within the 60–89 amino acid sequence. (iv) Conversely, intracellular PrP\(\text{d}\) in BSE-affected sheep seems to be truncated at different levels depending on the specific cell type; it would be within the 89–104 amino acid sequence in neurones, within the 94–105 sequence in glial cells and within the 100–111 sequence in dark zone TBMs. It will always be shorter than its scrapie counterpart, a notion that is in agreement with Western blotting studies showing that the molecular mass of brain-derived PrP\(\text{d}\) from ovine BSE is lower than that from ovine scrapie (Stack et al., 2002).

The precise amino acids at which truncation of these different PrP\(\text{d}\) forms described above occurs cannot be accurately determined from the present study, mainly because the epitopes recognized by the different antibodies are incompletely defined (Garssen et al., 2000).

The differences in intracellular truncation of PrP\(\text{d}\) between scrapie and ovine BSE most probably reflect differences in the conformation of the respective full-length extracellular PrP\(\text{d}\). This argument would explain why PrP\(\text{d}\) produced by a single cell type (e.g. ovine FDCs) is truncated differently when phagocytosed by another single cell type (i.e. ovine TBMs). Moreover, according to our IHC results, the conformational differences between scrapie-derived and BSE-associated PrP\(\text{d}\) would lie in the upstream segment of the N terminus of the PrP\(\text{d}\) molecule, i.e. the sequence reacting with P4, 521 and 505 antibodies. However, following the same argument, the different immunolabelling affinities of intracellular PrP\(\text{d}\) related to the cell type involved (light and dark zone TBMs in scrapie; light zone TBMs, dark zone TBMs, glial cells and neurones in BSE) are more difficult to reconcile with differences in conformation. These cell-related differences in intracytoplasmic truncation are more likely due to variation in the extent of enzymatic cleavage and digestion of PrP\(\text{d}\) between different cell types, at least within those in the same tissues, either LRS or CNS.

In conclusion, the results of the present study agree with our previous observations suggesting that different conformers of PrP\(\text{d}\) are produced following infection of sheep with either BSE or different scrapie sources. The precise amino acid at which truncation of these conformationally different PrP\(\text{d}\) proteins occurs cannot be accurately determined from the present study. In sheep BSE in particular, PrP\(\text{d}\) molecules of different sizes were inferred from variations in peptide labelling patterns in different cell types or in cells in different evolutionary stages. These variants may be due to differences in enzymatic digestion of PrP\(\text{d}\) within the endosomal–lysosomal compartments of different cell types, but could also be due to the production of specific PrP\(\text{d}\) conformers in different infected cell types.

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