Effect of Sinc genotype, agent isolate and route of infection on the accumulation of protease-resistant PrP in non-central nervous system tissues during the development of murine scrapie

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Introduction

Scrapie is a fatal neurodegenerative disease of sheep and goats. It is the best characterized of a group of transmissible spongiform encephalopathies (TSEs) which includes Creutzfeldt-Jakob disease (CJD) and bovine spongiform encephalopathy (BSE). These diseases have a long asymptomatic incubation period followed by a short clinical phase. Conventional infectious disease markers such as viruses, foreign nucleic acid or specific host immune responses have not been identified. Diagnosis is dependent on clinical signs and brain histopathology postmortem. Gross pathology is confined to the central nervous system (CNS). The development of a preclinical, antemortem diagnostic test has been made a priority by the epidemiological and commercial implications of the outbreak of BSE in Britain and its potential risk to human health (Tyrrell, 1990). Although the nature of the infectious agent is unknown, a host-encoded protein, PrP, has recently been shown by PrP gene ablation to be essential for disease development and for replication of the agent (Bueler et al., 1993). PrP is a highly conserved sialoglycoprotein of Mr 33K to 35K (Chesebro et al., 1985; Manuelidis et al., 1985; Oesch et al., 1985; Hope et al., 1986). PrP extracted from uninfected animals (PrP0) is soluble in detergents such as N-lauroyl-sarcosine and is completely hydrolysed by proteinase K (Meyer et al., 1986). PrP mRNA is detected in neurons within the CNS (Kretzschmar et al., 1986; Manson et al., 1992a) and has been found in a number of other adult and embryonic tissues (Oesch et al., 1985; Manson et al., 1992a) as is the PrP protein (Brown et al., 1990; Cashman et al., 1990; Bendheim et al., 1992). A fraction of PrP in scrapie brain extracts, PrPsc, is sedimentable after treatment with N-lauroyl-sarcosine and exhibits partial resistance to proteolysis in non-denaturing conditions, being cleaved to a polypeptide of Mr 27K to 30K (Bolton et al., 1985; Hope et al., 1986, 1988a; Somerville et al., 1989). PrPsc is a biochemical marker for all the TSEs (Bolton et al., 1982; Prusiner et al., 1982; McKinley et al., 1983; Hope et al., 1988b). PrPsc fibrils are associated with high titres of infectivity (Diringer et al., 1983; McKinley et al., 1983; Somerville et al., 1986). Detection of PrPsc in a tissue biopsy creates the potential for a preclinical diagnostic test for the TSEs. Validation of such a test is dependent on establishing relationships between PrPsc and infectivity. PrPsc is found in rodent spleen and lymph nodes from terminally affected and preclinical animals (Rubenstein et al., 1986, 1991; Shinagawa et al., 1986; Doi et al., 1988; Kitamoto et al., 1989; Race & Ernst, 1992) and in the same tissues of scrapie-infected sheep (Ikegami et al., 1991; Mohri et al., 1992; Race et al., 1992). PrPsc has not
been detected in the peripheral tissues from clinical, BSE-affected cattle (Mohri et al., 1992) or from human cases of CJD (Kitamoto et al., 1989).

In experimental mouse models of scrapie the incubation period is controlled by several parameters, primarily the strain of scrapie, the host Sinc genotype (Dickinson et al., 1968) and the dose and route of infection (Outram, 1976). Although the gross pathology is confined to the CNS, infectivity also accumulates in some peripheral tissues particularly those of the lymphoreticular system (LRS) (Eklund et al., 1967; Fraser & Dickinson, 1970, 1978; Fraser et al., 1992). This study is the first to examine whether the parameters known to control infectivity also control PrPsc deposition. Selected tissues of individual Sinc congenic mice infected intra-cerebrally (i.c.) or intra-peritoneally (i.p.) with either of two strains of scrapie were screened for PrP by immunoblotting.

The Sinc gene controls incubation period; we report here that Sinc also controls the time at which PrPsc can first be detected both intra- and extra-neurally. The time post-injection at which PrPsc is first seen in peripheral organs is also dependent on the infecting strain of scrapie and on the route of inoculation.

Methods

Scrapie mice. Animals were injected i.c. or i.p. with a 1% (w/v) homogenate (0.02 ml) of brain from a terminal case of mouse scrapie. Two inbred strains of mice, VM/Dk (Sincsc) and VM (Sincse) congenics (Bruce et al., 1991; Hunter et al., 1992) and their F1 cross (Sincscse), were inoculated with two scrapie strains, ME7 and 22A (Dickinson & Meikle, 1969). The ME7 strain of scrapie had been serially passed through Sincsc mice and the 22A strain through Sincse mice. VM/Dk mice were infected i.c. with a 1% (w/v) homogenate, and IM/Dk mice (also Sincsc) were infected with a 10% (w/v) homogenate of 87V scrapie brain (Bruce et al., 1976). The incubation period data are given in Table 1.

Animals were sacrificed by cervical dislocation and their tissues were frozen in liquid nitrogen before storage at −70 °C. Brain, spleen, pancreas, submaxillary glands, thymus, and pooled subcutaneous and cervical lymph nodes were collected from each animal. Organs were also collected from uninfected and normal brain homogenate-injected, age-matched, control animals.

Preparative method for PrPsc. Frozen tissues were weighed and pulverized in pre-cooled Potter homogenizers, then homogenized in 0.2 M-potassium chloride (2 ml) with 10 µl of each of the protease inhibitors, 100 mM-PMSF and 100 mM-E-ethylmaldehyde (NEM), both in propsan-1-ol. They were then centrifuged at 2000 g for 10 min at 4 °C. The supernatants were decanted and centrifuged for 30 min at 100000 g for 4 °C. The resultant pellets were resuspended in 2 ml of 100 mM-Tris-HCl at pH 7.4 and the suspension was divided into two equal parts. Twenty µl of 20 mg/ml proteinase K (Sigma) was added to one tube and the contents were incubated with shaking at 37 °C for 60 min; the other tube was held at 4 °C. One ml Sarkosyl (2%), 20 µl of PMSF and NEM, and 2 µl 2-mercaptoethanol were added to each tube before incubation at 37 °C for 60 min with shaking. The contents of each tube were then layered onto a cushion of 20% sucrose in 50 mM-Tris-HCl pH 7.4 and centrifuged for 2 h at 200000 g at 4 °C. Supernatants were precipitated with three volumes of 2% acetic acid in ethanol at 4 °C. Both supernatants and pellets were stored at −70 °C, prior to analysis.

Immunoblot detection of PrPsc. SDS-PAGE, electroblotting and immunostaining were carried out as previously described (Hope et al., 1988a; Farquhar et al., 1989). The tissue weights of most organs vary throughout pathogenesis depending on the model used (Outram, 1972; Carp et al., 1984). They can increase or decrease dramatically; this is at least partly due to behavioral changes in eating and drinking particularly during the clinical phase. As tissues are affected differently and at different times depending on the model, samples were run routinely as 50% of the total tissue wet weight. In some experiments doubling dilutions, starting from extracts of 25% of the initial tissue weight, were carried out to assess the relative amounts of PrPsc by semi-quantitative immunoblotting. Anti-mouse PrP serum (Farquhar et al., 1989) was used at a dilution of 1 in 1000.

Results

PrPsc detection in brain

PrPsc was detected in brain extracts from terminally affected animals from all the models investigated, made using the same preparative technique as for peripheral tissues. It was detected in individual brain extracts from Sincse mice infected with ME7 scrapie 32 days after i.c. injection. Immunoreactivity was seen in the equivalent of 0.4% (2 mg) of a brain (Fig. 1). Later in the incubation period, when infectivity has reached a plateau, PrPsc was demonstrated from the equivalent of less than 1 mg of brain tissue. No PrPsc was detected in up to 25% (110 mg) of brain tissue at 10 and 21 days post-infection (p.i.).

Based on the amount of PrPsc extracted from terminal-case brain from this model the sensitivity of immunoblot detection is of the order of 1 to 10 ng of PrPsc (Hope et al., 1988a; D. Armstrong, personal communication). The recovery of PrPsc from brain using the peripheral extraction procedure reported here is an order of magnitude less than with the CNS extraction method. The CNS extraction method is not appropriate for extraneural tissues.

Host and agent range of PrPsc detection in LRS tissues from terminally affected animals

PrPsc was detected on immunoblots of extracts of lymph node (Fig. 2) and spleen (Fig. 3) from individual, terminally affected, Sincsc, Sincse and Sincscse mice after i.e. infection with the scrapie strains ME7 or 22A. It exhibited a four band pattern, between Mr 20K and 30K, after reaction with anti-mouse PrP serum (Farquhar et al., 1989). PrPsc was not detected in sedimanted fractions from uninfected controls.

Supernatant fractions from spleens and lymph nodes
from terminally affected, ME7-, 22A- and 87V-infected animals contained proteinase K-sensitive PrP (PrPsc), as did this fraction from uninfected control tissue. Immunoreactivity was not seen with preimmune serum (data not shown).

After doubling dilution, PrPsc was easily identifiable on immunoblots with preparations equivalent to 3% (1 mg) of spleen or pooled lymph nodes from terminally affected animals. These titrations were not taken to their endpoints.

**Kinetics of accumulation of PrPsc in LRS tissues early in incubation**

In a short incubation model (Table 1), Sinc7 mice i.c. infected with ME7 either 10 or 21 days previously, no PrPsc was found in extracts from up to 50% (30 mg) of spleen or 50% (40 mg) of pooled lymph nodes. Shortly afterwards, from 27 or 32 days after injection, and at all time points thereafter, PrPsc was detected in spleen and in lymph nodes from individual mice (Fig. 1). A rapid, but variable, accumulation of PrPsc is indicated by the results of semi-quantitative immunoblotting. For example, spleens from two animals sacrificed 32 days p.i. showed an eightfold difference in amounts of PrPsc, whereas their lymph nodes showed only a twofold difference.

PrPsc was found consistently in extracts of thymus only after 88 days p.i. (Fig. 4) although one 50 day p.i. sample was positive.

**Extraneural deposition of PrPsc outside the LRS**

Extracts from the pancreas of terminally affected Sinc7, Sinc7 and Sinc77 mice infected with ME7 or 22A, and from preclinical mice whose lymph node preparations
Fig. 2. Immunoblot analysis of 7.5 to 20% gradient SDS-PAGE-separated proteins from the SAF fraction of pooled lymph nodes from individual clinically affected animals. Anti-PrP serum was used at a dilution of 1/1000. Each lane represents 25% of the starting wet weight (mg). Lanes 1 and 2 Sin<s>7</s> with ME7 (19 mg) and lanes 3 and 4 with 22A (12 mg); lanes 5 and 6 Sin<p>7</p> with 22A (18 mg) and lanes 7 and 8 with 87V (5 mg). Samples in lanes with odd numbers were not proteinase K-treated; those in even-numbered lanes were proteinase K-treated. M<sub>r</sub> standards are indicated on the left.

Smaller amounts were not tested. PrP<sub>sc</sub> was detected at all time points examined from 32 days p.i. onwards. An immunoblot from a preparation equivalent to 3% (only 4 mg) of starting tissue gave a positive signal from a terminally affected animal.

Although PrP<sub>sc</sub> was present on immunoblots of submaxillary gland samples from the 27 days p.i. time point, thereafter there were more negative than positive samples even though these individual mice had accumulated PrP<sub>sc</sub> in other tissues (Fig. 4). The immunoblot pattern of PrP reactivity both before and after proteinase K digestion closely resembled that of the brain, spleen and lymph nodes.

Effect of Sin genotype

The results from Sin<s>7</s> mice infected with ME7 have been given above. In Sin<sup>p7</sup> mice infected with ME7 (a longer incubation period model), protease-resistant PrP was not detected in 50% spleen preparations equivalent to up to 16 mg of tissue from time points between 55 and 98 days after injection (Fig. 4). However, PrP<sub>sc</sub> was present in a sample of 3% (2 mg) of spleen tissue from preclinical mice between 111 and 300 days p.i. and from 1.5% (0.25 mg) of spleen from terminally affected mice. From 14 spleens taken from 111 days p.i. onwards only one preparation (at 181 days p.i.) was negative. PrP<sub>sc</sub> was present in all lymph node samples tested from 86 days p.i. Thus, by changing the mouse Sin genotype to Sin<p>7</p>, there is a 60 to 80 day delay in the first detection of PrP<sub>sc</sub> in peripheral tissues after i.c. infection with ME7.

With Sin<s>7p7</s> (F1) mice infected with ME7 (Fig. 5) lymph node samples were first positive for PrP<sub>sc</sub> from 63 days p.i. and spleen and pancreas samples from 82 days p.i. This timing is intermediate between those of the two parental strains.

Table 1. Incubation periods for murine scrapie models

<table>
<thead>
<tr>
<th>Scapie strain</th>
<th>Sin genotype</th>
<th>Route of inoculation</th>
<th>Incubation period (days)</th>
<th>Range (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME7</td>
<td>s7s7</td>
<td>i.c.</td>
<td>181 (1.6)*</td>
<td>154–212</td>
</tr>
<tr>
<td></td>
<td>s7s7</td>
<td>i.p.</td>
<td>300 (3.4)</td>
<td>276–346</td>
</tr>
<tr>
<td></td>
<td>p7p7</td>
<td>i.c.</td>
<td>359 (2.8)</td>
<td>325–379</td>
</tr>
<tr>
<td></td>
<td>s7p7</td>
<td>i.c.</td>
<td>256 (2.5)</td>
<td>240–280</td>
</tr>
<tr>
<td>22A</td>
<td>s7s7</td>
<td>i.c.</td>
<td>461 (3.1)</td>
<td>397–518</td>
</tr>
<tr>
<td></td>
<td>p7p7</td>
<td>i.c.</td>
<td>216 (2.4)</td>
<td>180–277</td>
</tr>
<tr>
<td></td>
<td>p7p7</td>
<td>i.p.</td>
<td>345 (9.8)</td>
<td>315–364</td>
</tr>
<tr>
<td></td>
<td>s7p7</td>
<td>i.c.</td>
<td>533 (3.1)</td>
<td>439–551</td>
</tr>
<tr>
<td>87V</td>
<td>p7p7</td>
<td>i.c.</td>
<td>315 (1.3)</td>
<td>290–337</td>
</tr>
<tr>
<td></td>
<td>p7p7</td>
<td>i.c.†</td>
<td>272 (2.2)</td>
<td>269–283</td>
</tr>
</tbody>
</table>

* Values in parenthesis, S.E.M.
† 10% (w/v) brain homogenate inoculum.

were positive, contained PrP<sub>sc</sub> (Fig. 4 and 5). For individual Sin<s>7</s> mice infected with ME7 (the short incubation period model) up to 25% (115 mg) of tissue was negative for PrP<sub>sc</sub> at 21 days, but at 32 days PrP was detected (Fig. 1) in extracts from 3% (18 mg) of tissue.

Fig. 3. Immunoblot analysis of individual spleens from clinically affected animals. Lanes 1 and 2 represent 50% and lanes 3 to 8 25% of the starting tissue wet weight. Lanes 1 and 2, Sin<s>7</s> (8 mg), 3 and 4 Sin<p>7</p> (8 mg), 5 and 6 Sin<sup>p7</sup> (4 mg) infected with ME7 and 7 and 8 uninfected Sin<sup>s7</sup> mice (8 mg). Samples in lanes with odd numbers were not proteinase K-treated; those in even-numbered lanes were proteinase K-treated.

Effect of Sin genotype

The results from Sin<s>7</s> mice infected with ME7 have been given above. In Sin<sup>p7</sup> mice infected with ME7 (a longer incubation period model), protease-resistant PrP was not detected in 50% spleen preparations equivalent to up to 16 mg of tissue from time points between 55 and 98 days after injection (Fig. 4). However, PrP<sub>sc</sub> was present in a sample of 3% (2 mg) of spleen tissue from preclinical mice between 111 and 300 days p.i. and from 1.5% (0.25 mg) of spleen from terminally affected mice. From 14 spleens taken from 111 days p.i. onwards only one preparation (at 181 days p.i.) was negative. PrP<sub>sc</sub> was present in all lymph node samples tested from 86 days p.i. Thus, by changing the mouse Sin genotype to Sin<p>7</p>, there is a 60 to 80 day delay in the first detection of PrP<sub>sc</sub> in peripheral tissues after i.c. infection with ME7.

With Sin<s>7p7</s> (F1) mice infected with ME7 (Fig. 5) lymph node samples were first positive for PrP<sub>sc</sub> from 63 days p.i. and spleen and pancreas samples from 82 days p.i. This timing is intermediate between those of the two parental strains.
Effect of scrapie strain

The effect of scrapie strain on the kinetics of extraneural PrP deposition in the same mouse Sinc genotype can be seen by comparing Sinc<sup>87</sup> mice infected with ME7 or 22A (Fig. 4). As reported above, PrP<sup>sc</sup> was identified in lymph node and pancreas extracts from 27 days p.i. with ME7, and spleen from 32 days p.i., but no PrP<sup>sc</sup> was found in samples of all three tissues taken up to 87 days p.i. from 22A-infected individuals. From 192 days p.i. the only spleen, lymph node and pancreas extracts that were negative were samples representing 25% rather than 50% of the tissue weight and this may indicate the limits of detection with this technique.

In Sinc<sup>87</sup> mice infected with 22A, PrP<sup>sc</sup> was detected in spleen, lymph nodes and pancreas from approximately 100 days p.i., although one animal had PrP<sup>sc</sup> in spleen, pancreas and submaxillary glands, but not in lymph
nodes, at 62 days p.i. Interestingly, tissues from the same genotype infected with ME7 gave PrP\textsuperscript{Sc} -positive immunoblots from approximately 100 days p.i., although the incubation period is 150 days longer.

As reported above, in \textit{Sinc}\textsuperscript{Sp7} mice infected with ME7 (Fig. 5), the timing of PrP\textsuperscript{Sc} detection was intermediate to those of the two parental strains. However, with 22A, PrP\textsuperscript{Sc} was not detected until a time in excess of that of the longer incubation period parental strain, from 238 days p.i. for lymph node, pancreas and submaxillary glands and from 251 days p.i. from spleen. In this model although lymph node samples were consistently positive, a few spleen extracts were negative late in the incubation period (at 308 and 440 days p.i.).

In all the above models PrP\textsuperscript{Sc} was found outside the CNS in all clinical cases and to different degrees preclinically. However PrP\textsuperscript{Sc} was not found in spleens, lymph nodes or pancreas from \textit{Sinc}\textsuperscript{Sp7} mice injected with the standard dose (1%) of 87V scrapie (Fig. 2), even when they were clinically affected. It was present in their brains. PrP\textsuperscript{Sc} was identified from spleens, but not from lymph nodes, taken from terminally affected \textit{Sinc}\textsuperscript{Sp7} mice inoculated with a 10% (w/v) brain homogenate. PrP\textsuperscript{Sc} was present in pancreas and submaxillary gland extracts from 41 days p.i., when the single spleen tested at this time was negative. Spleen and lymph nodes were positive from 43 days p.i. There was a 40 day delay before PrP\textsuperscript{Sc} was found in brain extracts. In this series there was one anomalous individual mouse with negative spleen and lymph node samples 73 days p.i. This may indicate variation within pathogenesis. All the other peripheral tissues tested were positive from 43 days p.i., including all the extracts from pancreas and submaxillary glands. Thymus samples taken over a period from 10 to 62 days p.i. did not contain detectable amounts of PrP\textsuperscript{Sc}.

This pattern was repeated with 22A in VM/Dk mice (Fig. 6) after i.p. infection where PrP\textsuperscript{Sc} could be detected from 132 days in pancreas, 139 days in lymph node but not until 177 days p.i. in spleen. The individuals from which pancreas and lymph node preparations were first positive had no detectable PrP\textsuperscript{Sc} in their spleens. One animal culled at 177 days p.i. was PrP\textsuperscript{Sc}-negative for spleen, lymph node and pancreas. The first positive brain sample was found 240 days p.i., representing a delay of approximately 100 days between the accumulation of PrP\textsuperscript{Sc} in the periphery and its detection in the brain. Submaxillary gland extracts from this model displayed the same fluctuations in PrP\textsuperscript{Sc} detection as in the i.c. route models.

### Discussion

This study investigated the kinetics of PrP\textsuperscript{Sc} accumulation and whether the parameters that are known to control the development of disease and replication of infectivity also determine the timing and tissue location of this abnormally processed host protein. Host genetics, scrapie strain and route of inoculation were investigated using mice congenic for the \textit{Sinc} gene which controls the incubation period (Dickinson \textit{et al.}, 1968; Bruce \textit{et al.},
Two cloned scrapie strains, ME7 and 22A, were examined in mice of differing Sinc genotype because they have very different incubation period kinetics (Dickinson & Meikle, 1969). Intracerebral and i.p. inoculation routes were investigated to study the effects of different initial cellular events in the processing of infectivity (Outram, 1976).

The host/agent models used here were selected because of their very different incubation periods to maximize potential differences in the timing of PrPSc accumulation. Once it was established that PrPSc could be detected routinely in extracts from extraneural tissues from clinically affected experimental mice, a panel of tissues was examined from individual mice sacrificed at time points throughout the predicted incubation period. Organs were selected to represent the CNS, the LRS and organs outside the LRS. Submaxillary glands were chosen as an example of a tissue other than the LRS in which titre rises early (Eklund et al., 1967; Kimberlin & Walker, 1989). Pancreas was chosen because it is known to have a relatively low endpoint titre (Hadlow et al., 1982; Carp et al., 1989) and has never been implicated in pathogenesis. Emphasis was placed on the tissues of the LRS because of their importance in early pathogenesis not only in experimental murine models by any infection route but also in natural scrapie (Eklund et al., 1967; Fraser & Dickinson, 1970, 1978; Outram, 1976; Hadlow et al., 1982; Rubenstein et al., 1991; Fraser et al., 1992; Race & Ernst, 1992).

We report a simple subcellular fractionation technique that is applicable to all the selected tissues. In uninfected animals tissue weights vary with time and in some, but not all, scrapie models there is a substantial loss of tissue weight in terminally affected animals which makes quantitative comparisons of PrPSc on a tissue weight basis difficult (Outram, 1972; Carp et al., 1984). For this reason the presence of PrPSc was assessed by loading lanes with material equivalent to 50 or 25% of the total wet weight. The use of a high titre polyclonal rabbit antiserum gave a degree of sensitivity on immunoblotting which has allowed individual animals to be assayed for PrPSc. After proteinase K treatment immunoblots of all tissues displayed the same PrP polypeptide profile and Mr values as those found for brain (Hope et al., 1988a; Farquhar et al., 1989, 1990).

The investigation of a number of experimental murine scrapie models was an attempt to establish the generality of the findings. This is the first study to show that the timing and location of PrPSc accumulation varies with respect to Sinc, scrapie strain and route of inoculation, the parameters that control incubation period length.

Our results indicate that in most i.c. route models (ME7 in Sinc<sup>e</sup> and Sinc<sup>e</sup>v and 22A in Sinc<sup>e</sup> and Sinc<sup>e</sup>v) there is a delay between the detection of PrPSc in the LRS and the brain. In our shortest incubation model (Sinc<sup>e</sup>v mice i.e. infected with ME7), the delay could be only 5 days at most. This is in accord with infectivity studies, where even after i.c. infection, titre rises first in the spleen as most of the inoculum is rapidly dispersed from the CNS (Millson et al., 1979). Faster replication of the residual inoculum in the brain leads to shorter incubation periods than infection by peripheral routes (Dickinson & Fraser, 1969; Dickinson et al., 1969; Kimberlin & Walker, 1979). PrPSc in brain extracts from early time points is not thought to be due to residual inoculum as previous sampling points were negative.

In the short incubation period model (ME7/Sinc<sup>e</sup>v/i.c.), spleen PrPSc was detected 2 to 3 weeks after the titre is reported to start increasing, but in brain PrPSc was found before infectivity was measurable in cross-comparison with previous infectivity studies (Dickinson & Fraser, 1969; Dickinson et al., 1969). By changing the mouse genotype to Sinc<sup>e</sup>v, PrPSc detection in the LRS was delayed for a further 60 days. The timing in Sinc<sup>e</sup>v heterozygotes was intermediate to those of the two parental genotypes. This order is identical to that of their incubation period order, and may reflect not only the 1 month delay in the initiation of replication, but also the slower replication rate in the Sinc<sup>e</sup>v as compared with the Sinc<sup>e</sup>v genotype (Dickinson & Fraser, 1969; Dickinson et al., 1969; Kimberlin & Walker, 1989). Sinc is responsible for the major rate-limiting steps in murine scrapie pathogenesis; this work suggests it also determines when the abnormal processing of PrP<sup>c</sup> leads to PrP<sup>Sc</sup> accumulation both intra- and extra-neurally.

The timing of murine scrapie pathogenesis is also crucially dependent on the infecting scrapie strain. In contrast to the models described above, with 22A scrapie PrPSc was detected first in the LRS of Sinc<sup>e</sup>v mice, then in Sinc<sup>e</sup>v mice, with detection in the heterozygote even later. Again this order is identical to that of their respective incubation periods. The importance of the infecting strain was also seen in preliminary results from Sinc<sup>e</sup>v mice infected i.e. with a 1% homogenate of 87V-infected scrapie brain from a terminally affected animal. Although PrPSc was present in the extraneural tissues, as was the case for all animals tested whether infected or uninfected, no PrPSc was detected, even when the animals were clinically affected. This is despite replication in spleen occurring from very early in incubation and a plateau level being maintained until endpoint (Bruce, 1985). PrPSc was identified from spleens from the Sinc<sup>e</sup>v mice infected with a 10% homogenate of 87V brain, suggesting that in addition to the parameters described above the distribution of PrPSc is at least partly dependent on the infecting dose.

When and where PrP<sup>c</sup> was first detected was a reflection of the scrapie replication dynamics of that...
individual model, but the timing can not be predicted from the incubation period of the model.

Intracerebral infection was a more efficient way of initiating PrPsc accumulation in peripheral tissues than the i.p. route. This also correlates with the relative efficiency of infection by these two routes (Kimberlin & Walker, 1979). Intraperitoneal infection of Sinc77 mice with ME7 scrapie delayed the detection of PrPsc in the LRS by 2 weeks in comparison with i.c. inoculation, coinciding with infectious titre reaching a plateau (Dickinson & Fraser, 1969). There was a further 6 week delay before PrPsc was detected in brain. Very similar timing has been reported by Doi and colleagues for the Obihiro isolate of scrapie in Slc/ICR mice infected i.p. (Doi et al., 1988). However, the kinetics of PrPsc accumulation in our other i.p. infection model (22A in Sinc77 mice) were very different; PrPsc was not detected in spleen until approximately 20 weeks, and not in brain until approximately 30 weeks p.i. In another comparatively short incubation period model (139A in Sinc77 mice) after i.p. infection other workers report PrPsc fibrils in spleen within 2 to 3 weeks but none in brain until week 17 p.i., about 8 weeks after infectivity was detected (Merz et al., 1985; Rubenstein et al., 1991). Interestingly they did not find PrPsc fibrils in spleen until 50 days after i.c. infection.

An intact LRS facilitates entry of infectivity into the CNS after peripheral infection (Fraser & Dickinson, 1976, 1978; Kimberlin & Walker, 1989; Fraser et al., 1992) but PrPsc accumulation in both i.p. routed models occurs marginally earlier in pancreas and submaxillary glands than within the LRS. Although infectivity increases early in the submaxillary gland, it does so later than in the LRS with 139A scrapie (Ekland et al., 1967; Kimberlin & Walker, 1989) attaining much higher titres than are found in pancreas (Carp et al., 1989). We have yet to ascertain whether this is true for ME7 and 22A in our inbred Sinc congenics. Pancreas was consistently positive for PrPsc throughout incubation after the first detection whereas PrPsc was found only intermittently in submaxillary glands. No other tissue tested displayed such inconsistency. These results suggest either that PrPsc can be degraded in salivary glands or lost by export. Salivary gland is the only known experimental murine tissue where the titre has been reported to decrease substantially in the later stages of disease (Ekland et al., 1967), although this appears to be common in the extraneural tissues of naturally infected ruminants (Hadlow et al., 1982).

PrPsc accumulation may not be a good guide to the relative contributions tissues make in the peripheral phase of TSE pathogenesis. However, although it is unlikely that infectivity from pancreas plays much part in sustaining TSE infections, shedding of infectivity from salivary gland could establish secondary routes of pathogenesis within the gastrointestinal tract and could also be a source for horizontal spread, particularly in cattle which produce very large quantities of saliva. Such tissues may have the potential to act as reservoirs of infection.

Four published reports describe the kinetics of PrPsc accumulation after experimental scrapie infection in short incubation period models (Merz et al., 1985; Doi et al., 1988; Rubenstein et al., 1991; Race & Ernst, 1992). The data are disparate with respect to when and where PrPsc can first be detected even with comparable scrapie isolates (139A and Chandler) injected i.c. into Sinc77 mice. This may be due to their differing techniques and the use of spleens pooled from large numbers of random-bred mice (Rubenstein et al., 1991; Race & Ernst, 1992). Although ME7 and 139A are both short incubation period models their peripheral pathogenesis is different. ME7 titre increases earlier in spleen and lymph nodes, although 139A is more neuroinvasive, entering the CNS a month earlier (Kimberlin & Walker, 1979, 1988; Race & Ernst, 1992). This difference clearly extends to the dynamics of the cellular processing of PrPsc.

Our study supports the validity of using the presence of PrPsc in extraneural tissues as a diagnostic test for the TSEs. In some experimental models PrPsc can be detected by immunoblotting very early in the course of the disease, long before there is any gross pathology in the CNS and without the problem of differentiating PrPsc from subtle deposits of PrPsc at an immunohistochemical level.

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References


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