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Immunodetection of PrP^{Sc} in spleens of some scrapie-infected sheep but not BSE-infected cows

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The development of diagnostic tools for transmissible spongiform encephalopathies (TSEs) would greatly assist their study and may provide assistance in controlling the disease. The detection of an abnormal form of the host protein PrP in non-central nervous system tissues may form the basis for diagnosis of TSEs. Using a new antibody reagent to PrP produced in chickens, PrP can be readily detected in crude tissue extracts. PrP from uninfected spleen had a lower molecular mass range than PrP from brain, suggesting a lower degree of glycosylation. A simple method for detecting the abnormal form of the protein, PrP^{Sc}, in ruminant brain and spleen has been developed. PrP^{Sc} was detected in sheep spleen extracts from a flock affected by natural scrapie and was also found in spleens from some, but not all, experimental TSE cases. In spleens from cattle with bovine spongiform encephalopathy (BSE) no PrP^{Sc} was detected. It is therefore suggested that there is differential targeting of PrP^{Sc} deposition between organs in these different types of TSE infection which, with other factors, depends on strain of infecting agent.

Introduction

Scrapie in sheep and Creutzfeldt–Jakob disease (CJD) in man are examples of transmissible spongiform encephalopathies (TSEs). After long asymptomatic incubation periods those affected show progressive clinical signs of brain dysfunction ending inevitably in death. These diseases have been highlighted by the recent epizootic of bovine spongiform encephalopathy (BSE) and 10 cases of a new variant form of CJD (Will *et al.*, 1996). Diagnosis of TSEs traditionally depends on post-mortem neuropathological confirmation of clinical signs of the disease. There is no established preclinical diagnostic tool available.

TSEs are caused by a transmissible agent whose structure remains to be determined (Somerville, 1991). Replication of the infectious agent correlates with the genotype of a host gene, *PrP*, which encodes the glycoprotein PrP. In sheep several polymorphisms of the *PrP* gene are found which are associated with susceptibility and/or incubation period, depending on the source or isolate of the agent (Goldmann *et al.*, 1994; Hunter *et al.*, 1994*a*). Although there are some polymorphisms in the bovine *PrP* gene, none are associated with BSE incidence in cattle (Goldmann *et al.*, 1991; Hunter *et al.*, 1994*b*).

In tissues of scrapie-infected animals PrP is deposited abnormally in a form (PrP^{Sc}) which can be distinguished from the normal form of the protein (PrP^{C}) by its sedimentation after detergent treatment and partial resistance to protease digestion (Hope *et al.*, 1986; Meyer *et al.*, 1986; Somerville *et al.*, 1989). PrP^{Sc} has been found from early in the incubation period in some mouse models of the disease in brain, spleen and other peripheral organs. The timing and pattern of deposition depends on strain of infecting scrapie agent, *Sinc* (*PrP*) genotype of the recipient mouse and route of infection (Farquhar *et al.*, 1994). In one mouse model of the disease which was derived from the serial passage of BSE infection in mice, PrP^{Sc} was not detected in the peripheral organs examined until late in the incubation period, if at all (Farquhar *et al.*, 1996). PrP^{Sc} has also been found in brains, spleens and lymph nodes of sheep with natural scrapie (Mohri *et al.*, 1992; Race *et al.*, 1992; Rubenstein *et al.*, 1987), and in sheep spleens prior to the time when clinical signs were evident (Ikegami *et al.*, 1991;

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Muramatsu *et al.*, 1994; Schreüder *et al.*, 1996; Skarphedinsson *et al.*, 1994; van Keulen *et al.*, 1996).

Until recently, confirmation of diagnosis has solely depended on neuropathological examination post-mortem. The detection of PrP^{Sc} has been considered for use as a biochemical confirmation of post-mortem diagnosis (Farquhar *et al.*, 1989) or as a potential marker of infection in the living animal (Onodera *et al.*, 1994; Schreüder *et al.*, 1996; Shinagawa *et al.*, 1993). To develop a simple and sensitive detection system for PrP^{Sc} our strategy has been to use a recombinant source of sheep PrP with which to immunize chickens, thereby increasing antigenic differences between host and immunogen, and hence produce a high-titre antibody reagent. Using the new chicken polyclonal antibody reagents, immunoblotting procedures have been optimized for detecting PrP with high specificity and sensitivity. In addition, a simple extraction method was used in which PrP^{Sc} was concentrated about 1000-fold with respect to PrP^{C} by differential centrifugation. In a second sample PrP^{C} was also removed by digestion with proteinase K prior to differential centrifugation.

Using this methodology a survey was performed on the deposition of PrP^{Sc} in spleens of sheep infected with natural scrapie from the Neuropathogenesis Unit (NPU) flock, where certain *PrP* genotypes succumb to natural scrapie but other *PrP* genotypes do not, spleens from field cases of BSE, and available samples from experimental TSEs in sheep. The data show that deposition is model-specific, depending on either the source of infecting agent and possibly the route of infection or the *PrP* genotype. For example, all tested cases of natural scrapie contained PrP^{Sc}, while cattle with BSE had no detectable PrP^{Sc} in the spleen.

Methods

■ **Recombinant Met–PrP production.** The construction of DNA vectors, their expression in *E. coli* and the purification of recombinant methionyl–PrP (Met–PrP) will be described in detail elsewhere (C. R. Birkett & A. Bennett, unpublished results). Briefly, the DNA encoding amino acids Lys_{25}–Ala_{241} of the bovine *PrP* gene (six octapeptide repeat allele; Goldmann *et al.*, 1991) was expressed intracytoplasmically in *E. coli*. The 217 amino acid protein was extracted from bacterial paste by solubilization in guanidine–HCl, followed by cation exchange chromatography on SP–Sepharose at pH 8·0 in 6·0 M urea. Met–PrP at a purity of $> 95\%$ was dialysed exhaustively against water, and the resulting protein precipitate stored at -80 °C. After solubilization in 1% SDS, protein was quantified with bicinchoninic acid (micro BCA assay kit, Pierce Chemical) using BSA as standard.

■ **Antisera.** Female white leghorn or Rhode Island red fowl were inoculated subcutaneously at two sites each with 0·125 mg Met–PrP suspended in 0·1 ml PBS emulsified with an equal volume of Freund's complete adjuvant. Booster inoculations were done at 3–6 week intervals using the same amount of Met–PrP suspension emulsified with Freund's incomplete adjuvant (subcutaneously) or without any adjuvant intramuscularly into the breast muscle. Pre-immune and test sera were obtained by venepuncture of the wing vein. The birds were brought into lay with a light/dark cycle of 16/8 h and their eggs were stored at 4 °C.

■ **Antibody purification.** Egg yolk IgG was routinely separated (Akita & Nakai, 1992) from yolk lipoproteins by diluting the yolk with 6 vols ice-cold 10 mM HCl and standing for 16 h, followed by filtration through Whatman no. 1 paper and precipitation with 30% (w/v) $(NH_4)_2SO_4$, all at 0–4 °C. IgG was also prepared by precipitating the yolk lipoprotein with dextran sulphate (Jensenius *et al.*, 1981) or other polyanions (Yokoyama *et al.*, 1993), followed by precipitation with $(NH_4)_2SO_4$ as above. Further purification of the IgG was achieved by affinity chromatography or by twice precipitating with 14% Na_2SO_4 at 20 °C and subsequently either ultrafiltration in a stirred cell over a 100 kDa cut-off filter (Amicon) or by cation exchange chromatography. For cation exchange chromatography the IgG from the final salt precipitation was dissolved in 50 mM MES–NaOH pH 5·0 at between 2 and 5 mg/ml and applied to a 16 mm × 25 mm column of SP–Sepharose FF (Pharmacia Biotech) which was developed with a gradient of 0–0·3 M NaCl in 50 mM MES–NaOH pH 5·0, all at 4·0 ml/min and 4 °C. The major IgG peak was identified, by an antibody-capture ELISA using Met–PrP as the target, as a single peak eluting between 50 and 150 mM NaCl and was collected and dialysed exhaustively against PBS. Fab′ fragments were prepared according to the method of Akita & Nakai (1993).

■ **Affinity chromatography.** Met–PrP was dissolved at 0·25–2·5 mg/ml in 0·1 M sodium borate buffer pH 8·5 containing 0·1% (w/v) SDS (coupling buffer) by heating at 100 °C for 10 min. A water-equilibrated paste of 1,1′-carbonyldiimidazole-activated 6% cross-linked beaded agarose (Reacti-Gel 6X, Pierce Chemical) was dispersed in the Met–PrP solution at protein:gel ratios of 1–5 mg/ml gel and incubated, continuously rotating, at room temperature for 24 h. Residual coupling sites were blocked by suspending the drained gel in 10 vols 1·0 M Tris–HCl pH 9·0 for a minimum of 4 h. The Met–PrP–agarose was washed extensively with coupling buffer, transferred to a 9 or 16 mm diameter chromatography column and equilibrated with PBS. The difference in the amount of Met–PrP in solution before and after coupling, measured by a BCA assay (as above), was used to determine the coupling efficiency. Crude IgG from the ammonium sulphate precipitation stage (above), or that resulting from the polyanion precipitations, was dialysed against PBS and applied to the Met–PrP matrix at 0·1 ml/min with buffer re-circulation for 2–4 h. After removing any non-bound IgG with PBS, the PrP-reactive fraction was eluted with 0·1 M glycine–HCl pH 2·3, neutralized with 1·0 M Tris, buffer-exchanged and concentrated using centrifugal ultrafiltration (Centricon 100, Amicon). Solid BSA was added to give 1·0 mg/ml IgG, 10 mg/ml BSA in PBS for protection during storage at 4 °C or -80 °C.

■ **Tissue sources.** All sheep samples came from the NPU Cheviot flock (Hunter *et al.*, 1996), which has been selected into several lines according to susceptibility or resistance to experimental challenge with the SSBP/1 source of experimental sheep scrapie. Depending on *PrP* genotype, animals in two subgroups of the susceptible line ($VV_{136}RR_{154}QQ_{171}$ and $VA_{136}RR_{154}QQ_{171}$) spontaneously develop natural scrapie. Animals which are VA_{136} survive if they are also heterozygous at either codon 154 or codon 171 ($HR_{154}QQ_{171}$ or $RR_{154}RQ_{171}$) but remain susceptible to SSBP/1 experimental challenge. Animals in the resistant line ($AA_{136}RR/HR/HH_{154}QQ/RQ/RR_{171}$; Hunter *et al.*, 1996) never develop natural scrapie but are susceptible to experimental challenge with BSE or CH1641 if codon 171 genotype is QQ_{171} (Goldmann *et al.*, 1994). Challenged sheep and goats were injected by the subcutaneous (s.c.) or intracerebral (i.c.) routes with brain homogenates from sheep infected with the SSBP/1, CH1641 or spME7 sources of experimental sheep scrapie or field cases of BSE in cattle (Tables 1 and 2), challenged mice with the ME7 or 22A strains of mouse-passaged sheep scrapie (Dickinson, 1976) or BSE from confirmed field

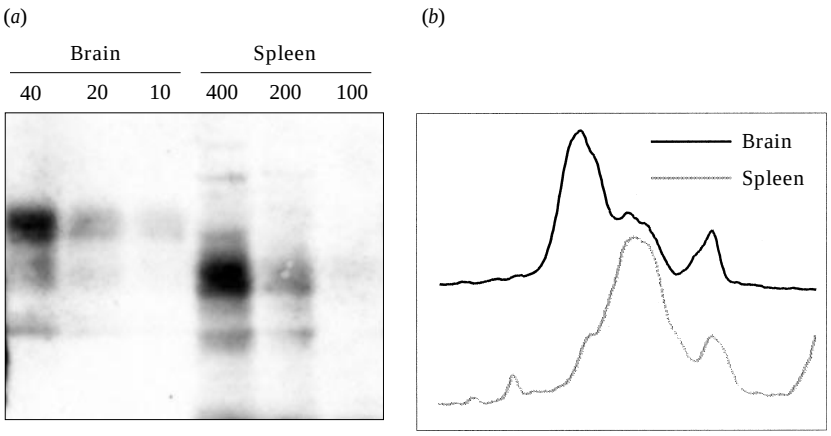

Fig. 1. (*a*) Immunoblot of PrP^{C} from sheep brain and spleen homogenates. Doubling dilutions of 40, 20 and 10 μg brain and 400, 200 and 100 μg spleen were subjected to immunoblotting. After processing, the membrane was exposed to X-ray film for 1 min. (*b*) Densitometric scan of brain (lane 1 of *a*) and spleen (lane 4 of *a*) samples.

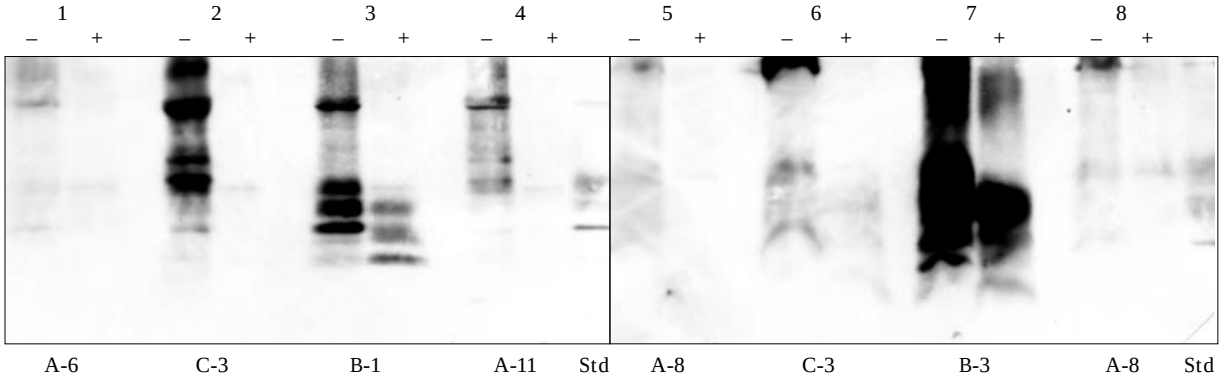

Fig. 2. Immunoblots of PrP^{Sc} extracts from sheep brain. The numbers of the groups (see Table 1) from which the samples were taken are indicated below the lanes. Samples were extracted with (+) or without (−) proteinase K treatment. Std, PrP^{C} immunostaining in bovine brain homogenate (25 μg). Samples 2 and 6 were from the cortex and medulla respectively of the same animal injected with SSBP/1. Samples 3, 6 and 7 were assessed as positive.

cases of the disease (Foster *et al.*, 1993). Genotypes were assigned using published methods (Hunter *et al.*, 1996). Cattle tissues were from field cases of BSE. Negative-control cattle tissues were taken from healthy animals at a slaughterhouse. All tissues were stored at −70 °C.

■ **Tissue extraction.** Spleen (⩾ 100 mg) was dissected free of connective tissue; brain was dissected free of connective tissue and most white matter. Tissue was homogenized in 9 vols 10 mM Tris–HCl pH 7·4 with 25 strokes in a Dounce homogenizer. Two aliquots (240 μl) of the homogenate were mixed for ⩾ 30 min with 24 ml Sarkosyl (1%), 100 mM Tris–HCl pH 7·4. PMSF (240 μl of 100 mM in n-propanol) was added to the first aliquot. Proteinase K (60 μl of 20 mg/ml) was added to the second aliquot and incubated for ⩾ 1 h at room temperature, after which PMSF (240 μl) was added. The samples were centrifuged at 265 000 ***g*** (60 000 r.p.m.) for 35 min at 20 °C in the 70 Ti rotor. Pellets were resuspended in 238 μl SDS sample buffer, 2 μl PMSF, heated at 100 °C for 30 min and 50 μl or 100 μl aliquots loaded onto the gel. Extracts from 5 mg brain or 10 mg spleen were found to be the maximum loads resolvable on the gels and therefore were used routinely. SDS–PAGE was performed according to the method of Neville (1971) using 7·5–15% acrylamide gradient slab gels and protein was transferred (Towbin *et al.*, 1979) to pre-wetted polyvinylidene fluoride membrane (Immobilon-P, Millipore) using a semi-dry blotter. The immunoblot was developed at room temperature using the Boehringer Mannheim chemiluminescent blotting substrate with the following modifications. Blocking was performed with Boehringer Mannheim blocking reagent (one-half of the manufacturer's recommended concentration) in 10 mM Tris–HCl pH 9·0, 100 mM NaCl (TBS) for 30 min (one-half of the manufacturer's recommended time); primary antibody (ch1659 at 1:2500) was applied overnight in blocking solution. The blot was washed twice with TBS plus 0·1% Tween 20 (TBST) for 10 min and twice with blocking solution for 10 min, incubated with horseradish peroxidase (HRP)-conjugated rabbit anti-chicken secondary antibody (Jackson) in blocking solution for 90 min and washed four times with TBST. HRP activity was detected using Boehringer Mannheim reagents according to the manufacturer's recommendation. Blots were assessed for the presence of PrP^{Sc} visually or with the aid of a Molecular Dynamics gel scanner. For a sample to be called PrP^{Sc}-positive a typical PrP banding pattern had to be discerned in both sample lanes, i.e. before and after proteinase K digestion. Samples which did not give a clear signal, e.g. because of background interference, were re-extracted.

Results

Immunoblotting of PrP using anti-PrP chicken antibodies

The chicken egg yolk IgG antibodies detected PrP at high titre on immunoblots but their utilization was compromised initially by a high degree of non-specific background staining of non-PrP proteins and of the support membrane. Affinity

Table 1. Detection of PrP^{Sc} in sheep brain and spleen extracts

Group no.	Infection	Route of injection	Codon*			Status†	PrP^{Sc}‡	
			136	154	171		Brain	Spleen
A-1	(Control)	–	AA/ND	ND	ND	neg	0/4	0/5
A-2	SSBP/1	i.c.	AA	HR	RQ	neg	0/1	0/1
A-3	CH1641	i.c.	AA	HR	RQ	neg	0/1	0/1
A-4	CH1641	i.c.	ND	ND	ND	neg	0/1	0/1
A-5	spME7	i.c.	AA	HR	RQ	neg	0/1	0/1
A-6	spME7	i.c.	AA	RR	RR	neg	0/1	0/1
A-7	spME7	i.c.	ND	ND	ND	neg	0/1	0/1
A-8	22A	i.c.	ND	ND	ND	neg	0/2	0/2
A-9	BSE	i.c.	VA	RR	RQ	neg	0/1	0/1
A-10	BSE	oral	AA	HH	QQ	neg	0/1	0/1
A-11	BSE	oral	AA	HR	RQ	neg	0/1	0/1
A-12	BSE	oral	AA	RR	QQ	neg	0/1	0/1
A-Total							0/16	0/17
B-1	Natural scrapie	–	VV	RR	QQ	pos	2/2	9/9
B-2	Natural scrapie	–	VA	RR	QQ	pos	1/1	3/3
B-3	Natural scrapie	–	ND	ND	ND	pos	3/3	4/4
B-Total							6/6	16/16
C-1	SSBP/1	i.c.	VA	RR	QR	pos	1/1	1/2
C-2	SSBP/1	s.c.	VA	RR	QQ	pos	0/0	1/1
C-3	SSBP/1	s.c.	VA	HR	QQ	pos	1/1	2/2
C-4	SSBP/1	s.c.	VA	RR	QR	pos	3/3	5/5
C-Total							5/5	9/10
D-1	CH1641	i.c.	AA	HH	QQ	pos	1/1	0/3
D-2	CH1641	i.c.	AA	HR	QQ	pos	1/1	0/2
D-3	CH1641	i.c.	AA	HR	QR	pos	1/1	0/1
D-4	spME7	s.c.	VA	RR	QR	pos	0/0	0/1
D-Total							3/3	0/7
E-1	BSE	i.c.	VA	RR	QR	pos	1/1	0/1
E-2	BSE	i.c.	AA	RR	QR	pos	3/3	0/3
E-3	BSE	i.c.	AA	HH	QQ	pos	1/1	1/1
E-4	BSE	oral	AA	HR	QQ	pos	1/1	1/1
E-Total							6/6	2/6

* *PrP* genotype is indicated at three codons which are polymorphic in sheep, shown by the amino acid one letter code. ND, Not done.

† Animals were killed with scrapie or BSE (pos) or showed no signs of the disease (neg), determined from an assessment of their clinical signs and brain pathology.

‡ The number of tissues which tested positive for PrP^{Sc} out of the total tested for brain and spleen is shown.

purification of the antibody (ch1659) from egg yolk minimized both types of background staining. The production of Fab′ fragments was also found to be effective (results not shown). PrP^{C} could be detected in 10 μg sheep brain and 100 μg sheep spleen when homogenates were resolved on immunoblots (Fig. 1). PrP^{C} from sheep brain had a higher proportion of higher M_r forms than that from spleen (Fig. 1).

Detection of PrP^{Sc} in ruminant brain extracts

Aliquots of 5 mg brain extract from NPU Cheviot sheep or goats with natural scrapie or from animals used in various TSE-challenge experiments were examined for the presence of PrP^{Sc}. Examples of brain extracts examined are shown in Fig. 2. PrP^{Sc} was found in brain extracts from all nine animals which were diagnosed scrapie-positive with natural scrapie, and six cases of experimental scrapie, based on clinical signs of disease and/or neuropathological confirmation of diagnosis (Table 1). In most cases a positive signal could be readily observed (e.g. Fig. 2, samples 3 and 7). However, in one case an animal injected subcutaneously with the SSBP/1 isolate of scrapie was diagnosed clinically positive but the standard pathological examination for vacuolation was negative. Small amounts of PrP^{Sc} could be detected by immunohistochemical staining in the medulla but not the cortex (J. Foster, unpublished results)

Table 2. Detection of PrP^{Sc} in goat and cattle brain and spleen extracts

Data are as described in the footnotes to Table 1 (except that no *PrP* genotype information is given).

Group	Infection	Route of injection	Status	PrP^{Sc} Brain	PrP^{Sc} Spleen
Goats					
F-1	SSBP/1	i.c.	neg	0/0	0/1
F-2	SSBP/1	i.c.	pos	1/1	0/2
Cattle					
G-1	(Control)	–	neg	0/1	0/1
G-2	Suspect BSE	–	neg	0/0	0/2
G-3	Natural BSE	–	pos	3/3	0/9
G-4*	Natural BSE	–	pos	12/12	ND

* Data from a previous experimental series (Mohri *et al.*, 1992).

of this animal. Similarly, PrP^{Sc} could be detected at low levels in extracts from the medulla (Fig. 2, sample 6) but not cortex (Fig. 2, sample 2) when brain extracts were immunoblotted. No PrP^{Sc} was detected in brains from any unaffected animals (Fig. 2, Table 1). A previous study had shown that brains from all cattle confirmed as having BSE by histopathological examination were PrP^{Sc}-positive (Table 2; Mohri *et al.*, 1992).

Detection of PrP^{Sc} in ruminant spleen extracts

Aliquots of 10 mg spleen extracts were analysed (Fig. 3). PrP^{Sc} was detected in extracts from all 16 cases of natural scrapie from the NPU Cheviot flock. Although a clear signal was observed in all samples, the strength of the signal varied (cf. Fig. 2 samples 3, 6 and 7). PrP^{Sc} was also detected in spleen extracts from nine out of ten animals infected intracerebrally or subcutaneously with the SSBP/1 scrapie isolate (Table 1). In several cases where a very weak reaction was observed the samples were re-tested to confirm the results. In one i.c.-injected case which was scrapie-positive (Table 1, group C1) PrP^{Sc} was not detected. The SSBP/1-challenged scrapie cases were from sheep with *PrP* genotypes which have not developed natural scrapie ($VA_{136}RR_{154}RQ_{171}$ or $VA_{136}HR_{154}QQ_{171}$) in the present outbreak in the NPU flock. PrP^{Sc} was not detected in spleen extracts from two goats injected i.c. with SSBP/1 (Table 2, Fig. 3).

No PrP^{Sc} was found in spleens from sheep (AA_{136}) injected i.c. with the CH1641 isolate (Foster & Dickinson, 1988). No PrP^{Sc} could be detected in spleen extracts from an animal injected s.c. with another scrapie isolate, spME7 (ME7 strain, passaged in sheep). All animals injected with TSE isolates which failed to succumb to the disease were negative for PrP^{Sc} (Table 1).

PrP^{Sc} could not be detected in spleen extracts from cattle with BSE (Table 2) or in four sheep experimentally injected i.c. with BSE. However, with one other animal injected i.c. and one infected orally, PrP^{Sc} was detected (Table 1). These two animals differed in their *PrP* genotype ($AA_{136}HH/HR_{154}QQ_{171}$) from the other four ($1 \times VA_{136}RR_{154}RQ_{171}$, $3 \times AA_{136}RR_{154}RQ_{171}$). BSE has been successfully transmitted to mice from both brain and spleen of the two sheep with PrP^{Sc}-positive spleens (Foster *et al.*, 1996). No tissues from the other BSE-infected animals have been assayed for infectivity. Four sheep which did not succumb to BSE after an oral or i.c. injection were negative for PrP^{Sc}. Because of their *PrP* genotypes none of these animals was expected to develop natural scrapie (Hunter *et al.*, 1996).

Discussion

PrP^{Sc} can be readily detected in extracts of brain and spleen from sheep infected with natural scrapie although the amounts found varied considerably between cases. PrP^{Sc} was also found routinely in spleens of sheep infected experimentally with one source of scrapie (SSBP/1 injected s.c. or i.c.) and spleens of

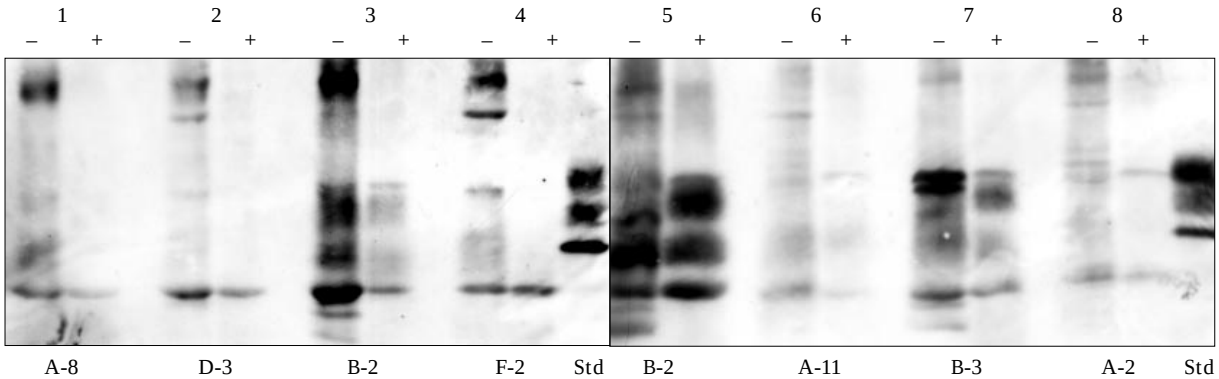

Fig. 3. Immunoblots of PrP^{Sc} from ruminant spleen. The numbers of the groups (see Tables 1, 2) from which the samples were taken are indicated below the lanes. Samples were extracted with (+) or without (–) proteinase K treatment. Std, PrP^{C} immunostaining in bovine brain homogenate (25 µg). A band with an M_r of 20000 reacts with the antibody in all samples. Samples 3, 5, 6 and 7 were assessed as positive.

two sheep infected with BSE. However, in spleens from other experimental models in sheep and from cattle infected with BSE no PrP^{Sc} was detected.

A simple extraction of infected tissue which operationally separates the PrP^{C} and PrP^{Sc} fractions is reported in this paper. The antibody reagent and the methodology described allow the sensitive and specific detection of PrP in ruminant brain and spleen extracts. The chicken antibody reagent could detect PrP^{C} in small amounts of tissue (< 10 μg brain homogenate, < 100 μg spleen homogenate) when used at high dilution with high specificity. However, affinity purification was required to avoid background and some non-PrP-specific reactions to other tissue components, particularly in spleen homogenate. The antibody reagent is produced from eggs in large quantities at high titre. In tests for the presence of PrP^{Sc}, tissue extracts of up to 5 mg brain or 10 mg spleen could be successfully resolved and accordingly these amounts were routinely analysed. The method concentrates PrP^{Sc} about 1000-fold with respect to PrP^{C}. In some cases (e.g. the brain sampled in Fig. 2, samples 2 and 6) the amounts of PrP^{Sc} detected are very low and specific to the region of brain tested. Thus, detection in brain extracts depends on the amount of PrP^{Sc} that has been deposited in the region of brain sampled, i.e. the targeting of lesions and appropriate sampling.

Previous studies of murine models of the TSEs showed that strain of agent, *PrP* genotype and route of infection could all affect whether and when during the incubation period PrP^{Sc} could be detected in peripheral tissues (Farquhar *et al*., 1994, 1996). In ruminants too these factors may well influence the timing and location of PrP^{Sc} deposition. The data herein, from TSE-infected animals showing clinical signs of disease at the time of death, show that the source of infecting agent was a major factor in determining whether PrP^{Sc} could be detected in sheep spleen extracts. There were insufficient samples available to determine whether the route of infection is of similar importance. However, it is worth noting that although PrP^{Sc} was found in spleens from most peripherally infected animals, in one s.c.-injected case it was not. Similarly PrP^{Sc} was found in spleens from two i.c.-injected cases (SSBP/1- and BSE-injected) but not in six spleens from animals challenged with CH1641 isolate or in five spleens from animals challenged with BSE or SSBP/1. There was also a correlation between *PrP* genotype and presence or absence of PrP^{Sc} in the spleen extracts from the six BSE-challenged sheep. Spleens from sheep with the genotype ($VA_{136}RR_{154}RQ_{171}$) were negative if challenged with BSE, but positive if challenged with SSBP/1. By contrast, spleens from sheep ($AA_{136}HR/RR_{154}QQ_{171}$) which were positive after BSE challenge, were negative after CH1641 challenge. Although these observations do not themselves demonstrate how these three parameters affect PrP^{Sc} deposition they support the concept demonstrated in murine studies (Farquhar *et al*., 1994, 1996) that these parameters act in concert to determine the time and location of PrP^{Sc} deposition in peripheral tissues.

Scrapie infectivity has been found in the lymphoreticular system of sheep infected with natural scrapie (Hadlow *et al*., 1982), but no infectivity has been found in peripheral tissues of cattle except distal ileum of experimentally infected calves (Fraser & Foster, 1993; Wells *et al*., 1994). However, infectivity has been found in the spleens of two BSE-infected sheep (Foster *et al*., 1996) in which we have also found PrP^{Sc}. The presence or absence of PrP^{Sc} in spleen correlates with these published results of the distribution of infectivity, but many more examples should be tested before an association can be confirmed.

Selection of particular peripheral tissues in which PrP is deposited and when deposition occurs may be a similar property to the targeting of lesions in the brain (Bruce *et al*., 1989). Lesions, and presumably infectivity, may be localized to only small areas of the brain or to selected organs. Diagnosis using PrP can presumably only be made by sampling those tissues or areas of the brain where infection or its sequelae occur.

It is not known what mechanisms control targeting, but presumably agent-specific encoded information interacts with host components to determine whether a cell becomes infected, or whether, once infected, it can replicate the agent. The degree and diversity of glycosylation of PrP (Endo *et al*., 1989) suggests that these carbohydrate moieties may play a role in target selection, in which context it is interesting to note the difference in degree of glycosylation of PrP in sheep brain and spleen. In contrast to data from brain PrP (Somerville & Ritchie, 1990), the gel migration data suggest that for most PrP^{C} in sheep spleen only one of the two *N*-glycosylation sites is utilized. This observation requires further investigation.

In experimental TSEs deposition of PrP in peripheral tissues may be of diagnostic value, but only when it has been ascertained that PrP is always deposited in the tested organ of affected animals in the model of the disease under study. With field scrapie in sheep it would be necessary to show that within an individual flock, assuming a single strain of scrapie is causing the outbreak, proven cases of the disease with similar *PrP* genotype do have PrP^{Sc} deposited in the organ selected for testing. The data indicate that any PrP deposition in the spleen of BSE-infected cattle, like infectivity, is not detectable and therefore of no potential diagnostic value with present techniques. The absence of infectivity in other peripheral organs of BSE-infected cattle (except distal ileum) suggests little hope for the detection of PrP^{Sc} in non-central nervous system organs in these cases. By contrast, detection of PrP^{Sc} in a lymphoid organ of sheep from the NPU flock with natural scrapie suggests some potential diagnostic value for this approach in sheep if the parameters controlling its deposition are better understood. It will be of value to determine if PrP^{Sc} is deposited in more accessible tissues, e.g. certain lymph nodes. It has recently been reported that PrP^{Sc} can be detected immunohistochemically in the tonsil and other organs in animals from a flock with natural scrapie (Schreuder *et al*., 1996;

van Keulen *et al*., 1996). However, it will be important to determine if PrP^{Sc} deposition is targeted to lymphoid organs such as spleen or tonsil in flocks in which natural scrapie affects other *PrP* genotypes (Hunter *et al*., 1994*a*).

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