

Distinct profiles of PrP^d immunoreactivity in the brain of scrapie- and BSE-infected sheep: implications for differential cell targeting and PrP processing

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Previous studies have shown that the patterns of disease-specific prion protein (PrP^d) accumulation in the brain (the 'PrP^d profile') of scrapie-affected sheep are mainly influenced by the source of scrapie agent. We have now extended those studies to investigate the effect of different PrP antibodies on the PrP^d profile of scrapie- and bovine spongiform encephalopathy (BSE)-affected sheep. Immunohistochemical examination of brains of 20 sheep was performed with four different PrP antibodies (P4, 521.7, 505.2 and R486), and the animals were allocated to four groups of five sheep each depending on the transmissible spongiform encephalopathy (TSE) agent source (two natural scrapie sources, SSBP/1 and BSE). Although the PrP^d profiles depended on the antibody used, the four TSE sources could always be differentiated. Natural Suffolk scrapie showed the highest levels of glia-associated PrP^d, natural Welsh Mountain scrapie uniquely had consistent vascular PrP^d plaques, SSBP/1 produced the highest intracellular accumulations of PrP^d and BSE led to moderate accumulation of all PrP^d patterns except for vascular plaques. The variations in PrP^d profile between TSE sources appeared to be the result of variations in cell tropism and in PrP processing. These processing differences are possibly associated with changes in PrP^d conformation, and are manifest as differences in intracellular truncation and in release to the extracellular space of the abnormal protein. Moreover, variations in PrP^d conformation would appear to be also influenced by the cell type supporting infection, arguing that it is modulated by the interaction between the infectious agent and the host.

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INTRODUCTION

Transmissible spongiform encephalopathies (TSEs) of animals and man are characterized by the accumulation in the central nervous system (CNS) of a disease-specific prion protein (PrP^d), which is partially proteinase-resistant (PrP^{res}). This abnormal protein is formed by a post-translational modification of the host-encoded, normal prion protein (PrP^c; Pan *et al.*, 1993). Detection of the abnormal protein can be achieved by several laboratory procedures and constitutes the basis for confirmation of TSE diagnosis. Some of those methods have been developed further to allow detailed analysis of the abnormal PrP produced during different TSE infections, and can contribute to the characterization of TSE agents. In sheep, such characterization is an important issue in view of the possibility that sheep may have become infected with the BSE agent and the repercussions that this could have for human health.

The PrP^{res} fragments generated during TSE infections have physical and chemical properties, such as electrophoretic mobility, glycoform profile and degree of proteinase

resistance, which may vary between different TSE agents (Bessen & Marsh, 1994; Collinge *et al.*, 1996; Somerville *et al.*, 1997; Kuczius *et al.*, 1998). The biochemical characterization of PrP^{res} can therefore aid in the discrimination between TSE isolates, although it has been reported that some biochemical properties of PrP^{res} are most likely to result from the interaction between the agent and the host (Somerville, 1999). A further antibody-based test, the conformation-dependent immunoassay technique, may be able to discriminate between different TSE agents by detecting specific hidden epitopes within PrP^{res}, which are only revealed after denaturation (Safar *et al.*, 1998).

Transmission and serial passage of scrapie sources in inbred mice have been employed to identify different murine scrapie strains, which may be distinguished by their incubation periods and their patterns of vacuolation in brain (the 'lesion profile'; Fraser & Dickinson, 1973). Although murine scrapie strains have been well characterized, the degree to which strain diversity might exist in sheep has not yet been determined. Experimental transmission of two highly passaged inocula derived from

different sheep scrapie sources, SSBP/1 and CH1641, target most efficiently sheep of different PrP genotypes (Hunter, 1998), and this contrasting behaviour has been considered indicative of the potential existence of different scrapie strains in the field. However, the patterns of vacuolation that have been described in natural sheep scrapie cases are highly variable (Wood *et al.*, 1997), and factors other than the PrP gene appear to have an effect on the vacuolar lesion profile in sheep (Begara-McGorum *et al.*, 2002). On present evidence therefore, it is unlikely that vacuolar lesion profiling per se can allow distinction between natural sheep scrapie sources.

In contrast to other methods for the detection of abnormal PrP, immunohistochemical (IHC) demonstration of PrP^d in tissue sections allows a detailed assessment of its cellular localization and morphological characteristics of accumulation. Brains of sheep with scrapie show marked variation in the morphology of PrP^d deposits (Miller *et al.*, 1993; van Keulen *et al.*, 1995; Foster *et al.*, 1996; Hardt *et al.*, 2000; Ryder *et al.*, 2001). We have recently indicated that these different PrP^d types and patterns can be used to obtain PrP^d profiles, which appear to be mainly influenced by the source of scrapie agent and very little, if at all, by the host breed and PrP genotype (González *et al.*, 2002). Based on these histological and other ultrastructural observations (Jeffrey *et al.*, 1990, 1994), the different PrP^d patterns are thought to represent infection and subsequent intracytoplasmic accumulation and/or release into the adjacent extracellular space of PrP^d by different cell types. Apart from providing a means of characterizing sheep TSE sources, the diversity of PrP^d profiles could also be indicative of variation in cell tropism by different agents.

Differences in neuronal tropism have been indicated in human and murine TSEs and can account for the observed differences in histopathological phenotypes. As an example, while parvalbumin-containing gamma-aminobutyric acid interneurons are preferentially lost in Creutzfeldt–Jakob disease (Guentchev *et al.*, 1997), glutamatergic neurons appear to be affected in murine scrapie after infection with the Rocky Mountain Laboratory strain (Díez *et al.*, 2001). Also, specific patterns of PrP^d accumulation characterize infection of mice of the same PrP genotype with different TSE sources (Bruce, 1993; Bruce *et al.*, 1991) and such differential targeting occurs following cross-species transmission with particular TSE agents (Bruce *et al.*, 1994; Telling *et al.*, 1996).

The differential diagnosis of ovine infections with a limited number of scrapie sources and the BSE agent can be achieved by IHC examination of brain and lymphoreticular tissues using a range of antibodies directed at different peptide sequences of PrP (Jeffrey *et al.*, 2001). The differences observed in the immunoreactivity of intramacrophage and intraglial PrP^d, presumably phagocytosed from the extracellular space, were attributed to intracellular truncation of the protein occurring at different amino acid residues of the PrP^d molecule in the two infections. It was

further suggested that these differences in PrP^d processing were probably due to differences in conformation of the PrP^d produced during sheep scrapie and ovine BSE.

In the present study we examined possible effects of the use of different antibodies on the PrP^d profile. In so doing we have attempted further validation of the PrP^d profiling as a system to characterize sheep TSE sources. In addition, the results are suggestive of differential TSE agent-specific cellular targeting and PrP^d processing, these possibly being pathogenetic mechanisms contributing to the variation in pathological phenotype of different sheep TSE infections.

METHODS

Animals. Twenty sheep showing clinical signs of TSE were examined for accumulation in the brain of different morphological types of PrP^d with four different antibodies, as described below. According to the source of TSE infection, the sheep were allocated to four groups of five animals each as follows.

- (i) Naturally infected ARQ/ARQ Suffolk sheep from a single flock in Scotland that had been kept as a closed flock for many years; the five animals examined developed clinical disease within a 5 week period.
- (ii) Naturally infected VRQ/VRQ Welsh Mountain sheep from a single commercial flock in Wales; the five animals examined developed clinical disease over a 20 month period.
- (iii) VRQ/VRQ Cheviot sheep experimentally infected with the SSBP/1 inoculum by the subcutaneous route, as described elsewhere (Goldmann *et al.*, 1994).
- (iv) Three Poll-Dorset and two Romney sheep, all of the ARQ/ARQ genotype and experimentally infected with BSE agent by the intracerebral route, as previously reported (Foster *et al.*, 1993).

The genotypes given indicate the polymorphisms of the PrP protein at codons 136, 154 and 171 in turn for each allele, in which amino acids are indicated by the appropriate single letter code. PrP genotyping was performed by sequencing with an ABI Prism 377 DNA sequencer according to the manufacturer's instructions (PE Applied Biosystems). The diagnosis of TSE in these sheep was based on clinical signs of neurological disease and histopathological confirmation of typical vacuolar pathology and PrP^d accumulation in the brain. Negative control sheep, either uninfected or of resistant ARR/ARR PrP genotype, were included in the study.

Immunohistochemistry. The brains of the sheep were fixed, trimmed and embedded following standard procedures, and subjected to IHC labelling for PrP^d according to previously described protocols (González *et al.*, 2002). Serial sections of each brain tissue sample were immunolabelled with four PrP antibodies, namely P4 (mouse monoclonal raised against the 89–104 amino acid sequence of ovine PrP; Hardt *et al.*, 2000), 521.7 and 505.2 (rabbit polyclonals raised against the 94–105 and 100–111 amino acid sequence of ovine PrP, respectively; van Keulen *et al.*, 1996) and R486 (rabbit polyclonal raised against the 221–234 amino acid sequence of bovine PrP; R. Jackman, unpublished).

Immunohistochemical examinations for a total of 11 different morphological types of PrP^d accumulation were done at five different neuroanatomical sites, i.e. frontal cerebral cortex and corpus striatum (telencephalon), thalamus/hypothalamus (diencephalon), midbrain (mesencephalon) and medulla oblongata at the obex (myelencephalon). Those types were grouped in six PrP^d patterns that included two

intracellular (intra-neuronal and intraglial) and four extracellular [glia-associated (stellate, perivascular and subpial PrP^d types), neuropil (coarse particulate, coalescing, linear and perineuronal PrP^d types), vascular and ependymal] accumulations of PrP^d. The designations as intra- or extracellular and the grouping of some types into particular patterns are based on previous immunohistochemical and ultrastructural studies (Jeffrey *et al.*, 1990, 1994). The 'PrP^d profile' of each animal represented the relative magnitudes of the six PrP^d patterns. A detailed description of these types and patterns, with the exception of the ependymal PrP^d, and of the scoring system to obtain the PrP^d profile has been given previously (González *et al.*, 2002). The ependymal type refers to PrP^d deposits on the apical border of the ependymal cells, and is different from and does not always coexist with the subependymal type previously reported (González *et al.*, 2002), which was not considered in this study. In order to ascertain eventual differences of intraneuronal PrP^d accumulation between specific neurone nuclei, this type was assessed in 15 neuroanatomical sites. These were: in the myelencephalon, the lateral cuneate, dorsal motor of the vagus, olivary, hypoglossal and midline raphe nuclei; in the mesencephalon, the red, dorsolateral geniculate, oculomotor, superior colliculus and tectum mesencephali nuclei; in the diencephalon, the ventrolateral thalamic nuclei; in the telencephalon, the caudate, putamen and globus pallidus nuclei, and the cerebral cortex neurones.

Statistical analysis. The scores obtained for the different PrP^d patterns, i.e. the PrP^d profiles, were compared between sheep groups and between antibodies by means of unpaired *t*-tests, using a statistics computer package (InStat; GraphPad Software). Parametric *t*-tests were used when the groups under comparison presented similar standard deviations (Bartlett test) and the data had Gaussian distributions (Kolmogorov and Smirnov test); otherwise, a non-parametric *t*-test (Mann–Whitney) was used.

RESULTS

PrP^d profiles with different antibodies

Details of the six PrP^d patterns scores with each of the four antibodies and for the four sheep groups studied are given in Table 1, in which statistical significance of differences in magnitude of PrP^d accumulation is also provided. The PrP^d profiles of the four sheep groups studied obtained with each of the four antibodies used are graphically represented in Fig. 1(a–d). These last show that, although the shape of the profiles varied with the antibody used, the four sheep groups could be distinguished by their respective PrP^d profiles regardless of the antibody.

No significant differences of immunoreactivity between the four antibodies were found for any of the PrP^d patterns of the Welsh Mountain sheep group. Also, each antibody provided similar scores for vascular and ependymal PrP^d in the four sheep groups. R486 was the most efficient antibody to label glia-associated PrP^d in all sheep groups (Fig. 2a–f) and was in fact the only antibody able to identify this pattern in SSBP/1-affected sheep. The differences in magnitude of glia-associated PrP^d between R486 and the other antibodies were also statistically significant in the BSE-affected sheep group.

Some antibodies showed reciprocal labelling intensities for some patterns when compared across different sheep groups. For example, neuropil PrP^d was best revealed by

505.2 and R486 antibodies in Suffolk scrapie and BSE-affected sheep, but in SSBP/1-affected sheep, 505.2 failed almost completely to reveal this PrP^d pattern. Similarly, P4 and 505.2 were respectively the most and the least efficient antibodies to detect intraneuronal PrP^d in SSBP/1-infected sheep, while the same antibodies behaved in the opposite way in Suffolk scrapie and especially in BSE-infected sheep (Fig. 3a–f). As far as intraglial accumulation of PrP^d is concerned, P4 was again the most efficient antibody in the SSBP/1-infected sheep group and 505.2 and R486 the least, whereas these last two antibodies revealed the highest levels of PrP^d in Suffolk scrapie and sheep BSE and P4 the lowest. Furthermore, 521.7 antibody did not reveal intraglial PrP^d in BSE-affected sheep (Fig. 4a–d).

Optimal PrP^d profile and cellular tropism

The above-described differences in the ability of the four antibodies to recognize particular cell-associated PrP^d patterns within specific sheep groups indicate that eventual differences in cellular tropism by different TSE sources cannot always be assessed by analysing the results obtained with a single antibody. As an example, the P4 antibody gave almost no intraneuronal labelling in BSE-affected sheep, but this cannot be interpreted as failure of the BSE agent to target neurones, as the other three antibodies demonstrated conspicuous intraneuronal labelling in these sheep. For this reason, 'optimal PrP^d profiles' were constructed (Fig. 5), which took into consideration the highest PrP^d score for each of the different patterns and sheep group regardless of the antibody used. The analysis of this 'optimal PrP^d profile' indicated that:

Welsh Mountain sheep scrapie was characterized by the accumulation in the brain of statistically similar, moderate amounts of all the different PrP^d patterns. It was also the only group in which consistent vascular plaques were found.

SSBP/1 scrapie was characterized by high levels of intracellular PrP^d, both intraneuronal and intraglial, significantly lower levels of extracellular glia-associated, neuropil and ependymal PrP^d patterns and absence of vascular plaques. By comparison with the other groups, it showed a significantly lower neuropil accumulation of PrP^d.

Suffolk sheep scrapie resulted in the accumulation of high amounts of glia-associated PrP^d, significantly lower but still moderate levels of intracellular and neuropil PrP^d patterns, low ependymal PrP^d deposits and very rare vascular plaques (a single plaque detected in the thalamus of a single sheep). By comparison with the other groups, it showed a significantly higher glia-associated accumulation of PrP^d.

Sheep BSE produced moderate to high levels of intracellular, glia-associated and neuropil PrP^d patterns, significantly lower ependymal PrP^d and no vascular plaques. It showed a significantly lower glia-associated accumulation of PrP^d than Suffolk sheep, but higher than Welsh Mountain and SSBP/1-affected sheep.

Table 1. PrP^d patterns scores with different antibodies

Immunolabelling scores for the different PrP^d patterns in four groups of TSE-affected sheep using four different antibodies. Results expressed as mean of five sheep \pm standard error. Statistical differences in magnitude of PrP^d accumulation ($P < 0.05$) are indicated by letters, so that values with no letter in common are statistically different; for each PrP^d pattern, differences can be read vertically (between antibodies for each sheep group) or horizontally (between sheep groups for each antibody). Values in bold are those selected to obtain 'optimal PrP^d profiles' represented in Fig. 5.

PrP ^d pattern/antibody	Sheep group			
	Suffolk	Welsh Mountain	SSBP/1	BSE
Intraneuronal				
P4	0.45 \pm 0.03 B	0.56 \pm 0.18 BC	1.50 \pm 0.14 E	0.06 \pm 0.02 A
521.7	0.98 \pm 0.09 DE	0.46 \pm 0.17 C	1.22 \pm 0.07 E	0.79 \pm 0.08 CD
505.2	1.03 \pm 0.12 DE	0.49 \pm 0.11 C	0.57 \pm 0.07 C	1.33 \pm 0.12 E
R486	0.66 \pm 0.07 C	0.86 \pm 0.14 CD	0.94 \pm 0.05 D	1.06 \pm 0.12 DE
Intraglial				
P4	0.15 \pm 0.07 A	0.68 \pm 0.05 C	2.03 \pm 0.21 E	0.03 \pm 0.02 A
521.7	0.81 \pm 0.19 C	0.65 \pm 0.05 C	1.69 \pm 0.26 D	0.08 \pm 0.03 A
505.2	1.03 \pm 0.18 C	0.70 \pm 0.04 C	0.83 \pm 0.15 C	1.08 \pm 0.23 CD
R486	0.94 \pm 0.23 CD	0.60 \pm 0.31 C	1.08 \pm 0.12 CD	1.51 \pm 0.18 D
Glia-associated				
P4	1.23 \pm 0.04 D	0.44 \pm 0.12 BC	0.06 \pm 0.03 A	0.76 \pm 0.11 C
521.7	1.35 \pm 0.06 D	0.31 \pm 0.09 BC	0.05 \pm 0.03 A	0.65 \pm 0.16 C
505.2	1.68 \pm 0.03 E	0.37 \pm 0.09 B	0.01 \pm 0.01 A	0.85 \pm 0.07 C
R486	1.94 \pm 0.10 E	0.64 \pm 0.09 B	0.43 \pm 0.10 B	1.26 \pm 0.10 D
Neuropil				
P4	0.62 \pm 0.08 C	0.79 \pm 0.11 C	0.29 \pm 0.09 B	0.95 \pm 0.15 CD
521.7	0.75 \pm 0.09 C	0.67 \pm 0.10 C	0.20 \pm 0.06 B	0.96 \pm 0.14 CD
505.2	1.11 \pm 0.10 D	0.75 \pm 0.13 C	0.02 \pm 0.01 A	1.12 \pm 0.07 D
R486	1.14 \pm 0.12 D	0.97 \pm 0.14 CD	0.20 \pm 0.04 B	1.29 \pm 0.10 D
Vascular				
P4	0.00 \pm 0.00 A	0.67 \pm 0.23 C	0.00 \pm 0.00 A	0.00 \pm 0.00 A
521.7	0.00 \pm 0.00 A	0.71 \pm 0.23 C	0.00 \pm 0.00 A	0.00 \pm 0.00 A
505.2	0.00 \pm 0.00 A	0.71 \pm 0.25 C	0.00 \pm 0.00 A	0.00 \pm 0.00 A
R486	0.05 \pm 0.05 A	0.68 \pm 0.20 C	0.00 \pm 0.00 A	0.00 \pm 0.00 A
Ependymal				
P4	0.47 \pm 0.15 B	0.56 \pm 0.13 BC	0.18 \pm 0.07 AB	0.31 \pm 0.19 B
521.7	0.35 \pm 0.13 B	0.32 \pm 0.07 BC	0.04 \pm 0.02 A	0.19 \pm 0.11 AB
505.2	0.56 \pm 0.12 B	0.45 \pm 0.13 BC	0.00 \pm 0.00 A	0.22 \pm 0.12 B
R486	0.28 \pm 0.09 BC	0.73 \pm 0.23 C	0.17 \pm 0.08 AB	0.13 \pm 0.06 AB

Intraneuronal PrP^d accumulation

As expected from the antibody-dependent variation observed in the magnitude of intraneuronal PrP^d accumulation (Table 1), the neuroanatomical distribution of this PrP^d type also showed substantial differences depending on the antibody used (results not shown). In order to test any eventual differences in neurone nuclei tropism between the four TSE sources studied, a single intraneuronal PrP^d profile was obtained for each of the sheep groups, by selecting the scores provided by the most efficient antibody in each case. These neuroanatomical profiles of intraneuronal PrP^d accumulation were compiled from 15 sites and are shown in Fig. 6, in which the scores for each neurone nucleus are expressed as percentages of the total intraneuronal PrP^d score. In all the four sheep groups, PrP^d

deposits were more consistent and conspicuous in the neurones of the myelencephalon, diencephalon and mesencephalon than in those of the telencephalon. Some relative differences were found in specific neurone nuclei, the most evident being in the olivary nuclei, in which Welsh Mountain sheep showed lower levels of intraneuronal PrP^d than the other groups, particularly the Suffolk sheep, and in the dorsal motor nucleus of the vagus, where the opposite situation was observed.

DISCUSSION

Utility of the PrP^d profile

The results of this study confirm previous observations which highlighted the effect of the TSE agent on the

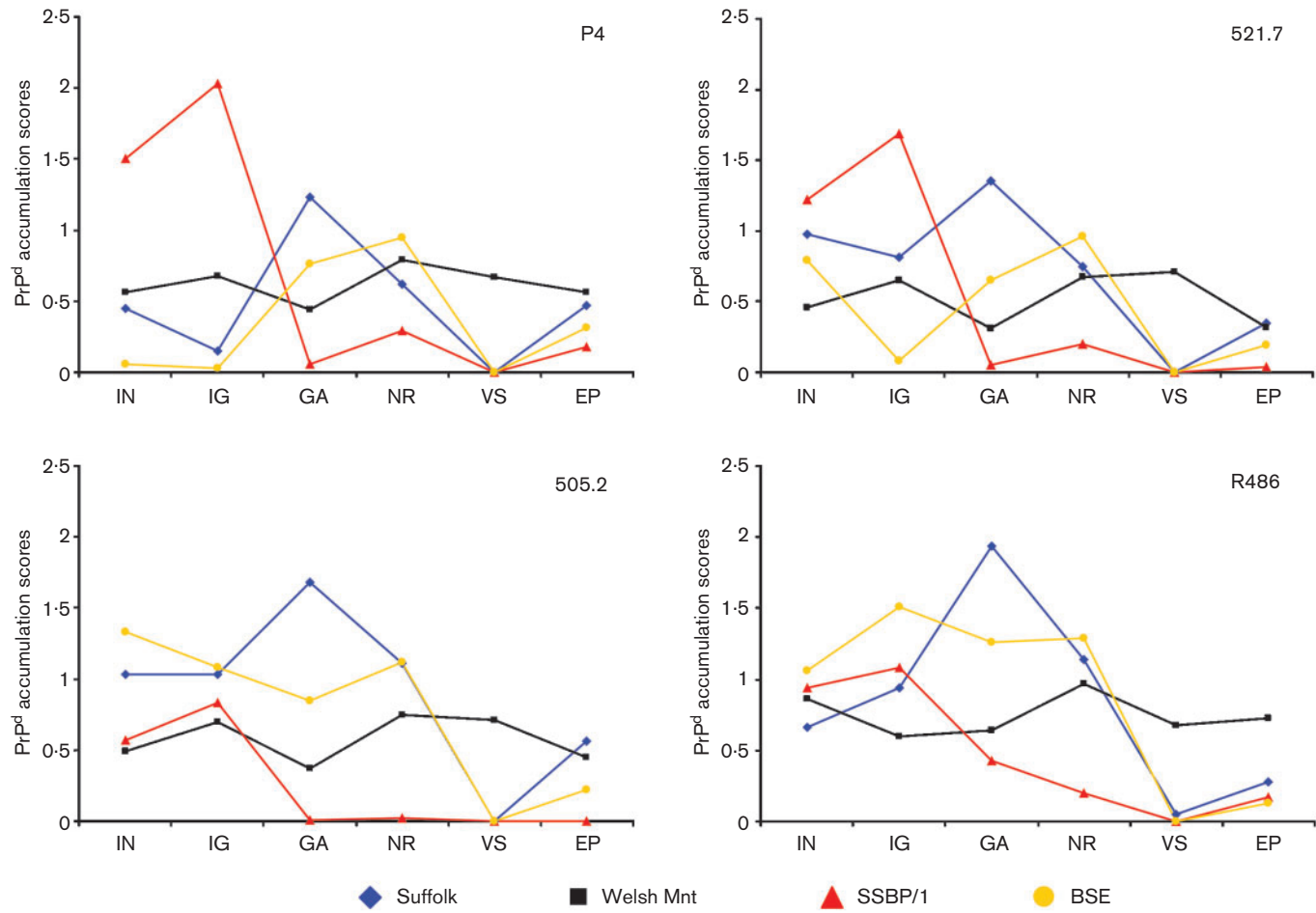


Fig. 1. PrP^d profiles of the four sheep groups with four different antibodies (as shown in the top right corners). IN, intraneuronal PrP^d; IG, intraglial PrP^d; GA, glia-associated PrP^d; NR, neuropil PrP^d; VS, vascular PrP^d; EP, ependymal PrP^d.

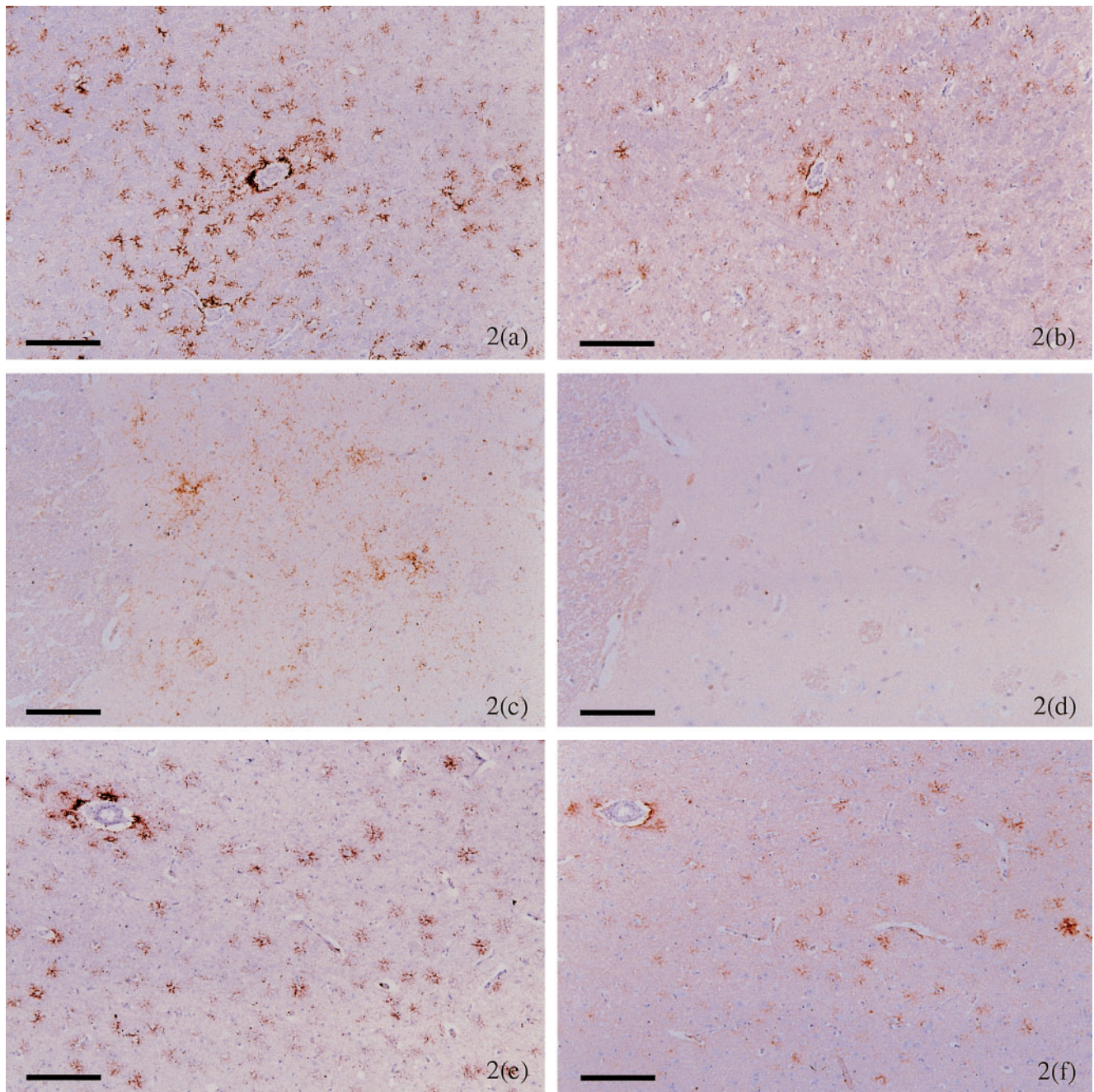


Fig. 2. Demonstration of glia-associated PrP^d accumulation in serial or semi-serial sections of brain of three sheep groups with two antibodies. Midbrain in natural Suffolk sheep scrapie with R486 (a) and P4 (b); corpus striatum in SSBP/1 scrapie with R486 (c) and P4 (d); cerebral cortex in experimental sheep BSE with R486 (e) and P4 (f). Note increased immunoreactivity in sections treated with R486 as compared to those treated with P4, occurring in all three sheep groups. Note also highest reactivity in Suffolk sheep and lowest in SSBP/1-affected sheep. All sections counterstained with haematoxylin; bars, 200 μ m.

proportion of different morphological patterns of PrP^d accumulation in the brain, i.e. on the PrP^d profile (González *et al.*, 2002). Also in agreement with that previous study, we have not detected, within the sheep groups examined, an effect of the PrP genotype on the IHC phenotype. This

is indicated by the contrasting PrP^d profiles observed in BSE-affected and scrapie-affected Suffolk sheep (both ARQ/ARQ PrP genotype) and in SSBP/1-affected Cheviot sheep and naturally affected Welsh Mountain sheep (both VRQ/VRQ). In the present report we also show that the PrP^d

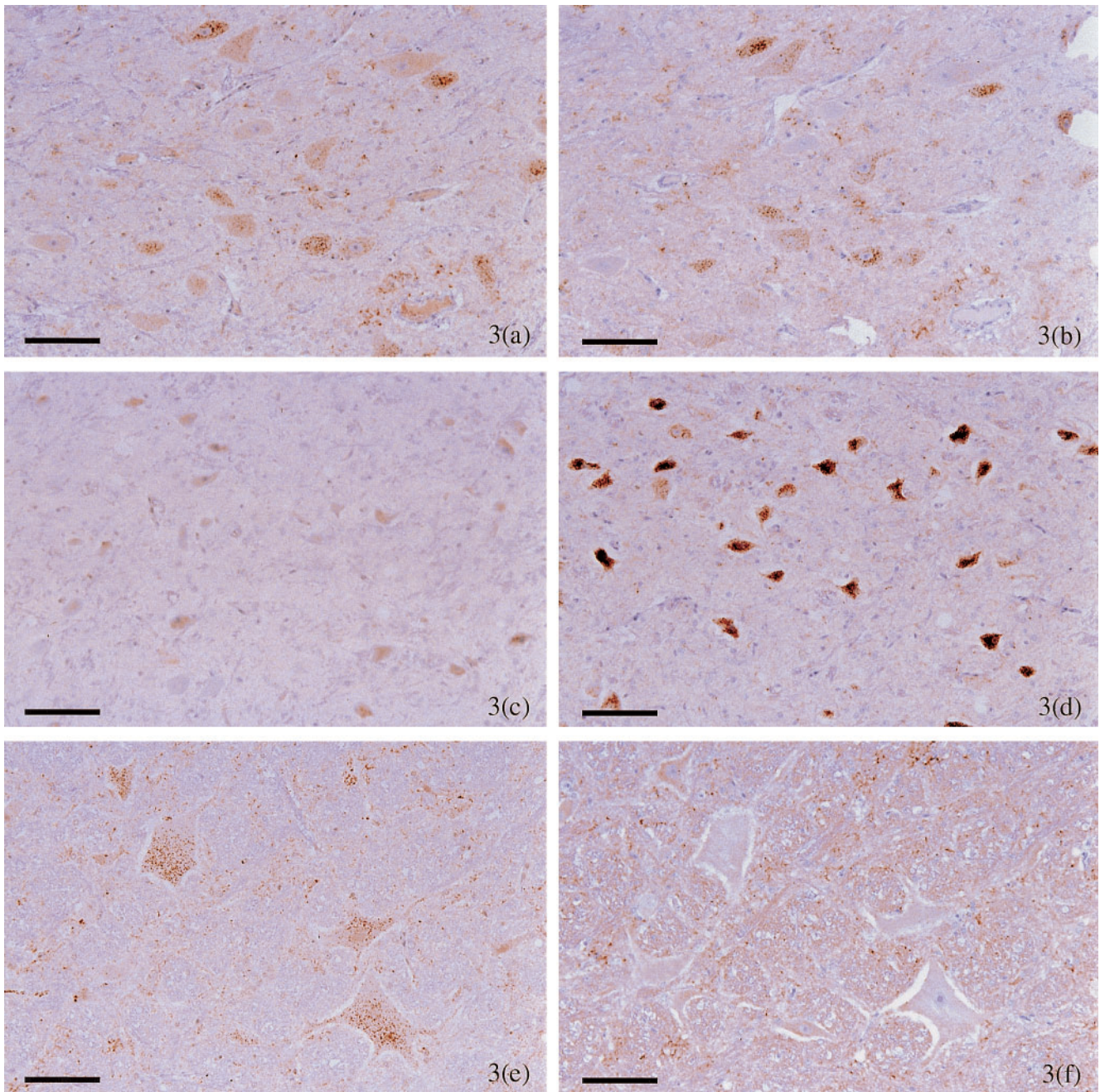


Fig. 3. Demonstration of intraneuronal PrP^d accumulation in serial or semi-serial sections of brain of three sheep groups with two antibodies. Hypoglossal nucleus in natural Welsh Mountain sheep scrapie with 505.2 (a) and P4 (b); olivary nucleus in SSBP/1 scrapie with 505.2 (c) and P4 (d); reticular formation in experimental sheep BSE with 505.2 (e) and P4 (f). Note similar immunoreactivity with the two antibodies in Welsh Mountain sheep scrapie, lack of and strong reactivity to 505.2 and P4, respectively, in SSBP/1-affected sheep and opposite results in BSE-affected sheep. Note also absence of PrP^d accumulation in the neuropil of SSBP/1-affected sheep in spite of high levels of intracellular PrP^d accumulation. All sections counterstained with haematoxylin; bars, 100 μ m.

profile may be a suitable means for characterizing not only scrapie sources, but also BSE agent infection in sheep. Moreover, although the profiles of the different groups varied according to the antibody used for the IHC

detection of PrP^d, phenotypic discrimination between the TSE sources was preserved (see Fig. 1).

In addition to confirming the utility of the PrP^d profile in

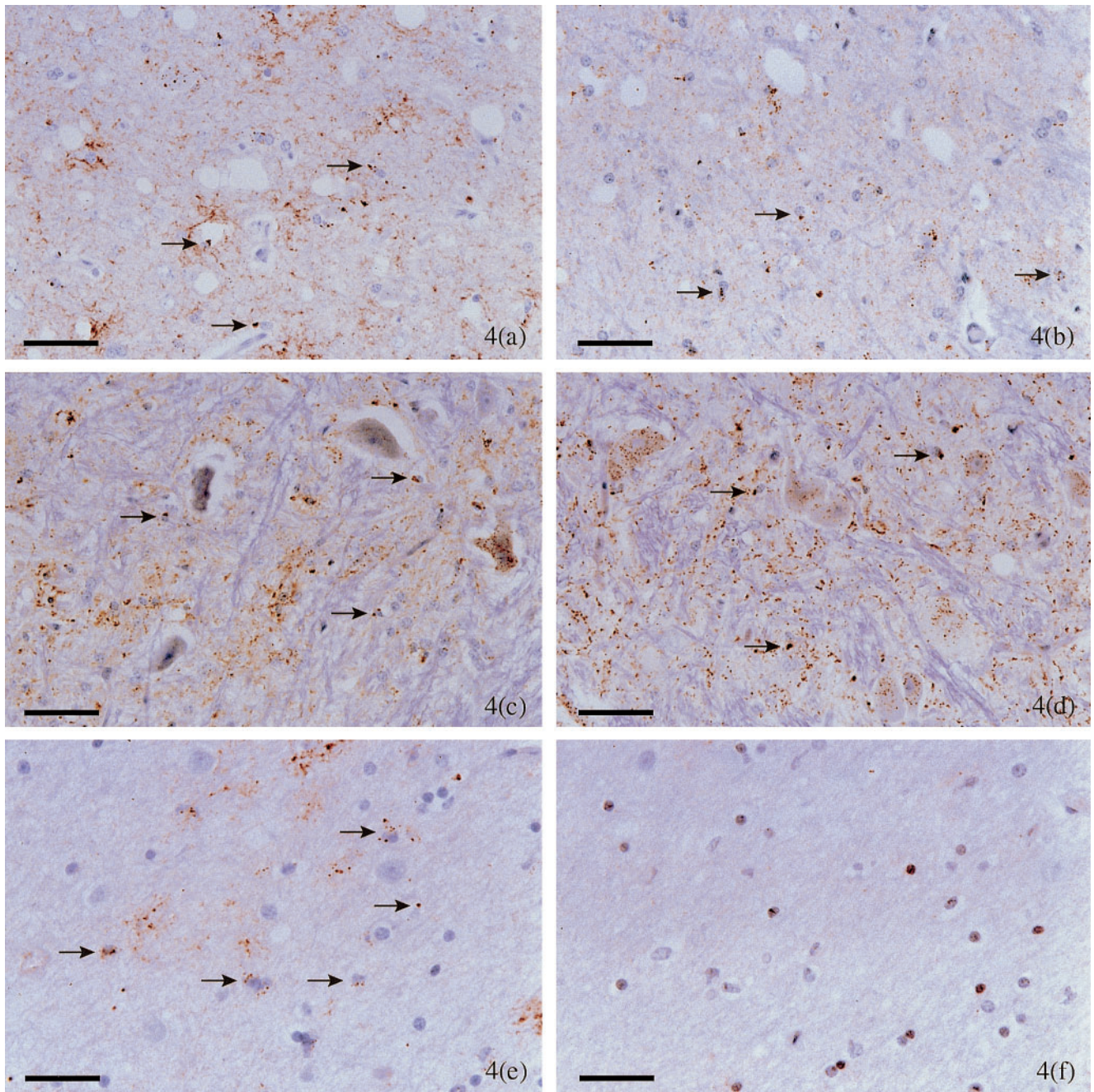


Fig. 4. Intense granular immunolabelling representing intragial PrP^d accumulation (arrows) in serial or semi-serial sections of brain of three sheep groups with two antibodies. Thalamus in natural Suffolk sheep scrapie with R486 (a) and 521.7 (b); obex in natural Welsh Mountain sheep scrapie with R486 (c) and 521.7 (d); cerebral cortex in experimental sheep BSE with R486 (e) and 521.7 (f). Note similar immunoreactivity to the two antibodies in Suffolk and Welsh Mountain sheep, while in BSE-affected sheep, R486 detects PrP^d accumulation but not 521.7. Note also higher extracellular glia-associated PrP^d accumulation revealed by R486 particularly in Suffolk and BSE-affected sheep. All sections counterstained with haematoxylin; bars (a–d), 50 µm; (e, f), 33 µm.

sheep brains as a means of characterizing sheep TSE sources, the study also revealed insights into the mechanisms that may be responsible for the PrP^d profile variation. We

hypothesize that the differences in PrP^d profile are founded on variation in the cellular tropism and PrP processing following infections by different TSE agents.

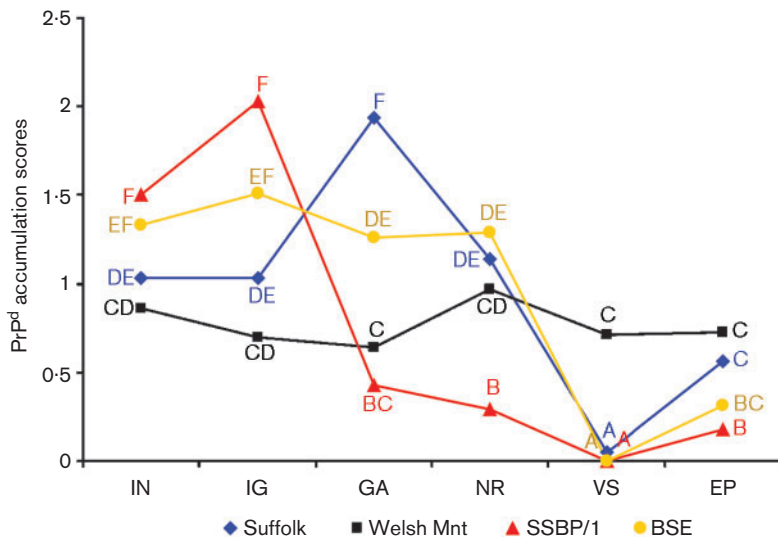


Fig. 5. Optimal PrP^d profiles of the four sheep groups. Values represented are those obtained with selected antibodies, as indicated in Table 1. Statistical differences in magnitude of the different PrP^d patterns are indicated by letters, so that values with no letter in common are statistically different ($P < 0.05$); differences can be read vertically (between sheep groups for each PrP^d pattern) and horizontally (between PrP^d patterns for each sheep group). PrP^d patterns in the x-axis are as described in Fig. 1.

PrP^d profile and cell tropism

To evaluate differences in tropism or cell targeting by different TSE agents it is assumed that PrP^d accumulation in and/or extracellular release by a particular cell type reflects infection and therefore susceptibility of that cell type. Due to differences of reactivity of different PrP antibodies, the assessment of tropism can most accurately be performed when comparing the PrP^d profiles obtained with the most efficient antibody for each TSE source and cell-associated PrP^d pattern ('optimal PrP^d profiles'; see Fig. 5). Examples of differential cell tropism can be qualitative, as with infection of endothelial cells (revealed as vascular PrP^d plaques) by the scrapie agent affecting Welsh Mountain sheep, which is negligible in infection by any of the other three sources examined. However, differences are more often quantitative, the best example

being the high affinity for glial cells, particularly astrocytes, shown by the scrapie agent infecting the Suffolk sheep group (highest glia-associated PrP^d pattern score). This scrapie agent would show higher tropism for astrocytes than the other three TSE sources examined and also higher affinity for this cell type than for neurones, ependymal or endothelial cells. In contrast, the scrapie agent affecting Welsh Mountain sheep would show a similar tropism for all cell types, while the BSE agent would show high tropism for neurones and glial cells but very low or none for ependymal and endothelial cells, respectively. These results, therefore, show differential targeting of CNS cells of different lineage by different TSE sources, in contrast with previous reports, in which differences in pathological phenotypes of PrP^d (or PrP^{sc}) accumulation were attributed almost exclusively to differences in neuronal targeting (DeArmond *et al.*, 1997, 1999). However, these latter reports suggested

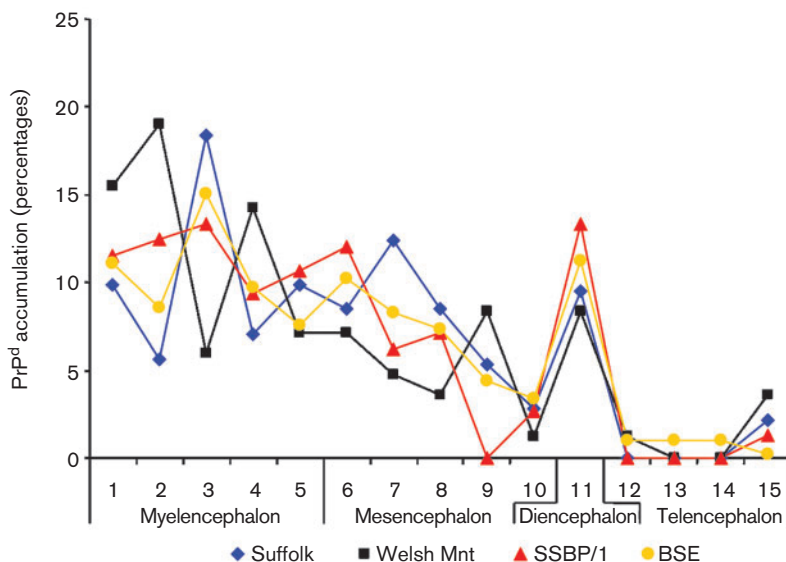


Fig. 6. Optimal profiles of intraneuronal PrP^d accumulation in the four sheep groups. Neuronal nuclei are: 1, lateral cuneate; 2, dorsal motor of the vagus; 3, olivary; 4, hypoglossal; 5, midline raphe; 6, red; 7, dorsolateral geniculate; 8, oculomotor; 9, superior colliculus; 10, tectum mesencephali; 11, ventrolateral thalamic; 12, caudate; 13, putamen; 14, globus pallidus; 15, cerebral cortex. Accumulation of PrP^d expressed as percentages generated from intraneuronal scores obtained with antibodies 505.2 (Suffolk scrapie and BSE), R486 (Welsh Mountain scrapie) and P4 (SSBP/1 scrapie).

that selective neuronal targeting would be a function of brain region and in that respect are in agreement with our own results. While we did not find differences in the targeting of specific neuroanatomical nuclei between TSE sources, regional tropic effects were evident when the myelencephalon, mesencephalon and diencephalon were compared with the telencephalon, irrespective of the TSE source (see Fig. 6).

PrP^d profile and PrP processing

Not all the variation in the PrP^d profile can be readily attributed to a greater or lesser tropism of different TSE agents for particular cell types. The significantly lowest neuropil PrP^d pattern score shown by SSBP/1 could initially be interpreted as indicative of a low tropism of this scrapie source for neurones, but this is difficult to reconcile with the high levels of intraneuronal PrP^d seen in SSBP/1 infection. Our interpretation is rather that SSBP/1 is highly neurone-tropic, but that PrP processing during this infection is different from that occurring during infections by the other three TSE sources studied. In SSBP/1 infection, most of the PrP^d produced would remain intraneuronal, with very little release to the neuropil (statistical differences shown in Table 1 and in Fig. 5), whereas in the other three infections there would be a balance between intraneuronal and extracellular neuropil PrP^d (lack of statistical differences shown in Table 1 and in Fig. 5).

Variability in PrP processing by neurones would seem to be the result not only of infection by different TSE sources, as discussed above, but could also be cell-dependent. Thus, because of the high levels of neuropil PrP^d in the dorsal motor nucleus of the vagus of Suffolk sheep (results not shown), the low levels of intraneuronal PrP^d at this site (see Fig. 6) are probably reflecting a quick and efficient release (or a low re-internalization) of PrP^d by infected neurones, rather than a low tropism of the corresponding scrapie agent for this neurone nucleus. A similar situation would take place in the olivary nuclei during scrapie infection of Welsh Mountain sheep, while the opposite (high intraneuronal and low neuropil PrP^d accumulation) would occur in the olivary nuclei of Suffolk sheep and in the dorsal motor nucleus of the vagus of Welsh Mountain sheep.

Cell tropism – PrP processing and PrP^d conformation

The precise mechanisms that account for the differences in PrP processing cannot be determined from the present data. However, our results suggest that they might be at least partially due to differences in conformation of the PrP^d that is produced after infection by different TSE agents. Differences in PrP^{res} glycosylation patterns have been described in infections by different TSE agents (Kasczak *et al.*, 1985; Somerville *et al.*, 1997) and the use of glycoform analysis for TSE agent typing has been advocated (Collinge *et al.*, 1996; Hill *et al.*, 1998). In our study, the variation in PrP^d conformation is revealed by the different affinity of

the protein to the four antibodies used. Thus, while scrapie infection of Welsh Mountain sheep would lead to the production of a PrP^d conformer that reacts similarly with all the antibodies used, the PrP^d produced during SSBP/1 infection would be of a different conformation, showing consistently low affinity for the 505.2 antibody and high for the P4 antibody. The differences in PrP^d conformation between the four TSE sources studied are particularly evident with intracellular accumulations, both intraneuronal and intraglial. Intraneuronal PrP^d produced during SSBP/1 infection showed a higher affinity for P4 antibody than the proteins produced as a result of the other three infections, and a lower affinity for 505.2 antibody when compared with Suffolk scrapie and sheep BSE. Also, BSE infection would lead to the production and intraneuronal accumulation of a PrP^d that reacts differently with P4 and R486 antibody than the intraneuronal protein produced during scrapie infection of Suffolk sheep. PrP^d accumulating in the cytoplasm of glial cells during SSBP/1 infection showed much higher affinity for P4 and 521.7 antibodies than intraglial PrP^d in the other three infections. Moreover, intraglial PrP^d in BSE-infected sheep was almost undetectable with 521.7 antibody, while it was readily detected with this antibody in infections with the other three sources.

We have evidence from separate studies to suggest that intraglial and intraneuronal PrP^d represent truncated, intralysosomal forms of the prion protein (Jeffrey *et al.*, 2001, 2003). Truncation at different amino acid sequences of the N terminus could explain the almost complete lack of detectable intraglial PrP^d with P4 in Suffolk scrapie and BSE-affected sheep, and also with 521.7 in the latter group. It would also account for the absence of intraneuronal PrP^d with P4 in the same BSE-affected sheep. Differences in intracellular truncation could be explained not only by differences in conformation of the PrP^d produced by, but also by differences in enzymatic ability of different cells during infections by different TSE sources. However, the detection of higher immunoreactivity for intracellular PrP^d with some of the antibodies to the N terminus than with R486, as is the case for intraneuronal PrP^d in Suffolk scrapie or for both intraneuronal and intraglial in SSBP/1 infection, is difficult to explain as a result of truncation. We consider that these findings are more likely to be due to differences in PrP^d conformation and not in enzymatic capability.

Variation in PrP^d conformation appears to depend not only on the infecting agent, but also on the infected cell type. This could explain why glia-associated PrP^d showed a consistently higher immunoreactivity with R486 than with any of the other three antibodies, regardless of the infecting agent. Another example would be the differences in intracellular PrP^d of BSE-infected sheep, in which intraglial PrP^d lacks both the 521.7 and P4 recognition epitope, while intraneuronal PrP^d lacks only the P4 recognition site. A dual effect on PrP^d conformation by the TSE agent and the cell type producing and/or releasing PrP^d as a result of

infection would be in agreement with previous studies on PrP glycosylation (Somerville, 1999). These pointed out differences in both PrP^c and PrP^{sc} glycoforms obtained from different brain sites, and others have made similar observations when comparing different tissues, such as brain and spleen (Rubenstein *et al.*, 1991) or brain and tonsil (Hill *et al.*, 1999). We think that these differences are best explained as being cell-dependent and that differences in PrP^d glycosylation are probably associated with differences in protein conformation, as suggested in the present report.

Conclusion

In conclusion, the results of this study indicate that different TSE sources show differences in their tropism for different cell lineages in the brain of affected sheep. It also appears that infection of sheep with different TSE agents results in differences in processing of the abnormal PrP, including greater or lesser release to the extracellular space and different intracytoplasmic truncation of the prion protein. We suggest that variation in PrP^d processing results from differences in conformation of the abnormal protein produced during infection by different TSE agents, although different conformers of abnormal PrP may exist for a single TSE agent depending on the cell type in which infection takes place.

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