Cellular prion protein status in sheep: tissue-specific biochemical signatures

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Expression of the cellular prion protein PrPSc is sine qua none for the development of transmissible spongiform encephalopathy and thus for the accumulation of the illness-associated conformer PrPSc. Therefore, the tissue distribution of PrPSc at the protein level in both quantitative and qualitative terms was investigated. PrPSc was quantified using a two-site enzyme immunometric assay which was calibrated with purified ovine recombinant prion protein (rPrP). The most PrPSc-rich tissue was the brain, followed by the lungs, skeletal muscle, heart, uterus, thymus and tongue, which contained between 20- and 50-fold less PrPSc than the brain. The PrPSc content of these tissues seems to be comparable between sheep. Other organs, however, showed different, but low, levels of the protein depending on the animal examined. This was also the case for tissues from the gastrointestinal tract. The tissue containing the lowest concentration of PrPSc was shown to be the liver, where PrPSc was found to be between 564- and 16000-fold less abundant than in the brain. PrPSc was concentrated from crude cellular extracts by immunoprecipitation using several monoclonal and polyclonal anti-ovine PrP antibodies. Interestingly, it was observed that the isoform profile of PrPSc was tissue-specific. The most atypical electrophoretic profile of PrPSc was found in the skeletal muscle, whereas two polypeptides of 32 and 35 kDa were detected.

Introduction

Prion diseases belong to a family of neurodegenerative disorders that affect both humans and animals. The mechanisms of prion transmission are not yet unravelled. Nevertheless, it is admitted today that one of the fundamental steps in pathogenesis is the conversion of the host-encoded cellular prion protein PrPC into its disease-associated conformer PrPSc. In addition, PrPSc has been shown to be crucial for the transmission of the disease and formation of PrPSc (Büeler et al., 1993; Prusiner et al., 1993; Weissmann, 1996). One of the emerging hypotheses is that the conversion phenomenon could take place at the site where the infectious agent meets PrPC. The latter has been shown to be expressed in various domains of the hamster brain (Bendheim et al., 1992; DeArmond et al., 1999; Somerville, 1999) and in several non-neuronal tissues from rodents (Bendheim et al., 1992), cows and humans, including the spleen and lymph nodes (McBride et al., 1992), squamous epithelia of the skin and upper gastrointestinal tract (Pammer et al., 1998, 1999). PrPSc has also been detected on the surface of lymphocytes in humans and mice (Cashman et al., 1990; Mabbott et al., 1997; Antoine et al., 2000). A PrP isoform truncated at the C terminus has been detected in mature human and bovine sperm (Shaked et al., 1999). In addition, PrPSc mRNA has been shown to be present in all tissues tested to date from humans, cattle and sheep (Goldmann et al., 1999). Nevertheless, precise qualitative and quantitative studies of PrPSc expression at the protein level have never been carried out in sheep. The difficulties for such an investigation are principally due to the lack of efficient and sensitive methods to concentrate, detect and quantify PrPSc from non-neuronal tissues, in which its expression is expected to be low. Horiuchi et al. (1995) made the first determination of PrPSc distribution in sheep organs and showed that PrPSc can be detected in several tissues other than the brain. The ratio between PrPSc expression in the brain and other tissues could not, however, be precisely determined at that time.

Here we report the first quantification of PrPSc in different tissues of sheep using a two-site enzyme immunometric assay (ELA). Furthermore, by use of a simple method to immuno-
precipitate PrP\textsuperscript{C} from a crude tissue extract of healthy sheep tissue, we have demonstrated that the isoform profile of PrP\textsuperscript{C} is tissue-specific. The most striking PrP\textsuperscript{C} glycoform profile was obtained from skeletal muscle.

**Methods**

*Antibodies.* The anti-PrP monoclonal antibodies (MAbs) used in the present work were 4F2 and 12F10 (Krasemann et al., 1996), which were obtained from Le Réseau Anticorps du Programme Français de Recherche sur les ESST, and SAF34 and SAF37, which were produced using hamster scrapie-associated fibrils as the immunogen and present a 4F2-like specificity (octarepeat region) (Rodolfo et al., 2001). Pe248 was obtained after immunization of PrP\textsuperscript{−/−} mice with PrP\textsuperscript{C} purified from sheep brain (unpublished data). The anti-PrP polyclonal antibody (PAb) MH44 was obtained after immunization of rabbits with ovine recombinant PrP (rPrP) expressed in *Escherichia coli* and purified to homogeneity by virtue of its intrinsic capacity to bind some divalent cations (Rezaei et al., 2000). Specific peroxidase-conjugated anti-rabbit immunoglobulins (IgGs) were purchased from BioSys.

**Tissue preparation.** Seven 2-year-old ewes (ewe I, PrnP genotype ARQ/ARR at positions 136, 154 and 171; ewe II, ARQ/ARR; ewe III, ARQ/ARR; ewe IV, ARQ/VRQ; ewe V, ARQ/ARR; ewe VI, ARQ/ARR; and ewe VII, ARQ/ARR) in the early stages of pregnancy were sacrificed under controlled conditions and the organs were rapidly removed and placed on ice for a maximum of 30 min. Before use, the organs were washed three times with wash buffer and incubated overnight at 4 °C with the tracer antibody. The latter consists of Fab' fragments obtained from 12F10 IgGs and coupled to a G4 tetramer of acetylcholinesterase (AchE) (Grassi et al., 1988). After three washes, AchE activity was assessed by adding Ellman reagent (Ellman et al., 1961) and measuring absorbance at 414 nm with an automatic reader (LabSystems).

**PrP\textsuperscript{C} immunoprecipitation.** Anti-PrP MAbs Pe248 or 4F2 were added to 500 µl (50 ng tissue equivalent) of the different clarified total extracts, obtained as described above. The samples were then incubated for 1 h at room temperature. To spin down the immunocomplexes, protein A-Sepharose beads (Pharmacia–Amersham) were added to the mixtures and incubated for 1 h at room temperature or overnight at 4 °C on a rotating wheel. The beads were then washed four times with the corresponding extraction buffer and once with double-distilled water before dissolving in 50 µl of 8 M urea and 20 mM DTT. The correct volume of 4 × Laemmli sample buffer was then added (Laemmli, 1970).

**Deglycosylation of PrP\textsuperscript{C}.** The immunoprecipitated PrP\textsuperscript{C} present in the urea/laemmli sample buffer was diluted in deglycosylation buffer (50 mM Tris–HCl, pH 8.0, 1% Nonidet-P40) and treated with 0.5 U/ml of *N*-glycosidase F (Boehringer Mannheim) for 5 h at 37 °C. Samples were precipitated with 10% trichloroacetic acid and washed twice with ethanol before dissolving in Laemmli sample buffer and analysis by Western blotting.

**Analytical methods.** SDS–PAGE was performed with the Mini-protein II Biorad system. Gel transfer of proteins separated by SDS–PAGE was carried out using the Minigel Transblot Cell system (Biorad), according to the manufacturer's instructions. Low molecular mass markers (Pharmacia–Amersham) were as follows: phosphorylase b, 94 kDa; BSA, 67 kDa; ovalbumin, 43 kDa; carbonic anhydrase, 30 kDa; soybean trypsin inhibitor, 20 kDa; and β-lactalbumin, 14 kDa. After immunoprecipitation with the anti-PrP MAbs Pe248 or 4F2, PrP\textsuperscript{C} was detected using either the purified IgG fraction of MH44 PAb or the biotinylated 4F2 MAb. PrP\textsuperscript{C} was then visualized using the ECL detection technique with specific goat anti-rabbit IgGs coupled to peroxidase or streptavidin-coupled peroxidase (Pierce), respectively. Protein content was determined by the Bradford method.

**Results**

**Quantification of PrP\textsuperscript{C} in different tissues**

PrP\textsuperscript{C} was quantified in crude tissue homogenates with a sensitive quantitative test for the prion protein (Rodolfo et al., 2001). In all cases, the quantification test was calibrated using ovine rPrP purified from *E. coli*. This allowed us to produce a reproducible linear standard curve with a detection limit of up to 2.5 ng/ml (data not shown; Rodolfo et al., 2001). The standard curve was similar whether rPrP was diluted in ELISA buffer or in brain homogenate obtained from PrP\textsuperscript{−/−} mice (Rodolfo et al., 2001). The detection limit obtained with the antibodies used in the present test is around 70 pg/ml. Two series of sheep were used to carry out this study. Fewer organs were removed from the first series (ewes I–III). The results for all sheep tested are presented as histograms in Figs 1 and 2. The overall distribution of PrP\textsuperscript{C} in ewes I–III (ARQ/ARR) gave comparable values with only few exceptions (Fig. 1). Aside from the brain, the other tissues from these animals that contained significant amounts of PrP\textsuperscript{C} were the lungs, heart and skeletal muscle, although in quantities of 20-, 36- and 34-fold less than in the brain, respectively. Ewe IV (ARQ/VRQ) showed some difference in PrP\textsuperscript{C} distribution compared with...
Fig. 1. PrP<sup>C</sup> quantification in different tissues from ewes I–III. The PrP<sup>C</sup> content in the brain is expressed in µg/g of tissue, while it is in ng/g of tissue for the other organs. The different tissues tested were as follows: tonsil (Ts), tongue (Tg), lung (Lu), heart (H), liver (Li), spleen (Sp), pancreas (Pa), kidney (Ki) and skeletal muscle (SM).

that found in the same tissues from ewes I–III (Figs 1 and 2). PrP<sup>C</sup> content in the tonsils of ewe IV was, for example, about three to four times greater than that in ewes I and II. Furthermore, the lungs of ewe IV contained less PrP<sup>C</sup> than ewes I–III. The uterus was found to be the most PrP<sup>C</sup>-rich extraneuronal tissue in ewe IV. PrP<sup>C</sup> was also detected in the salivary glands of ewe V and in the mammary glands of ewe IV (Fig. 2). However, because of the limited number of sheep studied, no conclusion regarding genotype effects on the level of PrP<sup>C</sup> can be drawn at this stage.

PrP<sup>C</sup> was quantified in different tissues taken from the digestive tract of ewes IV–VI. However, only values for ewes IV and V are shown (Fig. 2). PrP<sup>C</sup> was detected in all of the tissues tested. The values obtained for ewes V and VI along the digestive tract were, however, found to be two to four times less than what was quantified for ewe IV. Interestingly, we have observed that the liver is the tissue with the lowest PrP<sup>C</sup> content (between 0.3 and 4 ng/g of tissue) in all of the sheep tested.

### Tissue-specific isoform distribution of PrP<sup>C</sup>

To check whether the organs differed only by their content of PrP<sup>C</sup> or, in addition, by the biochemical signature of the protein, we developed an immunoprecipitation method to concentrate PrP<sup>C</sup> from whole tissue extracts. All tissues were homogenized to the same wt/vol ratio (10%). We first tried to detect PrP<sup>C</sup> by classical Western blot using several anti-PrP MAbs and PAbs. Only brain extract showed strong labelling of PrP<sup>C</sup> (data not shown). However, after lengthy exposure of the nitrocellulose membranes, we detected faint signals of PrP<sup>C</sup>, but always in the same tissues, in the skeletal muscle, tongue, lungs and heart (data not shown). We then decided to concentrate PrP<sup>C</sup> by immunoprecipitation directly from each clarified crude tissue extract before its detection by Western blot.

Fig. 3 shows an example of a comparative immunoprecipitation experiment with both MAb Pc248 IgG1 and an anti-PrP<sup>C</sup> unrelated mouse IgG1 on the same tissue extracts from ewe VII. Only results obtained with extracts prepared in TL1 buffer are shown, as they gave the best results (especially from non-neuronal tissues). The control IgG did not immunoprecipitate PrP<sup>C</sup> from the different extracts (Fig. 3C). PrP<sup>C</sup> was detected after immunoprecipitation with Pc248 using two different probes, PAb MH44 (Fig. 3A), which was obtained after injection of ovine rPrP into rabbits and had been shown to specifically recognize PrP<sup>C</sup> in a crude brain homogenate from different species (data not shown), and biotinylated MAb 4F2 (Krasemann et al., 1996) (Fig. 3B). In both cases, the same PrP<sup>C</sup> profile was obtained, although the intensity of PrP<sup>C</sup> bands differed slightly in a few tissues depending on the primary antibody used. Furthermore, pre-incubation of the primary antibodies used for Western blot with an excess of purified PrP abolished detection of the immunoprecipitated PrP<sup>C</sup> (data not shown). Altogether, these data showed that the signals obtained with Pc248 were specific for PrP<sup>C</sup>.

In order to be confident with the results obtained with Pc248, we repeated the immunoprecipitation experiment with 4F2 (purified IgG) on the same tissue extracts. As shown in Fig. 4, the same kind of PrP<sup>C</sup> profile was obtained, irrespective of the Western blot detection probe used to visualize the immunoprecipitated PrP<sup>C</sup> (biotinylated 4F2 IgG, Fig. 4A; MH44 IgGs, Fig. 4B).

It is evident from the Western blots carried out after immunoprecipitation that several other non-neuronal tissues showed significant levels of PrP<sup>C</sup>. A striking tissue specificity in the qualitative profile of the different isoforms of PrP<sup>C</sup> was obtained. Actually, the glycoform signature of PrP<sup>C</sup> in the brain, as obtained with most anti-PrP antibodies described in the literature, is in general characterized by the presence of three bands with decreasing intensity, representing the bi-
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Fig. 2. An example of PrP<sup>C</sup> content in a sheep from which gastrointestinal tract tissues were studied (ewes IV and V). The different tissues tested were as follows: tonsil (Ts), tongue (Tg), lung (Lu), heart (H), liver (Li), spleen (Sp), pancreas (Pa), kidney (K), skeletal muscle (SM), uterus (Ut), thymus (Tm), reticulum (Re), rumen (Ru), duodenum (Du), jejunum (Je), ileum (IL), mammary glands (Ma) and salivary glands (SG). nd, Not detected.

In the lungs, a complex profile with hyperglycosylated isoforms was always observed. Furthermore, the band migrating at the level of the unglycosylated isoform from the lungs migrated slightly faster than its corresponding band from the brain (Figs 4A, B and 5A). The second atypical PrP<sup>C</sup> electrophoretic profile was reproducibly observed in skeletal muscle, where a clear doublet of PrP<sup>C</sup> was detected from all seven sheep tested (Figs 3 and 4, lanes SM). The PrP<sup>C</sup> profile of skeletal muscle was specific, since it was not observed in other types of muscular tissue, such as the heart and tongue. In the uterus, either with or without myometrium, the PrP<sup>C</sup> profile was different from that observed in skeletal muscle and was more reminiscent of the heart PrP<sup>C</sup> profile (data not shown). The two bands from skeletal muscle migrated slower than the bi- and monoglycosylated isoforms from the brain: 32 kDa in the skeletal muscle instead of 30 kDa in brain for the monoglycosylated band and 35 kDa in skeletal muscle instead of 33 kDa in brain for the biglycosylated band (Fig. 5). The signals obtained from the spleen and kidney were often very low (Figs 1–3). PrP<sup>C</sup> was not significantly detected from liver tissue (Figs 3 and 4). This indicates that liver tissue contains the lowest amount of PrP<sup>C</sup>, if any, within the detection limits of the immuno-concentration method described in the present work.

Secondly, we investigated the distribution of PrP<sup>C</sup> along the digestive tract. Sections of tissue from the gastrointestinal tract were removed from the second series of sheep (ewes IV–VII). Only results from ewe VII are shown. We have observed that the signals obtained for PrP<sup>C</sup> from these tissues were often diffuse at the level of biglycosylated forms (Figs 3 and 4). The distribution of PrP<sup>C</sup> along the digestive tract was shown to be different from one sheep to another (Fig. 2). In ewe VII, PrP<sup>C</sup> was found all along the digestive tract, with more PrP<sup>C</sup> in the reticulum and ileum (Figs 3 and 4), whereas in ewe IV, PrP<sup>C</sup> was mainly detected in the reticulum, rumen and duodenum (Fig. 2). In ewe V, PrP<sup>C</sup> was concentrated in the reticulum and rumen (Fig. 2).

In order to check that the PrP<sup>C</sup> isoforms detected in some tissues corresponded to glycoforms, we carried out a deglycosylation experiment on PrP<sup>C</sup> immunoprecipitated from
the skeletal muscle, lung and brain (Fig. 5). Treatment of PrP<sup>C</sup> with N-glycosidase F resulted in simplifying the PrP<sup>C</sup> profile into one band, which probably corresponds to non-glycosylated PrP<sup>C</sup> (Fig. 5). Interestingly the nonglycosylated PrP<sup>C</sup> isoform obtained from the lung migrated faster than those obtained from the brain and skeletal muscle.

Discussion

PrP<sup>C</sup> expression is necessary for the development of TSE (Büeler et al., 1993; Prusiner et al., 1993). The spatial sequence of events after oral infection is still not completely understood. Following oral challenge, it is assumed that the gastrointestinal tract and its associated lymphoreticular system represent the first tissues in which PrP<sup>Sc</sup> could be detected, thus corresponding to tissues that support infection (McKinley et al., 1983; Race et al., 1998; van Keulen et al., 1999; Bons et al., 1999; Maigrien et al., 1999; Beekes & McBride, 2000; Andreoletti et al., 2000). PrP<sup>C</sup> has been found to be the substrate for the formation of its pathology-associated conformer PrP<sup>Sc</sup> (Weissmann, 1996). The distribution of the cellular form, PrP<sup>C</sup>, in non-neuronal tissues is not, however, well documented in sheep.

The quantification test used in the present work permitted the PrP<sup>C</sup> ratio between the brain and the extraneuronal tissues to be determined. The EIA test was able to detect PrP<sup>C</sup> in several crude non-neuronal tissue extracts, even after a tenfold dilution. We should mention here that the EIA was originally designed to detect PrP<sup>res</sup> for the post-mortem diagnosis of bovine spongiform encephalopathy. In this context, the EIA was evaluated by the European Commission, supervised by the Directorate XXIV (Moynagh & Schimmel, 1999; Moynagh et al., 1999) and it was concluded that this test was 'the most sensitive one' at that time. In general, there was a good correlation between the results obtained with the EIA and those observed using the immunoprecipitation/Western blot experiment.

The tissue distribution of PrP<sup>C</sup> in sheep was first studied by Horiushi et al. (1995). However, Horiushi and co-workers used microsomal preparations from different tissues to enrich PrP<sup>C</sup>. We have recently shown that the solubilized microsomal PrP<sup>C</sup> population correspond to 16% of total brain PrP<sup>C</sup> only (unpublished data). Altogether, these data prompted us to set up a different procedure which could better reflect the true PrP<sup>C</sup> content of different tissues in both qualitative and quantitative terms. In all cases, PrP<sup>C</sup> could be detected by loading only 5 mg of tissue equivalent per lane from almost all tissues except the liver. Serial dilutions made from the tissues richest in PrP<sup>C</sup> content (lungs, skeletal muscle and heart) showed that we could still detect PrP<sup>C</sup> in an equivalent of 0.5 to 1 mg of tissue (data not shown).

Similar results were obtained from immunoprecipitation/Western blot assays using several combinations of MAbs and PAb. Furthermore, we have observed that two different anti-PrP PAbs, MH44 and MH48, produced in our laboratory immunoprecipitated an additional faint band of about 66 kDa from skeletal muscle (data not shown). This band could correspond to a dimeric form of PrP<sup>C</sup> which is present specifically in this tissue. Comparable results from different animals were found in several tissues, such as the lungs, heart, skeletal muscle and tongue. Data from the gastrointestinal tract
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Fig. 4. PrP\textsuperscript{C} distribution in different tissues from ewe VII using 4F2 IgG for immunoprecipitation (IP) followed by Western blot (WB) detection with either biotinylated 4F2 IgG (A) or MH44 anti-PrP PAb IgG (B). The different tissues tested were as follows: brain (Br), tongue (Tg), tonsil (Ts), lung (Lu), heart (H), spleen (Sp), liver (Li), skeletal muscle (SM), reticulum (Re), rumen (Ru), duodenum (Du), jejunum (Je), ileum (Il) and mammary glands (Ma). (C) IP of PrP\textsuperscript{C} from H, SM and Br samples from ewes III and II, indicating the specific muscle profile of PrP\textsuperscript{C}. Note the difference in migration between the bi- and monoglycosylated isoforms of brain PrP\textsuperscript{C} and the PrP\textsuperscript{C} doublet observed in SM. Bi-, mono- and nonglycosylated (B, M and N) isoforms described in the brain are indicated on the right.

Fig. 5. Sensitivity of PrP\textsuperscript{C} from the brain, skeletal muscle and lungs to treatment with N-glycosidase F. PrP\textsuperscript{C} immunoprecipitated with MAb 4F2 from the brain (Br), skeletal muscle (SM) and lung (Lu) extracts were either (A) untreated (–PNGase) or (B) incubated with N-glycosidase F (+PNGase), as described in Methods. PrP\textsuperscript{C} was detected by Western blotting with biotinylated 4F2 MAb. Lanes SM and Lu were loaded with PrP\textsuperscript{C} immunoprecipitated from the same amount of tissue extract, while lane Br represents PrP\textsuperscript{C} obtained from a third tissue equivalent of brain than skeletal muscle and lungs. Note that the nonglycosylated PrP\textsuperscript{C} isoform in the lungs migrated with a reduced molecular mass.

tissues varied between individual sheep. Given the low number of sheep tested in the present work, we could not determine whether there is any relationship between animal genotype for the PrP locus and the tissue expression of PrP\textsuperscript{C}. Interestingly, in all organs from which PrP\textsuperscript{C} could be immunoprecipitated, bands of either bi- or hyperglycosylated isoforms were clearly detected. Nevertheless, a specific PrP\textsuperscript{C} isoform signature was systematically observed in the skeletal muscle (from either the thigh or the flank): two bands migrating more slowly than the brain mono- and biglycosylated isoforms (Fig. 5). These two bands correspond to PrP\textsuperscript{C} glycoforms, as deglycosylation resulted in one band migrating at the same level as that of the nonglycosylated protein obtained from the brain. However, differences in the composition and length of the oligosaccharide chains present on PrP\textsuperscript{C} could differ in the brain and skeletal muscle, and this could explain the difference in migration observed between the mono- and biglycosylated isoforms from these two tissues. Furthermore, we have shown that the nonglycosylated form obtained from the lungs migrated faster than its equivalents from the brain and skeletal muscle. Whether this is the result of N- or C-terminal truncation is not yet known. Indeed, a PrP isoform truncated at the C-terminal has been observed in mature human and bovine sperm (Shaked et al., 1999).

We should note here that pieces of skeletal muscle were removed with careful attention to avoid any residual nervous fibres. However, some neuromuscular junctions probably remained. Several arguments indicate that the typical muscular profile described here really belongs to muscle cells. PrP\textsuperscript{C} has indeed been detected in developing mouse muscle cells (Brown et al., 1998). Furthermore, PrP\textsuperscript{C} has been immunolocalized at the subsynaptic sarcoplasm of the neuromuscular junction of mammalian muscles (Gohel et al., 1999; Askanas et al., 1993). PrP\textsuperscript{C} has also been detected by immunoblotting in skeletal muscle from hamster (Bendheim et al., 1992) and in quadriceps muscle homogenates from non-transgenic mice (Westaway et al., 1994). Overexpression of hamster PrP\textsuperscript{C} in homozygote Tg(ShaPrP\textsuperscript{C}+/+)7 uninfected older mice resulted in spontaneous degeneration of the central nervous system (CNS) and skeletal muscle neuromyopathy (Westaway et al., 1994). Altogether,
these results underline a potential role of PrP\(^C\) in skeletal muscle cells.

The tissue distribution and developmental expression of sheep PrP mRNA has been published by Goldmann et al. (1999). No correlation between mRNA and protein levels can, however, be established. Altogether, these data reflect either a tissue-specific regulation of mRNA initiation, translation and/or stability, or a tissue-specific PrP\(^C\) catabolism control.

Our finding supports the idea that organs for which infectivity has been demonstrated (tonsil, thymus, intestine and spleen) do not contain more PrP\(^C\) than tissues shown not to support infection (skeletal muscle and heart) (Hadjow et al., 1979; Danner, 1993). Thus, like the ‘species barrier’, a ‘tissue barrier’ phenomenon for infection might also exist. Several hypotheses could be proposed: (i) it is possible that physical and/or physiological barriers may preclude the infectious agent from reaching some tissues, (ii) the existence of co-factors necessary for the achievement of PrP\(^C\) conversion in tissues that support infection or the presence of inhibitory molecules in uninfected organs could also explain this discrepancy, and (iii) a possible relationship between the biochemical features of PrP\(^C\) (e.g. glycoform diversity among tissues, unidentified tissue-specific PrP\(^C\) post-translational modifications and tissue differences in PrP clearance) and its susceptibility to be converted into the scrapie form still remains an open question. One of the intriguing phenomena in sheep is the dependence on the PmP locus genotype for susceptibility to scrapie (Bossers et al., 1996; Hunter et al., 1996). It has been demonstrated that animals which are highly susceptible to scrapie (homozygote VRQ/VRQ at positions 136, 154 and 171) accumulate PrP\(^S\) in the lymphoid tissue at early stages of the disease (Schreuder et al., 1996, 1998; van Keulen et al., 1996; Andreoletti et al., 2000). Later on, PrP\(^S\) is detected in the CNS. This sequence of PrP\(^S\) appearance is not found in sheep that are moderately susceptible to scrapie (heterozygote ARR/VRQ), where PrP\(^S\) deposits are observed only in the CNS. These results indicate that the nature of the protein itself might play the role of a ‘physiological bolt’ to control the conversion events.

In conclusion, the development of such biochemical and quantitative studies for tissue distribution of PrP\(^C\) might allow important advances in understanding the biochemistry and function of this protein. Furthermore, these studies should be combined with the immunocytochemical localization of PrP\(^C\). The latter point will precisely indicate which cell type expresses PrP\(^C\) in a given organ. It will be interesting then to look at the accumulation of PrP\(^S\) at the cellular level during the course of infection. This might aid the development of other pre-clinical diagnoses for TSE.

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