Abnormal prion protein in genetically resistant sheep from a scrapie-infected flock

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The central molecular event in transmissible spongiform encephalopathies, such as scrapie in sheep, is the accumulation in tissues of an abnormal isoform of the cellular prion protein. A previous investigation of 26 sheep showed that the accumulation of PrPres in brain correlated more with the prnp genotype than with the severity of the clinical disease. Here, the ability of a sandwich ELISA to detect PrPres distribution in the brain was demonstrated. Immunohistochemistry also strongly supported the hypothesis that the dorsal motor nucleus of the vagus nerve is the possible entry site in the brain for the scrapie agent. Remarkably, three asymptomatic (or possibly asymptomatic for scrapie) sheep carrying an allele known to be associated with clinical scrapie resistance (ARR), which were negative for the detection of PrPres by Western blotting and immunohistochemistry, were positive for the presence of PrPres by ELISA, raising the possibility of carriers resistant to the disease and possibly contributing to the persistence of scrapie in certain flocks.

Scrapie is a neurodegenerative disease that naturally affects sheep and goats (Pattison, 1988; Zlotnik, 1958). It belongs to the group referred to as transmissible spongiform encephalopathies (TSEs), or prion diseases, along with bovine spongiform encephalopathy (BSE) in cattle and Creutzfeldt–Jakob disease in humans (reviewed by Prusiner, 1997). The infectious agent responsible for these diseases has been termed a prion, and is thought to be composed entirely of a pathological form (PrPSc) of a host-encoded protein – the prion protein (PrPC) – although infectivity has also been demonstrated without detectable PrPSc accumulation in some cases (Lasmézas et al., 1997; Manuelidis et al., 1997). So far, PrPSc is the only specific molecular marker known for TSEs, and differs from its physiological isofrom by its insolubility in detergents and its partial resistance to proteases (PrPres). In natural scrapie, the accumulation of PrPSc has been reported in numerous tissues including the nervous system, lymphoid organs and placenta (Andréolletti et al., 2000, 2002; Foster et al., 1996; Hardt et al., 2000; Heggebo et al., 2002; Lezmi et al., 2001; Miller et al., 1993; Onodera et al., 1993; Race et al., 1998; Ryder et al., 2001; Schreuder et al., 1996). The current view is that the scrapie agent first replicates in lymphoid organs before reaching the central nervous system (van Keulen et al., 2000), as has also been shown in rodent models (Lasmézas & Coll, 1996). However, the age at which clinical signs develop in sheep is variable, and is at least partly determined by the primary sequence of the prion gene, which has also been shown to influence strongly the susceptibility of sheep to the disease (Belt et al., 1995; Bossers et al., 1996; Clouscard et al., 1995; Goldmann et al., 1994; Hunter, 1997; Hunter et al., 1997; Westaway et al., 1994).

Using Western blotting, we previously studied 26 sheep from one scrapie-infected flock (Madec et al., 2000) and demonstrated that PrPres levels in the brain varied according to their prnp genotype. All sheep sampled were 2 years of age, the only group showing symptoms in this flock at the time of this study. In the present work, we used immunohistochemistry (IHC) and a sandwich ELISA to investigate 23 of these 26 sheep further (Table 1). Initially, we considered a subset of 11 animals, all with detectable PrPres in the brain, as detected by Western blotting, which all carried at least one VRQ allele.

Following the same procedure as described previously (Bencsik et al., 2001a, b; Lezmi et al., 2001), specific PrPSc immunolabelling was found in the brainstem of all 11 sheep that were positive by Western blotting. PrPSc deposits were mostly found in the neuropil, typically in the dorsal motor nucleus of the vagus nerve (DMNV), and in the reticular formation, outlining the cell membrane of the cell body of neurons (Foster et al., 1996; Gonzalez et al., 2002; Hardt et al., 2000; Miller et al., 1993; Ryder et al., 2001; van Keulen et al., 1995). Outside the DMNV, but within the same obex sections, significant PrPSc accumulation was also observed in most structures for 9 of 11 sheep, but no staining or only very faint staining was detected for the two remaining sheep, even though these two animals (S9 and S14) were of very different clinical status. This latter observation strongly underlines the fact that severe clinical disease does not...
PrPres in sheep tissues, as reported elsewhere (Bennion & et al., 2002). The PrP res load was highest in the cerebellum, as reported previously (Ersdal et al., 2003), and the PrP res distribution in brain appeared to be highly consistent with that previously detected using Western blotting (Madec et al., 2000), showing a similar gradient in the levels of PrP res in positive animals from the cerebellum to the frontal cortex (Begara-McGorum et al., 2002; Gonzalez et al., 2002; Ligios et al., 2002; Wood et al., 1997; Zlotnik, 1958).

Next, we investigated the 12 sheep from the same flock (Table 1) that had previously had negative results for the detection of PrP res in Western blots (Madec et al., 2000), including sheep with possible early symptoms of scrapie (S18 and S22) and asymptomatic sheep (S15–17, S19–21 and S23–26). Two had the susceptible allele VRQ, but in both cases this was with the resistant ARR allele. Overall, 9 of these 12 sheep carried this ARR allele.

All 12 sheep were diagnosed as negative for the detection of PrP res by IHC. However, four animals were positive for the detection of PrP res in the cerebellum by ELISA, and two were also positive by ELISA for detection of PrP res in the brain stem and/or mesencephalon (giving a specific signal 2–5 times greater than the cut-off level in three separate experiments). All positive results were obtained using pure 20% homogenates and negative results were obtained at a 1:10 dilution, thus indicating low levels of accumulated PrP res. Two of these four sheep, including one VRQ/ARR sheep (S18), had been classified as having possible symptoms (Madec et al., 2000), including some wool loss and emaciation but no trembling, hyperexcitability or paresthesias. However, since marked clinical signs can be associated with a low amount of brain PrP res (as in sheep S9), it was reasonable to assume that a less obvious clinical status might also be associated with a very low level of PrP res accumulation, possibly only detectable by ELISA. By comparison, the two animals in which PrP Sc was only found in the DMNV (S9 and S14, see above) by IHC displayed OD values by ELISA at least 2.5-fold higher than those obtained for the two IHC-negative sheep with possible symptoms (S18 and S22). These results suggested that ELISA may have a higher sensitivity than IHC for the detection of PrP res in these sheep and under these experimental conditions, whereas Western blotting and IHC may have a comparable sensitivity, as also suggested by other studies (Jeffrey et al., 2002).

Table 1. Detection of PrP res in sheep brain using Western blotting, IHC and ELISA

Results were classified with regard to the prnp genotypes of the animals and their clinical status at the time of sacrifice, as defined previously (Madec et al., 2000). Three different combinations (+/+/+, −/−− and −/+−) were observed for the results obtained using Western blotting, IHC and ELISA, respectively (+, PrP res-positive sheep; −, PrP res-negative sheep). Genotypes are given using the single-letter code for amino acids corresponding to codons 136, 154 and 171 of the prnp gene, respectively.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Sheep</th>
<th>No. sheep</th>
<th>Clinical status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Highly affected</td>
</tr>
<tr>
<td>VRQ/VRQ</td>
<td>S4, S5</td>
<td>2</td>
<td>+/+/+</td>
</tr>
<tr>
<td>VRQ/ARQ</td>
<td>S6, S7, S8</td>
<td>3</td>
<td>+/+/+</td>
</tr>
<tr>
<td>VRQ/ARQ</td>
<td>S12, S13, S14</td>
<td>3</td>
<td>+/+/+</td>
</tr>
<tr>
<td>VRQ/ARH</td>
<td>S9</td>
<td>1</td>
<td>−/−−</td>
</tr>
<tr>
<td>VRQ/ARH</td>
<td>S10, S11</td>
<td>2</td>
<td>−/+/+</td>
</tr>
<tr>
<td>ARQ/ARQ</td>
<td>S15</td>
<td>1</td>
<td>−/−−</td>
</tr>
<tr>
<td>ARQ/ARH</td>
<td>S16</td>
<td>1</td>
<td>−/−−</td>
</tr>
<tr>
<td>ARQ/ARH</td>
<td>S17</td>
<td>1</td>
<td>−/−−</td>
</tr>
<tr>
<td>VRQ/ARR</td>
<td>S18</td>
<td>1</td>
<td>−/−/+</td>
</tr>
<tr>
<td>VRQ/ARR</td>
<td>S19</td>
<td>1</td>
<td>−/−/−</td>
</tr>
<tr>
<td>ARQ/ARR</td>
<td>S20, S21</td>
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<td>−/−/−</td>
</tr>
<tr>
<td>ARH/ARR</td>
<td>S22</td>
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</tr>
<tr>
<td>ARH/ARR</td>
<td>S23</td>
<td>1</td>
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</tr>
<tr>
<td>ARH/ARR</td>
<td>S24, S25, S26</td>
<td>3</td>
<td>−/+−</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>23</td>
<td></td>
</tr>
</tbody>
</table>

necessarily correlate with high PrP Sc deposition in the brain, as has been reported previously (Gonzalez et al., 2002). In addition, these data reinforced the hypothesis of the involvement of the DMNV in the very early stages of neuro-invasion (van Keulen et al., 1996, 2000).

PrP res was purified from the brain of all 11 sheep, following the protocol specified in the Bio-Rad BSE purification kit, and analysed by a sandwich ELISA using mAbs SAF-34 as the primary antibody (Demart et al., 1999) and 12F10 as the secondary antibody (Krasemann et al., 1996, 1999). The four brain sites previously found to be positive in Western blots in these 11 sheep (cerebellum, brain stem, mesencephalon and frontal cortex) were also positive for ELISA, confirming the suitability of the sandwich ELISA for the detection of PrP res in sheep tissues, as reported elsewhere (Bennon & Daggett, 2002). The PrP res load was highest in the cerebellum, as reported previously (Ersdal et al., 2003), and the PrP res distribution in brain appeared to be highly consistent with that previously detected using Western blotting (Madec et al., 2000), showing a similar gradient in the levels of PrP res in positive animals from the cerebellum to the frontal cortex (Begara-McGorum et al., 2002; Gonzalez et al., 2002; Ligios et al., 2002; Wood et al., 1997; Zlotnik, 1958).
asymptomatic for scrapie) and ELISA-positive animals carried the ARR allele (Table 1). The ARR allele is associated with a protective effect against, if not resistance to, clinical scrapie. Our results thus raise some concern that PrPSc can be found not only in preclinical stages of the disease in susceptible animals, but also in relatively resistant animals. It is generally considered that ARH/ARR animals are either not expected to develop scrapie during their life span or that they will develop the disease only after 70 months (Andréoletti et al., 2000; van Keulen et al., 1996). Therefore, these findings suggest that scrapie-infected sheep with genetic resistance may behave as healthy carriers or as only very slightly affected animals in flocks. The possibility of a carrier state has already been suggested in certain rodent models (Collis & Kimberlin, 1985; Race & Chesebro, 1998; Race et al., 2001) and this could help to explain the persistence of the natural disease in some cases. It may also have significance for breeding and lambing management programmes for the eradication of scrapie.

It is not known whether both PrPs encoded by the ARH and ARR alleles or only the PrP encoded by the most susceptible alleles (ARH or VRQ) are converted into a pathological form in these sheep during the natural process of scrapie infection. It is notable that the sheep PrP encoded by the ARR allele was successfully converted into its pathological counterpart in experimental BSE challenge of sheep (Houston et al., 2003). A recent study also reported that some atypical ELISA-positive sheep remained negative using either Western blotting or, in some cases, IHC (Buschmann et al., 2003), and this work also included a number of sheep carrying the ARR or AHQ allele associated with a relatively high resistance to clinical scrapie. Similar observations were also made by another group in sheep with so-called Nor98 scrapie carrying the AHQ allele, displaying clinical signs of scrapie (Benestad et al., 2003). The data presented here on asymptomatic animals with partial genetic resistance to the disease reinforce the possible association between these alleles and an unusual biochemical behaviour or low levels of PrPSc, according to the method used for its identification. Attempts to transmit the disease to ovine-transgenic mice, which have been shown to be more susceptible to scrapie than conventional rodents (Crozet et al., 2001; Vilotte et al., 2001), will help to determine the potential infectivity of these samples.

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References


